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USE OF HALLOYSITE FOR AGROCHEMICAL APPLICATIONS

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SCHOOL OF PHARMACY

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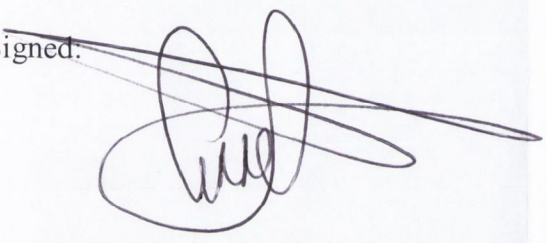

Anne Elizabeth Salter.

To my parents

To my sisters and brothers

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"There can not be no rainbow without a cloud and a storm"

Summary

The focus of this thesis was to develop two products for use in agrochemical applications, using the naturally occurring microtubular mineral, halloysite. Firstly, it was necessary to obtain a highly tubular grade of the mineral, which was achieved by sourcing a number of samples from different regions of the world. The halloysite samples were characterised using a number of techniques such as scanning electron microscopy, X-ray diffraction, helium pycnometry, surface area analysis and inductively coupled plasma-mass spectrometry. Various physical and chemical clean up procedures were applied to the mineral to remove debris and increase the proportion of intact tubules. From these studies it was found that the halloysite Goushan NZCC sample (provided by New Zealand China Clay Ltd.) had the highest tubular content.

Preliminary studies were performed on halloysite to ascertain whether it had potential for use in a number of different pharmaceutical and agrochemical applications that included: deflocculation studies, isolated film studies, tabletting studies and mercury porosimetry studies of pellets. Following these preliminary studies extensive work was carried out on the development of two agrochemical products.

The first agrochemical product developed was a pellet formulation used for vine weevil (*Otiorhynchus sulcatus*) control in primrose plants. Vine weevil attack a wide range of plants, resulting in considerable damage, which is of significant economic loss to commercial growers. The new sustained release chlorpyrifos pellets were cost effective and environmentally friendly in comparison to the commercially available *suSCon Green* pellets. Chlorpyrifos was loaded into the hollow internal lumen of the halloysite tubules to offer sustained release of the pesticide. Ethylene glycol was also loaded into the tubules to prevent premature decomposition of chlorpyrifos. The chlorpyrifos loaded halloysite was then formulated into pellets by extrusion-spheronization using liquid paraffin as a granulating liquid. A blue pigment was also incorporated into the pellets to aid uniform mixing in the peat mixes. The *suSCon Green* pellets gave the greatest sustained release of chlorpyrifos *in vitro*. However, an outdoor primrose pot

trial showed that the chlorpyrifos loaded halloysite pellets gave improved vine weevil control in comparison to the *suSCon Green* pellets over the time period of the trial.

The second agrochemical product developed was used for aphid control in barley crops. Aphids can cause stunted and distorted growth of flowers, shrubs and trees. Since aphids can move from one plant to another, they may transmit plant pathogens through their sucking mouthparts e.g. barley yellow dwarf virus (BYDV). Yield losses in cereal crops due to BYDV can be severe with considerable economic loss to the grower. A sustained release suspension concentrate of cypermethrin was thus developed. Halloysite was again utilised to give sustained release of the pesticide. The cypermethrin loaded halloysite suspension was successfully deflocculated using an anionic polyelectrolyte, *Dispex N40* (sodium polyacrylate), which prevented blocking of the sprayer. An adhesive agent, *Agrimer AL30* (poly(vinylpyrrolidone/1-eicosene)) was incorporated to ensure the cypermethrin loaded halloysite particles remained attached to the barley leaves for a prolonged period of time and also to prevent sedimentation. Finally, a suspending agent, xanthan gum was incorporated to aid anti-settling properties and enhance thixotropy. On dilution, the developed cypermethrin suspension concentrate remained attached to the leaves for a prolonged period of time and offered sustained release of the pesticide in comparison to the commercially available, emulsion concentrate, *Cyperkill*. Therefore frequent respraying of the crop was not necessary with the newly developed suspension concentrate. The cypermethrin loaded suspension concentrate gave improved sustained release of cypermethrin *in vitro*. Winter and spring barley field trials were carried out to ascertain which product gave improved control of aphids. However, the field trials did not show statistical difference between the two products.

Chapter 1

Introduction

1.1 ORIGIN AND SCOPE OF WORK

Halloysite is a naturally occurring aluminosilicate mineral, which is mined in several regions of the world. It is chemically similar to plate-like kaolin but differs morphologically in that it assumes a tubular structure. The surfaces are curved or rolled due to the misfit of the octahedral gibbsite-like sheets and the siloxane sheets (Frost *et al.*, 2000). Because of these lattice imperfections, the 'face' and 'edge' surfaces of the mineral normally carry a net negative and positive charge, respectively but it predominantly functions as a polyvalent anion. It is also capable of existing in a hydrated (endellite) and dehydrated state (metahalloysite), where kaolin only exists in a dehydrated state.

Because halloysite exists as a hollow tube, it has the unique ability to entrap active ingredients within the hollow internal lumen and also less extensively by chemisorption onto the surface, thereby providing a sustained release mechanism. The mineral's sustained release mechanism was firstly utilised for marine applications (Price and Gaber, 1997). Halloysite was used as a sustained chemical delivery system for antifouling agents, which were incorporated into a paint formulation and used for barnicle control on ships. From these findings Levis (2000) evaluated the mineral's potential for use in the pharmaceutical industry. This involved loading the halloysite tubules with cationic drugs (diltiazem HCl and propranolol HCl) and entrapping these drugs within the hollow lumen of the microtubules using retardant polymers, cationic coatings and other approaches to delay drug release. Kelly (2002) further developed this technology for use in two dental applications. The first product developed, involved extending the release of

tetracycline base by coating the drug loaded halloysite tubules with the cationic polymer chitosan, which were then incorporated into a thermoresponsive polymer system containing *poloxamer 407* for ease of application. This product was used in a clinical trial in dogs for the treatment of periodontal disease. It gave effective drug release over a six week period. Kelly (2002) also drug loaded halloysite tubules with sodium fluoride, which were incorporated into solid lipid microparticles to reduce the rate of drug release and then formulated into a bioadhesive bilayered tablet for fluoride supplementation.

However, despite the unique potential of halloysite as a sustained drug delivery system within the pharmaceutical industry, it needs regulatory approval. Also it is difficult to obtain a pharmaceutical grade of the material because it is a naturally occurring mineral with a high degree of batch to batch variability. From the above prior studies by my colleagues in the Department of Pharmaceutics and Pharmaceutical Technology, University of Dublin, it is the intention of this thesis to transfer the technology to a number of agrochemical applications. It was hoped that batch to batch variability would be less important in such applications and also regulatory approval would be easier to obtain for halloysite in the agrochemical industry.

The first product was developed for controlling vine weevil (*Otiorhynchus sulcatus*). At present chemical impregnated compost, *Intercept* (imidacloprid) and microencapsulated controlled release pellets, *suSCon Green* (chlorpyrifos incorporated in plastic resin) have proved to be the most effective products for controlling vine weevil. The two commercial products have a release period of approximately two years. It was hoped that halloysite could be used as a novel sustained delivery system for pesticides and then formulated into a pellet for ease of application to compost, to give vine weevil control for at least a 1 year period. Halloysite was loaded with chlorpyrifos, an organophosphate and formulated into pellets by extrusion-spheronization using liquid paraffin as the granulating liquid. These pellets were compared to the commercially available *suSCon Green* pellets *in*

vitro. An outdoor potted primrose trial was also performed using the two products for controlling vine weevil.

Secondly, a product was formulated to control aphids on barley crops. In Ireland aphids are usually controlled by spraying the infected crop with a dilution of an emulsion concentrate of a pyrethroid e.g. cypermethrin (*Cyperkill*). However the diluted emulsion concentrate version of pyrethroids can only give short term control of aphids as pyrethroids are subject to photolysis and are also easily washed off the cereal leaves by rain, which makes frequent respraying of the crop necessary. It was hoped that halloysite could again be utilised as a sustained delivery system and give prolonged release of the pesticide. Cypermethrin was loaded into halloysite and then formulated into a suspension concentrate. This was achieved by firstly deflocculating the pesticide loaded halloysite using a deflocculating agent, *Dispex N40* (sodium polyacrylate) to prevent blockage of the sprayer, then adding an adhesive agent, *Agrimer AL30* (poly(vinylpyrrolidone/1-eicosene)) to ensure the halloysite particles remained attached to the cereal leaves and to prevent sedimentation of particles. Finally a suspending agent, xanthan gum was added to also prevent sedimentation and enhance thixotropy. The commercially available emulsion concentrate, *Cyperkill* was compared to the newly formulated suspension concentrate *in vitro* and in a field trial to control aphids in a winter and spring barley crop. It was hoped the newly developed suspension concentrate of cypermethrin would give sustained release of the pesticide and remain on the cereal leaves for a longer period of time in comparison to the emulsion concentrate version of the pesticide.

1.2 HALLOYSITE

1.2.1 Introduction

Halloysite is a 1:1 type layer silicate being a member of the kaolin group of minerals (Levis, 2000). It contrasts markedly with the well-known pseudohexagonal plates of kaolinite [$Al_2Si_2O_5(OH)_4$], despite the chemical similarity of the two minerals. Unlike kaolinite, it is capable of existing in a hydrated (endellite) and dehydrated state (metahalloysite), the empirical formula of each being $Al_2Si_2O_5(OH)_4.2H_2O$ and $Al_2Si_2O_5(OH)_4$, respectively (Levis, 2000). Halloysite also differs from kaolinite in that it has a higher exchange capability and higher surface area (Harvey, 1996). According to Yuan and Murray (1997), it may be considered an extremely poor crystalline member of the kaolinite group. Alexander *et al.* (1943) and Bates (1971) showed that, unlike the platy structure of kaolinite, halloysite occurs in a number of different morphological forms.

Crystal structure, occurrence and mineralogical properties of long tubular halloysite have been reported by Honjo *et al.* (1954), Chukhrov and Zvyagin (1966), Parham (1969), Nagasawa and Miyazaki (1976), Kohyama *et al.* (1978), Noro *et al.* (1981), Churchman and Theng (1984) and Nagasawa and Noro (1985). Spherical and short tubular halloysite have been discussed by Sudo (1953), Sudo and Yotsumoto (1977), Tazaki (1979) and Churchman and Theng (1984). Kirkman (1977) described squat cylindrical and disk halloysite in rhyolitic tephra of New Zealand. Nagasawa and Karube (1975) reported ribbon-shaped halloysite in altered montmorillonite clay. Tazaki (1979) described that various morphologies of halloysite including spherical, walnut-shaped, acicular, crinkly, platy, tubular and square-tube were observed on the surface of altered plagioclase in volcanic ash. In addition, tabular halloysite was found in Texas soils by Kunze and Bradley (1964) and Carson and Kunze (1970). However, generally the most common morphology is that of the elongated hollow tubules (Dixon and McKee, 1974).

1.2.2 Worldwide sources of halloysite

The commercial development of high purity halloysite resources is restricted to New Zealand, South Korea, China, Turkey and perhaps Japan. The primary and largest source of halloysite deposits are found in New Zealand, where they are exploited by New Zealand China Clays Ltd. (a subsidiary company of Ceramco Corporation Ltd). These deposits were first discovered early in the 20th century but it was not until the 1960s that the systematic exploration and development program was carried out over various volcanic areas in the North Island. This led to the confirmation of these large deposits and several smaller deposits in the Coromandel region (Harvey, 1996). New Zealand China Clays Ltd. was established in 1969 to process the halloysite and develop both local and export markets in ceramics and other applications. Three commercially significant halloysite resources were confirmed and developed at Maturi Bay, Mahimahi and Maungaparerua. Currently 100% of the New Zealand halloysite is exported to make the world's finest porcelain and china creations due to its low iron and titanium content.

In Japan there are substantial deposits of halloysite in the Gaerome clays, which occur in Pliocene sediments in Seto (Aiche Prefecture) and Tajimi-Toki (Gifu Prefecture) areas in the vicinity of Nagoya, Central Japan. This area is one of the country's main centres of ceramic production. The Omura mine, to the north of Nagasaki in western Kyushu, produces material for refractories. In the north-western area of Kagoshima Prefecture in southern Kyushu, the Okuchi mine processes halloysite for paper manufacture. In the northern part of Gumma Prefecture, the Joshin mine exploits halloysite-bearing clays for use in refractories.

In South Korea a large number of deposits have been worked in the Sancheong, Danseong and Hadong areas within a region extending about 30 km north-south and 10 km east-west. There are many producers of halloysite in South Korea but the two largest are believed to be the Taemyung mine at Keumseo, Kyongsangnam and the Hankukyoeub mine at Samka, Kyongsangnam.

Other sources include the USA where the deposits are located 80 km north of Reno and Dragon mine in Utah (now closed). In France, Ste. Argiles Rares et Silice Industrielle du Perigord produces approximately 2,000 tonnes per annum of halloysite for use in ceramic porcelain. In the Czech Republic, halloysite is exploited from a deposit near Michalovce for the production of heavy clayware, refractories, ceramic insulators, paper and rubber filling. In the Philippines, halloysite of both weathering and hydrothermal origin are common components in many ceramics.

1.2.3 Formation of halloysite

Halloysite is the product of both subtropical or tropical weathering and hydrothermal alteration. During the weathering of rhyolitic tephras in New Zealand, volcanic glass altered to allophane, which on ageing crystallized to cylindrical halloysite (Kirkman, 1975). The rhyodacites were formed by either partial melting or differentiation of lower crustal material (Harvey, 1996). The volcanic heat has played a significant role in the formation of the halloysite deposits by providing a heat source for hydrothermal alteration. Kirkman (1977), observed that in New Zealand, allophane dominates clay fractions of tephras up to about 13,000 years of age, whereas the clay fractions of tephras between 13,000 and 42,000 years of age contain small but increasing amounts of halloysite. Similarly Aomine and Miyauchi (1963), reported that cylindrical halloysite had formed from allophane after 8000-9000 years in a buried soil on Mount Aso, Japan. Kirkman (1977), also observed that for rhyolitic tephras, most of the aluminium originally present in the allophane has been incorporated into the 1:1 lattice and that excess silica has been deposited as amorphous flakes. Farmer *et al.* (1979), showed that the extent to which halloysite is formed is dependent on concentration of silica in the soil and soil temperature.

1.2.4 Halloysite structure

1.2.4.1 Previous structural studies

Berthier described and named halloysite (metahalloysite) in 1826 and from that time until 1934 the mineral was regarded as an amorphous substance with no characteristic shape. An early worker (Mellor, 1908) described it in detail as irregularly shaped,

amorphous granules, with irregular surfaces and a sponge-like structure. Shaw and Humbert (1941) observed with an electron microscope that the mineral typically occurs as split rods and that the proposed crystal structure did not explain its observed features. In 1942, Kelley and Shaw assumed that halloysite (metahalloysite) particles were rod-shaped ellipsoids. In the same year, Kelley and Page (1942) described halloysite (metahalloysite) as "needle-like crystals". Bates *et al.* (1950) concluded that halloysite (metahalloysite) existed in the form of hollow tubes many of which had split longitudinally or collapsed to form laths or ribbons, which is the accepted structure to date.

1.2.4.2 Present concept and proposed structure

Figure 1.2a gives a diagrammatic picture of the structure of halloysite (endellite) proposed by Hendricks (1938). As in the other kaolin minerals, there is a 1:1 structure in which a modified gibbsite sheet is bonded to a silicon-oxygen sheet. In the mineral gibbsite, the six hydroxyl ions on one side of the unit cell occupy a distance of 8.62 Å, while in the silicon-oxygen sheet in kaolinite the corresponding six oxygen ions occupy a distance of 8.93 Å (Bates *et al.*, 1950). From this Pauling, (1930) pointed out that the two sheets, which make up the structure are not a perfect fit. It is proposed that the endellite halloysite as a result of the greater distance of 5.74 Å and the presence of water molecules between the 1:1 units, the hydroxyl ions are only slightly subject to "stretching" forces from opposing oxygen layers of neighbouring units. The six hydroxyl ions are therefore free to approach their normal spacing of 8.62 Å, while the six oxygen ions on the opposite side of the same unit occupy a distance of 8.93 Å.

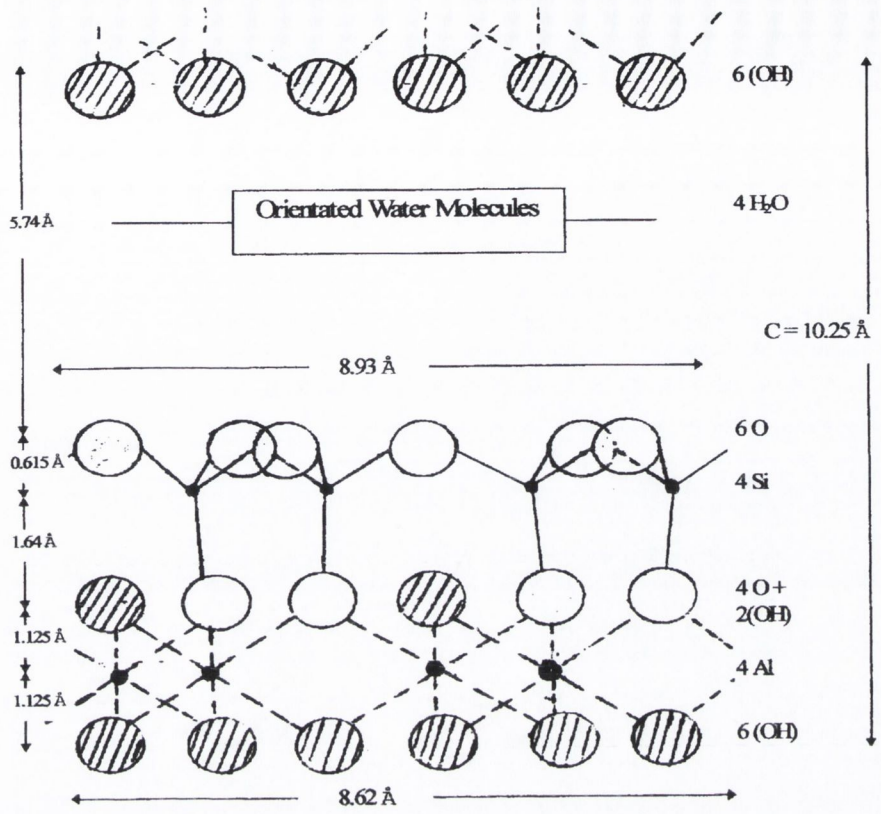


Figure 1.2a Structure of halloysite (endellite) according to Hendricks (1938).

If it is assumed that the "vertical" bonds within the unit remain of equal length relative to each other, a curvature will occur, as seen in Figure 1.2b (Bates *et al.*, 1950). When the halloysite dehydrates (metahalloysite), upon loss of the oriented water molecules between the layers, adjoining units come together from a distance of 5.4 Å to approximately 3 Å. The hydroxyl ions, which in the tubular form occupied approximately their normal spacing of 8.62 Å and were under little strain, now become subject to the forces of the closer oxygen ions and are "stretched" to a distance of approximately 8.93 Å (Bates *et al.*, 1950). The resulting strain manifests itself in the form of collapsed or split and partially unrolled tubes of metahalloysite. On the other hand, kaolinite with a similar layer composition to halloysite is almost invariably planar because in this instance the lateral misfit is corrected by the rotation of alternate silica tetrahedral in opposite directions (Radoslovich, 1963).

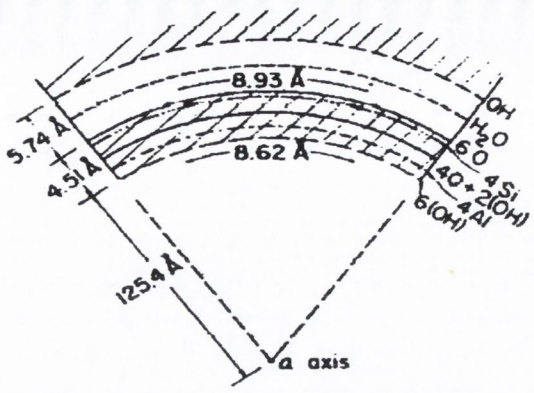


Figure 1.2b Diagrammatic representation of the structure of proposed curved halloysite (endellite) according to Bates *et al.*, 1950.

1.2.5 Surface charge of halloysite

The basal or 'face' and 'edge' surfaces of halloysite normally carry a negative and positive charge, respectively but the negative 'face' charges predominate. Therefore halloysite functions as a polyvalent anion. Bailey (1990) showed that the cation exchange capacity confirmed the net negative charge of the mineral. This was confirmed by electrophoretic experiments carried out by Tari *et al.* (1999) and Levis and Deasy (2002), using a zetasizer. Tari *et al.* (1999) confirmed that the permanent negative surface charge tends to electrostatically attract cations in order to ensure the electroneutrality of the system. Soma *et al.* (1992) further confirmed by XPS (x-ray photoelectron spectroscopy) that halloysite carries a negative charge on the 'face' surface of the crystal, occurring from the non-stoichiometric substitution of Al^{3+} by Fe^{3+}.

There are a number of proposed reasons for this net negative charge. Van Olphen (1963) stated that the clay lattice carries a net negative charge as a result of isomorphous substitutions of certain electropositive elements by elements of lower valency. Bailey (1990) suggested that it was due to the aluminium for silica substitutions in the tetrahedral sheet. The presently accepted view is that a combination of the substitution of octahedral Al^{3+} by Fe^{3+} and also the acidic and

amphoteric properties of the silica and gibbsite sheets lead to a net negative charge on the mineral's surface. The positive charge at the edge of the particle can be attributed to the disruption of the tetrahedral silica sheets and the octahedral alumina sheets (van Olphen, 1963).

The reasoning for the generation of the net negative charge is that the substitution of Fe^{3+} for Al^{3+} results in a positive layer deficiency. In addition, the silica 1:1 unit is mainly positioned on the outer surface of the tubule whereas the aluminium is present on the inner surface of the tubule, which gives an enhanced negative charge over a wide pH range. Levis and Deasy (2002) explained that surface hydroxyl groups (Sur-OH) are formed on exposure of these oxides to water, which are easily ionised (Equation 1.2 a):

$$Si-OH_2^+ \xleftarrow{H^+} Si-OH \xrightarrow{OH^-} Si-O^- \qquad \textbf{Equation 1.2a}$$

Because silica is an acidic oxide, the reaction to the right hand side predominates over a wide pH range. By contrast the net charge on the edges of halloysite, which are composed mainly of gibbsite (alumina) can be positive or negative depending on pH due to the amphoteric nature of alumina (Theng and Wells, 1995a). However as explained earlier since the surface is predominately silica, the net charge on the surface will be negative.

1.2.6 Effect of iron content on halloysite structure

Studies have shown that the morphology of halloysite mineral is not only affected by the mechanism involved in its generation, but can be influenced by the level of iron that has substituted the aluminium ions within the gibbsite (Levis, 2000). Soma *et al.* (1992), indicated by X-ray photoelectron spectroscopy (XPS) that Fe^{3+} substitutes for Al^{3+} in octahedral positions in approximately a 1:2 proportion. Further, halloysite layers within a crystal are generally non-homogeneous in composition. Built up like "onion skins", the surface layers would either be enriched or depleted in Fe depending on the chemical environment in which the crystal growth occurs. The

substitution of the larger Fe^{3+} ion for the smaller Al^{3+} ion in the octahedral sheet of halloysite alleviates the degree of lateral misfit.

Noro (1986) showed that above a certain level of substitution corresponding to a Fe_2O_3 content of 3.5-4% weight, the layers become planar, similar to kaolinite. This is reflected in the finding that the structural Fe content of halloysites generally increases from long tubules, through spheroidal and short tubular, to long tubular forms (Tazaki, 1982; Noro, 1986 and Bailey, 1990). On this basis, long tubular halloysites would be expected to have a lower concentration of Fe, as well as Al, in their surface layers than in their bulk structure, whereas platy halloysite crystals would show an enrichment of Fe at the surface. Therefore Soma *et al.* (1992), concluded that when the structural Fe_2O_3 content exceeds 4% weight, the lateral dimension of the octahedral sheet would become so large as to cause it to curl around the tetrahedral sheet.

1.2.7 Applications of halloysite

The principal use of halloysite is in the ceramics industry, which dates back to antiquity. Korea has used halloysite as a major component of its highest quality whiteware for many centuries (Harvey, 1996). The Japanese porcelain manufacturers subsequently adopted this technology. Halloysite was also amongst the earliest clay minerals to be used for catalysis. It has demonstrated the ability to act as a catalyst in the polymerisation of styrene (Njopwouo *et al.*, 1987). They observed that all clays, when pretreated with acid, effectively catalysed the thermal polymerisation of styrene, the catalytic activity of which depended upon its concentration in the reaction medium. It was concluded from the study that it was the ability of the clay to exchange cations that enabled it to easily fix protons on its surface and it was these fixed surface protons, which were responsible for its catalytic action. Halloysite from Dragon Mine, Utah was used as a base for some of the initial oil cracking catalysis studies in the early 20th century. Halloysite has sometimes been called a "poor man's kaolin", since its applications other than in ceramics are inferior to kaolin (Harvey, 1996). These applications include filling, paper coating, paint pigment and many

other applications. Theng and Wells (1995b) demonstrated that naturally acidic New Zealand clays, such as halloysite showed a commercial potential as a bleaching agent. Clays that had been severely leached by acids during their formation showed a relatively high propensity for decolourising oil and butterfat.

More recently Price and Gaber (1997) filed a patent on the use of hollow ceramics or organic tubules as sustained chemical delivery systems for antifouling agents. The antifouling containing material, is incorporated into a marine paint and used to combat barnacle growth on ships. In a preferred embodiment, the hollow ceramic or inorganic microtubule is a mineral microtubule, such as halloysite, cylindrite, boulangerite or imogolite, with an average diameter of less than 0.2 μm.

Price and Gaber (2001) described the use of halloysite for the entrapment and subsequent release of three compounds: oxytetracycline HCl (antibiotic); khellin (lipophilic vasodilator used transdermally to promote hair growth and as a narcoleptic agent for barnacle napuli in antifouling paints); and nicotinamide adenine dinucleotide (NAD) (an important co-enzyme in many biochemical processes including glycolysis).

Kelly (2002) described how the extended release of tetracycline base for a dental application was achieved over several weeks employing a coating procedure with the cationic polymer chitosan. A delivery system based on a thermoresponsive polymer, poloxamer 407, was developed as it offered ease of administration and retention in the periodontal pocket. The final system underwent clinical studies for the treatment of periodontal disease. It was tested *in vivo* in a dog model and showed good retention and positive antimicrobial activity, with effective drug release occurring over a 6 week period.

Kelly (2002) also developed a controlled release bioadhesive fluoride tablet, as there is currently a lack of products available that can be patient applied and offer controlled release. Halloysite was once again utilized, in conjunction with a solid

lipid microparticle, to improve tabletting and controlled release. A bioadhesive backing layer, containing Carbopol 974P was developed. Approximate zero order release of fluoride was observed over an 8 hr period, with the amount of drug released, suitable for the adult daily requirement of fluoride.

Lvov *et al.* (2002) used halloysite in an assembly by alternate adsorption with polyethyleneimine (PEI), which resulted in the formation of ordered multilayers containing from two up to twenty layers of the tubules glued together by polycation interlayers. An assembly of alcohol dehydrogenase (ADH) and halloysite loaded with nicotinamide adenine dinucleotide in alternation with PEI was achieved. This assembly could be used to provide a direct supply of ADH-cofactor to the enzyme immobilized in polyion films.

Levis and Deasy (2003) illustrated halloysite's potential in the pharmaceutical industry. They described how halloysite could be loaded with cationic drugs (diltiazem hydrochloride and propranolol hydrochloride) by chemisorption to the negatively charged surface, or more extensively by entrapment in the hollow lumen of the microtubules using retardant polymers, cationic coatings and other approaches to delay drug release.

1.3 VINE WEEVIL

1.3.1 Introduction

The vine weevil, *Otiorhynchus sulcatus*, is a European species, which is endemic to the temperate areas and the distribution of this species has extended to the United States, Australia, New Zealand and Japan (Moorehouse *et al.*, 1992). It is a member of the beetle family. It has a very wide host range and causes economic damage on various horticultural crops, glasshouse and outdoor ornamentals. A number of common names have been applied to *O. sulcatus*, such as 'cyclamen borer', 'strawberry root-weevil' and 'taxus weevil', however it is mostly referred to as 'black vine weevil'. The unique characteristic of this pest is that the adult is a flightless parthenogenic (all weevils are female and develop unfertilised eggs). It takes one year for this weevil to complete immature development in the field and it can reproduce more than once a year if it survives a hostile environment.

1.3.2 Host plants

Adult vine weevil will feed on over 100 different kinds of plants including trees, shrubs, vines and flowers. The preferred hosts seem to be yew (*Taxus*), hemlock, azalea, red maple, douglas fir, euonymus and various rhododendrons. *Taxus capitata* seems to be particularly susceptible to attack. It commonly infests containerised perennials in greenhouse and polyhouse production settings, where cyclamen, geranium, gloxinia, primula, carnation, ferns, begonia, orchids, sansevieria or lettuces are grown.

1.3.3 Description

(a) Adult

Vine weevil adults are slate grey to black with short, broad snouts, which are approximately 6 mm long. The head is narrow, the thorax is medium and rounded, and the wing covers are broad and well rounded. The wing covers have fine yellow hairs and conspicuous corrugations appear as lines down the back. Adults cannot fly as their wings covers are fused together. Since the weevils do not fly, they disperse

chiefly by walking. Figure 1.3a below illustrates an adult vine weevil, larvae and pupa.

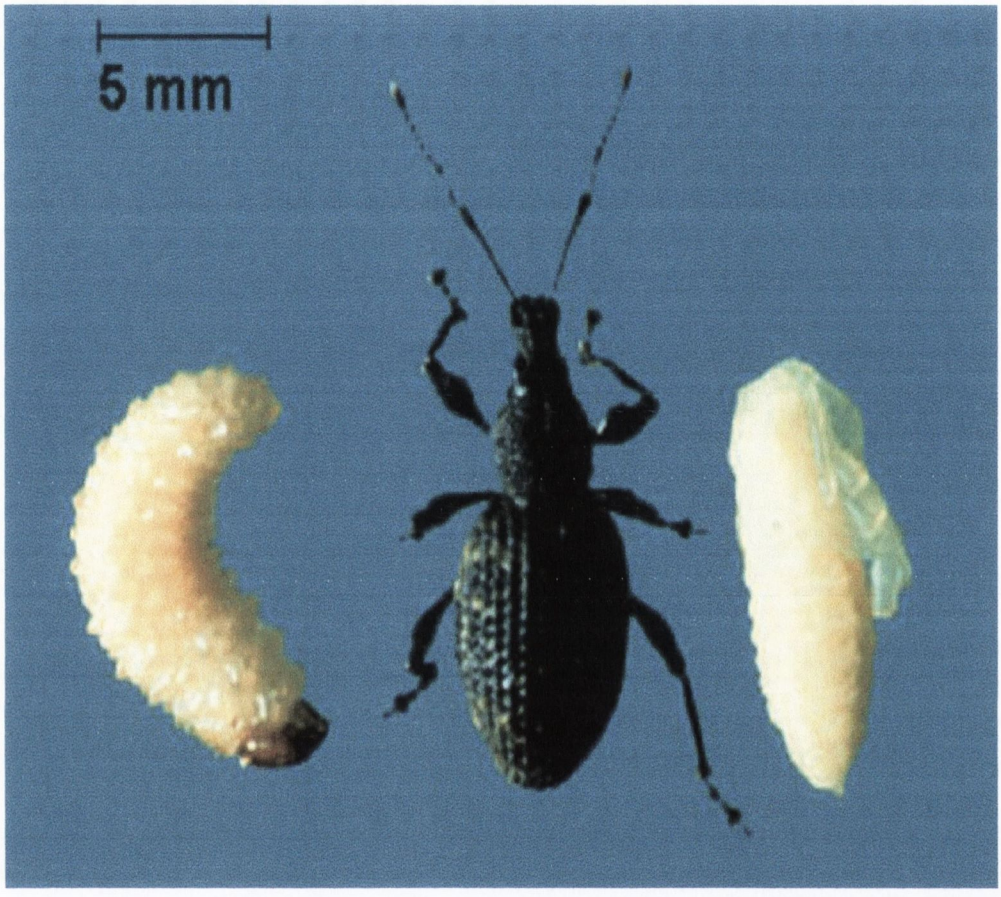


Figure 1.3a Photograph of a larvae, vine weevil and pupae (Cram, 2002).

(b) Egg

The egg is approximately 0.7 mm in diameter, with a smooth, shiny surface. It is white when first deposited but becomes brown as it ages.

(c) Larva

The larvae (grub) have a wrinkled c-shaped appearance. They are legless and white with a well-developed brown head. They grow to 10-12 mm in length in six stages or instars. The first three instars are root feeders, with stage four also feeding on stems and storage organs. The final two stages are immobile.

(d) Pupa

The pupa is white with predominant dusky spines on the head, abdomen and legs.

1.3.4 Life cycle

Adults usually emerge between mid-May and late-July, and live for several months. Adults require 21-28 days of foliage feeding prior to producing eggs. In indoor and heated glasshouses you can expect to see the first adults from the end of January onwards. They may lay as many as 500 eggs over a period of 14-21 days. Eggs are dropped indiscriminately to the ground near the base of host plants. Approximately 10 to 14 days later the larvae hatch and burrow down into the soil to feed on roots.

Larval feeding occurs through the growing season and when colder temperatures arrive they burrow deeper into the soil to overwinter. They resume feeding the following spring a short while before they enter the pupal stage. Pupation lasts 1 month and adults are most abundant from early June to mid-July. There are conflicting reports about overwintering as adults. Most adults die in the winter, while the larva survives the winter in the soil. Survival rates of adult vine weevil outdoors varied among geographical locations and shelter conditions; 90% in sheltered spots in Holland (Evenhuis, 1982), 25% in Vancouver (Garth and Shanks, 1978), 1% in Ohio (Nielsen and Dunlap, 1981). Oviposition in a group of overwintered adult weevils in Vancouver began 26th April and continued until late August with a peak in early August (Garth and Shanks, 1978).

Under laboratory conditions, the oviposition period is extended and adults have a potential life span of more than two years (Moorehouse *et al.*, 1992). It is generally known that the life cycle of this weevil is univoltine i.e. one generation per year. However, there may be considerable overlap between the different stages (Shread, 1972) and all stages can be present at the same time (Evenhuis, 1982). The life cycle (Figure 1.3b) shown is just an approximate simplified form because generally the life cycle of insects is influenced by many factors. Recently the developmental threshold for low temperature was determined for each stage: 6.3°C for eggs, 2.5°C for larvae, 6.1°C for pupae and 8.4°C for ovipositional stages (Masaki and Ohto, 1995).

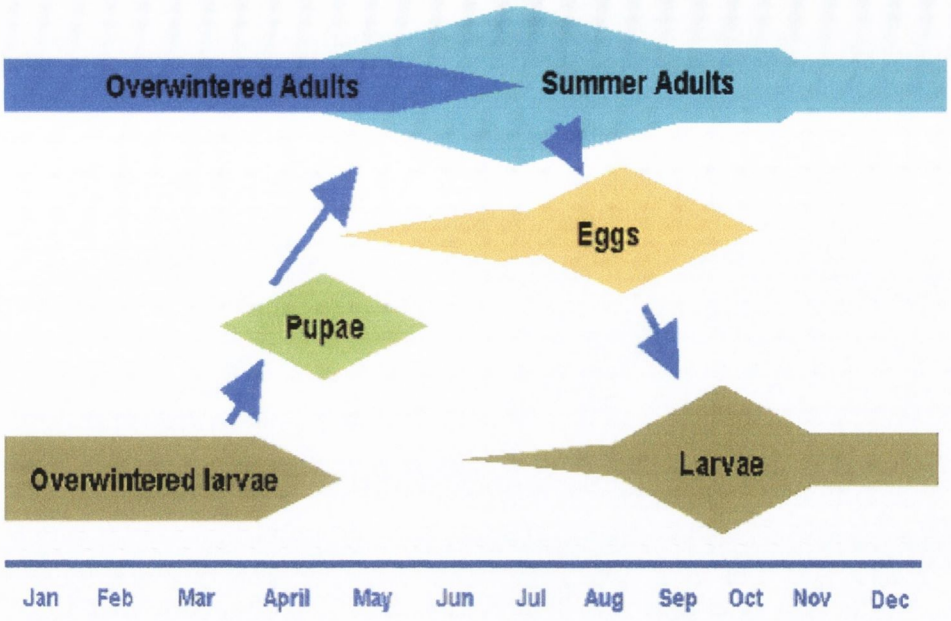


Figure 1.3b Life cycle of black vine weevil (Moorehouse *et al.*, 1992).

The timing for appearance of a population of vine weevil differs in indoor, sheltered outdoor and exposed outdoor crops, and is influenced by several factors. Among them temperature and also moisture availability are the most important factors to control the developmental rate for this pest. These are important considerations when timing treatments to hit a particular stage of the life cycle.

1.3.5 Damage

Larval damage by vine weevil is the most severe. The larvae feed on the roots and underground stems, sometimes girdling the root crown, which causes wilting and collapse of shoots and leaves (Figure 1.3c). The legless larvae prefer to feed on young tender roots. If young roots become scarce or the soil becomes overly moist the larvae will move to large roots near the base of the plant. Large larval populations or moist soils cause feeding on the plant stem and the plant may be girdled. Root feeding often results in death of the plant. Plants may fail to put out new growth in the spring as a result of the root injury, or may put out the first flush of new growth and subsequently die.

Figure 1.3c Photograph showing yellowing and wilting of primrose foliage caused by adult vine weevil and larvae.

Unfortunately, amateur gardeners often first notice the problem when plants are near death. Established crops are more resistant to larval feeding because of their larger root systems and can withstand a certain degree of feeding. Adults cut typical crescent shaped notches on the margins of leaves (Figure 1.3d). The adults are nocturnal, feeding at night and hiding under leaf litter or the soil during the day. When disturbed, the adults feign death. Therefore careful searches should be made to try and locate specimens since several other weevils and some caterpillars can produce the same type of notching. This foliar damage tends to be aesthetic and seldom impacts on plant health, even though it may be extensive. This may result in loss of saleability of plants.

Figure 1.3d Photograph shows adult vine weevil damage of a primrose.

1.3.6 Control

The failure of chemical pesticides, the banning of persistent chlorinated hydrocarbons and the increasing dispersal of the insect by infested potted plants has led to an urgent need to develop better and safer pest control methods (Zimmermann, 1996).

1.3.6.1 Cultural control

In practice, the main cultural controls that can be employed by the grower are good nursery hygiene such as the following:

- Destroy rather than compost dead plants.
- As far as possible, maintain a steady turnover of plants in the nursery.
- Eliminate weeds and plant debris in glasshouses, tunnels and nurseries.
- Remove hedgerows close to crop plants.
- Clean growing areas prior to restocking.
- Use plastic floor/soil cover in nurseries whenever possible.
- Discard old untreated plants from the nursery, as these may be a source of infestation.
- Check new stocks for weevil infestation and isolate or discard if infested.

- Monitor adult feeding by checking for damage or by searching for adults after nightfall (Dunne *et al.*, 2001).

The impact of some control methods was investigated:

- Compressed peat mats placed on the surface of the compost in a pot can reduce attack if tightly fitted to pots and kept moist. On the other hand, if not well fitted and allowed to dry out the weevils shelter under the mat and the level of attack increases.
- Using nylon mesh attached to both the stem and the pot to exclude egg laying adults also reduces attack but the mesh tended to be displaced by weeds.
- The ratio of peat to soil in the substrate does not consistently affect larval numbers.
- A plastic barrier coated with "Fluon" (Whitford Plastics Ltd) prevented adult weevils entering the protected area (Dunne *et al.*, 2001).

1.3.6.2 Biological control

Like all living things, vine weevil, have natural enemies such as hedgehogs, mice, birds, predatory insects, parasitic nematodes and fungal diseases. They do not seem to have many insect parasites. Fowl were used in the past to reduce vine weevil attack in nurseries, but the entities of greatest interest are the parasitic nematodes and fungal diseases. Probably the most promising biological control for vine weevil is entomopathogenic nematodes (e.g. *Heterorhabditis spp.*), which are minute worms that are free living in peat and soil compost, actively seeking out their prey. They are mainly used for control of vine weevil larvae and sciarid fly larvae, although other soil dwelling insects can be attacked. Once a host insect larva is found, one or more ineffective nematodes enter the body through natural openings. The nematodes carry symbiotic bacteria (*Xenorhabdus spp.*), which are released into the host. The bacteria multiply spreading rapidly throughout the insect causing septicaemia and death. The nematodes feed on the bacteria and host tissue, reproducing thousands of infective larval nematodes, which are released from the cadaver 10-14 days later.

The main drawback with this technique is that the vine weevil, are damagingly active at temperatures much lower than those required for effective action of nematode worms. This limits the use of nematodes, as they generally do not work well below 12°C (van Tol, 1996). However an advantage of nematodes is that one application should be sufficient to control all larvae present in the soil.

The entomopathogenic fungus *Metarhizium anisopliae* has shown great promise for the control of vine weevil in protected ornamentals. However it is not yet sufficiently reliable for commercial application. It is both expensive and has limited persistence in the soil. Also like the nematodes, its limiting factor is the temperature of the soil.

1.3.6.3 Chemical control

Available sprays against adult vine weevil are generally relatively ineffective and need to be applied frequently. Many common pesticides give no control e.g. chlorpyrifos (*Dursban*), chlorfenvinfos (*Birlane*, *Sapecron*), isofenfos (*Oftanol*), while others e.g. carbaryl may give 30-50% control on the first day of spraying. Pirimefos (*Blex*) may be the best option as it has been found to give 80-100% control in the first day. Synthetic pyrethroids e.g. deltamethrin (*Decis*), permethrin (*Ambush*), cypermethrin (*Cyperkill*), alpha cypermethrin (*Fastac*) do not kill adults even at high rates, but may have a repellant action. Again they are non persistent. The degree of population suppression with sprays aimed at adult control might be adequate for situations where low larval populations can be tolerated. There are several problems inherent in attempting to control adults. Adults emerge over an extended period and not all are active each night. They shelter in inaccessible places and some insecticidal resistance may be present.

Therefore chemical control of vine weevil has traditionally involved treatments making the soil toxic to the larvae, particularly the young larvae migrating to the plant roots after hatching from the eggs. The use of chlorinated hydrocarbon pesticides, such as aldrin and dieldrin, gave very long residual control of vine weevil; one treatment of potting medium before planting would have been sufficient for

protecting the plants until they were sold. However these pesticides have been withdrawn from the market because of persistence in the environment.

Therefore the current situation is that nearly all pesticides labelled for use on ornamentals have relatively short residual activity in soil. Because adult activity and egg laying can extend from spring through autumn, pesticides drenched into soil to control the larval stages may have to be repeatedly applied, or else used in early autumn to control mixed ages of larvae. Late instar larvae are notoriously difficult to control with conventional pesticides, so growers choosing to disinfect plants of late instar larvae currently must use root ball dips to meet strict phytosanitary standards (Evenhuis, 1982).

Examples of chemical drenches used to control larvae are chlorpyrifos (*Dursban*), carbofuran (*Yaltox*) and fonofos (*Cudgel*), which may be applied to foliage in mid-May to October. Spray should be allowed to run off foliage and into the soil so that the root system is also treated. Repeated spray applications will be needed to give adequate control. Some of these pesticides are available in granular form also. Gamma HCH (*Gammacol, Gama flow, Lindacol*) is used as a drench to kill established larval infestations. Sufficient liquid to wet the root ball must be applied. Fipronil, a phenyl pyrazole pesticide, has shown considerable promise in larval control as a granular formulation. It gave a level of control equal to *suSCon Green* in containerised plants and also lower than the standard rates were effective (Dunne *et al.*, 2001). Tefluthrin (*Force*), a synthetic pyrethroid pesticide suitable for soil application, gave excellent control of vine weevil in containers and in field grown strawberries, where the effect also persisted into the second season (Dunne *et al.*, 2001). However neither of these pesticides are registered in Ireland yet.

Two product advances, which have been shown to give long residual control of vine weevil larvae, are as follows:

(a) Chemical impregnated compost

This unique compost is an "anti vine weevil" compost which is treated with the pesticide imidacloprid (*Intercept*). The chemical imidacloprid works by interfering with the insect's nervous system, causing paralysis and eventually death to soil dwelling insect pests such as vine weevil for up to two years. This is also available in a drench but only gives protection for six months.

(b) Microencapsulated controlled release pellets

The formulation of chlorpyrifos as slow release pellets (*suSCon Green*) is used widely for control in hardy nursery stock. The active ingredient leaches slowly from the plastic resin, creating zones in the soil that are toxic to young larvae. An advantage in using this technology is that the formulation in the resin pellets makes the pesticide safe by releasing very little of the active ingredient at any one time (Figure 1.3e). Consequently, leaching of the active ingredient out of the pot and into surface run-off water is negligible compared with conventional pot drenches. In order to work, this material has to be distributed throughout potting soil, including the volume closest to the stem. In several trials (Buxton *et al.*, 1992), *suSCon Green* has been shown to last through two growing seasons. Imidacloprid (*Intercept*) was found to give better control than *suSCon Green* in the first year. However *suSCon Green* was also effective in the second year, where imidacloprid did not give satisfactory control (Dunne *et al.*, 2001). In this project *suSCon Green* pellets will be compared to chlorpyrifos loaded halloysite pellets for controlling vine weevil in primroses.

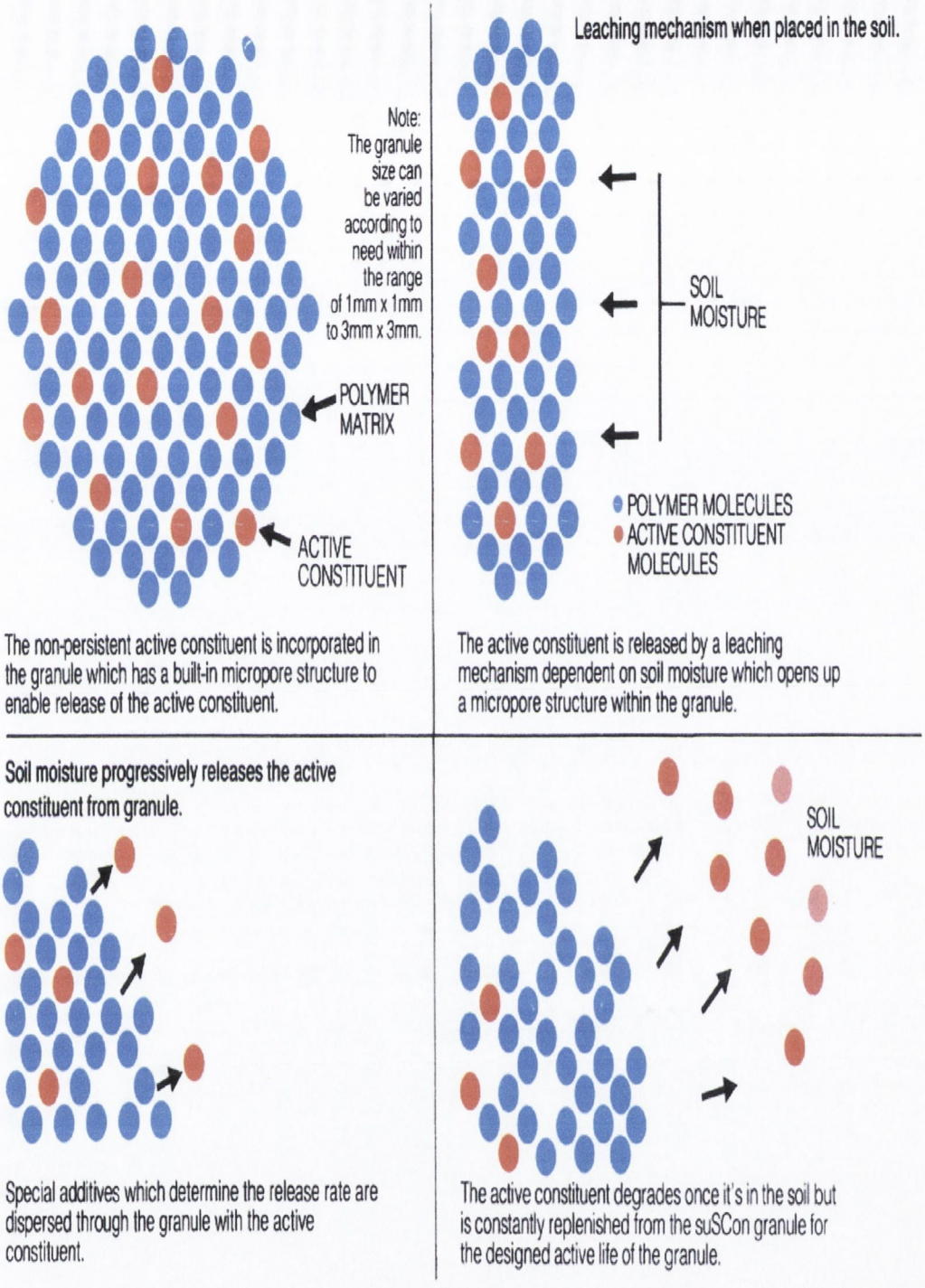


Figure 1.3e Explanatory cross-sectional diagram of a controlled release *suSCon Green* pellet (Product information leaflet, Consolidated Fertilizers Limited, An Incitec Company).

1.3.6.4 Future control strategies

It seems probable that total reliance on chemicals, constitutes part of the vine weevil problem. In the future wider strategies for the control of vine weevil will need to be considered, moving away from chemicals for control. Cultural measures, as outlined in Section 1.3.6.1 (i.e. good nursery hygiene), should be used to augment the action of pesticides and every effort should be made to achieve greater precision in application and timing of pesticides. Non-chemical control (parasitic nematodes and fungal diseases) probably will not reach its full potential until pesticides are reduced or phased out.

1.4 APHIDS

1.4.1 Introduction

Aphids are a group of about 4,400 species of small insects that belong to the superfamily *Aphidoidea* of which about 250 are serious pests. Although predominant in temperate regions, aphids have a worldwide distribution (Dixon, 1985). Aphids combine three traits - thelytokous parthenogenesis (obligate parthenogenesis where females give birth only to female offspring), short generation time (about 10 days on average) and telescoping of generations (where granddaughters begin developing directly within the daughters which are themselves not yet born) to reach very large population sizes very quickly (Moran, 1988). These reproductive characteristics allow aphids to quickly colonise ephemeral resources and growing plants, and therefore make them ideal enemies of monocultured crops, which are of major economic importance (Moran, 1992). Most aphids are monophagous, although some feed on more than one species of plant. Species like *Myzus persicae* feed on a number of different plant species, which is very rare.

1.4.2 Description

Aphids are 1-5 mm long with a soft pear-shaped body, long legs and antennae (Figure 1.4a). Their colour varies according to species, however the common aphids are usually green, yellow or black. The most distinguishing feature in the identification of aphids is the two short cornicles or tubes, which extend from the end of their body and secrete defensive substances (Kennedy and Stroyan, 1959). Adults may be winged or wingless and colonies often have both forms. They are weak fliers, but travel long distances on air currents.

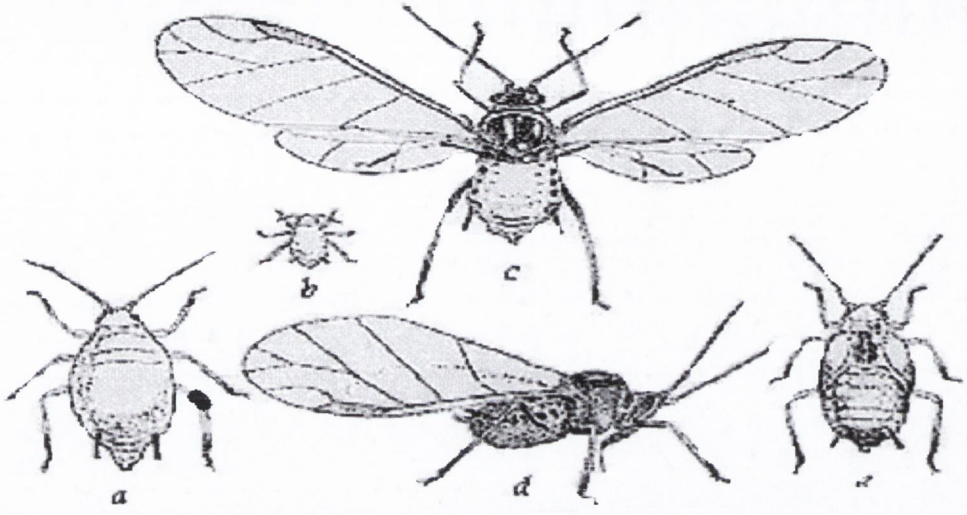


Figure 1.4a Aphids: **a**, wingless; **b**, newborn nymph; **c**, and **d**, winged; **e**, nymph (Day, 1996).

Although it is more important to recognise the economic potential of an infestation than to be able to identify the different species, the following are brief descriptions of the main species that damage cereal crops:

(a) English grain aphid (*Sitobion avenae*)

English grain aphids (Figure 1.4b) are usually grass-green in colour, but can be pinkish, especially in the spring. No toxins are injected during feeding, so injury is not apparent and usually not economic unless associated with barley yellow dwarf virus (BYDV) transmission. It is the most common species in the late spring, when it develops and feeds inside the upper leaf sheath (boot) and on the flag leaf. As the grain begins to head, the aphids move to the grain head and cluster among bracts and kernels. Feeding by large numbers causes the growing kernels to discolour and shrivel. Heavy head infestations can reduce yield by 13%. Scouting is especially valuable in predicting problem infestations because English grain aphids have only been found in heads of plants infested in the lower canopy earlier in the season. It appears that they move up plant stalks as grain heads develop, rather than fly in from

areas outside the field. English grain aphid vector BYVD and like bird cherry-oat aphids, can cause reduced yields if the virus is present in the populations at the time they are feeding (Herbert *et al.*, 2003).

Figure 1.4b English grain aphid (*Sitobion avenae*), (Hein *et al.*, 1996).

(b) Bird cherry-oat aphid *(Rhopalosiphum padi)*

Formerly known as the oat-bird cherry aphid, this species infests small grains (Figure 1.4c). These aphids are olive green to blueblack in colour and have reddish patches at the base of the cornicles. Like English grain aphids, bird cherry-oat aphids do not inject toxins while feeding, therefore, even though heavy infestations can develop, injury is not readily apparent and plants appear to be able to tolerate large infestations without economic yield losses. However, bird cherry-oat aphid is another known vector of BYDV and if the virus is present in aphid populations, feeding can vector the disease and yield losses can occur (Herbert *et al.*, 2003).

Figure 1.4c Bird cherry-oat aphid (*Rhopalosiphum padi*), (Hein *et al.*, 1996).

(c) Rose grain aphid (*Metopolophium dirhodum*)

Rose grain aphids are distinctly blue to dark green in colour with a darker area at the base of the cornicles (Figure 1.4d). They feed on small grains, wild grasses and corn but are most commonly found in sorghum whorls during vegetative growth stages. Large infestations can cause yellowish mottling of leaves, but injury is seldom an economic problem (Herbert *et al.*, 2003).

Figure 1.4d Rose grain aphid (*Metopolophium dirhodum*), (Hein *et al.*, 1996).

1.4.3 Life cycle

Many species of aphids display complex lifecycles with alternation of sexual and asexual generations and host plant alternation.

1.4.3.1 Alternation of generation

Aphids are one of the few groups of insects that undergo cyclical parthenogenesis i.e. the alternation of one or many generations of asexual (parthenogenetic) reproduction with a single generation of sexual reproduction. All asexual generations are entirely female. Species that produce both sexual and asexual morphs are holocyclic. When a species produces only asexual morphs it is anholocyclic.

In holocyclic life cycle, as illustrated in Figure 1.4e, both asexual and sexual morphs are produced at different times of the year. The males are present only in one part of the life cycle. They are produced by parthenogenetic females, usually only in the autumn.

In contrast, anholocyclic species (Figure 1.4f) only produce asexual females. They do not produce sexual females and males. Comparing the diagram of holocyclic with the diagram of the anholocyclic, the sexual males and females are missing from the anholocyclic species. In fact, anholocyclic species also lose the direct descendants of the sexual morphs, namely the egg and the fundatrix (a clone/colony foundress). Although many species are either holocyclic or anholocyclic, many other species possess both holocylic and anholocyclic populations (Stern, 1997).

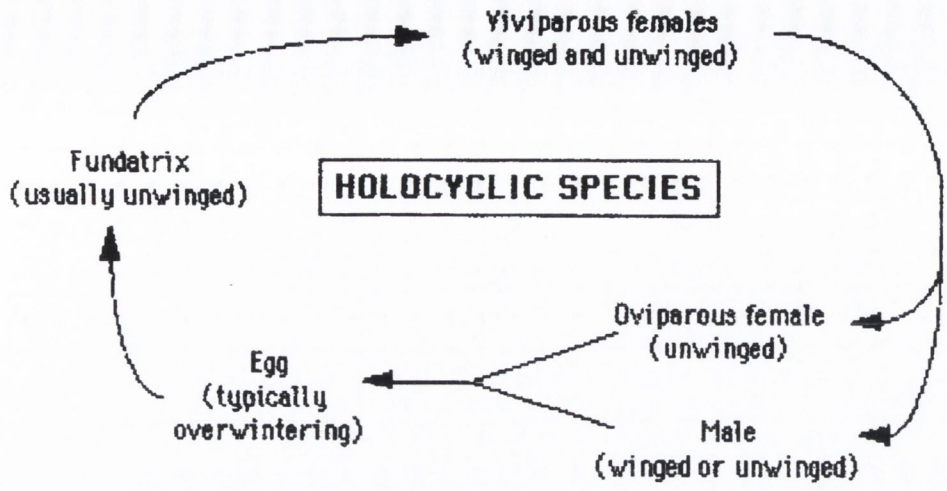


Figure 1.4e A typical life cycle of a holocyclic species (Stern, 1997).

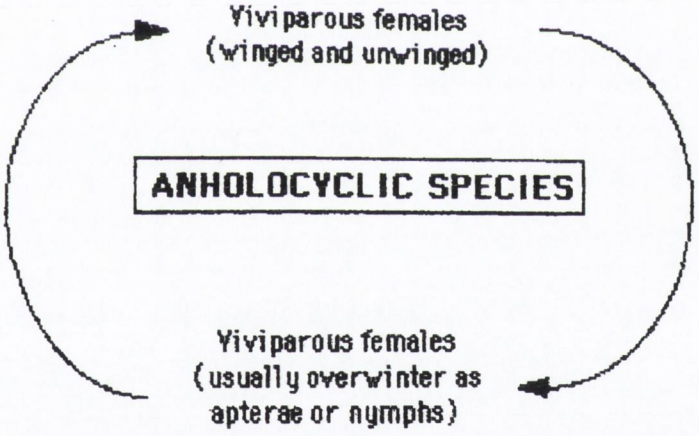


Figure 1.4f A typical life cycle of an anholocyclic species (Stern, 1997).

1.4.3.2 Host alternation

Many species of aphids alternate between two taxonomically different host plants at different times of the year. When an aphid species uses only a single host plant it is autoecious. Species that alternate between two host plants are called heteroecious.

Two distinct kinds of heteroecious life cycles are found in aphids. A typical life cycle of a heteroecious species is illustrated in Figure 1.4g. Aphids alternate between two taxonomically unrelated host plants. The primary host, a tree or shrub, is considered the "ancestral" host, in that the monoecious ancestor of aphids is thought to have attacked such trees. The secondary host is often an herbaceous plant. A specialized winged morph, the sexupara, flies from the secondary host to the primary host and deposits sexual males and females. The males and females mate and the female typically produces a single egg. In temperate regions the egg over winters and produces the fundatrix in the spring.

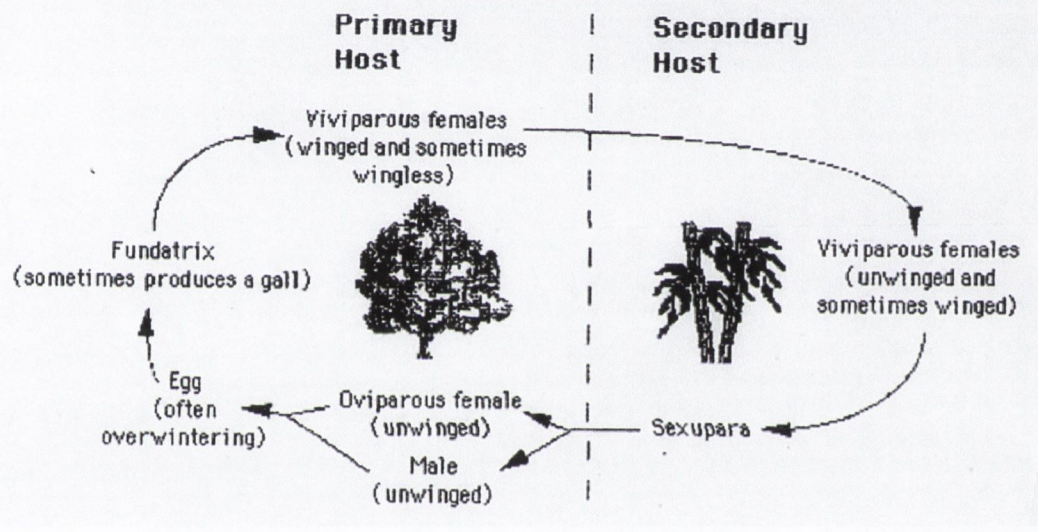


Figure 1.4g A typical life cycle of heteroecious species (Stern, 1997).

Some species have a different kind of host alternation. The primary difference is that sexuparae are not produced. Instead, winged males and specialized winged females depart from the secondary host. The females produce unwinged oviparous females that mate with the winged males (Stern, 1997).

1.4.4 Damage

Aphids feed by sucking up plant juices through a food channel in their beaks. At the same time, they inject saliva into the host. Light infestations are usually not harmful

to plants, but higher aphid populations cause leaf curl, wilting, stunting of shoot growth (Figure 1.4h) and delay in production of flowers and fruit, as well as general decline in plant vigour. Some aphids are also important vectors of plant diseases, transmitting pathogens, particularly viruses (e.g. BYDV as discussed in Section 1.4.5), in the feeding process.

Figure 1.4h Aphid feeding causes leaf curl and wilting (Day, 2001).

A distinctive feature of aphids is the production of honeydew, a sugary waste product (Figure 1.4i). Honeydew is the clear, sticky dropping that lands on the leaves or

anything below the plant or tree that the aphids are feeding upon. This honeydew attracts ants and promotes growth of black and brown fungi, appropriately called sooty moulds. The presence of honeydew or sooty mould is often an excellent indication that aphids are present. Sooty moulds are not in themselves harmful, but they prevent light getting to the leaves, causing premature leaf fall and spoilage in appearance.

Figure 1.4i Aphids excrete a sugary waste called honeydew – the clear drop highlighted on the left side of this photograph (Day, 2001).

1.4.5 Barley yellow dwarf virus

1.4.5.1 Introduction

BYDV is the most widespread viral disease of cereals (Plumb, 1983). It infects a wide range of cultivated grasses, such as wheat, oats, barley and rice, as well as many wild grasses (D'Arcy, 1995). The disease is transmitted by 25 aphid species (Halbert and Voegtlin, 1995), of which the three more important vectors in NW Europe are *Sitobion avenae*, *Metopolophium dirhodum* and *Rhopalosiphum padi* (Plumb, 1974; Hand, 1989; Henry and Dedryver, 1991; Leclercq-Le Quillec *et al.*, 1995 and Lowles *et al.*, 1996). Economic losses have been associated with BYDV in the U.S.A. (Oswald and Houston, 1953), U.K. (A'Brook, 1974) and New Zealand (Smith, 1993). Aphids transmit the disease in a persistent, circulative and non-propagative manner (Rochow, 1969). Several strains of BYDV have been characterised according to their transmission by different aphid vectors (Rochow, 1969), their cytopathology in host plants (Gill and Chong, 1979) and their molecular genetics (Martin and D'Arcy, 1995).

In spite of the economic importance of BYDV, few natural resistance genes have been identified in any of the major crops. A single well-characterized resistance gene, Yd_2, exists in barley (Schaller *et al.*, 1964), but it does not protect against all strains of BYDV (Skaria *et al.*, 1985). No natural resistance genes have been identified in oats and wheat. Researchers have begun using biotechnology to insert viral RNA into the genome of crops plants in order to induce resistance against BYDV (Miller and Young, 1995).

1.4.5.2 Symptoms

BYDV is sometimes difficult to diagnosis in the field and may go unrecognised in some cases. Symptoms of BYDV are highly variable and can be confused with wheat streak mosaic, nutrient deficiency, root and crown disease, and environmental stress.

Figure 1.4j Wheat with yellow dwarf virus in comparison to healthy wheat (Munkvold, 1999).

It is diagnosed in the field by the presence of yellowish to reddish stunted plants grouped singly or in small patches among normal plants (Figure 1.4j). Early infection of cereal grains may result in severe stunting, reduced tillering (reduced shoot numbers per plant), bright-yellowing or discolouration. The leaves of plants infected by BYDV are shorter than normal and the flag leaf may be severely shortened. Leaves often are stiffer and more erect. Roots systems are reduced and diseased plants are more easily pulled than healthy plants.

Stunted plants result from the failure of the stem internode to elongate. This leads to a "telescoped" plant where the leaves may unfurl before they have fully emerged from the sheath of the previous leaf. Infected plants are "dwarfs" and have lost their normal confirmation. Even the head, or panicle does not emerge fully or properly. Patterns of BYDV in a field may be seen as random within the crop or as circular or angular patches, which reflect the pattern of movement of the aphid vectors or carriers. Many of the infected plants ripen prematurely, after which they may be invaded by sooty moulds, which give a dirty appearance to the plant and may lower germination of harvested seed (Watkins and Lane, 1997).

1.4.6 Control

Aphid population outbreaks are largely dependent on weather and planting date of the grain. Mild temperatures during November and December increase the possibility of serious autumn infestations. Wheat and barley crops planted by 15th October are also more susceptible to aphid attack. These crops should be checked weekly during mild autumn weather to determine the presence of economically damaging populations of aphids and BYDV.

When sampling for aphids, all plants in at least 5 locations are examined and an estimation of the number of aphids is determined. Samples from the field edges should not be taken, where populations are usually highest. The field should also be examined for the presence of small yellowing patches of stunted plants (reddened areas in wheat and oats).

Continued mild weather during the winter makes spring outbreaks more likely. The beneficial insects that attack aphids reproduce slowly at temperatures below 65°F, whereas aphids can rapidly increase when temperatures exceed 50°F. Regular sampling should continue through April and May. If aphids begin to move into the grain heads, at least 50 heads from throughout the field should be examined and the average number per head should be determined. Treatment should be considered if an average of 20 or more aphids are found per head (Herbert *et al.*, 2003).

1.4.6.1 Cultural control

- Good sanitation should be practiced, such as removing discarded plant material and eliminating weeds around plant production areas. Weed host plants often serve as reservoirs for migrating aphids.
- Application of excessive amounts of nitrogen fertiliser should be avoided as it promotes soft plant tissue. New, soft plant tissue promotes higher aphid populations.

- Physical control methods could be used if appropriate. These include screens or other barriers. Screens are especially important in stock plant production areas to reduce the threat of virus transmission.
- All incoming plant material should be thoroughly inspected and spot treated if necessary.
- A hard jet of water from a hose could be used to dislodge the aphids. Periodic syringing will keep the populations from causing unnecessary damage and allow the parasites and predators to build up to effective levels.
- Trees, shrubs and flowers that are resistant to aphids could be sown at the outskirts of the crop.

1.4.6.2 Biological control

Aphids are attacked by a large number of predators, parasites and pathogens. Some of the common predators sold commercially include several species of lady beetles (*Coleoptera*: *Coccinellidae*), lacewing larvae (*Neuroptera*: *Chysopidae*) and syrphid fly larvae (*Diptera*: *Syriphidae*). Many small hymenoptera parasites infest aphids, most being species specific. Parasites of aphids include various braconid wasps, such as species of *Aphidius*. Parasitised aphids are easy to detect, their bodies turn a tan or cream colour, become hard and shiny and are commonly referred to as mummies. Pathogenic fungi such as *Beauveria bassiana* sometimes attack aphids if weather conditions are favourable. An infected insect becomes mummified and covered with white, grey, tan, pink or green powdery-looking mould. As the disease progresses, the insect disappears as mycelium expands over the body.

1.4.6.3 Chemical control

There a wide range of pesticides used for aphid control. They include organophosphates (e.g. chlorpyrifos), carbamates (e.g. bendiocarb), pyrethroids (e.g. deltamethrin and cypermethrin) and imidacloprid. Soaps and oils e.g. neem oil may also be used, which kill by contact so thorough coverage is needed.

1.4.6.4 Future control strategies

The control of aphids currently still involves using large amounts of pesticide in some countries, but other more ecologically friendly methods have been used. These generally involve biological control (as discussed in Section 1.4.6.2), mostly with a range of hymenoptera parasites, genetic manipulation for aphid resistance and forecasting to allow pre-emptive spraying in smaller amounts. Also it is important that farmers are educated in choosing alternatives to chemical sprays.

1.5 CHLORPYRIFOS

1.5.1 Introduction

Chlorpyrifos is a member of the organophosphate class of pesticides. This class has become one of the most used groups of pest control chemicals. In 1989, almost 40% of the £6.2 billion global pesticide market was composed of organophosphates (Phillips and McDougall, 1990). Although Clermont (1854) had first synthesised tetraethylpyrophosphate (TEPP), it was not until much later that the insecticidal properties of this organophosphate were described (Schrader, 1942). This milestone led to further synthetic work with insecticidal organophosphates and thus provided the impetus that would lead to the launch of an entirely new class of pesticides. Synthetic research with phosphorus containing pesticides began in 1947 within the Bioproducts Department at The Dow Chemical Company in Midland, Michigan (Kenaga, 1977). Early work focused on a series of substituted phenyl phosphoramidates (Blair *et al.*, 1963, 1965, 1966).

Continued research with O,O-dialkyl phenyl-substituted phosphates and phosphorothioates led to the development of the commercial pesticide '*ronnel*'. Replacement of the substituted phenyl ring with various substituted pyridinyl rings resulted in the discovery of a series of insecticidal O,O-dialkyl O-trihalo-2-pyridyl phosphates and phosphorothioates (Rigterink and Kenaga, 1966). The series was described by both French (1,360,901) and U.S. (3,244,586) patents (Kenaga, 1977 Rigterink, 1966). The most promising compound of this series [O,O-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate] was designated as *Dowco*^{TM} 179 and referred to at first as 'ethel'. The broad spectrum insecticidal properties of this compound, later designated "chlorpyrifos" indicated that it possessed substantial commercial potential (Kenaga *et al.*, 1965 and Whitney, 1967). Since the discovery of chlorpyrifos its broad spectrum insecticidal activity has been put to use against a wide variety of insect pests via a number of commercialised products.

1.5.2 Basic manufacture and formulation

Chlorpyrifos (trade name *Dursban*, *Lorsban* and others) is primarily produced by the US multinational Dow AgroSciences. Other manufacturers are Aimco, Agriphar, Excel, Ficom, Gharda, Lupin, Montari (all India), Frunol (Germany), Jim Hung (South Korea), Point Enterprises (Switzerland), Luxembourg and Makhteshim-Agan, (both Israel). Chlorpyrifos is formulated as a number of different commercial products (Table 1.5a). The most commonly available formulations include emulsifiable concentrates (EC), granular concentrates (GC) and wettable powders (WP).

Table 1.5a Selected commercial products containing chlorpyrifos.

Product trademark	Formulation type	Use pattern category	Maufacturer/ trademark holder
Dursban 2E	Emulsifiable concentrate	Indoor and horticultural pesticide	Dow AgroSciences
Dursban 50W	Wettable powder	Horticultural pesticide	Dow AgroSciences
Lorsban 15G	Granular	Agricultural pesticide	Dow AgroSciences
Empire	Microencapsulated concentrate	Indoor and outdoor pesticide	Dow AgroSciences
Lentrek	Emulsifiable concentrate	Wood protectant	Dow AgroSciences
Pyrinex	Emulsifiable concentrate	Agricultural and horticultural pesticide	Makhteshim-Agan

1.5.3 Physicochemical properties

The chemical structure is shown in Figure 1.5a. It has a molecular weight of 350.6 g/mole and empirical formula $C_9H_{11}Cl_3NO_3PS$. The chemical name of chlorpyrifos is O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl)phosphorothioate (Tomlin, 1997).

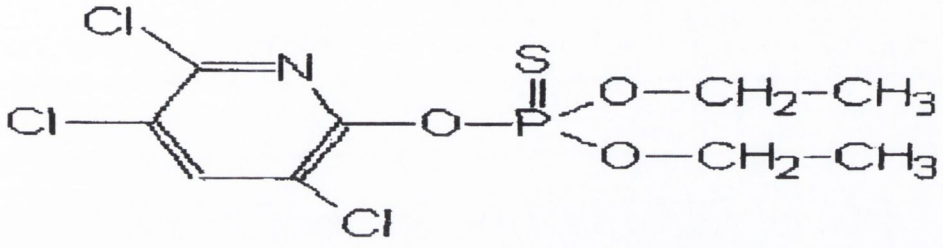


Figure 1.5a Chemical structure of chlorpyrifos.

a) State	***b) Odour***	***c) Boiling point***
Crystalline solid	Mild mercaptan	> 300°C
d) Colour	***e) Melting point***	***f) Vapour pressure***
White to tan	42-43.5°C	2.5 mPa at 25°C
g) Density	***h) Partition coefficient (n-octanol/water)***	
1.44 g/ml at 25°C	K_{ow} logP = 4.7	

i) Solubility

The solubility of chlorpyrifos in water is 2 ppm (McEwen and Stephenson, 1979) and in a variety of solvents (Kidd and James, 1991) is presented in Table 1.5b.

Table 1.5b Solubilities of chlorpyrifos

Solvent	Solubility (g/100 g at 20°C)
Benzene	790
Acetone	650
Chloroform	630
Carbon disulphide	590
Diethyl ether	510
Xylene	645
Methylene	714
Isoctane	79
Methanol	45

(j) UV absorption spectrum

Maximum absorption spectrum is at a wavelength of 280 nm.

(k) Metabolites

Chlorpyrifos and its metabolites are shown in Figure 1.5b. The two major metabolites are 3,5,6-trichloro-2-pyridinol (TCP) and 3,5,6-trichloro-2-methoxypyridine (TMP).

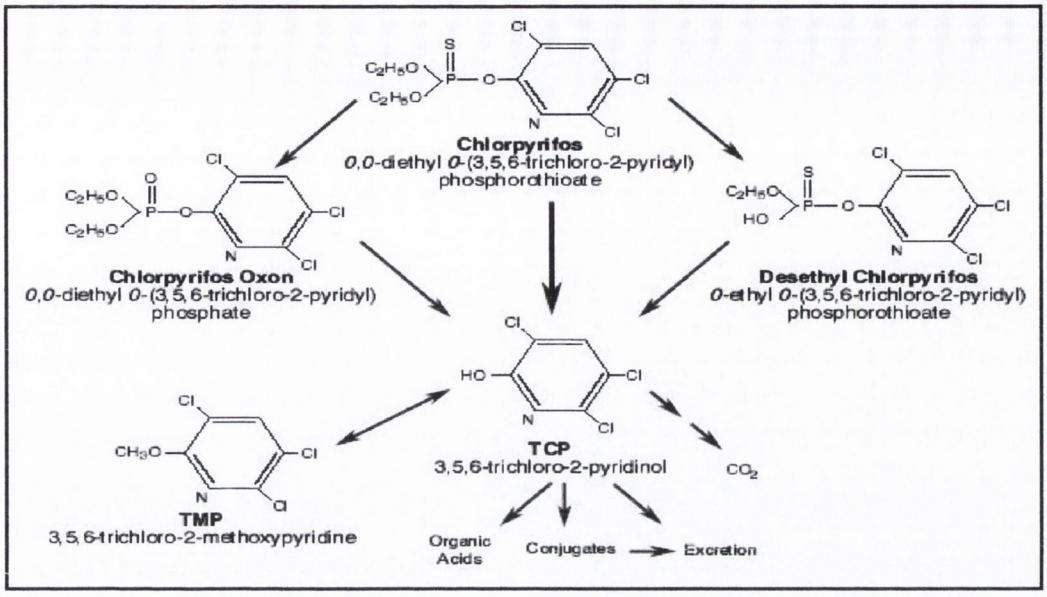


Figure 1.5b Illustration of Chlorpyrifos and its metabolites (Technical Profile, Dow AgroSciences).

1.5.4 Mode of action

Chlorpyrifos is an insecticidal organophosphorous compound, which inhibits the action of several enzymes. The major action *in vivo* is against the enzyme acetylcholinesterase, which controls the hydrolysis of acetylcholine, generated at the nerve junction, into choline.

In the absence of effective acetylcholinesterase, the liberated acetylcholine accumulates and prevents the smooth transmission of nervous impulses across the synaptic gap at nerve junctions. This results in the loss of muscular co-ordination, convulsions and death.

Acetylcholinesterase has two active sites, the anionic and the esteric site containing the primary alcoholic group, which attacks the electrophilic carbonyl atom of the substrate (COO). The structure of organophosphates (e.g. chlorpyrifos) mimics acetylcholine and by binding to the esteric site of the enzyme acetylcholinesterase, the molecule phosphorylates the enzyme (as opposed to esterifying it).

The P=O bond (found in organophosphates) is much stronger than C=O bond (found in acetylcholine), so the enzyme is converted back to its active form much more slowly when an organophosphorus molecule is "docked" on it, hence inactivating the enzyme (Cremlyn, 1990) and therefore blocks the degradation of the neurotransmitter acetylcholine.

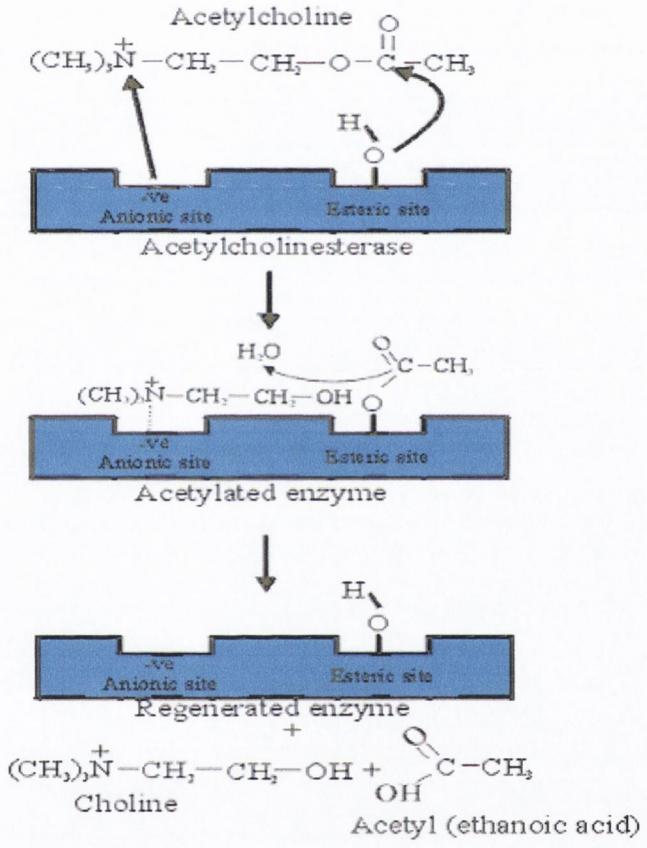


Figure 1.5c Illustration showing the mode of action of acetylcholinesterase (Cremlyn, 1990).

1.5.5 Adverse effects

The major effect of organophosphate poisoning (e.g. chlorpyrifos) is from the inhibition of acetylcholinesterase. Table 1.5c below is taken from a review article by Sultatos (1994) and gives a summary of adverse effects.

In the case of an overdose, antidotes such as pralidoxime chloride may be administered. Pralidoxine chloride is a strong nucleophilic agent that reactivates cholinesterase by reversing phosphorylation of the serine hydroxyl group at the active site of the receptor membrane. It should be used within 12-24 hr and may need to be repeated over a 2-3 week period. Anticholinergic agents such as atropine may be used to reduce the clinical manifestations of organophosphate toxicity.

Table 1.5c Adverse effects of chlorpyrifos

Nervous system location	**Receptor**	**Target tissue**	**Symptoms**
Central Nervous System	Muscarinic and Nicotinic	Brain	Hypothermia, lethargy, tremors, depression, convulsions, coma, paralysis
Peripheral Nervous System	Nicotinic	Heart and blood vessels	Tachycardia
		Skeletal muscle	Fasciculation, ataxia, weakness, convulsions, paralysis
	Muscarinic	Heart and blood vessels	Bradycardia
		Lungs	Excess secretion, bronchial constrictions
		Bladder	Incontinence
		GI Tract	Nausea, vomiting, diarrhoea
		Exocrine Glands	Salivation, miosis, sweating blurred vision

1.5.6 Acute toxicity

The acute oral LD50 (the dose required to kill half of a population of laboratory test animals) for chlorpyrifos is 135-165 mg/kg for rats (Tomlin, 1997). It is classified by the World Health Organisation as a Class II, moderately hazardous pesticide (WHO, 1996/1997).

1.5.7 Environmental fate

Chlorpyrifos is a degradable compound and a number of environmental factors may be active in its breakdown. In all systems (soil, water, plants and animals), the major pathway of degradation begins with the cleavage of the phosphorus ester bond to yield 3,5,6-trichloro-2-pyridinol (TCP). This first step is a detoxification, as TCP has no insecticidal activity and is considered toxicologically insignificant. In soil and water, TCP is further degraded via microbial activity and photolysis to carbon dioxide and organic matter. In animals, TCP may be excreted directly or following conjugation; in plants TCP conjugates are sequestered.

1.5.7.1 Fate in soil

Laboratory studies on its fate in soil have revealed it to be of moderate persistence and immobile to leaching. Aerobic soil degradation half-lives range widely (<10 to >120 days), but are most commonly in the order of 15 to 35 days (Davies and Kuhr, 1976; Tashiro and Kuhr, 1978; Getzin, 1981a). Both microbial degradation (Getzin, 1981a and Miles *et al.* 1983, 1984) and abiotic degradation (i.e. hydrolysis) are important factors in its dissipation from soil, with the latter being predominant in alkaline soils (pH>7.5), (Macalady and Wolfe, 1983), although a number of factors such as soil moisture and application rate (Racke *et al.*, 1996b) can influence the rate of degradation. Chlorpyrifos is hydrolysed under extremely dry soil conditions with half-lives of less than one week being observed (Getzin, 1981b).

Application of termiticidal rates results in initial soil residues of 1,000 ppm or greater (vs. 1 to 10 ppm for agricultural uses) and this results in extended persistence in soil, with half-lives of 175 to 1,576 days (Racke *et al.*, 1994). Under field conditions, dissipatation is somewhat dependent on placement within the soil. Soil surface applications exhibit typical dissipation half-lives of 1 to 2 weeks, whereas half-lives of 1 to 2 months are more common for soil incorporated applications. Chlorpyrifos exhibits a strong tendency to be adsorbed by soil and soil organic matter (K_{oc} = 8498) (Racke, 1993). Field tests have confirmed the negligible downward mobility of chlorpyrifos and in leaching terms it is categorised as "immobile". Field runoff

studies have demonstrated that even under relatively severe conditions, generally less than 1% of chlorpyrifos can move off the edges of treated fields with runoff water and eroding soil (Racke *et al.*, 1996b).

1.5.7.2 Fate in aquatic environments

Laboratory data on the fate of chlorpyrifos in pure water indicates that hydrolysis and photolysis occurs at moderate rates under neutral conditions (Racke *et al.*, 1996b). Hydrolysis half-lives of 29-74 days have been observed at pH 7 and 25°C (Howard, 1989). Photolysis half-lives of approximately 30 days have been noted under similar conditions (Howard, 1989). Under alkaline conditions, hydrolysis proceeds more rapidly; a 16-fold enhancement of hydrolysis rate has been observed in pond and canal water samples (Macalady and Wolfe, 1983). Results of field testing conducted in aquatic ecosystems corroborate the rapid dissipation of chlorpyrifos from natural waters (Racke, 1993). Half-lives in the water column of less than one day are typical, with dissipation rate from sediment similar to that observed in soil. Important dissipation pathways present under field conditions in natural waters include microbial degradation, volatility loss and entrainment in sediments.

1.5.8 Uses and usage

Because of its versatility, chlorpyrifos is used globally in a myriad of pest control situations. It has been widely used around the world to protect a number of important agricultural crops from pest insect attack. The major agricultural use of chlorpyrifos is on corn. Other major registered agricultural uses include alfalfa, almonds, pome fruit (apples and pears), cole crops, citrus fruits, cotton, onions, peanuts, pecans, grain sorghum, soybean, stone fruit (peaches, nectarines, cherries, plums and prunes), sugarberry, cranberry, dates, figs, grapes, leeks, mint, peppers, rappini, rutabaga, seed treatments, strawberries, tomatoes and turnips. It is also used to control over 250 non-agricultural insect and anthropod pests, including subterranean termites, cockroaches, fleas, ants and other insects and anthropods found in and around structures and on lawns, trees and shrubs (Dow AgroSciences, 1998-2000). However some uses of chlorpyrifos employed in the past are no longer commercially employed such as spray dip or pour-on applications of chlorpyrifos used on sheep and cattle.

Also the Environmental Protection Agency (EPA) determined in June 2000 that chlorpyrifos was sufficiently hazardous to human health as to warrant cancellation of nearly all household-use products in the United States.

1.6 CYPERMETHRIN

1.6.1 Introduction

Cypermethrin is a member of the pyrethroid class of pesticide. The pyrethroids form chronologically the fourth major group of pesticides after the organochlorines, the organophosphates and the carbamates. They are synthetic analogues of the pyrethrins, the naturally occurring insecticidal constituents of the flower *tanacetum cinerariaefolium* (National Pesticide Telecommunications Network, 1998). The pyrethroid class consists of 42 active ingredients, some of which differ only in relative stereoisomer content. This figure includes natural pyrethrums and four non-ester pyrethroids. Natural pyrethrums have seldom been used for agricultural purposes because of their cost and instability in sunlight. Their importance has been their role in stimulating intensive research, notably in Japan and in the UK, into the structural basis of their bioactivity. By 1965 a wide variety of analogues e.g. allethrin and tetramethrin had been prepared but none were superior in activity or photostability to pyrethrins. The later developments produced superior insecticidal activity (resmethrin in 1967) and photostability (phenothrin in 1971), as has been documented (Elliott, 1977). These efforts have continued to the present with some spectacular improvements in activity and photostability. At present the compounds in commercial use include e.g. deltamethrin, cypermethrin and fenvalerate, which are fourth generation pyrethroids. These pyrethroids are much more photostable with effectiveness in the range 0.01 to 0.05 lb per acre.

1.6.2 Basic manufacture and formulation

Cypermethrin is primarily manufactured by the US multinational Zeneca Agrochemicals. Other manufacturers are Aimco; Ankur; Atabay; BASF; Cyanamid; Dhanuka; Fitcom; FMC; Gharda; Krishi Rasayan; Mitchell Cotts; Mitsu; New Chemi; Rallis; Rotam; Sanachem; Searle India; Tagros and United Phosphorus (Tomlin, 1997). Cypermethrin is formulated as an emulsifiable concentrate (EC), wettable powder (WP), ultra-low volume concentrate (UL) and granules (GR).

1.6.3 Physicochemical properties

The chemical structure is shown in Figure 1.6a. It has a molecular weight of 416.3 g/mole and empirical formula $C_{22}H_{19}Cl_2NO_3$. The chemical name of cypermethrin is cyano(3-phenoxyphenyl)methyl 3-(2,2-dichloro=ethenyl)-2,2-dimethylcyclopropane carboxylate (Tomlin, 1997). It is a mixture of eight different isomers (Extension Toxicology Network, 1996).

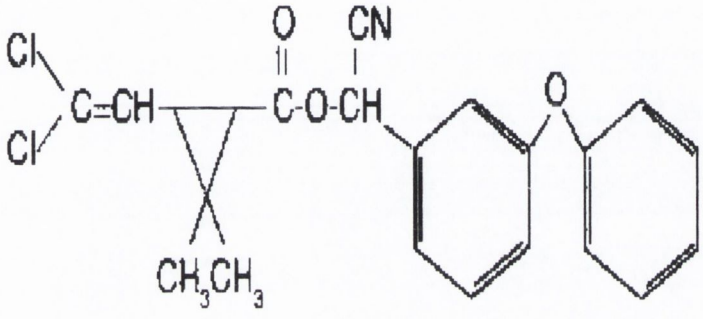


Figure 1.6a Chemical structure of cypermethrin

a) State	***b) Odour***	***c) Boiling point***
Viscous Liquid	Mild chemical odour	>200°C
d) Colour	***e) Melting point***	***f) Vapour pressure***
Clear amber	60-80°C	2.3×10^{-4} mPa at 20°C
g) Density	***h) Partition coefficient (n-octanol/water)***	
1.20 g/ml at 20°C	K_{ow} log P = 6.6	

i) Solubility

The solubility of cypermethrin in water and other solvents (g/l at 20°C) is illustrated in Table 1.6a (Heitzman, 2002).

Table 1.6a Solubilities of cypermethrin

Solvent	Solubility (g/L at 20°C)
Water	9.0×10^{-6}
Cyclohexane	> 600
Xylene	> 600
Ethanol	> 337
Hexane	103
Acetone	> 450

(j) UV absorption spectrum

Maximum absorption spectrum is at a wavelength of 254 nm.

(k) Stability

Relatively stable in neutral and weakly acidic media, with optimum stability at pH 4. Hydrolysed in alkaline media (Tomlin, 1997).

(l) Metabolites

The two principal metabolites of cypermethrin are 3-phenoxybenzoic acid (PBA) and cyclopropanecarboxylic acid derivatives (Sakata *et al.*, 1986), principally, 3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropanecarboxylic acid (DCVA) (Kaufman *et al.*, 1981).

1.6.4 Mode of action

Cypermethrin is a pyrethroid, which acts as both a stomach poison and contact pesticide affecting the insect's nervous system (Jin and Webster, 1998 and Klaassen *et al.*, 1996). Nerve cell membranes have a specific electrical charge and altering the amount of ions (charged atoms) passing through ion channels causes the membrane to depolarise, which in turn causes a neurotransmitter to be released. Neurotransmitters help nerve cells communicate. Electrical messages sent between nerve cells allow them to generate a response, like a movement in an animal or

insect. Pyrethroids affect the nervous system of insects by causing multiple action potentials in the nerve cells and therefore delay the closing of the sodium ion channel in the sense organs (Costa, 1997 and Vijverberg and van den Bercken, 1990). This repetitive activity results in paralysis and finally death of the animal or insect. Even though pyrethroids are nerve poisons, they are not cholinesterase inhibitors like organophosphates or carbamate pesticides (Tomlin, 1997).

1.6.5 Adverse effects

Early symptoms of poisoning include excessive salivation, nausea and vomiting; shortness of breath and laboured breathing; coarse tremors, hypersensitivity to external stimuli and general lethargy, hypotension and bradycardia. These may be followed by hypertension, tachycardia, convulsions, paralysis and death (International Programme on Chemical Safety, 1989).

There is no specific antidote, therefore treatment must be symptomatic. In cases of severe intoxication, therapy should include a sedative and anticonvulsant (e.g. barbiturates and diazepam). The use of antispasmodic drugs is of limited value. Atropine has been found to effectively alleviate the symptoms of pyrethroid poisoning in laboratory animals. If a large quantity has been swallowed, unless the patient is vomiting, gastric lavage should be performed using a 5% sodium bicarbonate solution, followed by powdered charcoal (International Programme on Chemical Safety, 1989).

1.6.6 Acute toxicity

The acute oral LD50 (the dose required to kill half of a population of laboratory test animals) for cypermethrin is >4920 mg/kg for rats (Tomlin, 1997). It is classified by the World Health Organisation as a Class II, moderately hazardous pesticide (WHO, 1996/1997).

1.6.7 Environmental fate

Cypermethrin shows active degradation in soil, water, vegetation and animals. However it is not found in air other than from drift that may occur with spray application. Experimental results indicate that there is practically no movement of cypermethrin from contaminated soils to the surrounding air (Bacci *et al.*, 1987).

1.6.7.1 Fate in soil

Hydrolysis and photolysis play major roles in the degradation of cypermethrin in soil. Hydrolysis of the ester linkage is the principal degradation route and leads to the formation of PBA and cyclopropanecarboxylic acid derivatives, prinicipally, DCVA (as explained in Section 1.6.3). Cypermethrin also photodegrades rapidly on soil surfaces to many byproducts, with half-lives of 8-16 days (Walker and Keith, 1992). As with hydrolysis, the principal photodegradation products of cypemethrin are PBA and DCVA, with >15% recovery of each after 32 days of irradiation (Hall *et al.*, 1981).

Cypermethrin is a non-polar pesticide, readily adsorbed onto the soil surface and bound there. According to Kaufman *et al*, (1981), very little cypermethrin pesticide should move through the soil profile, although all of the degradation products are more mobile than the parent product. The degradates, PBA and DCVA are organic acids which are often mobile in soil. These organic acids vary in their mobility from intermediate (in silty clay or loamy sand) to mobile (in silty clay loam) (Kaufman *et al.*, 1981). Cypermethrin is relatively non-persistent in soils with the typical half-life in sandy soils of 2-4 weeks (Chapman and Harris, 1981). Increased cypermethrin persistence was observed in soil with high organic matter, high clay content, reduced microbial activity and anaerobic conditions (Chapman *et al.*, 1981).

1.6.7.2 Fate in aquatic environments

Cypermethrin is extremely hydrophobic and will quickly move from an aqueous solution to suspended particulates. Thus, relatively small amounts of suspended matter in natural bodies of water may remove a significant amount of cypermethrin

from the aqueous phase. Soils and sediment are the main environmental reservoirs for cypermethrin (Bacci *et al.*, 1987). Under normal environmental temperatures and pH, cypermethrin is stable to hydrolysis with a half-life of >50 days. In sterile solution in sunlight, cypermethrin photodegrades slowly, with <10% lost in 32 days (Walker and Keith, 1992). In darkness, cypermethrin was fairly stable with 88.7% and 95.6% recovery after 10 days in river water and distilled water, respectively (Takahashi *et al.*, 1985). Kidd and James (1991) reported that in river water, rapid degradation occurred with a half-life of about 5 days, which is three to four times faster than degradation in distilled water. This suggests indirect photolysis involving naturally occurring substances results in enhanced photodegradation.

The partition coefficient of cypermethrin is very high (K_{ow} = 3.98 x 10^6), consequently it binds strongly onto organic matter (Crossland, 1982). Because of its affinity for soil, cypermethrin may be carried away to nearby water bodies in suspended sediment by rain and irrigation. Yet, once the pesticide is adsorbed to soil particles, availability is reduced, diminishing the toxicological risk to aquatic animals (Agnihorti *et al.*, 1986). In pond experiments, fish have survived in pond water that contained apparently lethal concentrations of cypermethrin (5 ppb) because the chemical was adsorbed onto suspended solids (Crossland, 1982).

1.6.8 Uses and usage

Cypermethrin is effective against a wide range of pests as shown in Table 1.6b (Tomlin, 1997 and Perrin, 1995).

Table 1.6b Leading markets for cypermethrin pesticide

Crop	Pests	Countries
Cereals	Aphids	Europe, USA
Cotton	Bollworm	USA, China, Pakistan, Central America
Vegetables	Leaf cutting caterpillars, aphids	Europe, South East Asia
Fruit	Leaf and fruit feeding caterpillars, aphids	Spain, Eastern Europe
Maize	Stem boring and ear feeding caterpillars	South America, France
Soybean	Leaf feeding caterpillars	Brasil, Argentina, South East Asia
Vector control	Mosquitoes	Middle East, Asia, South America
Professional Pest Control	Cockroaches, Termites	USA, Europe
Animals - Cattle	Ectoparasite	USA, Europe

Chapter 2

Materials

In general, reagent grade chemicals were used in the production and *in vitro/in field* testing of products. The high quality grades were reserved for HPLC analysis. The qualities or grades of materials listed here are those quoted by the manufacturer.

Description (Quality or grade)	**Batch**	**Supplier**
Agrimer 20% *AL30* (Poly(vinylpyrrolidone/ 1-eicosene))	Lot: 3452-01-01	International Speciality Products
Acetic acid (glacial)	Lot: 40K3474	Sigma Chemicals
Acetonitrile (HPLC grade)	Lot: 21270	Riedel-de-Haën
Brilliant blue lake pigment	B/N:AC1466	Colorcon Limited
Atlox 4913 (Methylmethacrylate graft copolymer)	B/N: 105B0142	Uniqema
Buffer soln. pH 4.00	Lot: 51910	Riedel-de-Haën
Buffer soln. pH 7.00	Lot: 71250	Riedel-de-Haën
Buffer soln. pH 9.00	Lot: 97F2	Riedel-de-Haën
Cetrimide	J106730H1	Aldrich Chemicals

Chlorpyrifos technical 98%	N/A	China Jiangsu International Technical Cooperation
Citric acid monohydrate	K91072444/749	Merck
Cyperkill (Cypermethrin emulsion concentrate)	N/A	Mitchell Cotts
Cypermethrin technical 98%	N/A	Barclay Chemicals
Dialysis tubing (inflated diameter 6.3 mm)	N/A	BDH Laboratory Supplies
Dichloromethane	Reagant Grade	Riedel-de-Haën
Diltiazem HCl	B/N: 150406	Profarmaco
Dispex N40 (Sodium polyacrylate)	Lot: 06962BR2	Ciba Speciality Chemicals
Emcocel 50 (Microcrystalline cellulose)	Lot: 530616	Penwest Pharmaceuticals OY
Ethylcellulose 46cps	Lot: 43,383-7	Aldrich
Ethylene glycol	Lot: 17574D0	Aldrich
Halloysite Balikesir	N/A	Balikesir, Central Anatolia, Turkey
Halloysite Konya	N/A	Konya, Central Anatolia, Turkey
Halloysite Goushan	N/A	Goushan, China
Halloysite G	B/N: 12/98	NZ China Clay Ltd.
Halloysite Goushan NZCC	N/A	NZ China Clay Ltd.
Helium Gas	N/A	Air Products
Hexane (HPLC grade)		Riedel-de-Haën
High pressure fluid		Micromeritics Instrument Corporation

High vacuum grease	N/A	Dow Corning
Hydrochloric acid	UNI789	Sparks
Kaolin	Lot: 41780	Riedel-de-Haën
Liquid paraffin, thick	N/A	Lennox Chemicals
Paracetamol	Granular	FMC Corp., USA
Proxel BD20 (1,2 --benzisothiazolin-3-one)	B/N: 0142	Avecia
Mercury	Triple distilled	BDH Laboratory Supplies
Methanol (HPLC grade)	B/N: 31950/2578	Scharlau
Nitrogen gas	N/A	Air Products
Reference material for Gemini 2370 (Kaolinite)	P/N 004-16819-00	Micromeritics Instrument Corporation
Reference material for PoreSizer 9320 (Silica alumina)	P/N 004-16822-00	Micromeritics Instrument Corporation
Rhodopol 23 (Xanthan gum)	B/N: 01.008.17	Rhodia
di-Sodium hydrogen phosphate-12-hydrate	Lot: K91671074/102	BDH Laboratory Supplies
Sodium sulphate anhydrous granulated	Lot: SO0667	Scharlau
Talc	Prod 33122	BDH Laboratory Supplies
Sodium citrate BP	3164	Aldrich Chemicals
Sodium hydroxide	B252582	Lennox Chemicals
suSCon Green (chlorpyrifos in plastic resin pellets)	N/A	Consolidated Fertilizers Limited
Water	Distilled, deionised	

Chapter 3

Methodology

3.1 METHODS USED FOR HALLOYSITE POWDER ANALYSIS

3.1.1 Scanning electron microscopy (SEM)

SEM was used to evaluate halloysite powder samples. The samples were mounted on aluminium stubs using double-sided sticky tape. The samples were coated with a thin film of gold in a Polaron SC500 sputter coater (UK) and examined using a field emission Hitachi S4300 scanning electron microscope (UK). The analysis allowed an examination of the size and surface appearance of the particles in the powder samples. The effects of certain mechanical processes on the appearance and size of fine particles could be observed and estimations of particle dimensions were made.

3.1.2 X-ray powder diffraction (XRD)

X-ray diffraction (XRD) patterns were obtained using a Siemens D500 X-ray powder diffractometer. Powdered samples were studied by placing a thin layer of the powder in conventional cavity mounts. The samples were scanned in all cases from 5 to 40° 2θ. A 1.00° dispersion slit, a 1.00° antiscatter slit and a 0.15° receiving slit were used. The Cu anode X-ray was operated at 40 kV and 20 mA in combination with a Ni filter to give monochromatic Cu Kα X-rays, λ=1.5418 Å.

3.1.3 Helium pycnometry

Helium pycnometry was carried out using a calibrated AccuPyc 1330 (Micromeritics, USA). The measurements were performed on powder halloysite samples (sieved through a 125 μm mesh) using a 1 cm^3 sample cup (sample weight 250 mg +/- 10%). Samples were dried to constant weight using a vacuum oven set to 50°C and removed prior to analysis. The pycnometer was calibrated prior to analyses using an empty 1 cm^3 sample cup to ensure accurate readings. Analyses were performed at least in duplicate using the run parameters shown in Table 3.1a.

Table 3.1a Analysis parameters used in helium pycnometry

Volume of sample cup (cm^3)	1
Number of purges	10
Purge fill pressure (psig)	19.5
Number of runs	50
Run fill pressure (psig)	19.5
Equilibration rate (psig/min)	0.005

3.1.4 BET surface area analysis

Surface area analysis was performed using a Micromeritics Gemini 2370 Surface Area Analyser with nitrogen as the adsorptive gas. The Gemini system is a balanced adsorption apparatus, which uses a technique known as SMART (a sorption method using adaptive rate technology) i.e. adsorbate gas is supplied to the sample at exactly the rate that the sample can adsorb it (Camp and Stanley, 1991). Moisture was removed from the samples using a vacuum oven set at 70°C for 24 hr prior to analysis. Subsequently, samples were degassed using a Micrometrics FlowPrep 060 Degasser. The FlowPrep uses a flowing gas (helium), which is passed over a heated sample (70°C) to remove moisture and other contaminants.

The Gemini analyser consists of two tubes of matched internal volume; one of these tubes contains the sample while the other is empty. Large-bulb tubes, containing longer filler rods were used with the powder samples. These filler rods (hollow glass rods) reduce the amount of gas above the sample and hence act to minimize the pressure 'noise' caused by the boiling of the liquid nitrogen and lead to more precise measurement. The filler rods achieve and improve baseline performance by reducing the random pressure fluctuations caused by slight temperature variations at or near the liquid nitrogen surface. By displacing most of the gas from this gradient region, the filler rods leave less gas to expand and contract in response to temperature changes. Without the random pressure fluctuations, the Gemini system can achieve a much closer balance of pressures and precise gas uptake measurement.

A powdered halloysite sample (sieved through a 125 μm mesh) was added to the large-bulb sample tube to within 1/8 inch of the top of the bulb. An equal volume of inert material having negligible surface area was added to the balance tube (3 mm solid glass beads were used). A long filler rod was worked into each tube through the materials until both rods were resting on the bottom of the tubes (Stephens, 1992). Approximately volume balance was attained, confirmed by the ΔFree Space value being –0.5 to +0.5 cm^3 in all cases. Helium was used for free space measurement, while nitrogen was used as the adsorptive gas. The saturation pressure (P_o) was measured each day. The operating conditions used in the determination of the BET surface area of the samples were an evacuation rate of 500 mm Hg/min and an evacuation time of 3 min with an equilibration time of 10 sec.

BET multipoint surface areas were determined. The volume of nitrogen adsorbed at six relative pressure points between 0.05 and 0.3 units was measured. This is the range to which the linearity of the BET plot is usually restricted (Sing *et al.*, 1985). The BET multipoint area was calculated using either five or six of the measured points (i.e. the surface area was calculated with all six points and then recalculated omitting the first

point or the last point; which ever result gave the highest correlation coefficient, for the fit of the BET plot, was selected). Analyses were performed at least in duplicate.

There were five different halloysite samples studied in this thesis and they are differentiated from one another as follows: halloysite G and halloysite Goushan NZCC (New Zealand China Clay) were supplied by New Zealand China Clay Ltd; halloysite Balikesir and halloysite Konya were supplied from Central Anatolia, Turkey and halloysite Goushan was supplied by Professor Haydn Murray from Indiana University, USA.

3.1.5 Inductively coupled plasma-mass spectrometry (ICP-MS)

A known weight of a halloysite G powder sample was digested with concentrated HCl and H_2O_2 with heat prior to analysis. The digested material was diluted with de-ionised water to a known final volume. A dilution of this digest was analysed. The instrument used was an Agilent 7500a ICP-MS in quantitative mode. Semi-quantitative analysis was initially carried out to determine the levels of the various elements present. The elements were then analysed quantitatively using six different standard curves with at least five individual standards per curve. The levels of aluminium and silicon in the samples were not determined due to i) their very high levels in the sample and ii) silicon could not be digested fully by the technique used. The levels of various contaminants in the halloysite G sample are shown in Appendix 1.

3.1.6 Evaluation of the physical strength of halloysite powder samples

A number of different mechanical processes were employed to determine the physical strength of halloysite Balikesir, halloysite Konya and halloysite G. The halloysite samples obtained were then evaluated by SEM to assess the differences obtained compared to the untreated halloysite samples.

3.1.6.1 Preparation of halloysite Balikesir and Konya samples

A sample of halloysite Balikesir rock (approximately 200 g) was weighed out and broken up into small pieces using a hammer. These pieces were then transferred into a metallic mixing bowl containing 500 ml of deionised water and were further size reduced using a multi-blade stirrer for 30 min by a process known as autogenous grinding. This process allowed the particles to impact with each other, the blades of the mixer and the walls of the container.

The above grinding process produced some small fragments of rock and also some pieces of rock, which were larger than desired. The mixture was therefore sieved through a 600 μm mesh to separate the coarse fraction from the finer fraction. The finer fraction was then placed in a beaker and brought back up to the original volume of 500 ml with deionised water, which was labelled the unmilled autogenous ground sample. The coarse fraction was then transferred to a Kenwood chef planetary mixer and milled for a further 10 min with the addition of 100 ml of water. This mixture was transferred to another beaker and brought up to the original volume of 500 ml with deionised water, which was labelled the milled autogenous ground sample.

A sample of halloysite Konya rock (approximately 65 g) was prepared and labelled in exactly the same manner as the halloysite Balikesir. The above samples were then transferred to 50 ml centrifuge tubes and centrifuged at 3000 rpm for 10 min. After centrifugation, the supernatant was discarded and the sediment removed and dried in a forced air circulation oven (Memmert, Type UL40, Germany) for at least 48 hr at 50°C to constant weight. The dried powder cake was ground to a fine powder using a mortar and pestle and sieved through a 125 μm mesh.

3.1.6.2 Preparation of halloysite G sample

The halloysite G sample was supplied as a fine powder, therefore it was only sieved through a 125 μm mesh. The halloysite G sample was then subject to different mechanical techniques as follows:

- **Mortar and pestle**

  5 g halloysite G samples were ground for either 5 or 10 min using a mortar and pestle.

- **Ball milling**

  5 g halloysite G samples were weighed out and transferred into a Retsch® vibratory ball mill and milled for 5 min at 60 and 90% vibratory speeds.

- **Rotar mill**

  5 g halloysite G samples were weighed out and transferred into a Retsch® rotar mill using different blade sizes (0.2 and 0.5 mm) and speed settings (14,000 and 18,000 rpm) for 5 min intervals.

- **Separation Techniques**

  The Alpine Air Jet Sieve A200 LS was used to facilitate separation of the large undesirable particle aggregates and debris from the tubular material of halloysite G. 5 g samples were transferred into to the air jet sieve and a 25 μm micromesh sieve was used for separation, at either 5 or 10 min settings.

3.2 EXTRUSION-SPHERONIZATION PROCESS FOR HALLOYSITE

3.2.1 Mixing

Halloysite G powder (80g) was placed in the bowl and mixed at speed 1.0 using a "K" shaped mixing arm (Kenwood Chef excel, UK). The water (20 ml) was added gradually to the halloysite G powder via a syringe and the mixer was stopped every 2 min to scrape any powder accumulating high on the side-wall of the mixing bowl back into the mix.

The wet mass was placed into an airtight container and allowed to stand for at least 12 hr to allow the powder and water to reach equilibrium.

3.2.2 Extrusion

The wet mass was extruded using a rotary gravity-fed cylinder-type extruder (Alexanderwerk Type GA65, Germany) fitted with a 7.0 cm diameter, 14.8 cm long perforated cylinder. The perforations were 1 mm in diameter and the cylinder wall was 4 mm thick. The perforations were spread evenly and centrally over 8.3 cm length of the cylinder wall. The perforated cylinder was located against a solid cylinder in the extruder and was capable of rotating from 20 to 100 rpm.

3.2.3 Spheronization

The extrudate was spheronized on a 4 inch (120 mm) diameter spheronizer (Caleva Model 120, UK) using a cross hatch friction plate. The spheronized pellets were collected and dried in a forced air circulation oven (Memmert Type UL40, Germany) for at least 48 hr at 50°C to constant weight.

3.3 GENERAL METHODS OF SPHERONIZED HALLOYSITE ANALYSIS

3.3.1 Mercury porosimetry analysis

The mercury porosimeter used was a Micromeritics 9320 Poresizer (semiautomated) with associated software. Measurements were performed on pellet samples using a 5 cm^3 "solid sample" penetrometer (sample weight ~1.0-3.0 g). These samples were placed in a vacuum oven set at 80°C and left to dry to constant weight and removed prior to analysis. Penetrometers were calibrated (by filling with mercury under pressure) prior to use to obtain an accurate measurement of penetrometer volume. Intrusion pressures between 0.5 and 30,000 p.s.i. were used, corresponding to pore diameters between 360 μm and 0.006 μm. The low pressure analysis (0.5 to ~20 p.s.i.) was semiautomatic,

while the high pressure analysis (~20 to 30,000 p.s.i.) was automatically controlled. The pressure Table used for the high pressure analysis is illustrated in Appendix 2.

3.3.2 Scanning electron microscopy (SEM)

SEM was used to evaluate selected pellets. The sample preparation prior to analysis and the SEM unit used in the analysis was similar to that described in Section 3.1.1. The analysis was used to characterize the surface and cross-sectional appearance of pellets.

3.3.3 SEM with energy dispersive x-ray analysis

This method of analysis applies to products containing an element with an atomic number ranging from 11 to 62 in the periodic Table. The samples of pellets were carbon coated using a carbon rope in a Polaron E6300 vacuum evaporator (UK). The samples were then examined using the same SEM unit described in Section 3.1.1 linked to an energy dispersive X-ray analysis system (Princeton Gamma Tech). The elemental analysis was performed at different spots on the samples so that the elemental content could be obtained.

3.4 EVALUATION OF FLOW PROPERTIES OF HALLOYSITE/KAOLIN POWDER MIXES

The formulation in Table 3.4a was used to produce the two powder mixes. 500 g powder mixes of the materials were blended together in a cube mixer for 15 min at 40 rpm.

Table 3.4a Formulation of the two powder mixes

Material	Quantity (% w/w)
Paracetamol, granular	50%
Halloysite G/Kaolin	25%
Emcocel 50M	24%
Talc	1%

3.4.1 Flowability (flodex tester) of powder mixes

The flodex determination of intrinsic flowability is based upon the ability of a powder to fall freely through a hole in a plate. For convenience the flowabilty index is given in millimeter diameter of the smallest hole the powder falls through freely on three successive attempts. This type of unit may be used to establish dry powder flow characteristics prior to setting up equipment such as tablet presses.

The tester was comprised of a stainless steel cylinder with approximate capacity of 200 ml. Also supplied was a series of stainless steel discs each with a precision, drilled hole in the centre (covering a range from 4 to 32 mm diameter). Each disc was attached to form a bottom for the cylinder. A shutter covered the hole during the filling operation. This could be removed smoothly, without vibration to allow powder to flow through the hole.

The test was started with the 16 mm hole or aperture. Once the disc was in position and the shutter closed, approximately 50 g of sample was introduced into the cylinder. The shutter was opened after approximately 30 sec.

The test was positive if the powder flowed through the hole leaving a residue in the cylinder in the form of an upside-down truncated cone. A powder that flocculates in bulk will fall abruptly forming a cylindrical cavity. If the powder did not flow through

the hole, the test was negative. In the case of a positive result the test was repeated with smaller apertures until the result was negative. For negative results, the size of the apertures was increased until the test was positive.

3.4.2 Static angle of repose of powder mixes

The static angle of repose is the angle between the horizontal and the slope of a cone of material. It is formed when excess feed material falls away from a cone of material supported on a stand. The apparatus compromised a stainless steel cylinder with an approximate capacity of 1 L mounted, which is open at both ends. This cylinder was mounted on a stand, which covered the bottom of the cylinder. Approximately 500 g of a powder sample was introduced into the cylinder. After approximately 30 sec the cylinder was slowly lifted off the powder sample. The powder sample formed a cone shape on the stand. The height of the cone was carefully measured. The static angle of repose of a powder sample was determined using Equation 3.4a.

$$\text{Static angle of repose } (\tan^{-1} \text{ degrees}) = 2h/D \qquad \textbf{Equation. 3.4a}$$

where h = axial height of cone (cm)

D = diameter of platform (cm)

The static angle of repose quoted was an average of six determinations. The static angle of repose is a direct indication of the potential flowability of a material. The lower the angle of repose of a dry material, the more flowable a material is.

3.5 TABLET PRODUCTION

100 g powder mixes of the materials in Table 3.4a were blended together in a cube mixer for 15 min at 40 rpm. The powder mixes were then compressed immediately to avoid segregation. The compression was achieved using a Manesty single station, hand operated tablet press (Model 1B 179) fitted with a 12.5 mm flat-faced punch and die set.

The tablet press was adjusted to produce a compression weight of 550 mg approximately (275 mg approximately of paracetamol). The average breaking strain was adjusted to the equivalent of 11 Kp units approximately. The adjusted die cavity depth and compression level were kept constant during the compression of the powder batches.

3.6 EVALUATION OF TABLETS

3.6.1 Uniformity of weight and thickness

Tablet weight was determined by using an electronic balance (Metler AE 100, Switzerland). Tablet thickness was determined by using an optical micrometer. The average of twenty tablets was measured and recorded.

3.6.2 Hardness

Tablet hardness was determined using a tablet hardness tester (Schleuniger Pharmaton Hardness Tester, Model 6D). The test was performed on ten tablets and the average calculated.

3.6.3 Friability

Friability was evaluated using an Erweka Copley Friabilator (TA 20). The test was performed using 20 tablets, which were weighed and then placed in a plastic drum rotated at 25 rpm for 4 min. The tablets were then dusted, reweighed and % friability calculated.

3.6.4 Disintegration

Tablet disintegration was performed on 6 tablets using a six-tube basket assembly surrounded by a water bath maintained at 37°C (Erweka Copley Disintegration Tester, ZT 44). The average of 6 disintegration times was recorded.

3.6.5 Dissolution testing

Dissolution testing was performed using Apparatus II of the BP (Erweka Copley Dissolution tester, DT 6). The dissolution rate was determined in 1000 ml of McIlvaine buffer pH 6.8 as shown in Table 3.6a (Pharmaceutical Codex, 1994), which was equilibrated at 37°C +/- 0.5 deg. and stirred at 100 rpm. The paddles were kept 23-27 mm above the base of the vessel. Samples were withdrawn every 5 min over the initial 15 min, every 15 min for the next 45 min and then every 30 min over a total dissolution time of 120 min.

Table 3.6a Composition of McIlvaine buffer system

pH	Disodium hydrogen-orthophosphate dodecahydrate (g/L)	Citric acid monohydrate (g/L)
6.8	55.3	4.8

Fresh dissolution medium was added in each case to replace the withdrawn samples. The sample was then filtered via a 5 ml syringe, through a 13 mm 0.45 μm pore size Gelman membrane filter (type Supor®-450). A 1 ml sample was diluted to 10 ml using the same buffer medium. 1 ml of this resulting solution was then diluted to 5 ml again using the same buffer. The first 1 ml of sample was used to rinse out the Hellma quartz ultra-violet (UV) cell, having a 1 cm light path and a sample of the remainder was read at 257 nm using a Shimadzu UV 160 spectrophotometer with McIlvaine buffer pH 6.8 as the reference solution.

For subsequent UV readings, the sum of X% of the previous values was added to the new value to give a corrected reading; this sum of the X% adjustments was used to account for the previous sample removed from the bulk medium. The calculation of the X value is shown by Equation 3.6a. Standards were prepared in the medium used to

cover the concentration of drug, which could be expected and a calibration curve was produced from which the absorbance readings could be converted to concentration values. The results of the dissolution represent the average of three determinations.

$$X = \frac{\text{Sample volume}}{\text{Volume of dissolution medium}} \times 100 \quad \textbf{Equation 3.6a}$$

3.7 EXPERIMENTS ON ETHYLCELLULOSE FILMS WITH SOLID INCLUSIONS (HALLOYSITE AND TALC)

3.7.1 Preparation of isolated films

The ethylcellulose was dispersed in dichloromethane by using an Ultra-Turax high shear mixer at a rate of 13,500 rpm for 10 min. Halloysite G or talc were added to the dispersion (Table 3.7a). Films were cast onto glass petri-dishes, covered with inverted funnels to ensure controlled evaporation of solvent and placed in a fume cupboard. After evaporation of the solvent, the films were removed and stored in a desiccator prior to use. The films were checked to ensure freedom from air bubbles or other imperfections. The thickness of the films was measured with a micrometer. All samples tested had a mean thickness of ~30 μm.

Table 3.7a Formulations used for the preparation of isolated films

Ethylcellulose (% w/w)	Dichloromethane (% w/w)	Halloysite G (% w/w)	Kaolin (% w/w)
5	95	0	0
4	95	1	0
4	95	0	1

3.7.2 Permeation study

The permeation study was performed by measuring the diffusion of diltiazem HCl through isolated films in a two-compartment glass diffusion cell. The film of 30 μm thickness and 2 cm diameter was mounted between the two-compartment cells and held in place with a clamp. 250 ml of deionised water and 250 ml of diltazem HCl solution were poured into the acceptor and donor compartment, respectively. The drug concentration for diltiazem HCl was 0.05 M. Continuous stirring was maintained by using stirring bars in both compartments with synchronous motors at 100 rpm.

The two-compartment cell was immersed in a water bath, maintained at 37°C. A 5 ml sample was withdrawn from the acceptor cell every 30 min and replaced with 5 ml of deionised water over a 6 hr period. The samples were taken from half way down the vessel. The samples were filtered through a 13 mm 0.45 μm pore size Gelman membrane filter (type Supor®-450) and were analyzed in a Shimadzu UV-160 spectrophotometer (Japan) at 234 nm wavelength. The repeated removal of samples from the acceptor medium was accounted for in subsequent calculations.

3.7.3 Measurements of mechanical properties

Mechanical properties of the films were evaluated using TA.XT2 texture analyzer equipment with 5 kg load cell (Stable Micro Systems). A film strip in the dimension of 50 x 10 mm and free from air bubbles or physical imperfections, was held between two clamps at a distance of 3 cm. Each test strip was clamped onto a metal flat faced grip after attaching a small piece of fabric (Elastoplast®) to the metal grip via double-sided tape. The cross-head speed for each test (strain rate) was set at 10 mm/sec. Results from film samples, which broke at and not between the metal grips were not included in calculations. Measurements were based on eight replicates for each film. Three mechanical properties, namely tensile strength, elastic modulus and elongation at break were computed from the load-time profile, cross-head speed and film dimensions as shown below:

$$\text{Tensile strength} = \frac{\text{Load at failure}}{\text{Film thickness x Cross - head speed}}$$

$$\text{Elastic modulus} = \frac{\text{Slope}}{\text{Film thickness x Film width x Cross - head speed}}$$

$$\text{Elongation at break} = \frac{\text{Increase in length}}{\text{Original length}} \times \frac{100}{\text{Cross - sectional area}}$$

3.8 DEFLOCCULATION STUDIES

3.8.1 Introduction

The aim of this experiment was to neutralise the charges on the halloysite particles, which form a flocculated system in suspension. This was achieved by using either deflocculating agents or pH changes and in doing so obtain a deflocculated system of separate halloysite particles. These studies involved the use of two deflocculating agents namely sodium citrate (trisodium-2-hydroxypropane-1,2,3-tricarboxylate dihydrate) and cetrimide (trimethyltetradecylammonium bromide), which are anionic and cationic in nature, respectively. The deflocculation study also observed the effect of changing the pH of the halloysite suspension using sodium hydroxide and hydrochloric acid to achieve a basic and an acidic environment, respectively. The deflocculation studies were performed on a halloysite sample from Balikesir, Turkey.

3.8.2 Preparation of halloysite Balikesir sample

The sample of halloysite Balikesir was prepared in exactly the same manner as explained in Section 3.1.6.1. The unmilled and milled samples were labelled A and B, respectively.

3.8.3 Deflocculation experiments on halloysite Balikesir sample

Deflocculation experiments were only carried out on the unmilled and milled halloysite Balikesir samples (sample A and B). All of the equipment used in these experiments was carefully washed with deionised water to avoid ion contamination of the samples. All deflocculation studies were carried out at room temperature (~20°C).

3.8.3.1 Using sodium citrate as a deflocculating agent

A 0.5% w/v suspension of samples A and B were prepared in 200 ml volumetric flasks using deionised water. A 100 mM stock solution of sodium citrate was prepared and a series of 1-in-10 dilutions were made up ranging from 100 mM to 1 nM. A 200 mM solution of sodium citrate was also prepared. 5 ml of sample A suspension was added to ten separate test tubes. The 0.5% w/v suspension was shaken vigorously between sample removal to ensure that the same amount of sample was added to each test tube. 5 ml of sodium citrate solution was then added to each of the test tubes, with concentrations ranging from 1 nM to 200 mM. The procedure was also repeated for sample B.

The test tubes were shaken vigorously at time zero, to ensure that a uniform suspension of particles was achieved. The test tubes were then observed after 30 min; 60 min; 180 min and 24 hr to visually determine if flocculation/deflocculation had occurred. The results of the above preliminary experiment were noted and subsequently a second deflocculation experiment was carried out to determine more precisely the actual concentration of sodium citrate needed to deflocculate the 0.5% w/v suspension of samples A and B. Hence, a second series of sodium citrate solutions were made up using deionised water (1 mM, 750 μM, 500 μM, 250 μM, 100 μM, 75 μM, 50 μM, 25 μM, 10 μM, 1 μM). A 2.5 mM solution of sodium citrate was also prepared. The same procedure was followed as above, but this time using sodium citrate solutions ranging from 1 μM to 2.5 mM, were added to each test tube. The test tubes were observed after

30 min, 60 min, 180 min and 24 hr to determine more accurately if flocculation/deflocculation had occurred.

3.8.3.2 Using cetrimide as the deflocculating agent

The above process was also repeated on both samples A and B with concentrations of cetrimide ranging from 30 pM to 300 mM.

3.8.3.3 pH effect on deflocculation

0.5% w/v suspensions of samples A and B were prepared in 200 ml volumetric flasks using deionised water. Aqueous solutions of 0.1 M hydrochloric acid and 0.1 M sodium hydroxide were also prepared. 5 ml of sample A was added to three test tubes, which also included the following:

Test tube 1: 5 ml of halloysite sample A + 5 ml of 0.1 M HCl.
2: 5 ml of halloysite sample A + 5 ml of deionised water.
3: 5 ml of halloysite sample A + 5 ml of 0.1M NaOH.

The above procedure was repeated for sample B. The test tubes were shaken vigorously at time zero and then observed after 30 min to visually determine if flocculation/deflocculation had occurred.

3.8.4 Preparation of halloysite Balikesir samples to be studied by SEM from deflocculation experiment using sodium citrate

The sediment and supernatant from the deflocculated suspensions (using sodium citrate) of samples A and B produced after 24 hr were removed and dried in a forced air circulation oven (Memmert, Type UL40, Germany) for at least 24 hr at 50°C to constant weight. The samples were prepared (Section 3.1.1) for SEM.

3.9 FORMULATION OF CHLORPYRIFOS LOADED HALLOYSITE PELLETS

3.9.1 Introduction

The aim of this work was to formulate a chlorpyrifos loaded pellet using halloysite to help provide sustained release properties of the pesticide for vine weevil control. The halloysite was firstly pesticide loaded with chlorpyrifos and then formulated into a pellet with liquid paraffin using an extrusion/spheronization technique. The final product was then used in a comparative outdoor pot trial with a commercially available product, *suSCon Green* (chlorpyrifos incorporated in plastic resin pellet), for the control of vine weevil in containerised potted primrose plants.

3.9.2 Chlorpyrifos loading of halloysite Goushan NZCC

2.0 g of halloysite Goushan NZCC sample (sieved through 125 μm mesh beforehand) was weighed out. 2 ml of chlorpyrifos solution (chlorpyrifos, 6.5% w/v in ethanol) and 0.26 ml of ethylene glycol was measured and mixed thoroughly with the halloysite powder. The wetted halloysite powder was placed in a sealed desiccator vessel and a vacuum applied (vacuum suction pressure approximately 30 mm Hg) for 60 sec, until all the air bubbles were removed, as illustrated in Figure 3.9a. The vacuum was removed and reapplied once more as above, to ensure that the pesticide solution had fully displaced all the air present in the mineral tubules. The pesticide loaded samples were dried at room temperature for 24 hr. The dried powders were ground down with a mortar and pestle and sieved through a 125 μm mesh to separate any large agglomerates. This procedure was repeated once again with the same powder samples but the ethylene glycol was omitted. The samples were dried at room temperature and sieved through a 125 μm mesh after each loading.

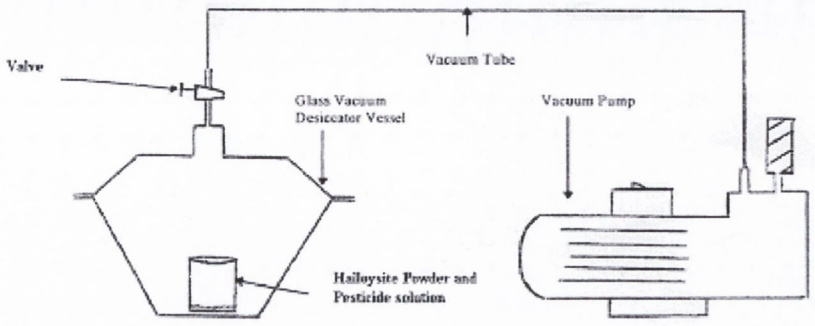


Figure 3.9a Illustration of apparatus used to pesticide load the halloysite powder.

3.9.3 Production of halloysite pellets

3.9.3.1 Introduction

A factorial experiment was designed to ascertain the optimum conditions necessary for producing pellets in the desirable size range of between 850 and 1180 μm. These pellets were produced with halloysite G and liquid paraffin alone. Chlorpyrifos containing halloysite was only incorporated in the final product (Section 3.9.5). The factorial experiment was carried out using halloysite G rather than halloysite Goushan NZCC as the latter was in short supply and also had a much higher tubular quality, therefore it was needed for the final product.

3.9.3.2 Factorial experimentation

This experiment was carried out to examine the effect of three variables i.e. liquid paraffin level, spheronization time and spheronization speed on the yield of pellets of a halloysite G mix (Table 3.9a). These formulation and processing variables were chosen as the most likely candidates to have an effect on the spheronized product properties. The halloysite G mixes were assessed by sieve analysis using a nest of standard sieves (Section 3.9.4). The values obtained for the spheronized product were evaluated by using an analysis of variance (ANOVA) i.e. 3^3 factorial design to determine the optimum

formulation and process variables for pellet formation. The experiment was carried out in random order and performed in triplicate. There were 81 runs completed in total.

Table 3.9a Variables used in the 3^3 factorial experimentation of the mixes and the levels employed

Variable		Level		
		1	2	3
Liquid paraffin level (% w/w)	F	19	21	23
Spheronization time (min)	P	5	10	15
Spheronization speed (rpm)	P	1000	1250	1500

F signifies a formulation variable; **P** is a process variable

3.9.4 Sieve analysis of halloysite pellets from factorial experiments

Sieve analysis was performed using a nest of standard sieves. The sieves were placed on top of each other, the largest aperture sieve at the top with decreasing aperatures as the sieve nest approached the base plate. The sieve aperatures used were 1700, 1180, 850 and 300 μm. A batch of pellets was placed onto a nest of Endecott sieves, which was securely mounted on an Endecott test sieve shaker (1 MK11, UK). The sieves were subsequently separated and their retained fractions weighed. The weight of each fraction was expressed as a percentage of the weight of dry solids added to the sieves. In this thesis the desirable size range of pellets was taken to be between the 850 and 1180 μm and any spheronized product occurring within this range is referred to as "pellets". Pellets occurring above this size are described as "large pellets", while any product occurring below this size range is referred to as "fines".

3.9.5 Production of chlorpyrifos loaded halloysite pellets

The pellets were produced as explained in Section 3.2 by extrusion-spheronization. However the pellets produced in this section were formulated using liquid paraffin rather than water as the granulating agent and dried at room temperature (20°C), as chlorpyrifos is water and heat degradable.

Table 3.9b Final formulation used for chlorpyrifos loaded halloysite pellets

Material	Content (% w/w)
Chlorpyrifos loaded halloysite Goushan NZCC*	78
Liquid paraffin	21
Brilliant blue lake pigment	1

* Halloysite Goushan NZCC was used to make the final product rather than halloysite G, which was only used in the factorial experiments. The halloysite Goushan NZCC used to make the pellets was double loaded with chlorpyrifos solution as explained in Section 3.9.2.

3.9.6 Selection of appropriate dissolution medium for product release studies

3.9.6.1 Introduction

The aim of this experiment was to choose an appropriate dissolution medium for observing the release of chlorpyrifos from the newly developed chlorpyrifos loaded halloysite pellets and the commercially available *suSCon Green* pellets. The medium was required to have a pH of 5 to successfully mimic the conditions of the peat mix used in the containerised pots for the outdoor pot trial. Also the medium needed to illustrate sink conditions and therefore allow the release of chlorpyrifos over an appropriate period of time.

3.9.6.2 Preliminary dissolution studies on unformulated chlorpyrifos

The dissolution studies were performed in 100 ml Duran flasks at room temperature (20°C). The various dissolution media were achieved by mixing McIlvaine buffer as shown in Table 3.9c below (Pharamceutical Codex, 1994) with acetonitrile (HPLC grade).

Table 3.9c Composition of McIlvaine buffer system

pH	Disodium hydrogen-orthophosphate dodecahydrate (g/L)	Citric acid monohydrate (g/L)
4	27.6	12.9

The solutions were thoroughly mixed together in the appropriate volumes as shown in Table 3.9d below and adjusted to pH 5 using an aqueous solution of 0.1 M sodium hydroxide, as confirmed with a calibrated pH meter. The dissolution media were transferred into the 100 ml Duran flasks. For the dissolution of unformulated chlorpyrifos, 10 mg samples were initially loaded into empty tea bags and sealed at the opened end by thread, before being placed into the flasks. The flasks were inverted 10 times immediately and then 10 times before and after sampling. 1 ml samples were withdrawn at intervals of 1, 2, 4, 5, 7, 10, 12 and 14 days and replaced with 1 ml of fresh dissolution medium. The samples were taken from a point half-way up the side of the flask. The samples were then filtered through a 13 mm 0.45 μm pore size Gelman membrane filter (type Supor®-450) and were analysed using a Shimadzu LC-5A liquid chromatograph system (Shimadzu Corp., Japan). The complete HPLC assay is described in Section 3.9.9. The repeated removal of samples from the dissolution medium was accounted for in subsequent calculations. The average of three determinations was reported for each dissolution medium.

Table 3.9d Composition of different dissolution media

McIlvaine buffer pH 4 (ml)	**Acetonitrile (HPLC grade)** (ml)
90	10
80	20
70	30
60	40

3.9.7 Chlorpyrifos content of halloysite pellets assay

5 mg quantity of the chlorpyrifos loaded halloysite pellets (Section 3.9.5) were accurately weighed and transferred to 10 ml volumetric flasks to which acetonitrile was added to the 10 ml mark. They were stirred continuously for approximately one week. 1 ml samples were then removed and the absorbance was measured by HPLC (high performance liquid chromatography) assay at 280 nm. The mean pesticide content in 3 samples was calculated. The concentration of chlorpyrifos within the pellets was calculated as % w/w.

3.9.8 Dissolution studies on chlorpyrifos loaded halloysite pellets, *suSCon Green* pellets and unformulated chlorpyrifos

The dissolution studies were performed in 100 ml Duran flasks at room temperature (~20°C). The dissolution medium was prepared as illustrated in Table 3.9e.

Table 3.9e Composition of dissolution medium

McIlvaine buffer pH 4	**Acetonitrile**
70	30

The samples of each product were weighed out so that they contained equivalent quantities of chlorpyrifos in each of the products present, as shown in Table 3.9f. The samples of each product were loaded into empty tea bags and sealed at the open end by thread, before being placed into the flasks.

Table 3.9f Quantities weighed out for each product in dissolution study

Product	**Quantity (mg)**
Unformulated chlorpyrifos	10
Chlorpyrifos loaded halloysite pellets (10.5% w/w*)	95
suSCon Green pellets (10% w/w**)	100

* Chlorpyrifos loaded halloysite pellets contain 10.5% w/w of chlorpyrifos.

** *suSCon Green* pellets contain 10% w/w of chlorpyrifos.

The dissolution procedure is the same as that described in Section 3.9.6.2. After the last samples were taken a known quantity of residue of each product was transferred into a 10 ml volumetric flask to which acetonitrile was added to the 10 ml mark. They were stirred continuously for approximately one week and were then analysed using a Shimadzu LC-5A liquid chromatograph system (Shimadzu Corp., Japan). The complete HPLC assay is described in Section 3.9.9.

3.9.9 Analysis of levels of chlorpyrifos

The concentration of chlorpyrifos was determined by reverse phase HPLC with UV detection (HPLC method supplied by Grosvenor Chemicals, UK). The HPLC system used in the quantitative analysis of the pesticide was a Shimadzu LC-5A liquid chromatograph system (Shimadzu Corp., Japan) equipped with a Shimadzu SPD-2A variable wavelength UV detector operated at ambient temperature. Output from the detector was plotted and analysed using a Shimadzu C-R3A chromatopac integrator. The

column used was a Keystone C18 column (25 cm x 4.6 mm) packed with *Hypersil*™ 5 μm octadecyl silica. A 50 μl loop-injection valve was used to introduce samples into the chromatographic column.

3.9.9.1 HPLC assay of chlorpyrifos

The mobile phase consisted of 82% acetonitrile (HPLC grade), 17.5% deionised water and 0.5% acetic acid (glacial). The mobile phase was filtered through a 47 mm Gelman membrane filter (with 0.2 μm pore-size) using a vacuum filter apparatus (Millipore Corp., USA) and degassed with the aid of an ultrasonic bath (Bran Sonic 220, USA) for 20 min prior to use. A flow rate of 1.3 ml/min of the mobile phase was used generating an operating pressure of 200 Kg/cm^2 and the absorbance readings were made at 280 nm. The retention time of chlorpyrifos was ~5 min. A sample chromatogram is shown in Appendix 3.

A calibration curve of chlorpyrifos concentration *vs.* area under the peak was prepared daily from stock solutions of chlorpyrifos. The chlorpyrifos stock solution (10 mg/10 ml) was prepared by dissolving the pesticide in 10 ml of acetonitrile. Four dilutions of chlorpyrifos and a blank were required to produce the standard concentrations in the range 0 to 100 μg/ml. The absorbance *vs.* their respective concentrations were subject to linear least square regression analysis, resulting in a linear calibration equation (Appendix 4), which was then used for calculating pesticide concentration in the unknown samples. The results were presented as percentage pesticide released.

3.10 OUTDOOR PRIMROSE POT TRIAL

3.10.1 Introduction

The main objective of the outdoor pot trial was to compare the efficacy of chlorpyrifos loaded halloysite pellets (Section 3.9.5) to the commercially available product, *suSCon Green* pellets for the control of vine weevil. This was determined by carrying out a fully

randomised outdoor pot trial using primroses as the model plants as they are easy to grow in containers and are highly susceptible to vine weevil larvae.

3.10.2 Details of primrose outdoor pot trial

The field trial took place at Kinsealy Research Centre, Teagasc, Dublin, Ireland. Primroses (*Primula*, cv. tempra) were grown in plastic pots (diameter 12 cm, volume 0.75 L) in July 2002. The peat mix used for growing the primroses consisted of Irish peat moss (Shamrock®), which was mixed with a slow release fertilizer (Osmocote®) and lime using a Podmore mixer to ensure uniform mixing.

There were four different treatments (64 pots per treatment) in the trial, which were numbered as follows:

1. *suSCon Green* pellets (10% w/w)*.

2. Chlorpyrifos loaded halloysite pellets (10.5% w/w)** at an equivalent rate to *suSCon Green* pellets.

3. Chlorpyrifos loaded halloysite pellets (10.5% w/w) at double the rate to *suSCon Green* pellets.

4. Control, untreated.

* *suSCon Green* pellets recommended application rate is 0.75 kg per cubic meter (1 m^3 = 1000 L) of peat mix. Therefore the recommended application rate for 0.75 L pots is 0.6 g.

** Chlorpyrifos loaded halloysite pellets recommended application rate for 0.75 L pots is 0.55g.

Figure 3.10a Potted primroses placed outside on a gravel bed.

The treatments were mixed with the peat mix and primroses sown as explained in Section 3.10.2.1. The pots were placed outside on a gravel bed (Figure 3.10a). The plot was surrounded by wooden planks that were covered in plastic, the top of which was coated with Fluon GP1 (Whitford Plastics Ltd. Runcorn, Cheshire, England). The experiment was set up in a fully randomised manner (Appendix 5). The plants were exposed to natural infestation of vine weevil and supplemented by artificial augmention with adult vine weevil.

3.10.2.1 Mixing of treatments with peat mix and sowing primroses

Treatments 1, 2 and 3 pellets were weighed out and transferred into 10 ml regina vials for each 0.75 L pot in the trial (treatment 1 – 0.6 g, treatment 2 – 0.55 g and treatment 3 – 1.1 g). This ensured that each 0.75 L pot received the required rate of product to peat mix. The chlorpyrifos loaded halloysite pellets (treatment 2 and 3) were soft 'sticky'

pellets because the granulating agent used to form the pellets was liquid paraffin, which does not evaporate and also the pellets could not be dried in an oven due to heat degradation of chlorpyrifos. Therefore the pellets were weighed out and sprinkled with talc to prevent them adhering together.

0.75 L of the peat mix was measured out and transferred into a bowl with the pre-weighed pellets from the regina vials and mixed at a speed 1.0 for 30 sec using a "K" shaped mixing arm (Kenwood Chef excel, UK). The peat mix and pellets was then transferred into the 0.75 L pot. This process was repeated for each of the three treatments. The colouring agents used in both pellet types facilitated visual confirmation of adequate mixing with the peat mix. Treatment 4, which was the control, did not involve the use of the mixer as no pellets were incorporated into the peat mix. A primrose seedling was then sown in each of the 0.75 L pots as shown in Figure 3.10b.

Figure 3.10a Primrose seedlings being sown in 0.75 L pots containing peat mix and treatments.

3.10.3 Assessment of primrose outdoor pot trial

3.10.3.1 Introduction

The primroses were assessed in December 2002 and February 2003 by determining the foliage vigour of each plant. In February the root vigour was also assessed and the number of larvae per pot were counted.

3.10.3.2 Assessment of foliage vigour

The foliage vigour was assessed by grading each plant from 10–0 (10 = excellent vigour, 0 = dead vigour) as shown in Figure 3.10c. The grades for the December 2002 and February 2003 assessment are tabulated in Appendix 6 and 7, respectively.

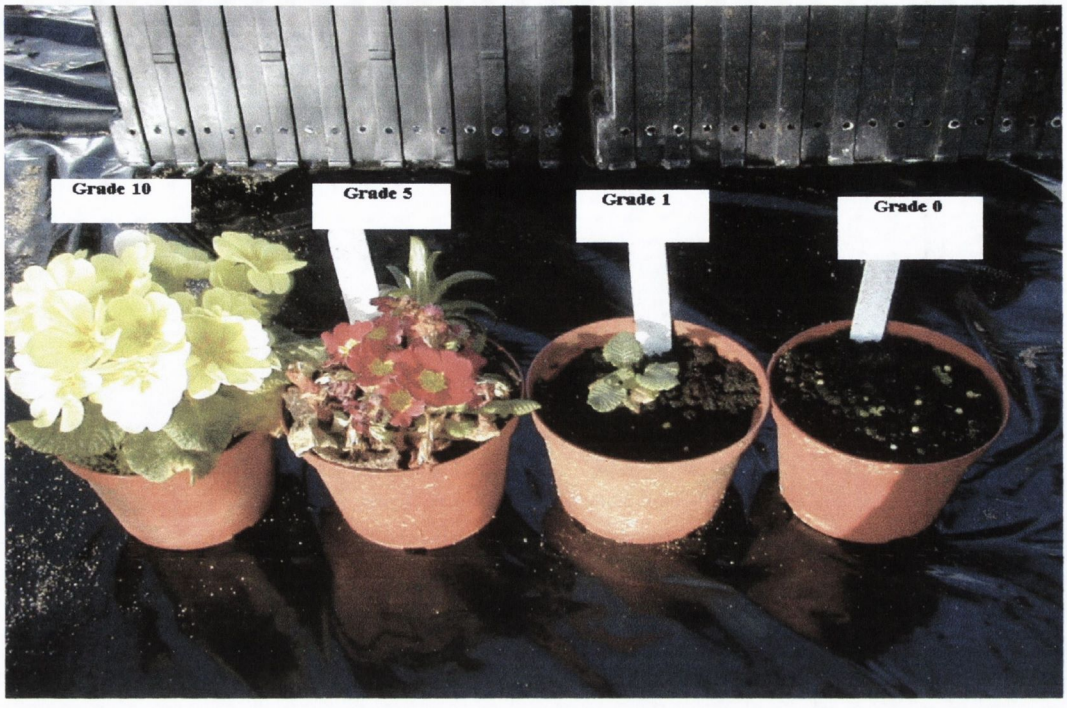


Figure 3.10c Foliage vigour assessment of plants graded from 10-0 (10 = excellent vigour, 0 = dead).

3.10.3.3 Assessment of root system vigour

The root system vigour was assessed in exactly the same manner as the foliage vigour by grading each root system from 10–0 (10 = excellent root system, 0 = no root system) as shown in Figure 3.10d and 3.10e. The grades for the February 2003 assessment are tabulated in Appendix 8.

Figure 3.10d Root system vigour assessment of plants graded from 10-1 (10 = excellent root system, 1 = very poor root system).

Figure 3.10e Root system vigour assessment of plants graded 0 (No root system).

3.10.3.4 Counting vine weevil larvae

Each plant was lifted carefully from the pot, so as not to disturb the root ball, placed on a ceramic tray and the peat mix was crumbled and searched for living larvae (Figure 3.10f and 3.10g). The number of larvae was counted and tabulated in Appendix 9.

Figure 3.10f Peat mix being crumbled by hand and searched for living larvae.

Figure 3.10g Larvae present in peat mix.

3.11 FORMULATION OF CYPERMETHRIN LOADED HALLOYSITE SUSPENSION CONCENTRATE

3.11.1 Introduction

The aim of this work was to formulate a suspension concentrate of cypermethrin using halloysite to provide sustained release of the pesticide. This was achieved by loading the pesticide into the centre of the hollow tubular material under vacuum. The cypermethrin loaded halloysite was then formulated into a suspension concentrate by using an adhesive agent, *Agrimer AL30* to ensure the formulated product adhered to plant leaves for a long period of time and to prevent sedimentation of the halloysite particles and a suspending agent, xanthan gum was also incorporated to prevent sedimentation and aid thixotropy. This suspension concentrate was then compared to a commercially available emulsion concentrate, *Cyperkill* in a field trial to control aphid numbers in a barley crop. The purpose of the newly developed product was to provide sustained release of pesticide and also hopefully remain on the plant leaves for a longer period of time in comparison to the emulsion concentrate version. These improved properties of the suspension concentrate would therefore avoid the need to respray the crop, which is necessary in the case of the emulsion concentrate.

3.11.2 Cypermethrin loading of halloysite Goushan NZCC

The halloysite was loaded with cypermethrin in exactly the same way as explained in Section 3.9.2. However, in this case, 2.0 g of halloysite Goushan NZCC sample (sieved through 125 μm mesh beforehand) was weighed out and 2 ml of cypermethrin solution (cypermethrin, 5% w/v in ethanol) was measured, mixed thoroughly with the halloysite powder and loaded under vacuum as illustrated in Figure 3.9a with the absence of ethylene glycol. The sample was sieved through a 125 μm mesh and then the procedure repeated.

3.11.3 Cypermethrin content of halloysite assay

A similar method was followed as shown in Section 3.9.7. However in this case the pesticide loaded halloysite was transferred to volumetric flasks to which methanol was added and the absorbance was measured at 225 nm. The average pesticide content in 3 samples was calculated. The concentration of cypermethrin within the halloysite powder samples was expressed as % w/w.

3.11.4 Deflocculation studies

3.11.4.1 Introduction

The aim of this study was to determine the minimum concentration of a suitable deflocculating agent needed to achieve a controlled deflocculated system of a halloysite suspension, which normally forms a flocculated system in suspension. A controlled deflocculated halloysite system was necessary to ensure the halloysite particles containing the pesticide were small enough to pass through a spray nozzle for application onto plant foliage. A flocculated system would result in blockage of a spray nozzle due to the presence of 'clumps' of halloysite particles and also result in inaccurate rates of pesticide delivered to the plant foliage. This was determined by using two deflocculating agents, which were *Dispex N40* (sodium polyacrylate) and *Atlox 4913* (methylmethacrylate graft coploymer).

3.11.4.2 Calculations

Dispex N40 and *Atlox 4913* are 40% w/w and 30% w/w solid solutions, respectively. 2% w/w of the solid deflocculating material to halloysite suspension (20% w/v) was chosen as a starting point for the study. Therefore in *Dispex N40* and *Atlox 4913,* 2% w/w of solid deflocculating material to 20 g of halloysite is equivalent to 0.4 g in 1 g and 1.33 g

short supply but had a much higher tubular quality, therefore it was needed for the final product.

3.11.4.3 Phase 1 : Preliminary deflocculation studies involving halloysite G

9 x 10 g of halloysite G samples were weighed (sieved through 125 μm mesh beforehand) into 100 ml jars. The required quantity of *Dispex N40* and *Atlox 4913* solution was added to each jar ranging from 0.5–4 g and 0.65–5.2 g of solution (2–16% w/w of solid deflocculating material) to 10 g halloysite G, respectively (Table 3.11a and 3.11b). A control containing no deflocculating agent was also used for comparative purposes.

Table 3.11a Quantity of *Dispex N40* solution weighed out

Solid deflocculating material (% w/w)	**Solid deflocculating material to halloysite (g/20 g)**	**Deflocculating solution to halloysite weighed into jars (g/10 g)**
2	0.4	0.5
4	0.8	1
8	1.6	2
16	3.2	4

Table 3.11b Quantity of *Atlox 4913* solution weighed out

Solid deflocculating material (% w/w)	Solid deflocculating material to halloysite (g/20 g)	Deflocculating solution to halloysite weighed into jars (g/10 g)
2	0.4	**0.65**
4	0.8	**1.3**
8	1.6	**2.6**
16	3.2	**5.2**

The contents of the jars were made up to 50 ml with deionised water. The halloysite G and deflocculating agent were dispersed in the water using an Ultra-Turax high shear mixer at a rate of 13,500 rpm for 30 sec. The jars were left standing in a beaker containing ice while they were being mixed to prevent foaming of the deflocculating agents. The halloysite suspensions were transferred to 50 ml graduated cylinders and allowed equilibrate for 18 hr.

After 18 hr the halloysite suspensions were visually examined to determine whether they were in a flocculated/deflocculated state. The halloysite suspensions containing *Atlox 4913* were all in a flocculated state so this deflocculating agent was not used for subsequent flocculation studies. *Dispex N40* gave a deflocculated system at the lowest level of 0.5 g (2% w/w) so this deflocculating agent was used in the following phase of studies.

3.11.4.4 Phase 2 : Deflocculation studies involving halloysite G

The same procedure was followed as explained in Section 3.11.4.3. *Dispex N40* was the only deflocculating agent used in phase 2, which ranged from 0.008–0.375 g (0.031–1.5% w/w of solid deflocculating material) as shown in Table 3.11c.

Table 3.11c Quantity of *Dispex N40* solution weighed out

Solid deflocculating material (% w/w)	Deflocculating solution to halloysite weighed into jars (g/10 g)
0.031	**0.008**
0.062	**0.016**
0.125	**0.032**
0.25	**0.064**
0.5	**0.125**
1	**0.25**
1.5	**0.375**

Dispex N40 gave a controlled deflocculated system at a level of 0.064 g (0.25% w/w solid deflocculating material), so quantities within the range of 0.032-0.25 g (0.125–1% w/w of solid deflocculating material) were used in the phase 3 of studies, which involved using halloysite Goushan NZCC.

3.11.4.5 Phase 3 : Deflocculation studies involving halloysite Goushan NZCC

The same procedure was followed as explained in Section 3.11.4.3. *Dispex N40* was added to halloysite Goushan NZCC at a range of 0.032-0.25 g (0.125–1% w/w of solid deflocculating material) as shown in Table 3.11c.

3.11.4.6 Phase 4 : Deflocculation studies involving cypermethrin loaded halloysite Goushan NZCC

The same procedure was followed as explained in Section 3.11.4.3. However in this phase *Dispex N40* was added to cypermethrin loaded halloysite Goushan NZCC at a range of 0.032-0.064 g (0.125–0.25% w/w of solid deflocculating material) as shown in Table3.11c.

3.11.5 Preparation of dispersion of 2% w/w master gel of xanthan gum

A 2% w/w master gel of xanthan gum was prepared by weighing out 98 g of deionised water, which was transferred into a 100 ml beaker containing 0.1 g of *Proxel BD20* (1,2 –benzisothiazolin-3-one), a preservative. The beaker was placed underneath a mechanical stirrer, such that the propeller was as near to the base of the beaker as possible. The xanthan gum powder (2 g) was added slowly to the water. The xanthan gum was not allowed to form a continuous film on the surface of the water. After all the xanthan gum was added, stirring was continued for another 15 min.

3.11.6 Rainfastness Studies

3.11.6.1 Introduction

The aim of this work was to ascertain the appropriate level of adhesive (*Agrimer AL30*) needed in the suspension concentrate to ensure that the final diluted product when sprayed, would remain in prolonged contact on the plant foliage compared to the commercially available emulsion concentrate, *Cyperkill* after simulated rainfall.

3.11.6.2 Preparation of 2% w/v cypermethrin loaded halloysite Goushan NZCC suspension concentrate containing varying levels of Agrimer AL30

For each of the systems examined, 50 ml quantities of suspension were made up using the following method:

5 x 10 g of cypermethrin loaded halloysite and 0.064 g of *Dispex N40* were weighed out and transferred into 100 ml jars with 15 ml of deionised water. The halloysite and *Dispex N40* were dispersed in the water by using an Ultra-Turax high shear mixer at a rate of 13,500 rpm for 30 sec. The recommended quantity of *Agrimer AL30* to be used was 0.1–2 g to 1:1000 diluted spray solution (Verbal communication with Dr. Narayanan, International Specialist Products, USA). The suspension concentrate is a 1:200 diluted spray, therefore giving a recommended adhesive range between 0.02–0.4 g

(0.02 g, 0.1 g, 0.2 g and 0.4 g). The required quantities of *Agrimer AL30*, as shown in Table 3.11d, were added to each jar.

Table 3.11d Quantities of *Agrimer AL30* added to each jar

***Agrimer AL30* added to suspension concentrate (g)**
No adhesive
1*
5
10
20

*A 1:200 diluted spray contains 0.02 g *Agrimer AL30*, therefore 1 ml of undiluted suspension concentrate contains 0.02 g *Agrimer AL30* (50 ml undiluted suspension concentrate contains 1 g *Agrimer AL30*).

The *Agrimer AL30* was mixed with the contents of each jar using the Ultra-Turax high shear mixer. 0.025 g of xanthan gum was mixed with the contents of each jar using a mechanical stirrer. The contents of each jar were transferred into 50 ml graduated cylinders and made up to the 50 ml mark using deionised water. Each graduated cylinder was shaken vigorously to ensure a uniform suspension concentrate.

3.11.6.3 Experiment in green house with simulated rain from a garden sprinkler

1 ml of each of the suspension concentrates (2% w/v) and emulsion concentrate, *Cyperkill* (10% w/v) were diluted to 200 ml and 1000 ml with deionised water, respectively and transferred into a hand operated garden sprayer. The garden sprayer had a nozzle that was adjusted to give a fine mist. The *Phormium tenax* var. yellow

wave was chosen as the plant most likely to resemble the barley plant foliage because the leaves on the plant had a similar waxy surface. Approximately four leaves were sprayed for each of the five suspension concentrates and the emulsion concentrate. Each area of the leaves was accurately sprayed twice from left to right to ensure the leaf surfaces received equal quantities of cypermethrin. After spraying, 1 hr was allowed to elapse for drying before the application of simulated rain.

A simple garden sprinkler, the Hozelock Lawn Queen Sprayer®, was selected for use as a rainfall simulator. Water is forced upwards through fifteen 15 mm orifices of the sprinkler and droplets formed then descend vertically to either side of the sprinkler. The sprinkler was adjusted into the position, which only allowed droplets to fall to the left hand side of the sprinkler. The sprinkler was connected by a hosepipe to a water tap and delivered simulated rain at 50 mm h^{-1}, which was estimated using four rain gauges.

The leaves were laid on a sieve and placed within the irrigation area of the sprinkler. 20 mm of simulated rainfall was applied 1 hr after spraying. 80 leaf discs (disc diameter = 0.12 cm) were punched at random after the treated leaves were allowed to dry and transferred into conical flasks. Four leaves were also sprayed with the suspension concentrate as explained above, but not exposed to rainfall and these acted as a control.

3.11.6.4 Extraction and analysis

The concentration of cypermethrin deposits on the leaves were determined by extraction and analysis by HPLC (Section 3.11.11.2). Cypermethrin was extracted from the leaf discs by placing 80 leaf discs in a conical flask and then adding 30 ml hexane (HPLC grade)/deionised water (5:1). The contents of the conical flask were sonicated for 30 min. The hexane was slowly decanted off (leaving water and leaf discs in the conical flask) and filtered through a glass funnel lined with a quantitative 12.5 cm diameter, No. 42 Whatman paper filter (Whatman International Ltd., Kent, U.K.), which contained 10 g of sodium anhydrous sulphate within the filter. The filtrate was collected in a 100 ml

round bottom flask. The filter was washed through with 25 ml of hexane, which was also collected in the round bottom flask.

The procedure was repeated again without adding the water. The hexane (approximately 100ml) from the extraction was evaporated to dryness on a rotary evaporator. The contents of the round bottom flask were reconstituted with 1 ml acetonitrile (HPLC grade) and vortexed on a Fison Whirlimixer for 1 min. Each sample was injected in triplicate and the entire extraction and analysis was repeated once. The results were expressed as % pesticide per cm^2 leaf surface.

3.11.7 Suspension stability study

3.11.7.1 Introduction

A suspension stability study was performed on the concentrate containing varying amounts of xanthan gum to determine the minimum concentration needed to ensure the cypermethrin loaded halloysite particles remained suspended and also that the product was easily redispersed.

3.11.7.2 Preparation of 2% w/v cypermethrin loaded halloysite Goushan NZCC suspension concentrate containing varying amounts of xanthan gum

The same method used to make the suspension concentrates in Section 3.11.6.2 was also used here except the xanthan gum level varied. The adhesive level remained constant at 20 g. The quantities of xanthan gum within the suspension concentrates (50 ml) are shown in Table 3.11e.

Table 3.11e Quantities of xanthan gum added to 50 ml of suspension concentrates

Quantities of xanthan gum* (g) added to suspension concentrates
0.025
0.05
0.075
0.1

*Added from a 2% w/w master gel of xanthan gum.

The suspension concentrates containing varying quantities of xanthan gum were transferred to 50 ml graduated cylinders. Each cylinder was sealed and after thorough redispersal was observed over a six month period of undisturbed storage at room temp. (~20°C).

3.11.7.3 Assessment

a) Sedimentation volume

Sedimentation volume was calculated as the ratio of the height of the sedimented material (H_u) to the original height of the suspension concentrate (H_o). This measure could be used to determine the concentration of xanthan gum needed in the suspension concentration.

b) Ease of redispersion

The ease of redispersion was deduced from the number of times each cylinder had to be inverted, by hand, under reasonably controlled conditions, before the sediment was apparently completely resuspended. If complete redispersion was not achieved after 12 inversions of the cylinder it was shaken vigorously. If after shaking vigorously the deposit was still present, it was described as "caked".

3.11.8 Preparation of the final 2% w/v cypermethrin loaded halloysite Goushan NZCC suspension concentrate

The final formulation for the suspension concentrate was determined from the deflocculation, rainfastness and suspension stability studies and is shown in Table 3.11f. The suspension concentrate was prepared as described in Section 3.11.6.2.

Table 3.11f Quantities of each material added to final suspension concentrate formulation

Material added	Quantity
Cypermethrin loaded halloysite Goushan NZCC	10 g
Dispex N40	0.064 g
Agrimer AL30	20 g
Xanthan gum	0.025 g
Deionised water	to 50 ml

3.11.9 Rheometry

3.11.9.1 Introduction

Rheological properties of the suspension concentrate were evaluated using a Carri-Med rheometer CSL^{2}500 (TA Instruments, UK), which is a controlled stress/controlled rate rheometer capable of handling numerous types of samples and a range of geometry types and sizes. This rheometer has an air bearing, i.e. it uses air as its lubricating medium, which permits virtually friction free application of torque, allowing it to move under the smallest of forces. The pressurised air is passed through a coarse filter desiccant dryer system and a double filter regulator assembly, to remove particulate matter. A peltier plate is temperature controlled via a constant water supply that allows rapid and accurate heating and cooling of samples. An automatic gap was determined by the instrument

that was 42 μm. The automatic gap setting mechanism was used in order to minimize manual operating errors and reduce operating time.

3.11.9.2 Methods

Two types of rheometry were performed namely continuous shear and oscillatory. Continuous shear is a destructive test, which was performed on a number of different systems outlined in Table 3.11g. The oscillatory technique is theoretically a non-destructive test, which measures the viscous and elastic behaviour of a sample simultaneously. When performing oscillatory frequency sweeps, the linear viscoelastic region of the system must be first determined by a torque sweep. Oscillatory measurements were performed on the systems outlined in Table 3.11h.

All experiments used cone and plate test geometry (angle 4°), which is ideally suited for single-phase homogeneous samples or samples with sub-micron particles. Sample size is determined by the geometry used, as it is spread to the edges of the geometry in use. Proper loading of the sample is not only based on sample size but also on correct positioning of the sample on the peltier plate. An equilibration time of 15 min was allowed after loading the sample on the peltier plate before each experimental run, in order to allow internal stresses caused by sample loading to dissipate and to ensure a uniform temperature throughout the sample. Each experiment was run in triplicate, with fresh sample loaded for each run.

Table 3.11g Suspension concentrate systems on which continuous shear determinations were performed

Sample no.	**System**
1 (Examined at 0, 30, 90 & 180 min)	Suspension concentrate with 0.025 g of xanthan gum (Table 3.11f) *final product*
2	Suspension concentrate with 0.05 g of xanthan gum (Table 3.11e)
3	Suspension concentrate with 0.075 g of xanthan gum (Table 3.11e)
4	Suspension concentrate with 0.1 g of xanthan gum (Table 3.11e)
5	Suspension concentrate with 20 g of *Agrimer AL30* (Table 3.11f) *final product*
6	Suspension concentrate with 10 g of *Agrimer AL30*
7	Suspension concentrate with 30 g of *Agrimer AL30*

Table 3.11h Suspension concentrate systems on which oscillatory rheometry was performed

System	Frequency range (Hz)	Oscillatory torque (μN.m)
Suspension concentrate with varying xanthan gum level (Table 5.1e)	0.01-10	1000
Suspension concentrate with 20 g of Agrimer *AL30 - final product* (Table 5.1f)	0.01-10	1000
Suspension concentrate with 10 g of Agrimer *AL30*	0.01-10	1000
Suspension concentrate with 30 g of Agrimer *AL30*	0.01-10	1000

3.11.10 Dissolution studies comparing the cypermethrin suspension concentrate and the emulsion concentrate (*Cyperkill*)

The dissolution studies were performed in 100 ml Duran flasks at room temperature (20°C). The dissolution medium was achieved by mixing McIlvaine buffer as shown in Table 3.9c (Pharmaceutical Codex, 1994) with methanol (HPLC grade) to give a 70:30 composition, respectively.

100 ml volumes of dissolution medium were transferred into the 100 ml Duran flasks. The dissolution study for the emulsion concentrate was also repeated using 100 ml of dissolution medium and an extra 7 ml of deionised water to take into account the

difference in concentration gradient and volume between the emulsion concentrate and the suspension concentrate.

The liquid samples of each product were measured out so that they contained equivalent quantities of cypermethrin in each of the products present, as shown in Table 3.11j. Dialysis tubing was used to hold the liquid samples of each product, which is permeable to water, with a molecular weight cut off in the range 12,000 to 14,000, inflated diameter of 6.3 mm and a pore size of approximately 2.4 nm. The tubing was cut into 15 cm strips and was then thoroughly wetted by immersion in deionised water. An elastic band was tied around one end of the dialysis tubing and the liquid sample was introduced via the open end. Another elastic band was tied around the open end before being placed into the flasks. The total length of dialysis tubing exposed to the dissolution medium was 10 cm. The excess tubing on both sides of the elastic bands was cut off and discarded.

Table 3.11j Volumes measured out for each product in dissolution study

Product	Volume (ml)
Suspension concentrate (2% w/v*)	10
Emulsion concentrate (10% w/v**)	2

* Suspension concentrate contains 2% w/v of cypermethrin.

** Emulsion concentrate contains 10% w/v of cypermethrin.

The flasks were inverted 10 times immediately and then 10 times before and after sampling. 2 ml samples were withdrawn at intervals of 1, 2, 4, 5, 7, 10, 12, 14 and 21 days and replaced with 2 ml of fresh dissolution medium. The mean of three experiments was reported for each release study. After the last samples were taken a known volume of residue (250 µl) of each product was transferred into a 10 ml

volumetric flask to which acetonitrile was added to the 10 ml mark. They were stirred continuously for approximately 3 days and were then all analysed using a Shimadzu LC-5A liquid chromatograph system (Shimadzu Corp., Japan). The complete HPLC assay is described in Section 3.11.11.2.

3.11.11 Analysis of levels of cypermethrin

The concentration of cypermethrin was determined using the same HPLC system and UV detector as explained in Section 3.9.9. The column used was a Symmetry® C8 column (15 cm x 4.6 mm, packed with 5 μm particles). A 50 μl loop-injection valve was used to introduce samples into the chromatographic system. Metwally *et al.*, (1997), developed the two HPLC methods used. The method described in Section 3.11.11.1 was used for analysis of cypermethrin in the pesticide loaded halloysite to determine the % w/w. The method described in Section 3.11.11.2 was originally used to analyse cypermethrin in vegetables. Therefore in this thesis it was deemed suitable for analysing pesticide content on leaves. Also it was used to determine pesticide levels in the release studies rather than using the method described in Section 3.11.11.1, as components from the suspension and emulsion concentrate gave interfering peaks around the same retention time as the cypermethrin peak at 3.3 min.

3.11.11.1 HPLC assay of cypermethrin in halloysite powder

The mobile phase consisted of 90% methanol (HPLC grade) and 10% deionised water. The mobile phase was filtered as explained in Section 3.9.9.1. A flow rate of 1.0 ml/min of the mobile phase was used, generating an operating pressure of 200 Kg/cm^2 and the absorbance readings were made at 225 nm. The retention time of cypermethrin was ~3.3 min. A sample chromatogram is shown in Appendix 10.

A calibration curve of cypermethrin concentration *vs.* area under the peak was prepared from a stock solution of cypermethrin. The cypermethrin stock solution (10 mg/10 ml) was prepared by dissolving the pesticide in 10 ml of acetonitrile. Four dilutions of

cypermethrin and a blank were required to produce the standard concentrations in the range 0 to 100 μg/ml. The absorbance *vs.* their respective concentrations were subject to linear least square regression analysis, resulting in a linear calibration equation (Appendix 11), which was then used for calculating pesticide concentration in the halloysite. The results were presented as % w/w.

3.11.11.2 HPLC assay of cypermethrin in suspension concentrate and emulsion concentrate used in rainfastness and dissolution studies

The mobile phase consisted of 75% acetonitrile (HPLC grade) and 25% deionised water. The mobile phase was filtered as explained in Section 3.9.8.1. A flow rate of 1.0 ml/min of the mobile phase was used, generating an operating pressure of 200 Kg/cm^2 and the absorbance readings were made at 225 nm. Cypermethrin produces two peaks with retention times of ~10.5 and ~10.9 min using this mobile phase. Sample chromatograms are shown in Appendix 12 and 13.

A calibration curve of cypermethrin concentration *vs.* area under the peak was prepared from a stock solution of cypermethrin as explained in Section 3.11.11.1. The absorbances vs. their respective concentrations were subject to linear least square regression analysis, resulting in a linear calibration equation (Appendix 14).

3.12 WINTER AND SPRING BARLEY FIELD TRIALS

3.12.1 Introduction

The main objective of the field trials was to compare the efficacy of cypermethrin loaded halloysite suspension concentrate (2% w/v) to a commercially available cypermethrin emulsion concentrate, *Cyperkill* (10% w/v) at equivalent dilution of active in winter and spring barley crops for aphid control.

3.12.2 Details of field trials

The field trials took place at the University College Dublin farm at Lyons Research Farm, Newcastle, Co. Dublin in the cropping year 2002-2003. The winter and spring barley crops were located in the Canal field and Kearneystown 1 field, respectively.

There were eight different treatments in the field trials, which were replicated four times (32 plots) (Appendix 15). The recommended application rate (undiluted) of the commercially available cypermethrin emulsion concentrate, *Cyperkill* for aphid control on cereal crops is 1.25 L/ha. The equivalent application rates of the cypermethrin emulsion concentrate, *Cyperkill* to the cypermethrin loaded halloysite suspension concentrate are shown in Table 3.12a. The eight different treatments used in the field trials are shown in Table 3.12b.

Table 3.12a Equivalent application rates of the emulsion concentrate of cypermethrin, *Cyperkill* to the cypermethrin loaded halloysite suspension concentrate for aphid control

	Emulsion concentrate, *Cyperkill*	**Suspension concentrate**
Product	100 g/L	20 g/L
Application rate to crop (undiluted)	0.25 L/ ha	1.25 L/ha
Application rate to crop (diluted)	250 L/ha	250 L/ha

ha - hectare (1 hectare = 10,000 square meters)

Table 3.12b Different treatments used in winter and spring barley field trials

Treatment no.	Treatment name	Application rate to crop (undiluted) (L/ha)
1	Control, untreated	N/A
2[a]	Emulsion concentrate	0.5
3[b]	Emulsion concentrate	0.25
4[c]	Emulsion concentrate	0.125
5[a]	Suspension concentrate	2.5
6[b]	Suspension concentrate	1.25
7[c]	Suspension concentrate	0.625
8[d]	Suspension concentrate	0.3125

Treatments with the same superscript have equivalent active content.

3.12.3 Winter barley crop

3.12.3.1 Cultivation and sowing

The field was ploughed to a depth of 25 cm with a Kverneland reversible plough, which was followed by two passes of a Kuhn power harrow and a front mounted furrow press. This produced a fine, firm and level seed bed.

Trial plots were sown with winter barley cv. *Antigua* on the 8th October 2002 using an Oyjord plot seed drill, which was fully mounted on a customised plot tractor to fit row widths. The plot size in all cases was set at 15 m x 1.5 m. The plots were rolled immediately afterwards with a ring roller.

3.12.3.2 Weed control

The herbicides used were *Cougar* (diflufenican and isoproturon) and *Arelon* (isoproturon). They were applied at a rate of 1.5 L/ha and 2.5 L/ha, respectively. A

tractor mounted Hardi sprayer with an operating pressure of 3 bars and an application rate of 200 L/ha was used to apply the herbicides on the 25th March 2003 at growth stage 24 (GS). Cereal growth and development was monitored by a growth stage identification key using a decimal code system, which is shown in Appendix 16 (Tottman and Broad, 1987).

3.12.3.3 Growth regulation

Growth regulators (Table 3.12c) were applied by a tractor mounted Hardi sprayer, with an operating pressure of 3 bars and an application rate of 200 L/ha on the 16th April 2003.

Table 3.12c Growth regulator programme for winter barley crop

Date	Growth stage	Growth regulator and rate
16th April 2003	31	*Moddus* at 0.2 L/ha and *CCC* at 1 L/ha

Moddus contains the active ingredient triexapac-ethyl.

CCC contains the active ingredient chloromequat choline chloride.

3.12.3.4 Fertilisation control

No basic compound fertilisation was applied as the soil levels of phosphorus and potassium were indicated to be satisfactory by soil analysis. Two applications of nitrogen were applied with a calibrated Sissis plot spreader (Table 3.12d). The nitrogen was applied in the form of calcium ammonium nitrate (C.A.N.) 27.5% nitrogen.

Table 3.12d Fertilisation programme for winter barley crop

Date	Growth stage	Rate
26th March 2003	24	50 kg /ha
9th April 2003	30	70 kg /ha

3.12.3.5 Disease control

A two spray fungicide programme was applied to the winter barley crop on the 16th April 2003 and the 9th June 2003. The first fungicide application was applied by a tractor mounted Hardi sprayer, with an operating pressure of 3 bars and an application rate of 200 L/ha. The second fungicide application was applied by a handheld Azo air compressed sprayer at an application rate of 250 L/ha. The fungicide spray programme is shown in Table 3.12e.

Table 3.12e Fungicide spray programme for winter barley crop

Date	Growth stage	Fungicide and rate
16th April 2003	31	*Stereo* at 1.2 L/ha and *Acanto* at 0.7 L/ha
9th June 2003	75	*Acanto* at 0.8 L/ha, *Opus* at 0.6 L/ha and *Bravo* at 1.0L/ha

Stereo contains pyrimidinamine.

Acanto contains picoxystrobin.

Opus contains epoxiconazole.

Bravo contains chlorothalonil.

3.12.3.6 Pest control

The pesticide used for aphid control was the cypermethrin loaded halloysite suspension concentrate and the cypermethrin emulsion concentrate, *Cyperkill* (details of different treatments shown in Table 3.12b). They were applied by a handheld Azo air compressed sprayer at an application rate of 250 L/ha on the 19th December 2002 and 9th June 2003 at GS 22 and 75, respectively.

3.12.4 Spring barley crop

3.12.4.1 Cultivation and sowing

The field was ploughed to a depth of 25 cm with a Kverneland reversible plough. Trial plots were sown with spring barley cv. *Tavern* on the 10th April 2003 using a one pass Kuhn Accord power harrow/seed drill combination which was fully mounted on a customised plot tractor to fit row widths. The plot size in all cases was set at 10 m x 3 m. The plots were rolled immediately afterwards with a ring roller.

3.12.4.2 Weed control

The herbicide used was *Starone 2* (fluroxypyr), which was applied at a rate of 1.0 L/ha. A tractor mounted Hardi sprayer with an operating pressure of 3 bars and an application rate of 200 L/ha was used to apply the herbicide on the 11th June 2003 at GS 37.

3.12.4.3 Fertilisation control

No basic compound fertilisation was applied as the soil levels of phosphorus and potassium were indicated to be satisfactory by soil analysis. Two applications of nitrogen were applied with a calibrated Sissis plot spreader (Table 3.12f). The nitrogen was applied in the form of calcium ammonium nitrate (C.A.N.) 27.5% nitrogen.

Table 3.12f Fertilisation programme for spring barley crop

Date	Growth stage	Rate
8th May 2003	14	62 kg/ha
13th June 2003	39	85 kg/ha

3.12.4.4 Disease control

A two spray fungicide programme was applied to the spring barley crop on the 4th June 2003 and the 2nd July 2003. The fungicides were applied by a tractor mounted Berthoud 17 m sprayer, with an operating pressure of 3 bars and an application rate of 180 L/ha. The fungicide schedule is shown in Table 3.12g.

Table 3.12g Fungicide spray programme for winter barley crop

Date	Growth stage	Fungicide and rate
4th June 2003	32	*Punch C* at 0.4 L/ha and *Corbel* at 0.3L/ha
2nd July 2003	65	*Allegro* at 0.8 L/ha and *Bravo* at 1.2 L/ha

Punch C contains flusilazole and carbendazim.

Corbel contains fenpropimorph.

Allegro contains epoxiconazole and kresoxim-methyl.

Bravo contains chlorothalonil.

3.12.4.5 Pest control

The pesticide used for aphid control was the cypermethrin loaded halloysite suspension concentrate and the cypermethrin emulsion concentrate, *Cyperkill* (details of different treatments shown in Table 3.12b). They were applied by a handheld Azo air compressed sprayer at an application rate of 250 L/ha on the 27th May 2003 at GS 24.

3.12.5 Harvesting

All plots were harvested using a Sampa Rosenlew 2025 combine harvester. Plot yield was weighed on the combine using an electronic weigh cell. A sample of grain (approximately 1.5 kg) was taken from each plot for quality assessment in the laboratory. All plots were harvested under good conditions and at satisfactory grain moistures. The winter and spring barley were harvested on the 5th August and 27th August 2003, respectively.

3.12.6 Plant observations and measurements

3.12.6.1 Barley yellow dwarf virus (BYDV)

BYDV infection was assessed on the winter barley crop on the 28th May 2003 by visually determining the percentage of the crop infected with BYDV within each plot (symptoms of BYDV explained in Section 1.4.5.2).

3.12.6.2 Aphid counts

Aphid numbers were assessed on the winter and spring barley crops (Table 3.12h). The number of aphids on five main shoots were counted within each plot. This process was repeated five times in random locations throughout the plot and the average obtained.

Table 3.12h Dates of aphid number assessments on winter and spring barley crops

Date	Barley crop	Assessment no.	Days after pesticide spraying
28th May 2003	Winter barley	1st (after 1st pesticide spraying)	160
17th June 2003	Winter barley	1st (after 2nd pesticide spraying)	8
26th June 2003	Winter barley	2nd (after 2nd pesticide spraying)	17
6th June 2003	Spring barley	1st	10
17th June 2003	Spring barley	2nd	21
26th June 2003	Spring barley	3rd	30
3rd July 2003	Spring barley	4th	37
11th July 2003	Spring barley	5th	45
27th July 2003	Spring barley	6th	61

3.12.7 Yield and yield components

3.12.7.1 Yield

The plot weight was recorded at harvest by the electronic weigh cell on the combine harvester. The moisture content was determined using an SM10 Foss electrical chondrometer and all the yields were adjusted to 15% moisture content. Plot weight was converted to yield (expressed in tonnes per hectare) by a multiplication factor to transform plot yield to yield per hectare. This conversion from plot weight to grain yield (t/ha) allows for a plot edge row effect of 0.25 m (width) per plot.

3.12.7.2 Thousand grain weight

A random sample of one thousand grains per plot were counted using a Pfeuffer Contador seed counter and then weighed to determine the thousand grain weight.

3.12.8 Grain quality analysis

3.12.8.1 Moisture content

The moisture content was determined by placing 300 g samples into a SM 10 Foss electrical chondrometer.

3.12.8.2 Hectolitre weight

The hectolitre weight (kg/hl) was determined by placing 300 g samples into a SM 10 Foss electrical chondrometer.

3.12.8.3 Grain size

Grain size was tested by placing 100 g samples into the Herbst Sortimat screening machine for 2 min. The sieves in the machine were positioned on top of each other, the largest aperature sieve at the top with decreasing aperatures as the sieve nest approached the base plate. The sieve aperatures used were 2.25 mm and 2.0 mm. The weight of each grain fraction was expressed as a percentage of the total weight of grain added to the sieves.

3.13 STATISTICAL METHODS USED IN EXPERIMENTS

3.13.1 Introduction

Statistical analyses used in the experiments were compiled with the aid of a statistical package (Minitab Release 10.1 for Windows, Minitab Inc., USA). The "randomisation" of experimental runs has been found to guarantee inferential validity in the face of unspecified disturbances and "blocking" eliminates the unwanted sources of variability

(Box et al., 1978). Hence all the runs within an experiment were conducted in a fully randomised order and whenever appropriate, "blocking" was used to reduce error.

3.13.2 Simple comparative experiments

Simple comparative t-tests were applied to situations where two independent variables were involved. All tests were conducted at 0.05 level of significance. The standard error was calculated using a pooled estimate of the sample variance. The hypothesis was tested with a t-ratio complying with a student's t-distribution having n-1 degrees of freedom, where n is equal to the sample number for each sample group. The t-ratio for two sample means is given by:

$$t = \frac{\overline{X}_2 - \overline{X}_1}{S_D}$$

where t is the t-ratio

$\overline{X}_1$ is the mean of sample 1

$\overline{X}_2$ is the mean of sample 2

S_D is the standard error of the mean difference.

3.13.3 Analysis of variance (ANOVA)

In the above section, the two sample t-test was used to compare the means of two different groups. The t-test can however be generalised to permit comparisons between more than two sample means. This generalization is called analysis of variance (ANOVA). Hence ANOVA is the statistical procedure used for an overall test for more than two sample means for quantitative data. The ANOVA tables encountered in the result sections are divided into the following columns:

Source is the source of variation,

df is the degrees of freedom,

SS is the sequential sum of squares,

MS is the adjusted mean squares,

F is the F-statistic and

p is the p-value, which is the probability associated with the F-statistic.

The F-statistics in the ANOVA table are based on the adjusted sums of squares, which are equivalent to the adjusted mean squares multiplied by the respective degree of freedom. The F-statistic is calculated by dividing the mean square value for the respective term by the mean square value for residual error. A high F-statistic associated with a low p-value (<0.05) would be considered significant.

3.13.4 Factorial experiments

Full factorial experiments were designated by the following nomenclature:

$N = L^K$

Where K is the number of variables,

L is the number of variable levels and

N is the number of experiment runs.

The experiments allowed the determination of the main effects of each variable and interaction between factors.

Chapter 4

Physicochemical characterisation of halloysite samples from different regions

4.1 INTRODUCTION

The main aim of this Chapter was to both physically and chemically characterise halloysite samples from different regions around the world. This was achieved by microscopic analysis (scanning electron microscopy), X-ray diffraction to identify whether the halloysite samples were present in a hydrated or dehydrated form, skeletal density using helium pycnometry, surface area analysis using nitrogen adsorption technique and inductive coupled plasma-mass spectrometry to identify the major trace elements within the samples.

The physical strength of selected halloysite samples was also determined by employing a number of different mechanical techniques: autogenous grinding using a multi blade stirrer (unmilled); Kenwood chef planetary mixer (milled); mortar and pestle; ball mill and rotar mill. A separation technique was used to ascertain if the large agglomerates of halloysite particles and debris could be removed to give a higher tubular content.

It was hoped that from the preliminary characterisation studies of halloysite from different regions, a highly tubular sample of the material with minimal debris and batch to batch variability could be obtained. This halloysite sample could then be employed in the pesticide sustained release agrochemical applications described in Chapters 6 and 7 for loading chlorpyrifos and cypermethrin into the internal lumen of the microtubules, respectively.

4.2 SOURCING HIGH QUALITY TUBULAR HALLOYSITE

Commercially it is well known that significant quantities of high quality halloysite deposits are available in New Zealand and to a lesser extent in the Republic of Korea, China and Japan. Poorer grades are available in the United States (Indiana), Czech Republic, France, the Phillipines and Morocco (Harvey, 1996).

In our study halloysite samples were obtained from a number of different regions around the world. Small samples of halloysite were obtained from the Balikesir and Konya regions, Central Anatolia, Turkey and the Goushan region of China, which were kindly provided through Hacettepe University, Turkey and Indiana University, USA (Professor Haydn Murray), respectively. The halloysite samples from Balikesir, Konya and Goushan regions were referred to as halloysite Balikesir, halloysite Konya and halloysite Goushan, respectively. The primary sources of halloysite were provided by New Zealand China Clay Ltd (a subsidiary company of Ceramaco Corporation, Ltd). The two samples were referred to as halloysite G and halloysite Goushan NZCC with the latter sample believed to have originated from the Goushan region of China.

4.3 MICROSCOPIC ANALYSIS OF HALLOYSITE

As explained in Section 4.2, five halloysite samples from different regions of the world were examined by scanning electron microscopy. SEMs of these five halloysite samples from different regions are shown in Figure 4.3a, 4.3b, 4.3c, 4.3d and 4.3e. From these SEMs it can be seen that there is considerable difference between all the samples in relation to their tubular dimensions and appearance. Figure 4.3a shows an SEM of halloysite G, with tubular dimensions of approx. 0.1 μm in diameter and 0.5 μm in length, which gives a very small tubular volume of only 0.004 μm^3 (Table 4.3a). These tubules are much smaller in comparison to the tubules seen in the other four SEMs of the halloysite samples. There is also a significant amount of debris present in this sample. Therefore it can be concluded

that halloysite G might be inappropriate for pesticide sustained release studies because of the small tubular dimensions and the presence of debris, which would give this sample a poor ability to entrap and retain pesticides for an extended period of time.

(a) **(b)**

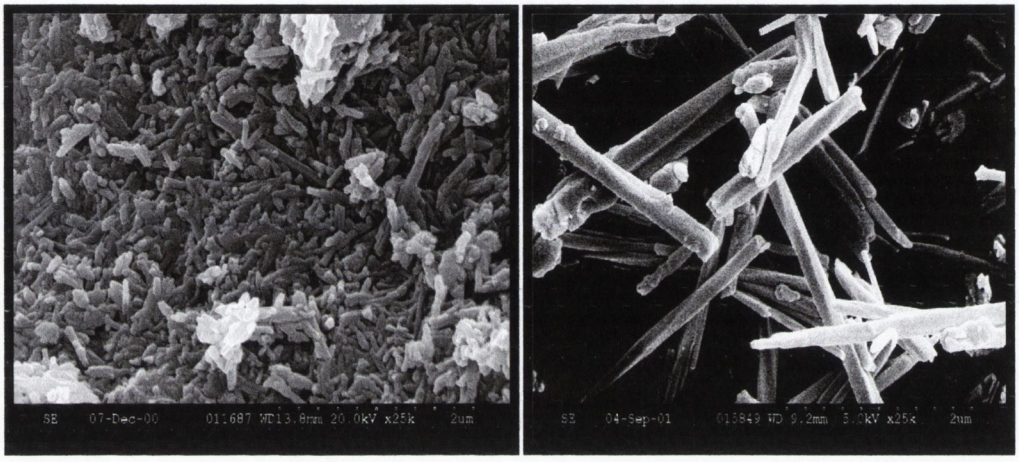


Figure 4.3 SEM of **(a)** halloysite G, **(b)** halloysite Goushan (magnification x 25,000).

Table 4.3a Typical volume measurements of halloysite samples

Halloysite sample	**Volume (μm^3)**
Halloysite G	0.004
Halloysite Goushan	0.314
Halloysite Goushan NZCC	0.057
Halloysite Balikesir	0.074
Halloysite Konya	0.075

Halloysite Goushan, which is shown in Figure 4.3b had significantly larger tubular dimension to halloysite G, with an approx. diameter of 0.4 μm and a typical tubular length of 2.5 μm. Therefore as seen in Table 4.3a, the halloysite Goushan tubules

had a much greater volume (0.314 μm^3) in comparison to the halloysite G tubules (0.004 μm^3) i.e. 78.5 times bigger, based on volume. However there was a large degree of variability within the sample with a significant presence of smaller fragmented tubules. Therefore it was also concluded that this sample would probably be unsuitable for pesticide release studies due its' wide size distribution.

Figure 4.3c illustrates an SEM of halloysite Goushan NZCC. The tubular dimensions of this sample are approx. 0.2 μm in diameter and 1.8 μm in length, which give a tubular volume of 0.057 μm^3 (Table 4.3a). The tubule lengths are much longer than the halloysite G sample in Figure 4.3a. This is further confirmed by the volume measurements of both tubules (Table 4.3a), which indicates that the halloysite Goushan NZCC sample is 14 times bigger, based on volume. Also, qualitatively the internal lumens of halloysite Goushan NZCC were quite prominent from the SEM in Figure 4.3c, which is not the case for the halloysite Goushan sample. In the halloysite Goushan sample there was a significant presence of very large tubules with a wide diameter range, but the tubules did not appear to have adequate internal lumen to significantly entrap pesticides. The halloysite Goushan NZCC tubules were very consistent in shape and size with small sample variability. Therefore this sample should definitely be suitable for further pesticide sustained release studies.

Figure 4.3d shows a SEM of halloysite Balikesir. The tubular dimensions of this sample are approx. 0.25 μm in diameter and 1.5 μm in length, with a tubular volume of 0.074 μm^3 (Table 4.3a). However the halloysite Balikesir sample shows the presence of a large amount of debris. Therefore the halloysite Baliksir sample should give a smaller internal tubular capacity for loading pesticide.

(c) **(d)**

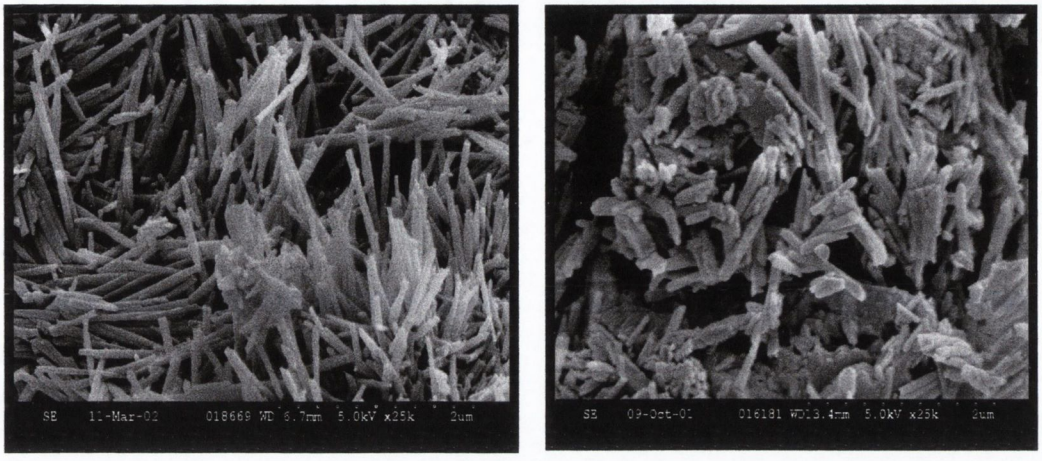


Figure 4.3 SEM of **(c)** halloysite Goushan NZCC, **(d)** halloysite Balikesir (magnification x 25,000).

Figure 4.3e shows an SEM of halloysite Konya. The tubules had typical dimensions of a diameter of approx. 0.2 μm and a length of 2.4 μm, with a tubular volume of 0.075 μm^3 (Table 4.3a). However the tubules in this sample vary considerably from very small fragments to very large tubules. The extent of this variability would be less acceptable for pesticide loading and therefore it might be very difficult to attain uniformity in loading.

(e)

Figure 4.3e SEM of halloysite Konya (magnification x 25,000).

By assessing the halloysite samples visually, the colour of the halloysite Balikesir sample varied considerably in sub-sections compared to the other halloysite samples. It had a distinctive 'pink' colour unlike the other samples, which were varying shades of white. The halloysite Goushan NZCC sample was the whitest in colour. The halloysite mined in New Zealand is known to produce the whitest samples in the world (New Zealand MIA, 2001), which have a very low iron content of approx. 0.25% (Harvey, 1996 and Technical information, New Zealand China Clay, 2001). However halloysite mined in Korea, which was 'pink' in colour, gave a much higher iron content of 1.22% for a typical sample (Harvey, 1996). Therefore this evidence indicates that the colour of the halloysite samples can give an indication of the iron content, with the whiter samples containing lower levels of iron.

Soma *et al.* (1992) showed using X-ray photoelectron spectroscopy that halloysite particle morphology is influenced by structural iron content. The long tubular halloysite had very little surface iron and it's concentration tends to increase with the proportion of non-tubular particles in the sample. This is also reflected in the findings that the structural iron content of the halloysites generally increases from long tubular through spheroidal and short-tubular, to tabular form (Tazaki, 1982; Noro, 1986 and Bailey, 1990). Thus the halloysite sample containing the least amount of iron should be the most tubular in structure.

Therefore from this microscopic and visual study, which examined the samples qualitatively, the halloysite Goushan NZCC sample as seen in Figure 4.3c was regarded as the most suitable sample to use in the pesticide loading studies, because it showed the least amount of sample variability. The tubules were of a sufficient length and diameter to probably give an appropriate level of sustained pesticide release. Also as explained above, the colour of the samples can give a good indication of tubular content with the whitest sample, which was the halloysite Goushan NZCC, giving the more superior tubules.

4.4 XRD IDENTIFICATION OF HALLOYSITE

SEMs can only be used to examine the appearance of the tubular mineral and its dimensions. However XRD was used to identify if the halloysite samples from different regions were present in a hydrated, partially hydrated or dehydrated states. Halloysite as mentioned before is a member of the kaolin group, however it differs from kaolin in that it has a tubular rather than plate-like structure. Also it exists in a hydrated and dehydrated form unlike kaolin, which only exists in a dehydrated form.

In the fully hydrated state the interlayer spacing of halloysite is 10.1 Å. On dehydration the spacing decreases to 7.2 Å (Frost *et al.*, 1997). According to Brindley and Brown (1980), the 10 Å-form is recognised by the 10.1 Å 001 diffraction peak (hydrated), which collapses to 7.2 Å (7 Å-form) when heated for an hour at approx. 100°C. This also concurs with Wada and Mizota (1982), who stated that the basal spacing collapsed to 7.2 Å on heating the sample to 300°C. This collapse of the basal spacing differentiates 10.1 Å halloysite from other clays with basal spacings near 10.1 Å. The 003 reflections (d = 3.40 Å) of the 10 Å form is then replaced by the 002 reflection (d = 3.60 Å) of the 7 Å-form.

Figure 4.4a shows the XRD plots of the five different halloysite samples. These plots show a number of features, which determine whether the halloysite samples are present in a hydrated or dehydrated form. All five halloysite samples show very similar plots with some distinctive features. There is a sharp peak at 12.05° 2θ (7 Å-form) in Figure 4.4a, which translates to a 001 basal spacing of 7.35 Å using Bragg's Law. Also there is a peak at the 002 basal reflection at 24.55° 2θ (d = 3.63 Å) . These two features in the XRD plots are indicative of dehydrated halloysite (7 Å-form). There is a complete absence of a peak at 8.76° 2θ, which is the hydrated 10 Å-form. Therefore it can be concluded that the five samples examined in this thesis are all in the completely dehydrated 7 Å-form.

It can also be observed that the XRD plot of the halloysite Balikesir sample has a number of different peaks to the other four samples. In Section 4.3 under visual examination, this sample was a 'pink' colour unlike all the other samples, which were off-white in colour, indicating that other minerals may be present in the Balikesir sample. Under closer examination of the XRD plot, there are two distinctively large peaks, which are not present in the other samples. These two peaks are at 17.8° 2θ (d = 4.98 Å) and 28.5° 2θ (d = 3.13 Å) on Figure 4.4a, which indicate that traces of sepiolite, a magnesium silicate were also present in the halloysite Balikesir sample.

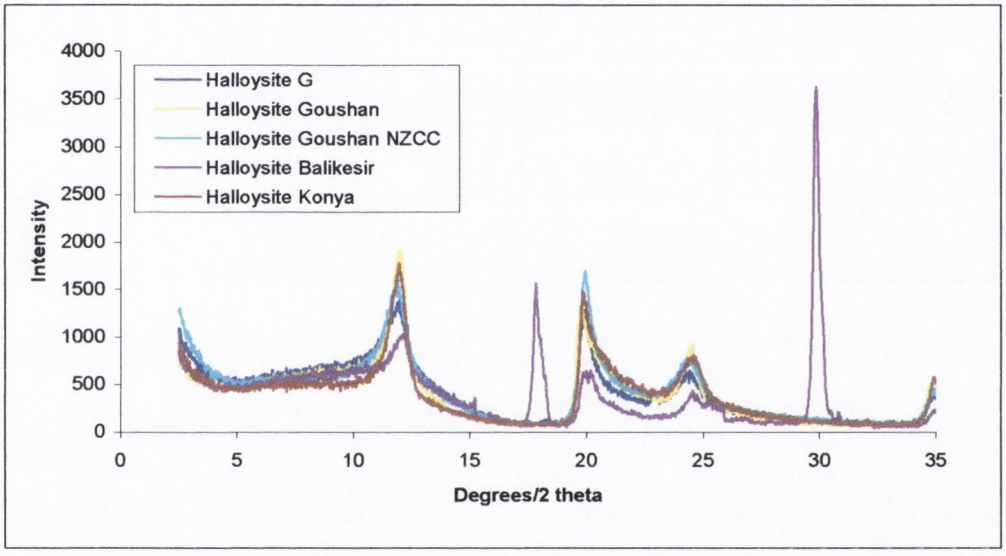


Figure 4.4a X-ray diffraction plots of halloysite samples.

The presence of the dehydrated form for halloysite G (provided in powder form) would be expected as it had previously undergone a beneficiation process by the suppliers, which involved a treatment and heat process. However the other halloysite samples were all supplied in rock form, so it is surprising that they were not present in a hydrated or semi-hydrated form. Despite that, it was unknown when these halloysite samples were excavated. Studies carried out by Hughes (1966) found that halloysite dehydrated rapidly in the 70-30% relative humidity region. Harrison and Greenburg (1962) noted that fully hydrated halloysite underwent some dehydration,

even at relative humidities of 90%. The stability of the interlayer water may be related to the age of formation (Nagasawa, 1969). Therefore it may be deduced that these samples were stored in relatively low humidity conditions and probably for a significant period of time.

4.5 HELIUM PYCNOMETRY OF HALLOYSITE

Helium pycnometry is a technique that can be used to measure the skeletal density of different clays. It can further help in the identification of different clays along with microscopic and XRD analysis. Skeletal density is an inherent property of clay materials. Therefore if the skeletal densities of the different samples varied considerably then it could be deduced that the samples contained unrelated minerals. The pycnometer determines density and volume by measuring the pressure change of helium in a calibrated volume. The analysis measures sample volume, from which density can be derived automatically if the sample weight has been entered.

The skeletal density measurements of the five halloysite samples are shown in Table 4.5a. The measurements range from 2.4593 to 2.6029 g/cm^3. Halloysite G, halloysite Goushan, halloysite Goushan NZCC and halloysite Konya show very similar skeletal densities ranging from 2.4593 to 2.4978 g/cm^3 +/- 0.0385. This was expected because if the samples were halloysite, which was confirmed previously by microscopic and XRD analysis, they should all give the same skeletal densities, approx..

However this is not the case for the halloysite Balikesir sample because it had a higher skeletal density of 2.6029 g/cm^3 in comparison to the other four samples (i.e. 0.1050 g/cm^3 higher than the nearest skeletal density measurement, which is for the halloysite Goushan sample). Therefore it can be concluded that the halloysite Balikesir sample contains other contaminants, which result in the higher skeletal density measurement compared to the other four halloysite samples. This was further

confirmed by the XRD plot in Section 4.4, which showed the presence of another mineral within the sample, that was probably sepiolite.

Table 4.5a Skeletal density measurements of halloysite samples using a helium pycnometer

Halloysite sample	Skeletal density (g/cm^3)
Halloysite G	2.4763 +/- 0.0038
Halloysite Goushan	2.4978 +/- 0.0014
Halloysite Goushan NZCC	2.4593 +/- 0.0006
Halloysite Balikesir	2.6029 +/- 0.0037
Halloysite Konya	2.4837 +/- 0.0005

4.6 SPECIFIC SURFACE AREA MEASUREMENTS OF HALLOYSITE

A nitrogen adsorption technique was used to perform surface area analysis on the five halloysite samples from different regions. Values for the specific surface area of the five samples, as determined using the BET equation are compared in Table 4.6a.

Table 4.6a shows, that the specific surface area for the halloysite samples varies considerably from the lowest of 15.34 m^2/g to the highest of 65.51 m^2/g for halloysite Goushan and halloysite G, respectively. The surface area obtained for halloysite Goushan NZCC is similar to the surface area value of 57.28 m^2/g reported by Levis and Deasy (2002), which were from similar sources of halloysite in New Zealand. It is also similar to a value of 60 m^2/g reported by Price, 1999 (correspondence) and 56.2 m^2/g determined by Churchman *et al.* (1995) on a tubular material from Jarrahdale, Western Australia. The BET surface area value for halloysite Konya is similar to a value of 30.3 m^2/g determined by Churchman *et al.* (1995) for a halloysite sample obtained from Dunedin, New Zealand. Also the surface area of

halloysite Balikesir and halloysite Konya corresponds with the surface area reported by New Zealand China Clay Ltd for a premium grade halloysite (Technical Information, New Zealand China Clay, 2001), which was 20 m^2/g.

Table 4.6a Comparison of specific surface area of halloysite samples using a nitrogen adsorption technique

Halloysite sample	**Surface area (m^2/g)**
Halloysite G	65.51 +/- 1.80
Halloysite Goushan	15.34 +/- 0.76
Halloysite Goushan NZCC	60.68 +/- 1.30
Halloysite Balikesir	20.55 +/- 0.86
Halloysite Konya	25.85 +/- 1.20

There is an approx. four-fold increase in specific surface area between the halloysite Goushan and halloysite G sample as shown in Table 4.6a. This can be explained qualitatively by the SEMs, shown in Figure 4.3a and 4.3b. Halloysite G (smaller tubules) and halloysite Goushan (larger tubules) have average dimensions of approx. 0.5 x 0.1 μm and 2.5 x 0.4 μm, respectively. The nitrogen adsorption technique has measuring units in square metres per gram. Therefore the high concentration of very small halloysite tubules in the halloysite G (Figure 4.3a) sample would give a much higher specific surface per gram in comparison to halloysite Goushan (Figure 4.3b).

A similar explanation may be given for halloysite Balikesir and halloysite Konya, which gave surface area measurements of 20.55 m^2/g and 25.85 m^2/g, respectively. Halloysite Balikesir (Figure 4.3d) and halloysite Konya (Figure 4.3e) have approx. tubular dimensions of 1.5 x 0.25 μm and 2.4 x 0.2 μm., respectively. The tubular dimensions of these two samples are slightly smaller than the dimensions of halloysite Goushan tubules, therefore as explained above they would be expected to give a higher specific surface per gram compared to halloysite Goushan.

The halloysite Goushan NZCC (Figure 4.3c) sample has a surface area of 60.68 m^2/g, which is comparable with the halloysite G sample surface area of 65.51 m^2/g. However it is an unusual finding as the tubular dimensions of halloysite Goushan NZCC (1.8 x 0.2 μm) are approx. four-fold larger than the tubular dimensions of halloysite G (0.5 x 0.1 μm). Therefore it would be expected that the specific surface area of the halloysite G sample would be significantly larger than that of the halloysite Goushan NZCC sample because as explained above there would be a higher concentration of smaller halloysite G tubules compared to halloysite Goushan NZCC tubules.

4.7 TRACE ANALYSIS OF HALLOYSITE G SAMPLE

The levels of various trace elements present in a sample of halloysite G were determined quantitatively using an inductively coupled plasma-mass spectrometre (ICP-MS). The levels of aluminium and silicon in the sample were not determined due to their very high levels in halloysite (aluminosilicate mineral) and also because silicon is not digestable by the technique used. These would be regarded as the two most significant elements in a halloysite sample because the mineral is composed essentially of an alumina sheet in conjunction with a silicon–oxygen sheet. Table 4.7a shows the six major trace elements found in halloysite G. The other trace elements found in smaller amounts in this sample are shown in Appendix 1.

Iron was the main trace element with a quantity of 1360 ppm. This is not surprising because as mentioned in Chapter 1 studies have shown that iron can substitute for aluminium ions within the gibbsite sheet of halloysite (Noro, 1986). However iron content can vary considerably between different halloysite samples. Soma *et al.* (1992), showed that long tubular halloysite had very little surface iron and its concentration tended to increase with the proportion of non-tubular halloysite.

In addition sodium, magnesium, barium, potassium and manganese were also present within the halloysite sample. These elements agree with the elements found in the chemical analysis carried out by New Zealand China Clay Ltd (Technical Information, New Zealand China Clay, 2001). A typical premium grade of halloysite from New Zealand contained traces of all the above elements in oxide form.

Table 4.7a Major trace elements in the halloysite G sample

Trace element	Quantity (ppm*)
Iron	1360
Sodium	470
Magnesium	391
Barium	385
Potassium	119
Manganese	76

*ppm is parts per million

The mineral is chemically similar to other pharmaceutically acceptable silicates such as kaolin, talc and bentonite, which are widely used in pharmaceutical products both internally and externally, but it has not received regulatory approval. As seen in Appendix 1 there is a vast array of unwanted elements in minute amounts present in halloysite such as lead, uranium, arsenic and mercury.

4.8 CHARACTERISATION OF THE PHYSICAL STRENGTH OF HALLOYSITE SAMPLES

4.8.1 Introduction

Halloysite is a relatively strong microtubule mineral being a 1:1 silicon-oxygen and aluminium-hydroxyl structural layer. Therefore the aim of this Section was to determine the physical strength/robustness of the mineral to various mechanical

processes typical of those likely to be involved during compounding and use. The samples of halloysite were compared to an untreated sample using SEM to assess the differences. A number of different techniques were employed to determine the physical strength of halloysite.

The halloysite Balikesir and halloysite Konya were examined using the following mechanical techniques:

- Autogenous grinding, using a multi-blade stirrer (unmilled).
- Kenwood chef planetary mix (milled).

The halloysite G sample was examined using the following mechanical techniques:

- Mortar and pestle treatment for 5 and 10 min.
- Ball milling for 5 min at 60 and 90% vibratory speeds.
- Rotar mill using different blade sizes (0.2 and 0.5 mm) and speed settings (14000 and 18000 rpm) for 5 min.

A separation technique (Alpine air jet sieve with a 25 μm micromesh sieve) was also employed to determine if large agglomerates and debris could be removed from the halloysite G sample, to give a more uniform tubular material.

4.8.2 Results and discussion

Figure 4.8a shows an SEM of the untreated halloysite Balikesir sample. The average dimensions of these tubules are approx. 1.5 x 0.25 μm. Figure 4.8b shows an SEM of halloysite Balikesir after the autogenous grinding process. This process allowed the particles to impact with each other, the sides of the metallic container and the blades of the multi-blade stirrer mixer. From this SEM it is seen that fully intact tubules remained after this severe mechanical process. The tubules did not differ appreciably in size and shape compared to those seen in the untreated halloysite Balikesir sample (Figure 4.8a). The tubules were reasonably uniform in size and shape, and also very little debris was present within the sample after this potentially highly destructive process.

As seen in Figure 4.8c, the average dimensions of the tubules obtained via both the autogenous grinding and milling process were similar to the tubules shown in Figure.4.8b, which only involved the autogenous grinding process. Therefore this finding indicated that the milling process, which involved using a Kenwood chef planetary mixer, did not adversely affect the structure of the halloysite Balikesir tubules, confirming the robustness of these extracted tubules to the vigorous grinding procedures.

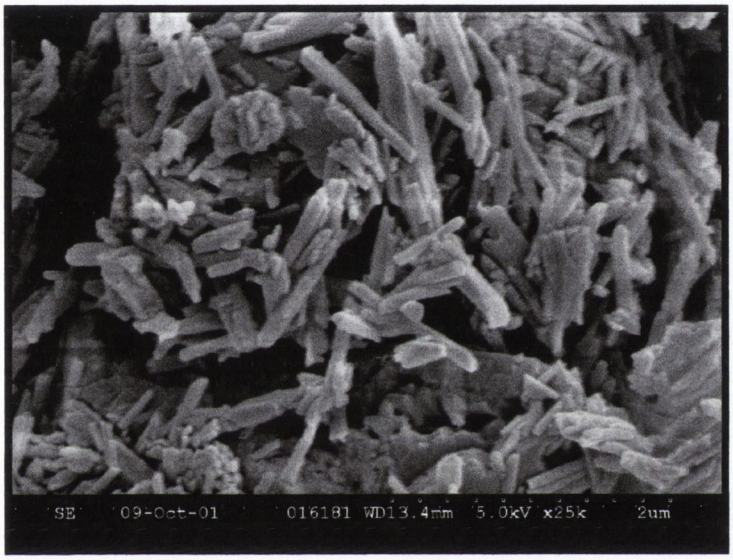


Figure 4.8a SEM of halloysite Balikesir; untreated sample (magnification x 25,000).

Figure 4.8b SEM of halloysite Balikesir extracted from the rock using an autogenous grinding process (magnification x 25,000).

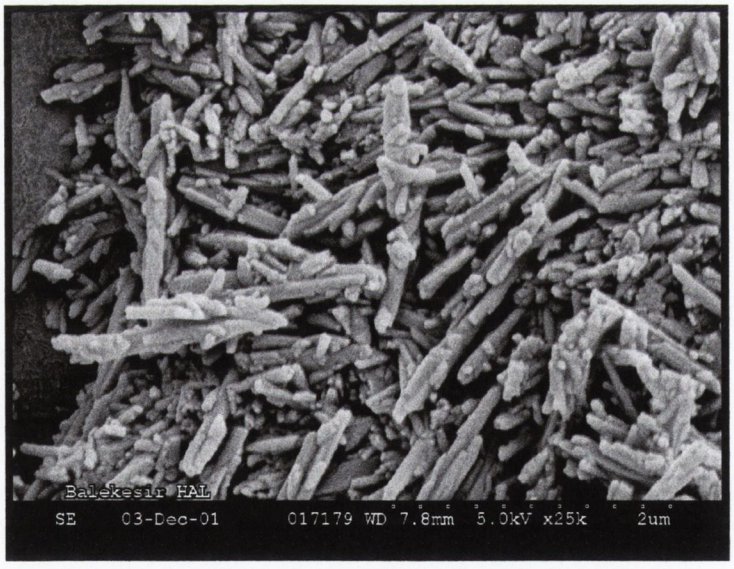


Figure 4.8c SEM of halloysite Balikesir extracted from the rock using an autogenous grinding and milling process (magnification x 25,000).

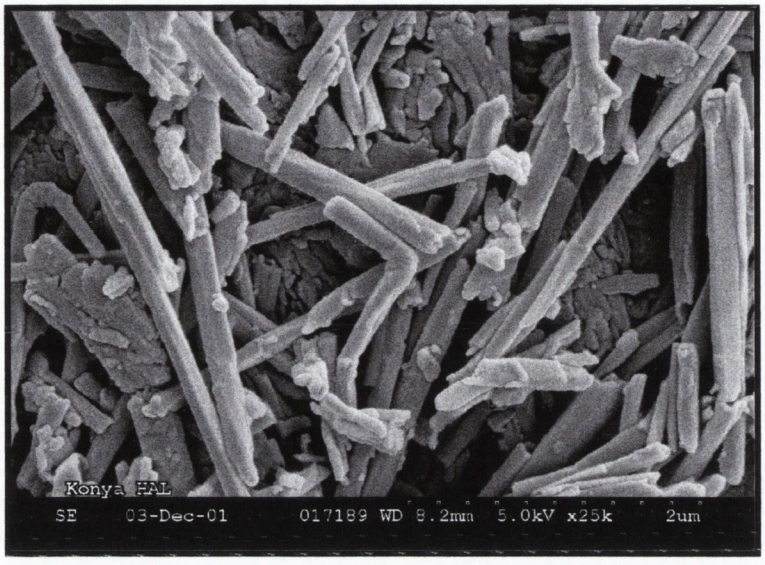


Figure 4.8d SEM of halloysite Konya, untreated sample (magnification x 25,000).

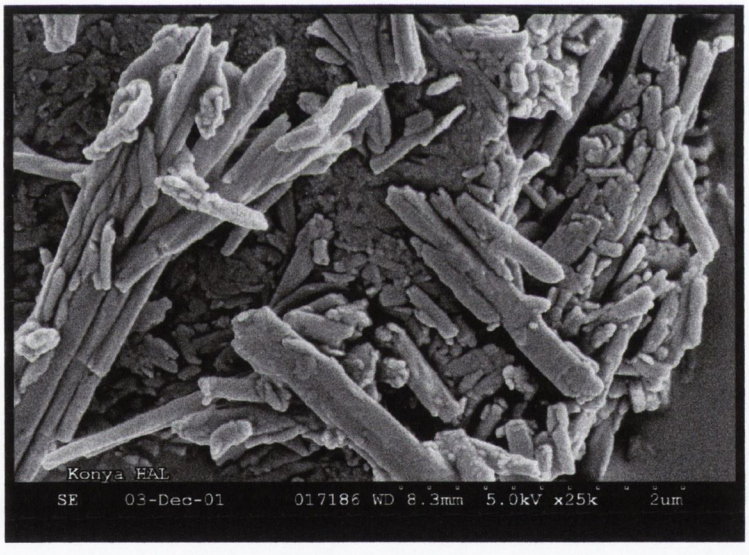


Figure 4.8e SEM of halloysite Konya extracted from the rock using an autogenous grinding process (magnification x 25,000).

Figure 4.8d illustrates an SEM of the untreated halloysite Konya sample, which has tubular dimensions of 2.4 x 0.2 μm. These tubules appear to be much more varied in size and structure than those of the untreated halloysite Balikesir sample as seen in

Figure 4.8a. Some of the tubules in this sample are as long as 3 μm, while other tubules in the sample are <0.5 μm in length. Also there is more debris and fragmented tubules present in the untreated halloysite Konya sample than in the untreated halloysite Balikesir sample (Figure 4.8a).

The halloysite Konya sample, after the autogenous grinding process (Figure 4.8e) shows tubules, which vary widely in size in comparison to the untreated halloysite Konya sample (Figure 4.8d). In Figure 4.8d the majority of the tubules are approx. 2 μm in length. However in Figure 4.8e, after the autogenous grinding process the majority of the tubules are <0.5 μm in length with a very small minority of the tubules approx. 2 μm in length. However in Figure 4.8f, the average dimensions of the tubules after both the autogenous grinding and
milling process are all <0.5 μm in length with a complete absence of tubules approx. 2 μm in length, which were seen in Figure 4.8d and 4.8e.

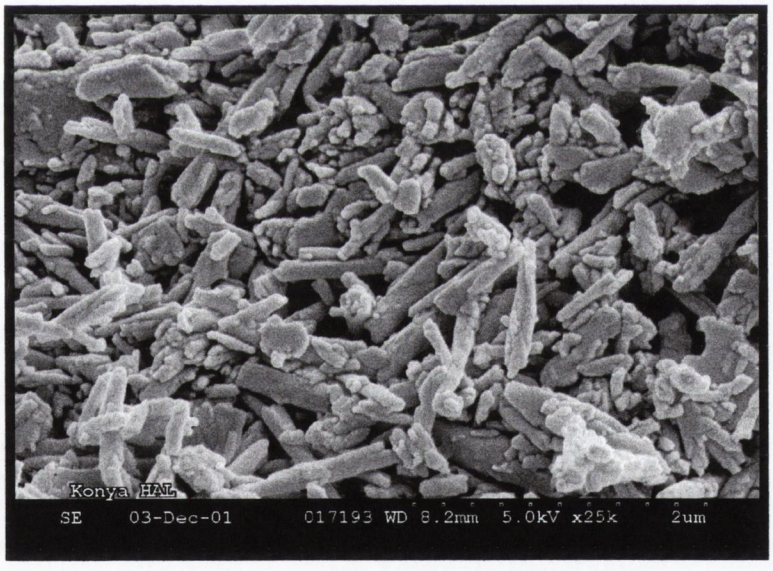


Figure 4.8f SEM of halloysite Konya extracted from the rock using an autogenous grinding and milling process (magnification x 25,000).

Therefore the large degree of fragmentation of the halloysite Konya sample may be attributed to the vigorous mechanical processes used in this experiment. It may have been more appropriate to use a more gentle extraction process such as sonication, which may have produced more intact tubules. This indicates that the halloysite Konya sample was unable to withstand the extraction process in comparison to the halloysite Balikesir sample. Therefore the halloysite Konya recovered from this simple mechanical processing would be unsuitable to be used for an agrochemical or pharmaceutical loading application due to the high level of fragmented tubules. However the halloysite Baliksir sample would show definite potential in this area due to the tubules remaining intact throughout the mechanical processing. Despite that, as explained in Section 4.3 there is a greater presence of debris in the untreated halloysite Balikesir sample in comparison to the halloysite Goushan NZCC sample, therefore making the latter more suitable for pesticide loading applications.

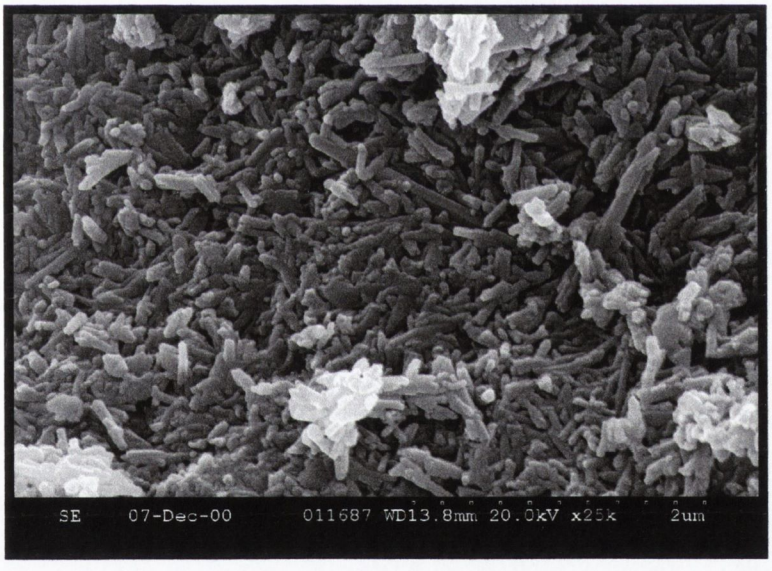


Figure 4.8g SEM of halloysite G, untreated sample (magnification x 25,000).

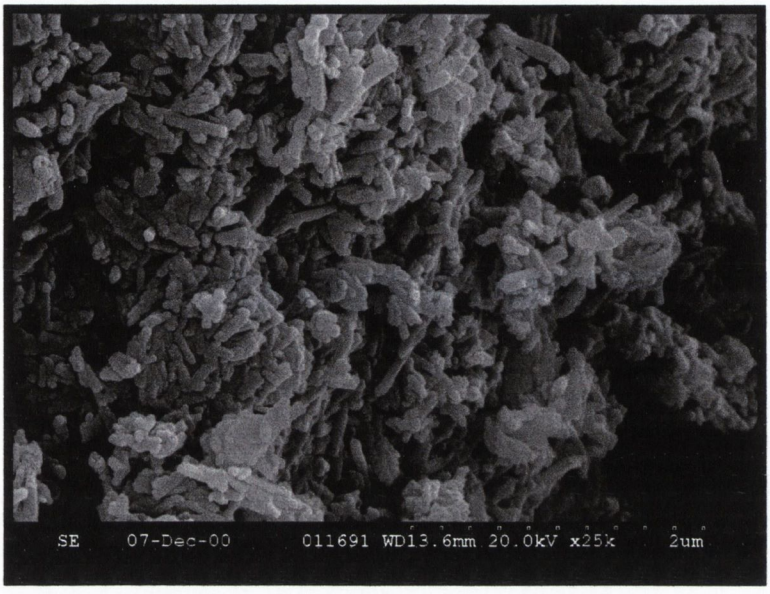


Figure 4.8h SEM of halloysite G treated with a mortar and pestle for 5 min (magnification x 25,000).

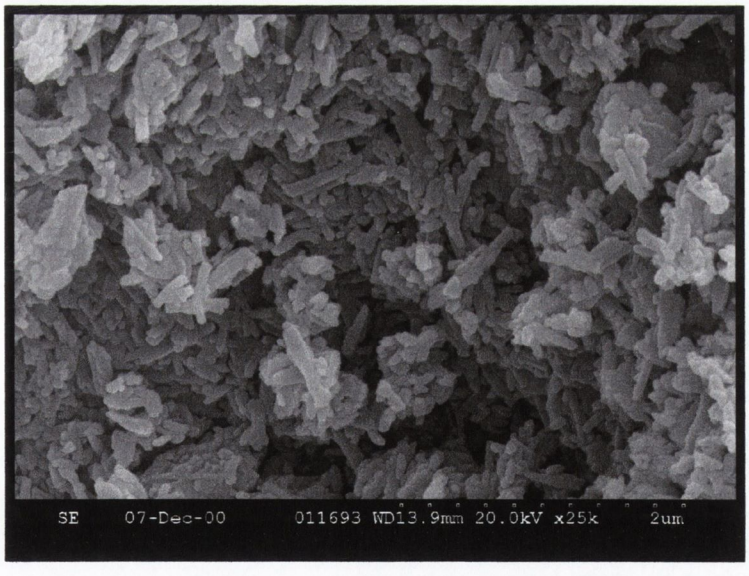


Figure 4.8i SEM of halloysite G treated with a mortar and pestle for 10 min (magnification x 25,000).

Figure 4.8g illustrates an SEM of an untreated halloysite G sample, which has tubular dimensions of approx. 0.5 μm in length and 0.1 μm in diameter. From the SEMs in Figure 4.8h and 4.8i it can be observed that there was no apparent destruction of the tubular structure when the samples were ground with a mortar and pestle for varying lengths of time (5 and 10 min). From these SEMs it is seen that intact tubules remained after this process. The tubules do not differ appreciably in size, shape or appearance to those seen in the untreated sample of halloysite (Figure 4.8g).

Other mechanical methods were also employed, which involved using a ball mill and a rotar mill. The ball mill technique involved a circular stainless steel ball set at different vibrations, which would give a much higher level of impaction compared to a mortar and pestle. Figure 4.8j illustrates that there was no apparent destruction of the halloysite tubules using this technique at the lower vibration setting of 60%. The tubules had similar dimensions to the untreated halloysite sample (0.5 x 0.1 μm). However for the halloysite sample exposed to ball mill vibrations of 90% (Figure 4.8k), there was a much larger presence of debris with a significant quantity of tubules in the <0.25 μm size range. The increased vibration setting of 90% resulted in a much greater level of impaction on the material, consequently resulting in significant destruction of the tubules.

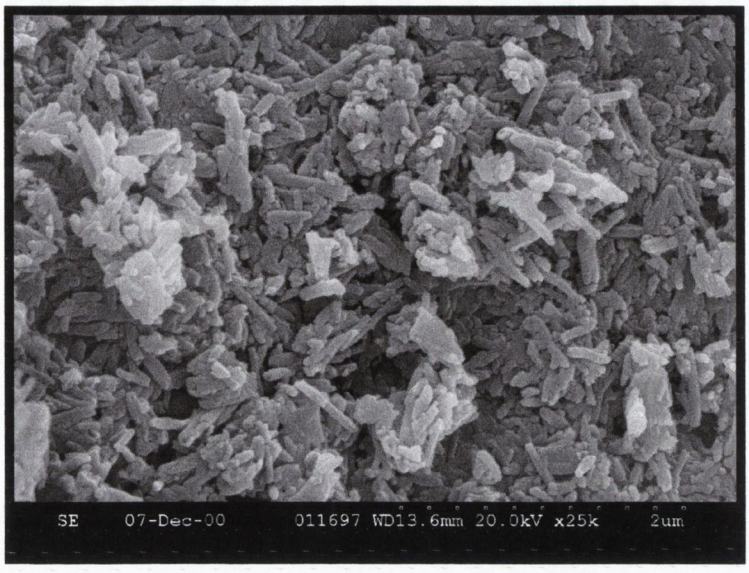


Figure 4.8j SEM of halloysite G treated with a ball mill at 60% max. vibration (magnification x 25,000).

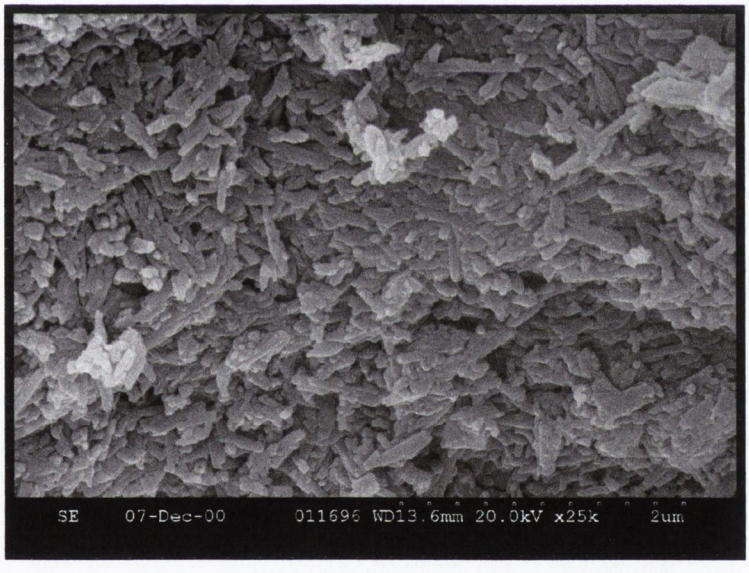


Figure 4.8k SEM of halloysite G treated with a ball mill at 90 % max. vibration (magnification x 25,000).

Figure 4.8l, 4.8m, 4.8n and 4.8o illustrates SEMs of the halloysite tubules after exposure to the rotar mill technique, which used elevated levels of shear and impaction with high speeds of 14000 and 18000 rpm compared to either of the other techniques. Figure 4.8l and 4.8m show the SEMs of halloysite samples after using the 0.5 mm blade. The halloysite tubules in Figure 4.8m using the higher speed of 18000 rpm show a greater presence of smaller tubules of <0.25 μm compared to Figure 4.8l using the lower speed of 14000 rpm. This is self-explanatory with the higher speed leading to greater destruction of the tubular material.

The halloysite tubules exposed to the 0.2 mm blade (Figure 4.8n and 4.8o) show a large amount of debris compared to the untreated halloysite sample (Figure 4.8g). The smaller blade size of 0.2 mm would be expected to cause a further reduction in tubule size. However there is no major difference in tubule size between the SEMs of the 0.5 mm blade size using the higher speed of 18000 rpm and the 0.2 mm blade size.

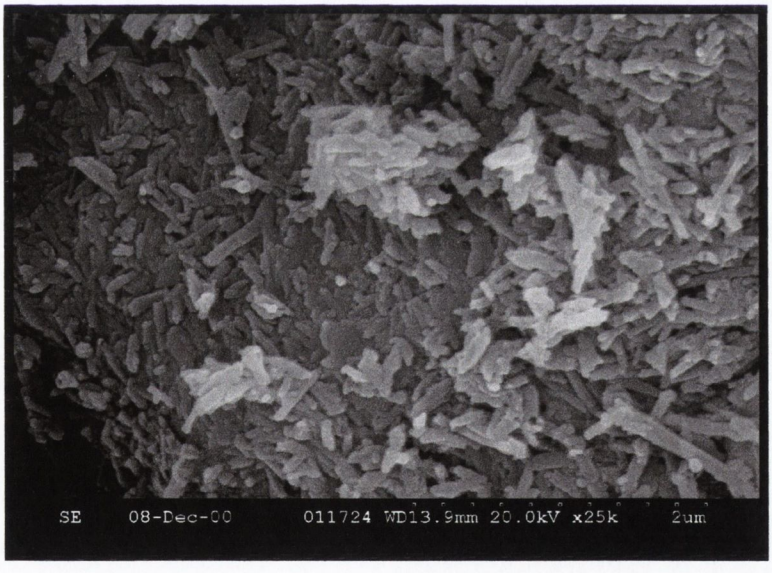


Figure 4.8l SEM of halloysite G treated with a rotar mill (0.5 mm blade size) at a speed of 14000 rpm (magnification x 25,000).

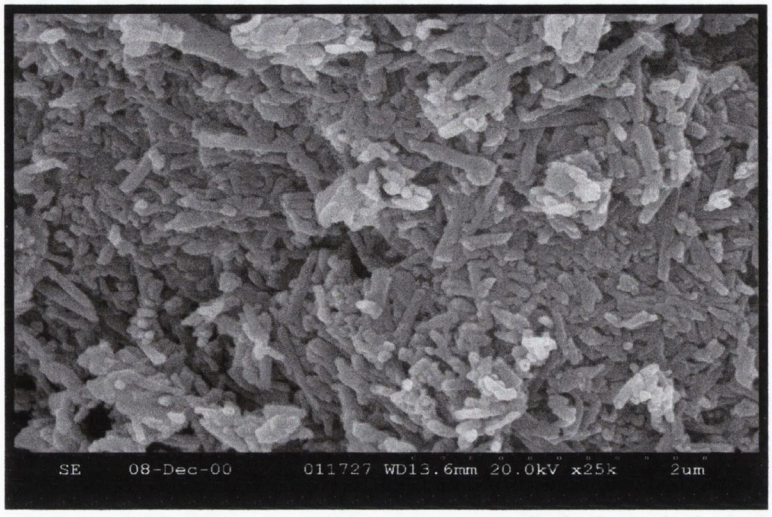


Figure 4.8m SEM of halloysite G treated with a rotar mill (0.5 mm blade size) at a speed of 18000 rpm (magnification x 25,000).

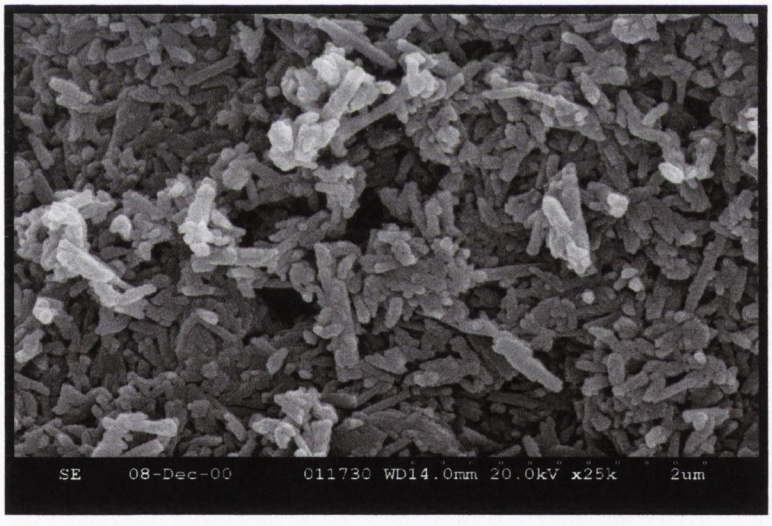


Figure 4.8n SEM of halloysite G treated with a ball mill (0.2 mm blade size) at a speed of 14000 rpm (magnification x 25,000).

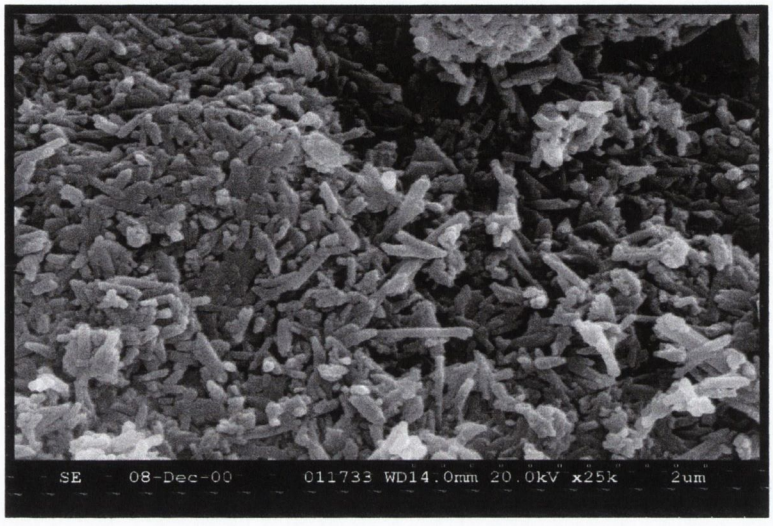


Figure 4.8o SEM of halloysite G treated with a rotar mill (0.2 mm blade size) at a speed of 18000 rpm (magnification x 25,000).

The alpine air jet sieve was used to determine if the unwanted debris and agglomerates could be separated from the highly tubular material of the untreated halloysite G sample (Figure 4.8g). As shown in Figure 4.8p there was a definite increase in tubular content with significantly less debris and agglomerates of halloysite compared to Figure 4.8g, therefore giving a more uniform tubular material.

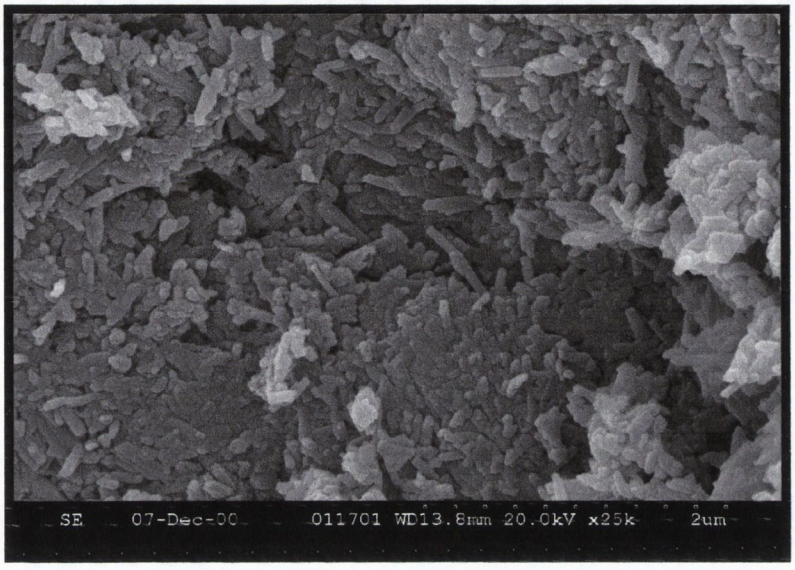


Figure 4.8p SEM of halloysite G after air jet sieving using a 25 μm sieve mesh, (magnification x 25,000).

Further work in this area of separation might involve the use a finer mesh sieve, to increase the yield of better quality tubular material. From this part of the study it can be concluded that halloysite G is a very robust material, which can withstand a high level of force and impaction without showing significant destruction of the tubules.

4.9 CONCLUSION

Halloysite Goushan NZCC proved to be the best source of a suitably large and uniform tubular halloysite. Microscopic analysis using SEM showed that the sample consisted of an essentially uniform material with average dimensions of 0.2 μm in diameter and 1.8 μm in length. The other halloysite samples were either too small in dimension or varied significantly in size with a large presence of debris, which would probably give these samples a poor ability to entrap and retain pesticides for an appropriate period of time.

XRD analysis confirmed that the halloysite samples were all present in a dehydrated state with sharp peaks at 12.05° 2θ (7 Å-form) and 24.55° 2θ. These two features in the XRD plots are indicative of dehydrated halloysite (7 Å-form). It was also observed that the XRD plot of halloysite Balikesir sample had two distinctively different peaks to the other four samples, which indicated traces of sepiolite.

Halloysite G, halloysite Goushan, halloysite Goushan NZCC and halloysite Konya showed very similar skeletal densities ranging from 2.4593 to 2.4978 g/cm^3, which was supported by microscopic and XRD analysis. The halloysite Balikesir sample had a higher skeletal density of 2.6029 g/cm^3 in comparison to the other four samples. However this was expected as the sample was a completely different colour to the other samples and the XRD plot indicated that sepiolite was also present.

The specific surface area of the halloysite samples varied considerably from 15.34 m^2/g to 65.51 m^2/g for halloysite Goushan and halloysite G, respectively, which can be explained qualitatively by the SEMs. For example halloysite Goushan (larger tubules) had an approx. four-fold decrease in surface area in comparison to halloysite G (smaller tubules), which as seen from the SEMs had average dimensions of approx. 2.5 x 0.4 μm and 0.5 x 0.1 μm, respectively. Consequently the high concentration of very small halloysite tubules in the halloysite G sample would give a much higher specific surface area in comparison to the halloysite Goushan sample.

The levels of trace elements were determined using inductively coupled plasma-mass spectrometry (ICP-MS). Iron was the main trace element (1360 ppm), which was expected as it can substitute for aluminium ions within the gibbsite sheet of halloysite. Sodium, magnesium, barium, potassium and manganese were also present in the halloysite sample but in smaller amounts. However there was a significant amount of unwanted trace elements present in halloysite such as lead, uranium, arsenic and mercury.

The physical strength of halloysite Balikesir and halloysite Konya was assessed using an autogenous grinding and milling process. Microscopic examination was used to ascertain the affect of different mechanical processes on the halloysite tubules. The halloysite Balikesir tubules remained fully intact after the autogenous grinding and milling process in comparison to the untreated sample. However this was not the case for the halloysite Konya sample, which initially had tubular lengths of approx. 2 μm, but after the milling process the majority of the tubules had a tubular length of <0.5 μm.

For halloysite G the physical strength was assessed using a pestle and mortar, ball mill and rotar mill. It was observed that there was no apparent destruction of the tubular structure when the samples were ground with a pestle and mortar. There was a greater presence of debris in halloysite G samples exposed to the ball mill at the higher vibration setting of 90%, rotar mill at the higher speed of 18000 rpm using the 0.5 mm blade and at both speed settings using the 0.2 mm blade. However overall the halloysite G sample proved to be able to withstand high levels of mechanical stress without showing a great degree of tubule size reduction. The Alpine air jet sieve proved to be a successful technique for minimising unwanted debris and agglomerates from the highly tubular material of the untreated halloysite G sample.

Based on the above findings, the halloysite Goushan NZCC sample was chosen as the halloysite sample for use in the subsequent pesticide sustained release agrochemical applications because the sample proved to have the highest tubular quality and also minimal batch to batch variability. Because it was available in limited quantity, certain development work was also undertaken using other grades of halloysite (e.g. halloysite G) not restricted by supply issues.

Chapter 5

Preliminary studies on halloysite powder and pellets

5.1 INTRODUCTION

The main aim of this Chapter was to carry out preliminary studies on halloysite for pharmaceutical and agrochemical applications. The work in Section 5.2 of this Chapter was carried out to ascertain the most effective method to deflocculate a halloysite suspension. Two deflocculating agents, sodium citrate and cetrimide, which are anionic and cationic in nature, respectively and pH changes were used to deflocculate halloysite in suspension. The information gained from this preliminary study will be used to develop a suspension concentration for an agrochemical application as described in Chapter 7 of this thesis. Stabilising the suspension concentrate by using appropriate deflocculating agents is vital in obtaining long term dispersibility of the system. Also deflocculating agents may be used as a method for separating the high quality halloysite tubules from the unwanted debris.

Isolated ethylcellulose films were studied in Section 5.3, using halloysite and talc as different filler types. Talc is chemically similar to halloysite and is widely used in pharmaceutical applications to minimise tackiness in film coatings. Therefore talc would prove useful in the isolated film study for comparative purposes to halloysite. The ethylcellulose films alone and in combination with halloysite and talc were assessed in a permeation and mechanical study. It was hoped that halloysite could be adopted for use in film coatings for a number of different uses e.g. bulking agent, anti-tack agent or opacifier.

The work on Section 5.4 was carried out to ascertain whether halloysite could be used as a tablet excipient for a pharmaceutical application. It was compared with the widely used pharmaceutical excipient, kaolin for binding, filler and disintegration properties. It was hoped that halloysite could have the potential for use as a cheap and cost effective alternative tablet excipient.

Halloysite pellets were formulated in Section 5.5 and then exposed to varying temperatures of 100°C, 200°C, 300°C and 1200°C. The pellets were examined visually by SEM and also by mercury porosimetry to ascertain if the individual halloysite tubules retained their structure and porous distribution. It was hoped that the information gained from this preliminary study could be used to help design a sustained pesticide delivery pellet as described in Chapter 6.

5.2 DEFLOCCULATION STUDIES

5.2.1 Introduction

The aim of this study was to carry out preliminary work on deflocculating halloysite particles in suspension. Sodium citrate and cetrimide were selected as deflocculating agents of choice as they are widely used and accepted in the pharmaceutical industry. Work was also carried out on the ability of varying pH to cause deflocculation of halloysite suspensions. Most of the research to-date has involved deflocculating kaolin, which is chemically similar to halloysite but differs in morphological structure. They have similar properties in relation to surface charge on the particles. Halloysite is negatively charged along the tubule ('face') and positively charged at the ends of the tubules ('edge'), where kaolin is negatively charged along the flat surface and positively charged on the 'edges' of the plate-like stacked structure. Deflocculating agents such as the two mentioned above and also sodium hexamethaphosphate (Yuan and Murray, 1997), sodium chloride (van Olphen, 1963) and sodium polyacrylate (Pabst *et al.*, 2000) have been used to deflocculate kaolin.

Therefore from these studies it was hoped that the above deflocculating agents (sodium citrate and cetrimide) will also successfully deflocculate halloysite.

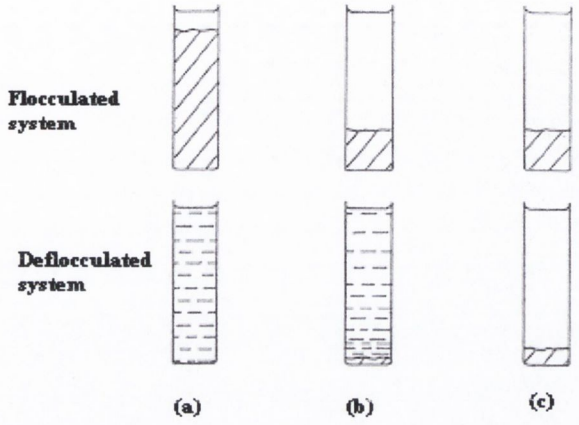
5.2.2 Theory of flocculation/deflocculation

If a suspension is deflocculated the dispersed particles remain as discrete units, and since the rate of sedimentation depends on the size of each unit, settling will be slow. The repulsive forces between individual particles allow them to slip past each other as they sediment. The slow rate of sedimentation prevents the entrapment of liquid within the sediment, which thus becomes compacted and can be very difficult to redisperse. This phenomenon is also called 'caking' or 'claying' and is among the most serious of all the physical stability problems encountered in suspension formulations (Figure 5.2a).

Aggregation of the particles in a flocculated system will lead to a much more rapid rate of sedimentation or subsidence because each unit is composed of many individual particles and is therefore larger than the deflocculated particles above. The rate of settling will also depend on the porosity of the aggregate since, if porous, the dispersion medium can flow through as well as around each aggregate or 'floccule' as it sediments.

The nature of the sediment of a flocculated system is also very different from that of a deflocculated system. The structure of each aggregate is retained after sedimentation thus entrapping a large amount of the liquid phase. Although aggregation in the primary minimum will produce compact floccules while a secondary minimum effect will produce loose or 'fluffy' floccules of higher porosity, the volume of the final sediment will still be large and easily redispersed by moderate agitation. The supernatant of a deflocculated system will remain cloudy for an appreciable time after shaking due to the very slow settling rate of the smallest particles in the product many of which may be retained in indefinite suspension due to Browian motion. In a flocculated system, the supernatant quickly becomes clear

as the 'flocs', which settle rapidly, are composed of particles of all sizes (Figure 5.2a).



The sedimentation behaviour of flocculated and deflocculated suspensions. Within a few minutes of manufacture **(a)** there is no apparent change within the deflocculated system compared with its initial appearance. Even after several hours **(b)** there is still little obvious change except that the concentration of solids in the lower layers has increased at the expense of the upper layers due to slow particle sedimentation. There is a small amount of a compact sediment. After a prolonged storage **(c)** depending on the physical stability of the system, the supernatant has cleared leaving a compact sediment. However there may be a slight haze due to very fine particles suspended indefinitely due to Browian motion. In the flocculated system at **(a)** there is some clear supernatant with a distinct boundary between it and the sediment. At **(b)** there is a large volume of clear supernatant with a relatively large volume of porous sediment, which does not change further even after prolonged storage **(c)**. The fine particles in a flocculated system are bound into the 'flocs' and therefore the supernatant is completely clear.

Figure 5.2a Diagram depicting the sedimentation behaviour of flocculated and deflocculated suspensions (Aulton, 2002).

5.2.3 Deflocculation experiments using sodium citrate as a deflocculating agent

The aim of this experiment was to ascertain if halloysite particles, which form a flocculated suspension on addition to water, could be deflocculated using sodium citrate (tri-sodium 2-hydroxy propane-1,2,3-tricarboxylate dihydrate). Sodium

citrate, an anionic trivalent deflocculating agent, works mainly by neutralising the positive 'edge' charges of halloysite particles and thus promotes deflocculation of the tubules.

As explained in Section 5.2.2, in a deflocculated halloysite suspension, the rate of sedimentation of the tubules is much slower than in a flocculated suspension. The sedimentation of the halloysite tubules in a deflocculated suspension can be 'graded', so that the larger tubules sediment first, followed by the medium-sized tubules and finally the smallest fragments of tubules. From this study it was hoped that the smaller tubular fragments (<1 μm), would be indefinitely suspended in the supernatant of the deflocculated system. Since tubules of <1 μm are less suitable for pesticide loading studies, these less desirable tubular fragments could be removed by discarding the supernatant. In contrast, in a flocculated suspension the rate of sedimentation is much faster and so all of the tiny tubular fragments are 'trapped' in the floccules and thus cannot be successfully removed from the system.

The theory outlined above in Section 5.2.2 regarding the difference in the appearance of a flocculated and deflocculated system was used to establish the concentration of sodium citrate required to deflocculate a 0.25% w/v halloysite Balikesir suspension. A sodium citrate concentration in the range of 1 nM to 200 mM was selected to roughly determine the concentration needed to deflocculate the halloysite suspensions (Table 5.2a and 5.2b). There was no appreciable change in appearance of the test tubes between the 30 min, 60 min and 180 min intervals. Table 5.2b shows the results of the deflocculation study after 24 hr, which indicated that sodium citrate in the concentration range of between 10 μM to 1 mM, should be studied further.

Table 5.2a Deflocculation experiment results of halloysite Balikesir (sample A - unmilled fraction) using 1 nM to 200 mM sodium citrate solutions (after 30 min*) at room temp. (~20°C)

Test tube	Sodium citrate concentration	Sodium citrate final concentration	Flocculated/ deflocculated	Appearance of supernatant
1	200 mM	100 mM	Most deflocculated	Very cloudy
2	100 mM	50 mM	Deflocculated	Very cloudy
3	10 mM	5 mM	Deflocculated	Very cloudy
4	1 mM	0.5 mM (500 μM)	Least deflocculated	Cloudy
5	0.1 mM (100 μM)	50 μM	Partially flocculated	Nearly clear
6	10 μM	5 μM	Least flocculated	Clear
7	1 μM	0.5 μM (500 nM)	Flocculated	Clear
8	0.1 μM (100 nM)	50 nM	Flocculated	Clear
9	10 nM	5 nM	Flocculated	Clear
10	1 nM	0.5 nM	Flocculated	Clear

*There was no appreciable change in the appearance of the test tubes after 60 and 180 min.

Table 5.2b Deflocculation experiment results of halloysite Balikesir (sample A – unmilled fraction) using 1 nM to 200 mM sodium citrate solution (after 24 hr) at room temp. (~20°C)*

Test tube	Sodium citrate concentration	Sodium citrate final concentration	Flocculated/ deflocculated	Appearance of supernatant
1	200 mM	100 mM	Most deflocculated	Very cloudy
2	100 mM	50 mM	Deflocculated	Very cloudy
3	10 mM	5 mM	Deflocculated	Very cloudy
4	1 mM	0.5 mM (500 μM)	Least deflocculated	Top half clear and bottom half cloudy
5	0.1 mM (100 μM)	50 μM	Difficult to determine if flocculated or not	Top two thirds clear and bottom one third cloudy.
6	10 μM	5 μM	Least flocculated	Clear
7	1 μM	0.5 μM (500nM)	Flocculated	Clear
8	0.1 μM (100nM)	50 nM	Flocculated	Clear
9	10 nM	5 nM	Flocculated	Clear
10	1 nM	0.5 nM (500 pM)	Most flocculated	Clear

*All of above observations that were noted for sample A (Table 5.2a and 5.2b) were similar to those observed with sample B (milled fraction) of halloysite Balikesir.

Table 5.2c Deflocculation experiment results of halloysite Balikesir (sample A – unmilled fraction) using 1 μM to 2.5 mM sodium citrate solutions (after 30 min*) at room temp. (~20°C)

Test tube	Sodium citrate concentration	Sodium citrate final concentration	Flocculated/ deflocculated	Appearance of supernatant
1	2.5 mM	1.25 mM	Deflocculated	Very cloudy
2	1 mM	0.5 mM (500 μM)	Deflocculated	Very cloudy
3	750 μM	375 μM	Deflocculated	Very cloudy
4	500 μM	250 μM	Deflocculated	Cloudy
5	250 μM	125 μM	Deflocculated	Cloudy
6	100 μM	50 μM	Least deflocculated	Cloudy
7	50 μM	25 μM	Flocculated	Top one fifth clear and bottom four fifths cloudy
8	25 μM	12.5 μM	Flocculated	As test tube 7 above
9	10 μM	5 μM	Flocculated	As test tube 7 above
10	1 μM	0.5 μM	Most flocculated	Top one third clear and bottom two thirds cloudy

*There was no appreciable change in the appearance of test tubes after 60 min.

Table 5.2d Deflocculation experiment results of halloysite Balikesir (sample A - unmilled) using 1 μM to 2.5 mM sodium citrate solutions (after 180 min) at room temp. (~20°C)

Test tube	Sodium citrate concentration	Sodium citrate final concentration	Flocculated/ deflocculated	Appearance of supernatant
1	2.5 mM	1.25 mM	Deflocculated	Very cloudy
2	1 mM	0.5 mM (500 μM)	Deflocculated	Very cloudy
3	750 μM	375 μM	Deflocculated	Very cloudy
4	500 μM	250 μM	Deflocculated	Cloudy
5	250 μM	125 μM	Deflocculated	Cloudy
6	100 μM	50 μM	Least deflocculated	Cloudy
7	50 μM	25 μM	Flocculated	Top two fifths clear and bottom three fifths cloudy
8	25 μM	12.5 μM	Flocculated	As test tube 7 above
9	10 μM	5 μM	Flocculated	As test tube 7 above
10	1 μM	0.5 μM	Most flocculated	Top half clear and bottom half cloudy

Table 5.2e Deflocculation experiment results of halloysite Balikesir (Sample A - unmilled) using 1 μM to 2.5 mM sodium citrate solutions (after 24 hr) at room temp. (~20°C)*

Test tube	Sodium citrate concentration	Sodium citrate final concentration	Flocculated/ deflocculated	Appearance of supernatant
1	2.5 mM	1.25 mM	Deflocculated	Very cloudy
2	1 mM	0.5 mM (500 μM)	Deflocculated	Very cloudy
3	750 μM	375 μM	Deflocculated	Very cloudy
4	500 μM	250 μM	Deflocculated	Cloudy
5	250 μM	125 μM	Deflocculated	Cloudy
6	100 μM	50 μM	Least deflocculated	Cloudy
7	50 μM	25 μM	Flocculated	Top half clear and bottom half cloudy
8	25 μM	12.5 μM	Flocculated	Clear
9	10 μM	5 μM	Flocculated	Clear
10	1 μM	0.5 μM	Most flocculated	Clear

*As in the first part of this deflocculation experiment, all of above observations that were noted for sample A were similar to those observed with sample B (milled fraction).

Table 5.2c, 5.2d and 5.2e show the results of the deflocculation studies using the narrower sodium citrate concentration range to ascertain more closely, deflocculation of the halloysite suspension. There was no appreciable difference in appearance of test tubes in the 30 min and 60 min time intervals. As in the first part of the deflocculation experiment, all of the observations that were noted for sample A were similar to those observed with sample B. Therefore, this showed that the extra

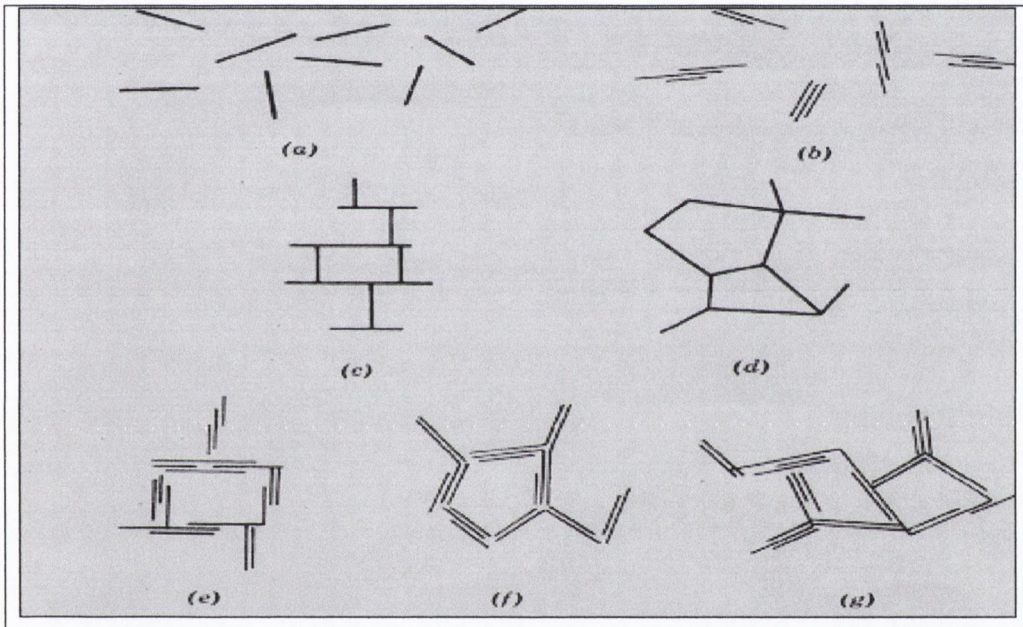
milling process carried out on sample B did not significantly effect the concentration of sodium citrate needed to deflocculate the system. Consequently from this study, test tube 6 in Table 5.2e indicated that a concentration of sodium citrate of 100 μM was needed to deflocculate the 0.25% w/v suspension of halloysite Balikesir (sample A or B).

The deflocculation of halloysite using sodium citrate may be explained in the following manner:

Because of the differences in charge distribution on halloysite particles, the opposite charges on the 'edges' and 'faces' (as explained earlier) will cause the particles to associate in different ways. van Olphen (1963) explained that when a suspension of clay particles flocculate, three different modes of particle association may occur: face-to-face (FF), edge-to-face (EF) and edge-to-edge (EE). The physical results of these three types of association will be quite different. FF association merely leads to thicker and possibly larger floccules, whereas EE and EF association will lead to a three-dimensional, voluminous house of card structure. The various modes of particle association are shown in Figure 5.2b. A proposal was made by van Olphen (1963), which stated that only the dissociation of the edge-to-face and the edge-to-edge linked particles could be described as 'deflocculation' and that the splitting of the face-to-face associates or 'aggregates' into thinner flakes could be described as dispersion of the clay. Halloysite tubules normally tend to associate in an edge-to-face arrangement because of the electrical attraction of one tubule for another caused by the difference in the charge of the edges and faces of the particles.

Sodium citrate is a salt of citric acid, which dissolves in water to form both citrate and sodium ions. Trivalent citrate ions are negatively charged and so will associate with the positively charged 'edges' of the halloysite particles. Each particle would then be completely or nearly completely negatively charged. If association of the citrate ions with the 'edges' of the particles were possible, as seems to be the case from the results obtained above in Table 5.2e, then little or no edge-to-face

flocculated associations between the halloysite particles can occur because the positively charged 'edges' of the particles would no longer be present for association. This would indicate deflocculation of the halloysite suspension occurred, as was observed in practice. The edges of the particles seem to have a higher affinity for the citrate ions than the faces of the other particles since deflocculation of the halloysite occurred. An explanation for this is that there is far less positive edge charges than the extensive negative surface charge, so effects on the edge charge by added electrolyte would be more evident at this region of the halloysite tubule.



Modes of particle association in clay suspensions and terminology (a) "Dispersed" and "deflocculated". (b) "Aggregated" but "deflocculated" (face-to-face association, or parallel or oriented aggregation). (c) Edge-to-face flocculated but "dispersed" (d) Edge-to-edge flocculated but "dispersed" (e) Edge-to-face flocculated and "aggregated" (f) Edge-to-edge flocculated and "aggregated" (g) Edge-to-face and edge-to-edge flocculated and "aggregated".

Figure 5.2b Diagram depicting the particle association in clay suspensions, according to van Olphen (1963).

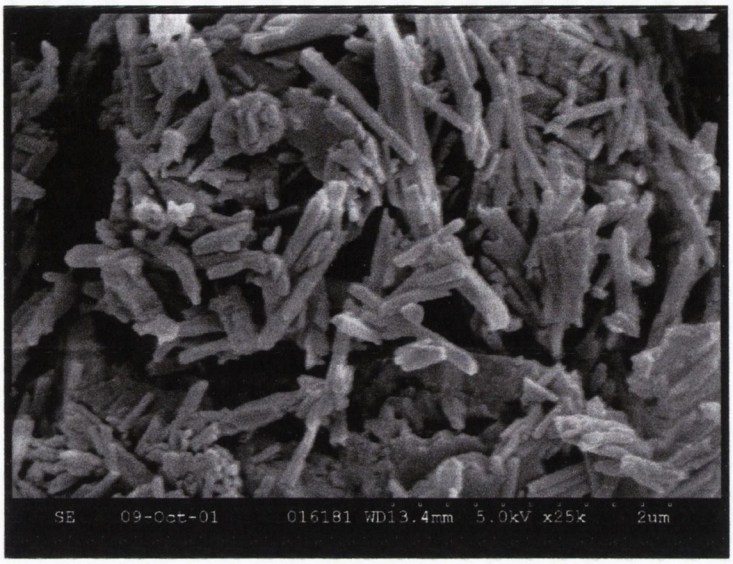


Figure 5.2c SEM of halloysite Balikesir, untreated sample (magnification x 25,000).

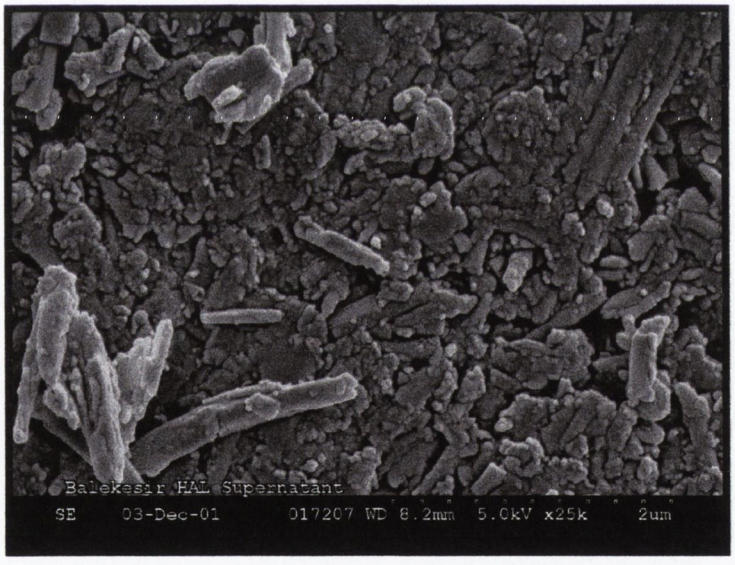


Figure 5.2d SEM of the supernatant of the deflocculated halloysite Balikesir suspension (sample A), which was produced using a sodium citrate concentration of 100 μm (magnification x 25,000).

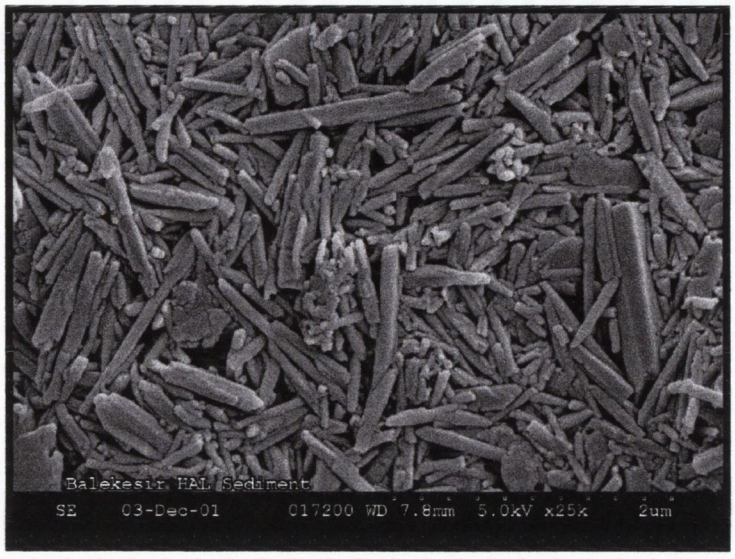


Figure 5.2e SEM of the sediment of the deflocculated halloysite Balikesir suspension (sample A), which was produced using a sodium citrate concentration of 100 μm (magnification x 25,000).

Figure 5.2c shows a SEM of the untreated halloysite Balikesir sample. Figure 5.2d and 5.2e show that deflocculation of halloysite Balikesir suspension by sodium citrate (100 μm) made it possible to improve the separation of the halloysite tubules from the debris in the sample. The sediment of the suspension (Figure 5.2e) has significantly less debris present in comparison to the untreated halloysite Balikesir as seen in Figure 5.2c, which was not treated with the deflocculation process. In Figure 5.2d it can be seen that the supernatant of this deflocculated suspension contained large amounts of irregular-shaped particles, which are considered to be mainly unwanted debris that can be removed from the clay sample by discarding the supernatant. This further indicates that the debris may be separated from the halloysite tubules by a deflocculation procedure.

Figure 5.2f SEM of the supernatant of the deflocculated halloysite Balikesir suspension (sample B), which was produced using a sodium citrate concentration of 100 μm (magnification x 25,000).

Figure 5.2f and 5.2g show the supernatant and the sediment, which were obtained from a deflocculated suspension of sample B. Again the process of deflocculating the halloysite suspension appeared to be effective in separating the desired tubules from the debris in the halloysite Balikesir sample. However there appeared to be less debris in the supernatant obtained with sample B (milled fraction), than in sample A (unmilled fraction). Since both of these samples were taken from the one sample of rock, it would appear that the content of debris in the Balikesir rock sample is very variable depending on the region sampled as would be expected for this naturally occurring material.

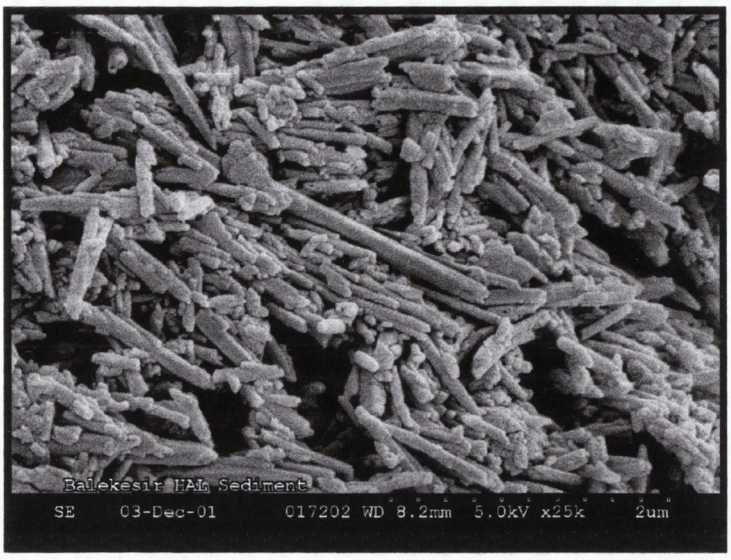


Figure 5.2g SEM of the sediment of the deflocculated halloysite Balikesir suspension (sample B), which was produced using a sodium citrate concentration of 100 μm (magnification x 25,000).

5.2.4 Deflocculation experiments using cetrimide as a deflocculating agent

The aim of this experiment was to determine if a suspension of halloysite Balikesir could be deflocculated using cetrimide, which is a cationic deflocculating agent. Since the halloysite suspension could be deflocculated via neutralisation of the positive edge charges using sodium citrate above, it was hoped that deflocculation would also be possible via neutralisation of the more extensive negative 'face' charges of the tubules using cetrimide. Therefore similar to what occurred using sodium citrate, the tubules would settle into a sediment leaving the smaller unwanted debris dispersed in the supernatant, which could be removed by decanting off.

However from the results seen in Table 5.2f, the addition of cetrimide did not cause deflocculation. All of the test tubes still appeared flocculated after 30 min. There was no distinguishable difference between the highest concentration and the lowest concentration of cetrimide added. Therefore no further observations were made on this series of test tubes. A 300 mM cetrimide solution was added to the halloysite

suspension but again there was no evidence of deflocculation at this elevated concentration of cetrimide.

Table 5.2f Deflocculation experiment results of halloysite Balikesir (sample A) using 30 pM to 30 mM cetrimide solutions (after 30 min) at room temp. (~20°C)

Test tube	Cetrimide concentration	Cetrimide final concentration	Flocculated/ deflocculated	Appearance of supernatant
1	30 mM	15 mM	Flocculated	Clear
2	3 mM	1.5 mM	Flocculated	Clear
3	0.3 mM (300 μM)	150 μM	Flocculated	Clear
4	30 μM	15 μM	Flocculated	Clear
5	3 μM	1.5 μM	Flocculated	Clear
6	0.3 μM (300 nM)	150 nM	Flocculated	Clear
7	30 nM	15 nM	Flocculated	Clear
8	3 nM	1.5 nM	Flocculated	Clear
9	0.3 nM (300 pM)	150 pM	Flocculated	Clear
10	30 pM	15 pM	Flocculated	Clear

A possible explanation for this may be that cetrimide (trimethyltetradecylammonium bromide – $CH_3(CH_2)_{15}N^+(CH_3)_3Br^-$) as a molecule contains a long hydrocarbon tail (14 carbon atoms) with a positive end charge that associates with the negatively charged 'faces' of the halloysite tubules and this would have helped neutralise the charge. Thus this process would have been expected to cause deflocculation of the tubules, as was the case with sodium citrate, which produced charge neutralisation mainly of the positive 'edges' of the tubules. However, the hydrophobic nature of the long carbon chain of cetrimide may have caused these 'complexes' to associate together, and in effect cause flocculation of the tubules. Thus it seems that the desired effect of the neutralisation of the negative 'face' charge of the tubules was overcome by the tendency of the long chains of the cetrimide molecules to associate

together. Therefore the use of cetrimide as a deflocculating agent was less effective. Sodium citrate does not contain this long hydrophobic carbon chain and so the desired effect of the neutralisation of the positive edge charges occurs. The magnitude of charge on the 'faces' of the halloysite tubules is also much greater than that of the 'edges'. Therefore it seems that trying to deflocculate the tubules via neutralisation of the negatively charged 'faces' would be much more difficult to achieve, particularly with cetrimide.

The other deflocculating agents mentioned above: sodium chloride, sodium polyacrylate and sodium hexamethaphosphate all have much shorter chain lengths than cetrimide, and probably little or no association of their chains occurs. Therefore this indicates that the association of the long hydrophobic chains of the cetrimide makes it unable to act as an effective deflocculating agent for halloysite particles.

5.2.5 pH effects on deflocculation

The aim of this experiment was to ascertain the effect varying pH would have on a halloysite suspension and to determine if pH could be used as a method to aid deflocculation of a flocculated system. A concentrated hydrochloric acid solution (HCl) and sodium hydroxide (NaOH) solution was used to give a low and high pH, respectively, while water gave a neutral pH. As seen from Table 5.2g the addition of equal amounts of 0.1 M HCl to the halloysite suspension caused flocculation, whereas the addition of 0.1 M NaOH caused the suspension to deflocculate in both samples A and B of halloysite Balikesir.

Table 5.2g Deflocculation experiment results of halloysite Balikesir (samples A and B) using varying pH after 30 min at room temp. (~20°C)

Test tube	Halloysite sample	Solution added	pH of suspension	Flocculated/ deflocculated	Appearance of supernatant
1	Sample A	0.1M HCl	1.86	Flocculated	Clear
2	Sample A	De-ionised water	7.30	Partially flocculated	Slighty cloudy
3	Sample A	0.1M NaOH	13.99	Deflocculated	Cloudy
4	Sample B	0.1M HCl	1.86	Flocculated	Clear
5	Sample B	De-ionised water	7.30	Partially flocculated	Slighty cloudy
6	Sample B	0.1M NaOH	13.99	Deflocculated	Cloudy

The change from a flocculated to deflocculated system as the pH is increased, may be explained in terms of the suppression of the double layer, which is present on the edges of the halloysite particles that occurs on increasing pH. This double layer is present because of the exposed alumina sheet. At very high pH its sign may even be reversed (to negative).

The 'faces' of halloysite particles carry a negative charge arising from the non-stoichiometric replacement of Al^{3+} by Fe^{3+} in the octahedral sheet (Soma *et al.*, 1992). Since this charge (on the faces of the particles) is intrinsic to the layer structure, its sign and magnitude are independent of pH (Theng and Wells, 1995a). By contrast Theng and Wells (1995a) also stated that the charge on the crystal edges of the particles, which is essentially composed of gibbsite (alumina), varies with the pH of the ambient solution or suspension. According to Theng and Wells (1995a),

the amphoteric nature of the edge surface may be ascribed to the protonation and deprotonation of hydroxyl groups coordinated with exposed Al ions under acid and alkaline pH conditions, respectively. This process is represented by the scheme in Figure 5.2h (Theng and Wells, 1995a), where the vertical line denotes the extent of the coordination sphere of Al.

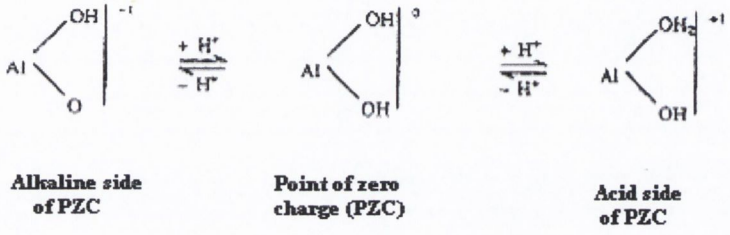


Figure 5.2h Illustration of amphoteric nature of alumina, present on the edge surfaces of halloysite tubules (Theng and Wells, 1995a).

At the PZC (point of zero charge), the edge surface is essentially uncharged, while at pH values above and below the PZC it will be negatively and positively charged, respectively. When the pH is equal to PZC the charge on the particle edges is zero, edge-to-edge association by van der Waals forces is apparently the preferred mode of particle interaction (Theng and Wells, 1995a). At the alkaline side of the PZC, the particles tend to repel each other since the charge on both the edge and the face surface is negatively charged. As a result, the system deflocculates (Theng and Wells, 1995a). The results in Table 5.2g agree with this hypothesis, where a high and low pH resulted in deflocculation and flocculation of the halloysite suspension, respectively.

van Olphen (1963) stated that the PZC does not necessarily occur at neutral pH, it is known to vary with the crystal structure of the alumina particle. By increasing the pH of the suspension of halloysite, the ionisation of the positive end charge is first suppressed to neutral. This is analogous to the situation above where the halloysite suspension was deflocculated by the citrate ions via neutralisation of the positively charged edges. Both of these processes prevented the edge-to-face flocculation of the

particles. At later stages, by further increasing the pH, anions will be adsorbed on to the neutralised edges of the tubules, thus causing the tubules to have a negative charge all over the surface. If the total surface of the tubule is negatively charged, then each particle will repel the other electrostatically and deflocculation will occur.

Other researchers have used this phenomenon of deflocculation or flocculation by increasing or decreasing the pH, respectively. Austin and Challis (1999) used this process of deflocculation by increasing pH in a kaolin suspension. Soma *et al.* (1992) also used this phenomenon in the process of acidifying a clay suspension to pH 6 in order to render it flocculated.

5.3 EXPERIMENTS ON ISOLATED FILMS

5.3.1 Introduction

Insoluble solids (fillers or pigments) are incorporated in film coatings, both pharmaceutical and non-pharmaceutical, for a variety of reasons. They serve as opacifiers, colorants, bulking agents and reinforcers. They may also minimise tackiness (e.g. talc). Sometimes the desire may be to achieve altered permeability and mechanical properties. No matter what the intended use, the fundamental properties of coatings are likely to be affected to varying degrees when fillers are incorporated (Okhamafe and York, 1984).

Generally, the effect of insoluble solids on film properties such as permeability and strength depends on the volume concentration of the filler, it's chemical composition, size, size distribution and shape, degree of dispersion in the film and the extent of polymer-filler interaction (Elm, 1953; Michaels, 1965 and Okhamafe and York, 1984). It is difficult to determine precisely the contribution of each of these factors to any particular property.

Ethylcellulose was chosen as the polymer of choice for these films because it is probably the most widely used water-insoluble polymer in film coating (Rowe, 1985 and Porter, 1989) due to its good film-forming properties that enable tough, flexible coatings to be produced. Dichloromethane was employed as a solvent of choice. The solubility parameter of dichloromethane is 19.8 $MPa^{0.5}$, indicating that it is a good solvent for ethylcellulose (solubility parameter is 21.1$MPa^{0.5}$) (Brandrup and Immergut, 1975 and Kent and Rowe, 1978) and therefore films cast from dichloromethane as a sole solvent should be homogeneous. Diltiazem HCl was selected as a model drug for this investigation because of its high water solubility (Kao *et al.*, 1997).

The aim of this study was to investigate the effect of the composition of ethylcellulose alone and in combination with different filler types (i.e. halloysite and talc) on the drug diffusivity and mechanical properties of isolated films. Talc is chemically similar to halloysite, which was used for comparative purposes only, as it is widely used in films to reduce tackiness. Permeability through isolated films and mechanical properties using load-time profiles were the methods of evaluation for this study.

5.3.2 Permeation Study

Films were prepared with ethylcellulose and varying filler types (halloysite and talc) using dichloromethane as a solvent. Figure 5.3a shows the amount of diltiazem HCl diffused through the films as a function of time.

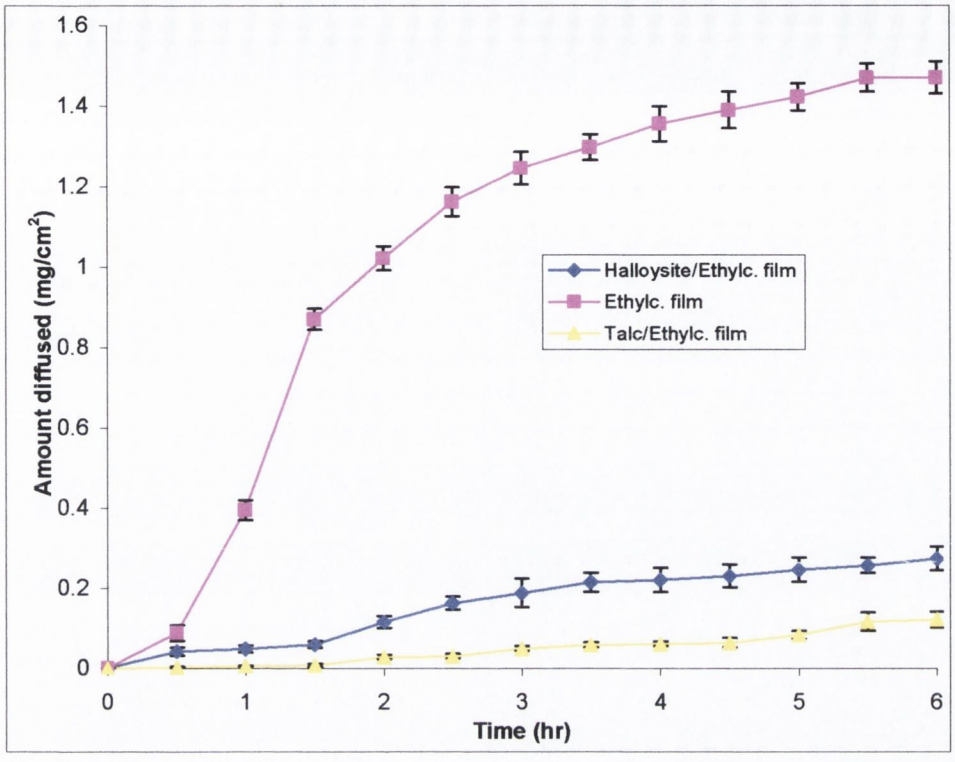


Figure 5.3a Diffusion profile of diltiazem hydrochloride through isolated films (~30 μm) of ethylcellulose alone and in combination with halloysite G or talc.

It is seen from Figure 5.3a that as fillers are added to the ethylcellulose films there is a significant decrease in the amount of diltiazem HCl diffusion through the films, indicating that on addition of fillers (halloysite and talc), the film becomes less permeable. This suggests that adding different fillers can readily modify the permeability of the film. Filler particles are expected to reduce diffusion by making the diffusion channels more tortuous (Okhamafe and York, 1984). An ANOVA factorial was performed at each of the time points of the three diffusion profiles. It revealed that diffusion profiles were all statistically different ($p<0.05$). There was an approx. ten-fold decrease in the amount of diltiazem HCl diffusion through the film on addition of halloysite and a further reduction of diltiazem HCl diffusion on the addition of talc to the film. This is supported by Delporte and Jaminet (1978 a,b)

who found a decrease in water vapour permeability of free cellulose acetate phthalate and methyl/ethylcellulose films loaded with talc.

By comparing halloysite and talc as fillers in ethylcellulose films, it can be observed from Figure 5.3a that talc causes a greater retardation (i.e. approx. one-fold decrease) of diltiazem HCl diffusion than halloysite. This can be explained by focusing on the particle shape, specific surface area of the filler and the filler-polymer interaction, assuming the degree of dispersion of the solids in the polymer matrix to be the same in all cases.

Halloysite and talc differ in structure, having a tubular rather than platelet-like structure, respectively as seen in Figure 5.3b and 5.3c. Therefore considering particle structure, talc particles should present a more tortuous barrier to drug diffusion than halloysite. The incorporation of platelet-shaped fillers into organic coatings is well known in the steel industry to protect steel against corrosion (Salensky, 1986 and Bieganska *et al.*, 1988). The plate-like particles show a tendency to align parallel to the substrate surface and thus build a roof-like barrier, rendering the penetration of water difficult. With talc, similar effects have been reported (Heine, 1992).

However, the difference between the specific surface areas of fillers is also an important factor. In general, talc exhibits a large surface area in relation to its mass because of its platelet-like structure. The values, to a large extent, depend on the fineness of the product, typically 12 m^2/g (Handbook of Pharmaceutical Excipients, 2000). Halloysite G and talc used in this experiment have a specific surface area of approximate 65.51 m^2/g and 15 m^2/g, respectively. However it must be taken into consideration that halloysite has a large internal lumen due to its hollow tubular morphology. Therefore a large proportion of the tubule surface area is not in contact with the polymer. However talc, which is a flat plate-like structure will be in greater contact with the polymer. Also with halloysite, there may be increased diffusion of drug through the hollow internal lumen. Drug diffusion is probably also influenced by reversible binding of the cationic drug to the polyanionic faces of the insoluble

fillers, where the larger specific surface area of the halloysite might be expected to help retard drug release to a greater extent than kaolin. However this effect is probably minor and is not in the overall release shown in Figure 5.3a.

(a) **(b)**

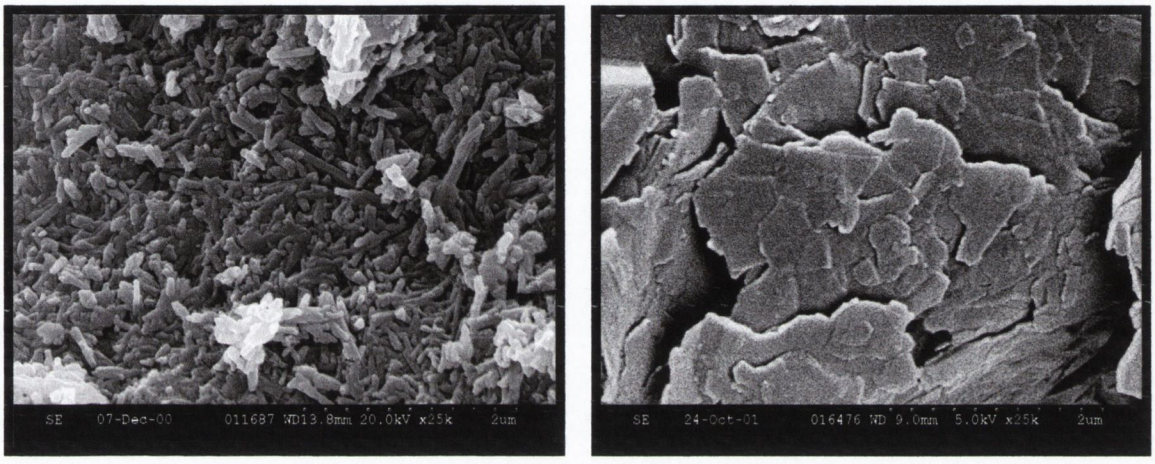


Figure 5.3 SEM of (b) halloysite G and (c) talc (magnification x 25,000)

5.3.3 Mechanical Properties

Tensile testing gives an indication of the strength and elasticity of the films, reflected by the parameters tensile strength, elastic modulus and elongation at break. However, independent interpretation of these parameters may not always lead to meaningful conclusions. Therefore all parameters need to be taken into consideration to determine film properties. Table 5.3a shows the tensile strength measurements for the different films.

Overall the filler loading has a detrimental effect on the tensile strength of the films. The behaviour of fillers suggests disruption of some of the bonds (mainly hydrogen bonds) holding the polymer chains together. Since halloysite and talc are polar in character some form of interaction, mostly of the dipole-dipole type, will occur between the filler and the polymer matrix; but these bonds are considerably weaker than the inter-segmental hydrogen bonds of the polymer. In addition, the filler-

polymer interfaces constitute stress locations or concentrations, which contribute further to lower film strength (Okhamafe and York, 1984).

Table 5.3a Mechanical properties of isolated films of ethylcellulose alone and in combination with halloysite G or talc

Formulations	Tensile strength (MPa)	Elastic modulus (MPa)	Elongation at break (%/m^2)
Ethylcellulose 5%	35.72[a]	37.12[a]	2.725[a]
S.D.	3.68	2.20	0.296
Ethylcellulose 4%, Halloysite G 1%	10.25[b]	37.86[a]	1.192[b]
S.D.	1.12	1.29	0.220
Ethylcellulose 4%, Talc 1%	22.65[c]	38.41[a]	1.647[b]
S.D.	2.27	3.82	0.192

Values with similar superscripts are statistically different.

When halloysite was added to the film there was a significant decrease in tensile strength (10.25 MPa) in comparison to the ethylcellulose films alone (35.72 MPa) i.e. three-fold decrease. The talc-filled films showed a decrease in tensile strength (22.65 MPa), but at a comparatively lesser extent to the films containing halloysite. An ANOVA test was performed on these results, which revealed that they were all statistically different ($p<0.05$). It is difficult to compare tensile strength results between different groups of workers because of differences in both film preparation and testing method. Okhamafe and York, (1984) reported that hydroxypropyl methylcellulose films containing talc resulted in a decrease in tensile strength.

There is a considerable difference in tensile strength between halloysite and talc containing films, which may be explained by comparing particle morphology and filler-polymer interaction. The talc particles tend to align parallel to the substrate due to the plate-like structure and therefore interact more effectively with the polymer as explained earlier. The structural formation of talc with the polymer results in a

restraint of volume shrinkage parallel to the plane of coating (Rowe, 1981) and may also account for slightly stronger films. The halloysite particles will not necessarily align parallel to the surface and are unable to form the close intertwined network associated with talc particles. Therefore this will cause considerable disruption of the filler-polymer film. For this reason it can be deduced that since halloysite is tubular and talc plate-like in structure, stress concentrations will be much greater in halloysite-filled films than talc-filled films and therefore tensile strength results will be superior for talc-filled films (Table 5.2a).

Table 5.3a shows that generally elastic moduli of the films were increased on addition of fillers due to an increase in stiffness of the polymer phase. This occurs as a result of physical restriction of the polymer matrix by the hard, rigid filler particles (Okhamafe and York, 1984). The halloysite-films and talc-films gave elastic modulus readings of 38.41 MPa and 37.84 MPa, respectively and the ethylcellulose films alone gave a reading of 37.12 MPa. An ANOVA test was performed on these results, which revealed that they were not statistically different ($p = 0.769$). Aulton and Abdul-Razzak (1984); Funke *et al.* (1969); Toussaint (1973, 1974) and Rowe (1982, 1983) observed an increase in elastic modulus on addition of fillers and pigments to films. There is a slight increase in elastic modulus between talc and halloysite filled films, which may be explained by focusing on filler-polymer interaction. There is an improved filler-polymer interaction with talc-filled films resulting in an increase in rigidity of the polymer phase and therefore improved elastic modulus as explained earlier under tensile strength.

The results in Table 5.3a indicate that filler inclusion causes a decrease in elongation. The halloysite and talc filled films gave lower elongation readings (1.192% /m^2 and 1.647% /m^2) compared to ethylcellulose films alone (2.725% /m^2). Elm (1953); Funke *et al.* (1969) and Okhamafe and York (1984) have reported similar observations with addition of fillers. An ANOVA test was performed on the above results for elongation at break, which revealed that they were statistically different ($p<0.05$). However the halloysite and talc films were not statistically different (same

superscripts). Elongation at break is a measure of the ductibility of a film and elongation can be regarded as a characteristic, which defines the ability of a film to deform before failure occurs. Consequently on addition of halloysite or talc, the film is less capable of yielding to allow deformation. Okhamafe and York (1984) hypothesized that for films including fillers, failure was brought about in two ways. Either solid particles, such as talc or titanium dioxide, caused excessive stress concentration, or a filler-polymer interaction caused a reduction in the mobility of the polymer chains. Therefore with regard to the halloysite or talc films, failure originates from a flaw (stress concentration) or an inadequate filler-polymer interaction. These factors are probably slightly greater in halloysite-filled films as indicated earlier under tensile strength.

5.4 TABLETTING STUDIES

5.4.1 Introduction

The aim of this study was to determine the role of halloysite as a tablet excipient and compare it to kaolin, which is widely used as a pharmaceutical excipient (Handbook of Pharmaceutical Excipients, 2000). Halloysite was compared to kaolin for binding, filler and disintegration properties. Microcrystalline cellulose, a direct compression base was used in conjunction with halloysite or kaolin in the tablet mixes because alone they produced tablets of poor quality.

The formula below was used to produce the two powder mixes:

Paracetamol, granular	50%
Halloysite G/Kaolin	25%
Emcocel 50M	24%
Talc	1%

5.4.2 Flowability and static angle of repose

The flow properties of the two powder mixes were determined before tabletting by using flowability index and static angle of repose measurements (Table 5.4a). The results obtained were used to give an indication of the ability of the powder mixes to flow through the tablet hopper and into the die cavity.

The flowability index gives a determination of intrinsic flowability, which is based upon the ability of a powder to fall freely through a hole in a plate. It is a direct indication of the potential flowability of powder mixes i.e. the lower the flowability index the more flowable the material will be. Table 5.4a shows that the halloysite and kaolin mixes gave flowability indexes of 14 mm and 34 mm, respectively, indicating that the halloysite mix had superior flowability properties in comparison to the kaolin mix in this tabletting application.

Table 5.4a Flowability index and static angle of repose of halloysite and kaolin powder mixes

	Flowability index (mm)	**Static angle of repose (degrees)**
Halloysite mix	14	50
Kaolin mix	34	64

The static angle of repose is the angle between the horizontal and the slope of a cone of material when the excess feed material falls away from the cone of material supported on a circular place. Also like the flowability index, it gives a direct indication of the flowability of a material i.e. the lower the angle of a dry material, the more flowable a material will be (Carr, 1965). Table 5.4a shows that the halloysite and kaolin mixes gave static angle of repose values of 50 degrees and 64 degrees, respectively, demonstrating that the halloysite mix had improved flowability properties. A static angle of repose of 25 degrees was considered the optimum and was given a point score of 25 with 'excellent' flow properties (Carr, 1965).

Therefore using this point score developed by Carr (1965), the halloysite mix gave a point score of 12 and would need 'agitation' in the tablet hopper. The kaolin mix gave a lower score of 7 and would need 'more agitation and vibration' in the tablet hopper to ensure the powder mix flowed adequately.

From these results (Table 5.4a) obtained it can be seen that the halloysite mix gave improved flow properties in comparison to the kaolin mix. However as explained above, the halloysite mix would still need agitation in the tablet hopper. Several factors may be taken into consideration when explaining the improved flowability of the halloysite mix such as particle shape, surface charge and size, porosity, bulk density and surface area.

As explained in Section 5.2.3, halloysite and kaolin are both highly electrically charged particles with a predominantly anionic charge. Therefore this strong electrostatic attraction (cohesive forces) between the particles would directly result in a tendency of the particles to bridge together in the tablet hopper for both powder mixes and therefore reduce flowability.

The halloysite and kaolin particles will stack in a house of card-like or layered arrangement within the powder bed. However the kaolin particles, which are plate-like will not stack as efficiently as the halloysite particles. Also taking surface area into consideration, halloysite particles have a surface area of approx. 65 m^2/g (Table 4.6a) while the kaolin particles have a surface area of approx. 9 m^2/g (Besra *et al.*, 2000). The increased surface area of the halloysite particles is attributed to the hollow internal lumen of the microtubules. However the kaolin particles have a larger available external surface area because of the plate-like structure of the individual particles, thus increasing the difficulty in arranging the kaolin particles. Therefore the kaolin particles will have increased interparticulate voids between the individual particles.

The halloysite and kaolin particles both have an individual particle size of approx. 0.5-1 μm. As explained above because of their highly charged surface they will form agglomerates. However the halloysite particles have a higher exchange capacity (Harvey, 1996), therefore they will rarely exist as single particles and more likely as agglomerates with an approx. size of 50 μm. These agglomerates will be tightly packed because of their interlocking non-isometric tubular structure. The tightly packed halloysite particles will have a low electrostatic charge because the individual particles will effectively have neutralised each other within the agglomerate arrangement, which will result in agglomerates acting as individual coarse particles of ~50 μm with a low cohesive attraction and improved compatibility with the microcrystalline cellulose particles size range of also ~50 μm. Also the microcrystalline cellulose particles of the ~50 μm size range (Emcocel 50M) used in this formulation are matchstick-like particles (Bolhuis and Lerk, 1973), which are more compatible with the isometrically similar microtubular structure of the halloysite particles. The halloysite agglomerates and microcrystalline cellulose particles are coarse particles, which will result in improved flow properties of the halloysite mix in comparison to the kaolin mix.

The kaolin particles however may exist individually and also as agglomerates of varying sizes because of their inability to form closely packed agglomerates. The particle's charge will be more exposed because of their loosely packed arrangement and also there is an increased likelihood of particles existing as single highly electrostatically charged particles. Therefore the kaolin mix will have a more cohesive charge, which promotes bridge formation within the powder bed. The void spaces in the powder bed between the coarser microcrystalline particles and the kaolin agglomerates may become filled with the individual kaolin particles, resulting in a more closely packed cohesive powder with reduced ability of the powder mix to flow efficiently. The difference in particle shape of the kaolin particles and the microcrystalline particles will result in interlocking between irregularly shaped particles and therefore bridging within the powder bed. This tendency for irregular particles to produce open structures supported by small or large powder arches causes

them to have a larger difference in porosity between loose packing and tight packing geometries than more regularly shaped particles (Aulton, 2002)

From this information it can be concluded that the halloysite mix would flow more easily than the kaolin mix, because the halloysite particles form agglomerates with a similar size range to the composite microcrystalline cellulose particles.

5.4.3 Uniformity of weight and thickness

Table 5.4b and 5.4c illustrate the uniformity of weight and thickness results. The two sample t-test with a confidence interval of 95% indicated that the tablet weight and thickness are statistically different ($p<0.05$). However both the tablets were not considered acceptable for uniformity of weight (average tablet weight deviates <5%, British Pharmacopoeia (2000).

Table 5.4b Uniformity of weight of paracetamol tablets using halloysite or kaolin (average of 20 tablets)

	Average (mg)	**S.D.**	**C.V. (%)**
Halloysite mix	553.4	30	5.42
Kaolin mix	540.3	45	8.33

Table 5.4c Uniformity of thickness of paracetamol tablets using halloysite or kaolin (average of 20 tablets)

	Average (mm)	**S.D.**	**C.V. (%)**
Halloysite mix	3.76	0.04	1.06
Kaolin mix	3.67	0.20	5.45

The higher tablet weight and thickness recorded for the halloysite mix may be explained by considering the flowability of the powder mix. As explained in Section 5.4.2, the halloysite mix had considerably better flowability of the powder feed

compared to the kaolin feed. The improved flowability of the halloysite mix should directly result in more efficient flow of the powder through the tablet hopper and shoe into the die cavity, which will result in more uniform tablets of similar weight and thickness. The smaller standard deviation and coefficient of variation for the uniformity of weight and thickness of the halloysite tablets confirm this. The kaolin mix has a higher porosity and therefore a lower bulk density in comparison to the halloysite mix. The higher porosity of the kaolin mix would result in less efficient packing of plate-like particles and therefore a lower bulk density. Thus comparable volumes of powder will result in compressed tablets of reduced weight for the kaolin mix.

5.4.4 Mechanical strength of tablets – hardness/ friability

The mechanical properties of both batches of tablets were tested. The results for uniformity of hardness and friability are shown in Table 5.4d and 5.4e, respectively. A two sample t-test with a confidence interval of 95% illustrated that the values obtained for both tests were statistical different ($p<0.05$). From the results obtained, it is seen that the halloysite tablets produced the harder and superior tablets.

Table 5.4d Uniformity of hardness of paracetamol tablets using halloysite or kaolin (average of 20 tablets)

	Average (Kp)	**S.D.**	**C.V. (%)**
Halloysite mix	11.5	0.02	0.17
Kaolin mix	10.1	1.24	12.27

The average hardness value for the kaolin tablets was lower and the coefficient of variation was much greater. This is again a reflection of the poor flowability of the kaolin feed, which as previously discussed, resulted in consistently lower average tablet weight and thickness. The lower tablet weight and thickness directly translates to less powder being present in the die cavity at the time of compression and

therefore overall tablet hardness will be lower when using identical compression settings.

Also the mechanical interlocking of the particles within the powder mixes makes a considerable attribute to tablet hardness. Mechanical interlocking is the term used to describe a situation where strength is provided by interparticulate hooking. This phenomenon usually requires that the particles have an atypical shape such as needle-shaped or highly irregular and rough particles (Aulton, 2002). Microcrystalline cellulose used in this formulation is a direct compression excipient with superior bonding properties attributed to the filamentous structure of the cellulose micro-crystals (Bolhuis and Lerk, 1973). It is composed of typical matchstick-like particles that are mechanically interlocked and bonded by hydrogen bridges under compression. These matchstick-like particles are similar in structure to the tubular halloysite particles, therefore directly resulting in improved packing of the particles within the powder bed, which mechanically interlock and form tablets of increased strength. The kaolin will not mechanically interlock as efficiently with the microcrystalline particles because of the dissimilar structures.

Table 5.4e Friability test of paracetamol tablets containing halloysite or kaolin (average of 3 determinations of 20 tablets)

	Average (%)	**S.D.**	**C.V. (%)**
Halloysite mix	2.5	0.15	6
Kaolin mix	17	2.3	13.52

The friability results obtained support those of hardness with the kaolin tablets producing the highest friability values. However, both the tablets reached friability values, which were unacceptable (>1%, British Pharmacopoeia, 2002). Despite that the kaolin tablets gave a friability value, which was approx. 7-fold larger than the halloysite tablets. This may be explained further by visual inspection of the tablets, which revealed that there was a significant presence of capping in the kaolin tablets

before being added to the friabilator. Therefore the kaolin tablets would have a greater tendency to break up quicker in the friabilator. This reflects the lower hardness values for these tablets, which have been explained previously in terms of poor flow properties and bonding of this tablet mix.

5.4.5 Disintegration of tablets

Disintegration times for the halloysite and kaolin tablets are shown in Table 5.4f. Both tablets complied with the British Pharmacopeia (2002) disintegration test with disintegration times significantly shorter than the 15 min limit for uncoated tablets. The very low disintegration times are attributed to the microcrystalline cellulose. It has been shown to take up an amount of water, which is significantly greater than the initial void volume of the compacts. This rapid water uptake is not only due to capillary forces but also illustrates that pore widening and extensive swelling of the powder bed occurs (Carmella *et al.*, 1984a and Ek *et al.*, 1995). The halloysite tablets disintegrated at a slightly slower rate than the kaolin tablets and a two sample t-test with a confidence interval of 95% revealed that they were statistically different ($p<0.05$). Disintegration of the halloysite tablets is reduced in water due to higher tablet hardness and also the improved mechanical interlocking between the microcrystalline cellulose and halloysite particles. Therefore the water is unable to penetrate into the halloysite tablets as quickly as into the kaolin tablets.

Table 5.4f Disintegration times of paracetamol tablets using halloysite or kaolin (average of 6 determinations)

	Average (sec)	**S.D.**	**C.V. (%)**
Halloysite mix	13	0.82	6.31
Kaolin mix	9	0.91	10.11

5.4.6 Dissolution

The dissolution profiles for the halloysite and kaolin tablets are plotted in Figure 5.4a, which are very similar for both tablets. A two sample t-test with a confidence

interval of 95% ($p<0.05$) was performed on the results at 5 and 10 min and gave p-values of 0.006 and 0.142, respectively, therefore indicating that after 10 min there was no significant difference observed between the two dissolution profiles.

The increased initial dissolution rate for the kaolin tablets may be due to the increased porosity between the kaolin particles because the kaolin particles do not align as effectively as the halloysite particles with the microcrystalline cellulose particles. Therefore there are increased interparticulate voids within the kaolin tablets. This increased porosity will increase capillary action, thus increasing the initial penetration of the dissolution medium into the tablet.

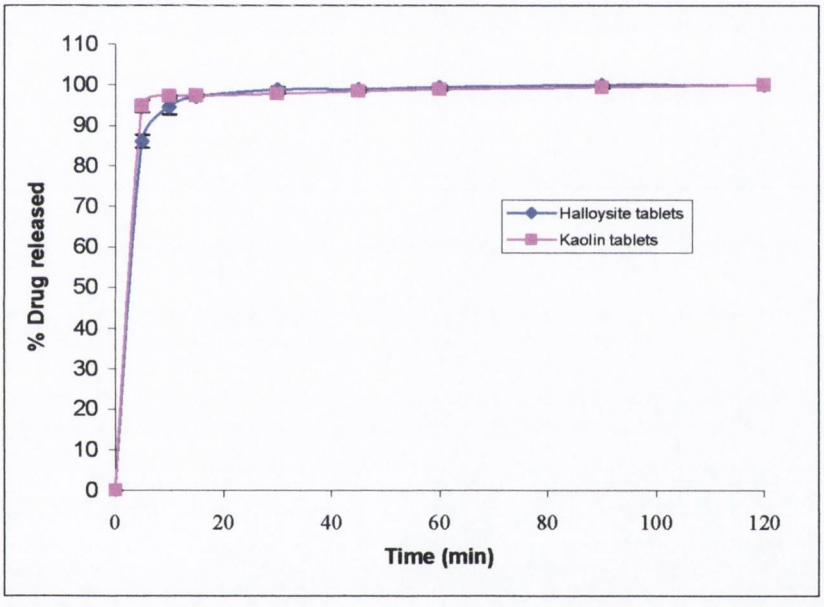


Figure 5.4a Dissolution profiles of paracetamol tablets prepared using halloysite or kaolin.

5.5 MERCURY POROSIMETRY OF HALLOYSITE PELLETS

5.5.1 Introduction

Mercury porosimetry was used to determine the porous distribution of pellets made from halloysite G powder. Porosity studies were performed on five halloysite pellet samples. Each of the pellet samples were heated to varying sintering temperatures i.e. 100°C, 200°C, 300°C in an oven for 3 hr and another sample was fired in a kiln to 1200°C for 12 hr using a similar technique as for pottery manufacture. These pellet samples were then compared to an unheated pellet sample.

The aim of this study was to compare the porous distribution of pellet samples at different temperatures and from this information, decide which pellets would be best suited to pesticide loading without losing the structure of the individual halloysite tubules and also to prevent disintegration on addition to water. The porous distribution of the pellet samples was determined by plots of cumulative pore volume intruded by mercury *vs* the pore diameter and plots of the incremental intrusion volume *vs* pore diameter. The findings of this study could be further developed for an application of sustained pesticide delivery in pellet form.

5.5.2 Mercury porosimetric study

Table 5.5a and 5.5b illustrate the total mercury intrusion for the pellet samples. The unheated pellet samples and pellet samples heated to 100°C, 200°C and 300°C show very similar mercury intrusion values ranging from 0.206 ml/g to 0.225 ml/g. However the pellet samples heated to 1200°C have a statistically lower mercury intrusion volume of 0.005 ml/g compared to the other pellet samples (ANOVA analysis gave p-value <0.05). This is also reflected in the overall porosity of the pellet samples, the values of which range from a minimum of 3.04% for the pellets heated to 1200°C, to a maximum of 70.76% for the unheated pellet samples.

Table 5.5a Quantitative results of porosimetric study of halloysite pellets exposed to varying temperatures

	Unheated	**100°C**	**200°C**
Hg total intrusion volume (ml/g)	0.206 +/- 0.003	0.225 +/- 0.007	0.224 +/- 0.067
Median pore diameter volume (μm)	0.012 +/- 0.0001	0.014 +/ 0.0003	0.014 +/ 0.0004
Bulk density (g/ml)	3.435 +/- 0.012	2.896 +/- 0.042	2.851 +/- 0.035
Porosity (%)	70.76 +/- 0.26	65.16 +/- 1.56	64.03 +/- 1.78

Table 5.5b Quantitative results of porosimetric study of halloysite pellets exposed to varying temperatures

	300°C	**1200°C**
Hg total intrusion volume (ml/g)	0.219 +/- 0.064	0.005 +/- 0.003
Median pore diameter volume (μm)	0.013 +/-0.0003	48.641 +/- 0.014
Bulk density (g/ml)	2.843 +/- 0.056	6.338 +/- 0.053
Porosity (%)	62.18 +/- 0.87	3.04 +/- 0.58

The lower mercury intrusion volume and porosity reading of the 1200°C pellet samples are evident qualitatively by SEMs as illustrated in Figure 5.5a and 5.5b. Figure 5.5a illustrated that there is transformation in the internal structure of the pellets when heated to a temperature of 1200°C. There is visually a complete loss of the characteristic tubular structure of the individual halloysite particles (intraparticulate voids) and voids between the halloysite particles (interparticulate voids) as seen in Figure 5.4b, which is a SEM of an unheated pellet sample. The individual halloysite tubules within the pellet samples heated to 1200°C fused to form large compact masses, which resulted in a very low mercury intrusion volume (0.005 ml/g) and porosity (3.04%). The other pellet samples heated to 100°C, 200°C and 300°C (Table 5.5a and 5.5b) gave much higher mercury intrusion volumes and porosity readings similar to the unheated pellets, which illustrated that the interparticulate and intrapaticulate voids remained intact on exposure to these lower temperatures. For example the pellet sample heated to 300°C and 1200°C resulted in

a porosity loss of 8.58% and 67.72%, respectively, compared to the unheated pellet sample.

(a) **(b)**

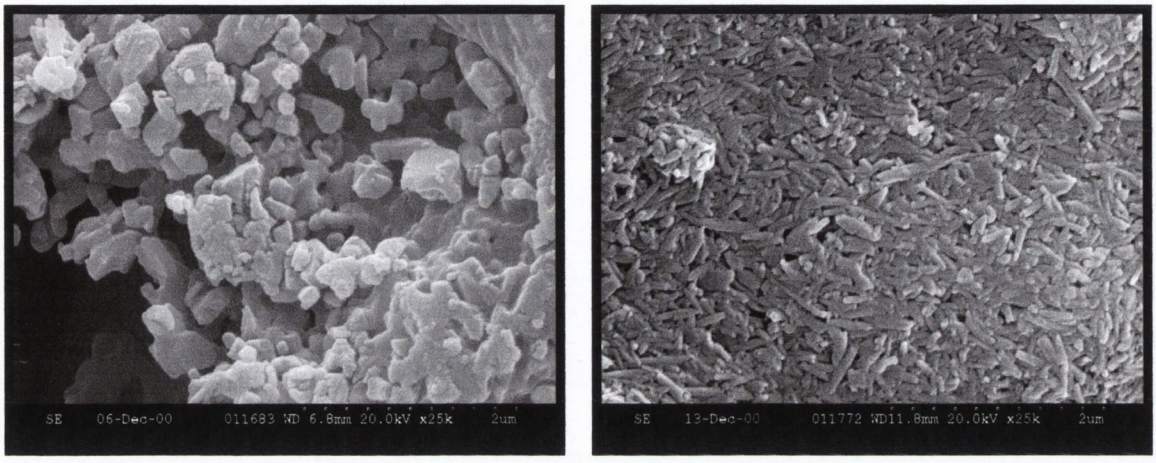


Figure 5.5 SEM of a) halloysite pellets heated to 1200°C, b) unheated halloysite pellets (magnification x 25,000).

There is also an increased powder bed packing for the halloysite pellets heated to 1200°C as illustrated by the increased bulk density measurements (6.338 g/ml) compared to the other pellet samples, which ranged from 2.843 g/ml to 3.435 g/ml for the pellets heated to 300°C and unheated pellets, respectively (Table 5.5a and 5.5b). The main reason for this increased bulk density measurement is that as explained above the individual halloysite particles fused to form large masses with very few interparticulate voids and also nearly a complete absence of intraparticulate voids. This further explains the significantly lower porosity and mercury intrusion measurements for the pellet samples heated to 1200°C.

As shown in Table 5.5a and 5.5b the median pore diameters vary from 0.012 μm to 48.641 μm for the unheated and heated pellet samples to 1200°C, respectively. There is a statistical difference between the pellets heated to 1200°C compared to the other four pellet samples, which correlates with the previous measurements of intrusion

volume and porosity (ANOVA analysis gave p-value <0.05). As explained earlier, there is virtually a complete loss of intraparticulate pores for the pellet samples heated to 1200°C, resulting in much greater median pore diameters as seen in Figure 5.5g where pores in the ~10-100 μm size range predominate. For the other four pellet samples much smaller pores predominate as seen in Figure 5.5c, 5.5d, 5.5e and 5.5f (~0.01 to 0.1 μm) due to significant presence of intraparticulate pores within the individual halloysite tubules, which resulted in the low median pore diameters.

Similar porosimetric studies were performed by Levis (2000) on halloysite powders rather than halloysite pellets. It was reported that halloysite powder had a porosity ranging from 57.99% for the 'sieved only' to 66.26% for the 'sieved and milled'. These results correlated with the observed results in this study with pellet sample porosities ranging from 62.18% to 70.76% for the 300°C and 100°C heated samples, respectively.

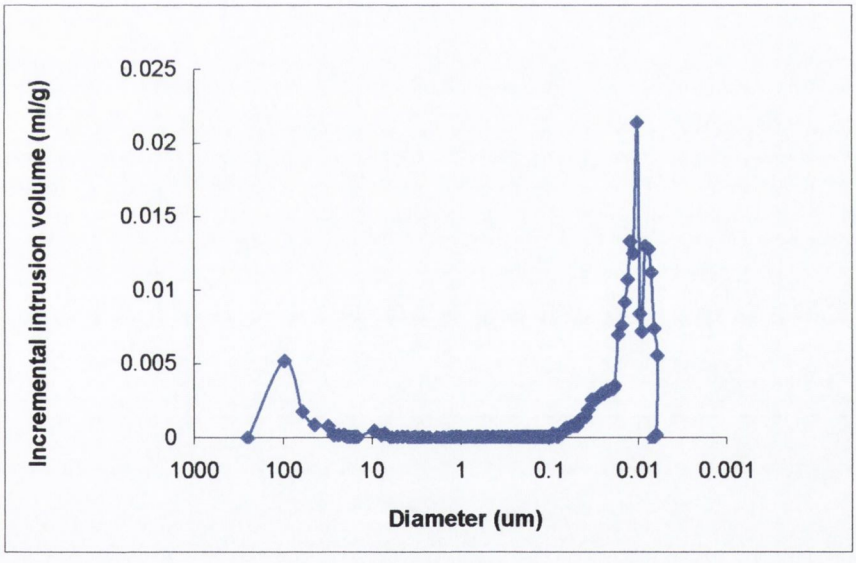


Figure 5.5c Incremental mercury intrusion curve for unheated halloysite pellet.

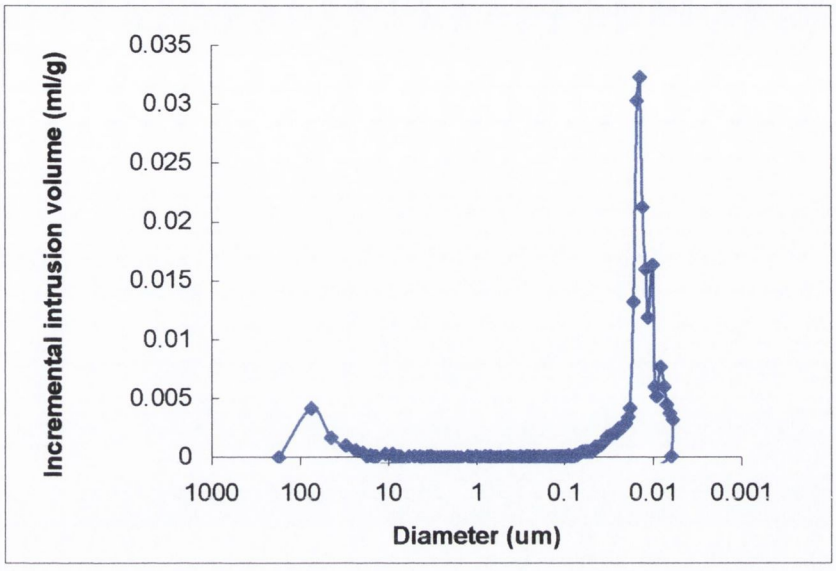


Figure 5.5d Incremental mercury intrusion curve for halloysite pellets heated to 100°C.

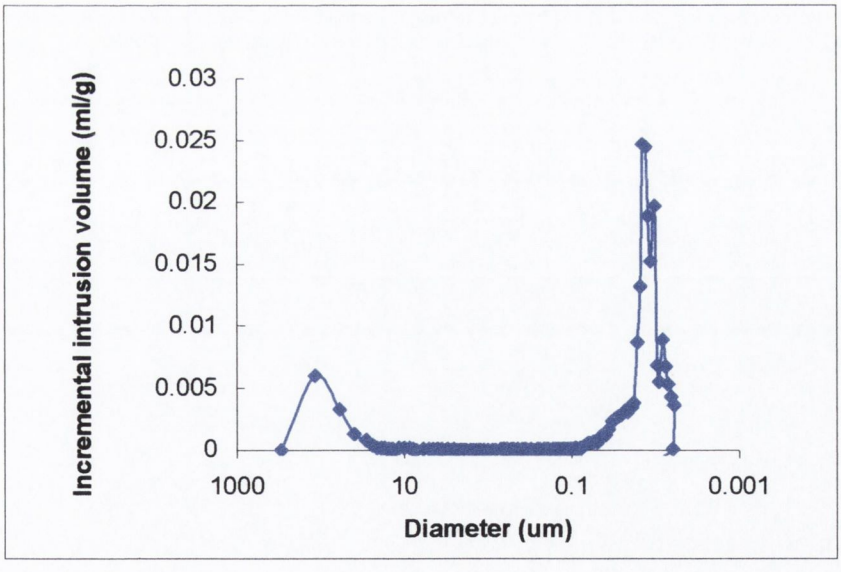


Figure 5.5e Incremental mercury intrusion curve for halloysite pellets heated to 200°C.

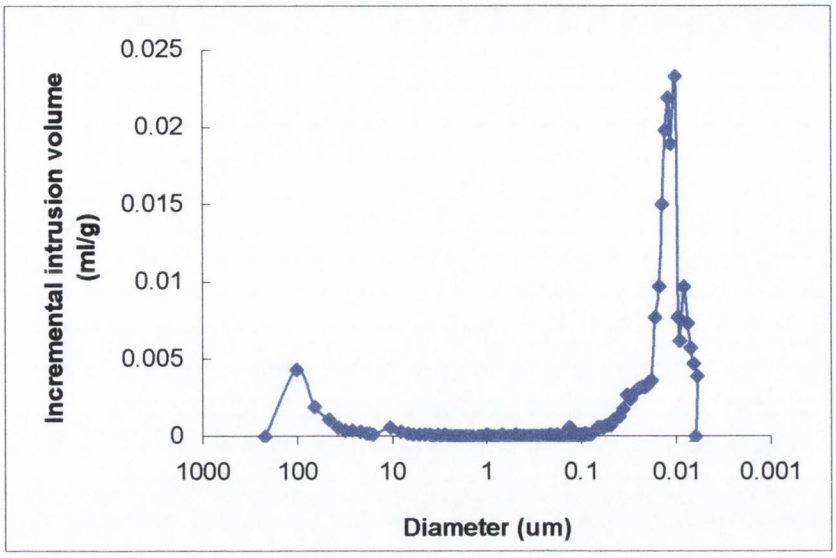


Figure 5.5f Incremental mercury intrusion curve for halloysite pellets heated to 300°C.

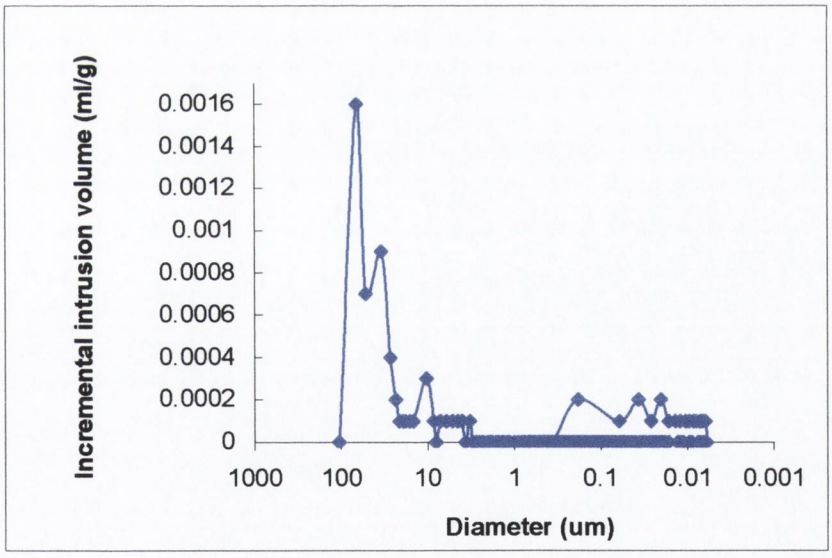


Figure 5.5g Incremental mercury intrusion curve for halloysite pellets heated to 1200°C.

The incremental mercury intrusion curves for the five pellet samples are illustrated in Figures 5.5c, 5.5d, 5.5e, 5.5f and 5.5g. All the pellet samples illustrate bi-modal pore distributions. The unheated pellets and the pellets heated to between 100 to 300°C all show similar pore distributions. Fine pores predominate for the unheated halloysite pellets and pellets heated to100°C, 200°C and 300°C as seen in Figures 5.5c, 5.5d, 5.5e and 5.5f, which are observed in the range of ~0.01 to 0.1 μm with a mercury intrusion volume of approx. 0.025 ml/g. The coarser pores in the range of ~50 to 300 μm are present in a much smaller proportion with a lower mercury intrusion volume of approx. 0.005 ml/g. The fine pores (~0.01 to 0.1 μm) correspond to the intraparticulate pores within the individual halloysite particles. Levis (2000) observed similar results with fine pores ranging from between 0.01 to 0.1 μm in halloysite powder samples. Diamond (1970), Churchman and Payne (1983) and McCrea *et al.* (1990), all using mercury intrusion porosimetry, found that pores in the <50 nm range provide most of the pore volume. Jackson *et al.* (1971) using isotherms of nitrogen and also from water sorption studies, found a sharp peak at 2-3 nm in the pore size distribution, due to the presence of mesopores. Pores within the tubes consist predominantly of the luminal space and also voids created by mineral dehydration. Using an electron microscope with an environmental cell, Kohyama *et al.* (1982) showed that dehydration of halloysite caused groups of layers to apparently shrink as a block, creating slit shaped pores between each group. The pores within the ~50 to 300 μm range correspond to interparticulate pores.

The halloysite pellet samples heated to 1200°C as illustrated in Figure 5.5g had a completely different pore distribution. The bi-modal pore distribution of these pellets is in the reverse of the other four samples with the coarser pores predominating (~10 to 100 μm) with a mercury intrusion volume of 0.0016 ml/g and the smaller pores (~0.01 to 0.5 μm) present at a low level, which is evident due to a very low mercury intrusion volume of < 0.0002 ml/g. Thus these pellets could be regarded as having a single pore distribution of coarse pores only, which is representative of the interparticulate pores. This would support the earlier findings of the low intrusion volume, low porosity and high bulk density as there was virtually a complete absence

of intraparticulate pores due to fusion of the individual tubules. The interparticulate pores (coarse pores) are only present in small amounts (mercury intrusion volume of 0.0016 ml/g) compared to the interparticulate pores in the other four samples (mercury intrusion volume of approx. 0.025 ml/g) because of the individual tubules fusing together on application of excessive heat (1200°C) forming large masses with minimal interparticulate spacing.

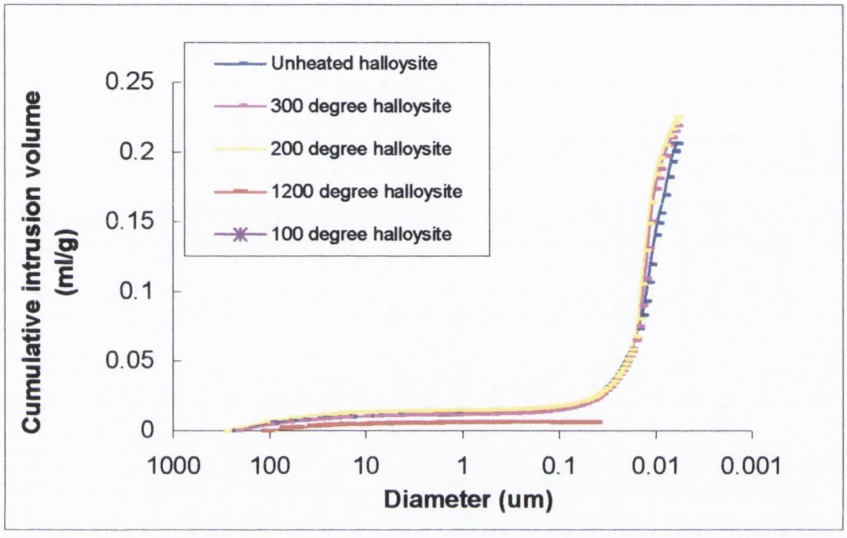


Figure 5.5h Cumulative mercury intrusion curves for the unheated, 100°C, 200°C, 300°C and 1200°C halloysite pellet samples.

The cumulative intrusion plots are illustrated in Figure 5.5h. There is a gradual increase in the mercury intrusion volume, corresponding to the filling of the coarser pores (50 to 300 μm) for the unheated pellets and pellets heated to 100°C, 200°C and 300°C. A levelling off period follows this and then a very rapid increase in intrusion filling in the range 0.01 to 0.1 μm corresponds to the filling of the finer pores. For the halloysite pellets heated to 1200°C, there is also a gradual increase in the mercury intrusion volume, which is representative of the filling of the coarser pores in the range of 10 to 100 μm. There is no sharp increase in intrusion in the 0.01 to 0.1 μm range as seen with the other four pellet samples because as explained previously there

was virtually a complete fusion of the individual halloysite tubules (Figure 5.5a), which resulted in the absence of pores within this size range.

5.5.3 Energy dispersive analysis

Energy dispersive analysis was also performed on the unheated pellet sample and the pellet sample heated to 1200°C as shown in Appendix 17. This technique is a semi-quantitative method, which involves bombarding the elements present within a sample with high voltage electrons. These electrons have an associated energy and emit X-rays. The X-rays detected behave as markers for particular elements and are plotted as peaks. Analysis of both samples showed similar results with two sharp peaks for aluminium and silicon, which is expected as halloysite is composed of an alumina sheet in conjunction with a silica-oxygen sheet. There are also very small peaks for carbon and calcium, which may be present in oxide form (Technical information, New Zealand China Clay, 2001). Calcium may also be present in its carbonate form. A noticeable change in colour occurred on heating the pellets to 1200°C, which resulted in the formation of hard white pellets compared to the 'cream' coloured and relatively soft unheated pellets. This change in colour is reported to occur at temperatures in excess of 1000°C causing impurities to volatilise or dissolve in the resultant amorphous glass (Deasy *et al.*, 2001).

5.6 CONCLUSION

The preliminary deflocculation studies illustrated some interesting findings, which show potential in pharmaceutical and agrochemical applications. Section 5.2 showed that the use of sodium citrate as a deflocculating agent for halloysite Balikesir samples proved to be very successful. It was established that 100 μM solution of sodium citrate was sufficient to deflocculate a 0.25% w/v halloysite suspension. Also it was effective in separating the desired halloysite tubules from the debris in the halloysite Balikesir sample. However use of cetrimide as a deflocculating agent was ineffective as it did not cause deflocculation of the halloysite suspensions even at

levels as high as 300 mM. The use of pH could be used as a method to deflocculate a flocculated system, with a basic pH causing deflocculation of the halloysite suspension. From this study the use of the anionic deflocculating agent, sodium citrate and pH changes proved to be the most effective methods at causing deflocculation of a halloysite suspension. However pH changes would be very difficult to adopt as a deflocculating tool in an agrochemical suspension concentrate. Therefore the use of an anionic deflocculating agent would be the method of choice for deflocculating a halloysite suspension preferably using a polyvalent ion like *Dispex N40* (sodium polyacrylate).

The results from the permeability and mechanical testing on ethylcellulose films in combination with halloysite or talc suggest that an interaction between the fillers and ethylcellulose was occurring. The permeability study showed a significant reduction in diffusion of diltiazem HCl across the films on addition of the fillers. The fillers reduced the tensile strength of the films, shortened the elongation at break and increased the elastic modulus i.e. the films became more brittle. However in this study talc was only used for comparative purposes because it is widely incorporated in coatings to reduce tackiness in the pharmaceutical industry. Therefore halloysite, which gave similar values to kaolin in the mechanical properties study for elastic modulus and elongation at break, however it gave increased permeability of diltiazem HCl and also increased tensile strength and may have the ability to be used also as a filler in film coatings.

From the tabletting studies, it can be concluded that the halloysite produces the more superior tablets in comparison to kaolin. The halloysite tablets showed improved uniformity of weight, thickness and hardness, which is a result of the improved flow properties of the powder mix. The halloysite tablets also showed a reduction in disintegration and a slight reduction in dissolution testing in the first 5 min. This may be attributed to the increased hardness of the tablets, which is a consequence of greater compatibility between the halloysite and microcrystalline particles because of their similar particle shape. This resulted in the particles aligning more closely

within the tablet die with reduced porosity and increased bulk density, which was not the case for the kaolin particles. Therefore halloysite could be used as an alternative to kaolin as a tablet excipient with improved binding characteristics.

Based on the mercury porosimetry results, the pellet samples heated to 200°C could be used for further pesticide loading studies because their structure remained intact on addition to water. This was not the case for the unheated pellet samples and pellets heated to 100°C as they disintegrated rapidly on addition to water. Levis (2000) reported similar findings with unheated halloysite pellets disintegrating rapidly when added to water. It was suggested that halloysite did not possess the ability to form adequate hydrogen bonds, which are capable of retaining the matrix structure. Also the pellet samples heated to 200°C retained good porosity compared with the unheated pellet samples with porosities of 64.03% and 70.76%, respectively, which is vital to ensure maximum pesticide loading. The pellet sample heated to 300°C remained intact on addition to water, but gave lower porosity measurements of 62.18% with the pellet sample heated to 1200°C giving a very low porosity measurement of only 3.04%. Therefore it would not be advantageous to use the pellet samples heated to 300°C and 1200°C for pesticide loading studies.

Chapter 6

Formulation of chlorpyrifos loaded halloysite pellets for vine weevil (*Otiorhynchus sulcatus*) control

6.1 INTRODUCTION

The vine weevil is a serious pest in nurseries and established landscape plantings. The host range of vine weevil is extensive with at least one hundred species of both indoor and outdoor plants being attacked. The damage caused by vine weevil can result in poor growth and in some cases plant death, reduced plant quality and uneven plant size and condition, resulting in significant financial loss to commercial growers. This pest is the most destructive and widely distributed species of root weevils in the genus *Otiorhynchus*. Injury caused by the larval stage feeding on the roots is most destructive to plants. Feeding by larvae occurs from mid-summer through autumn and in early spring. At first larvae feed on small tender roots, but in early spring, they move to the bark of large roots or the stems. Damage to the roots may go undetected in nurseries, but infested plants that are placed in landscapes often die. Injury caused by adults is in the form of marginal notching of broad leaved evergreen foliage and other host plants. This foliar damage seldom impacts on plant health, even though it may be extensive.

As discussed in Chapter 1, chemical control has proved to be the most effective method in reducing vine weevil successfully as non-chemical control (parasitic nematodes and fungal diseases) has drawbacks e.g. nematodes do not work effectively at temperatures below 12°C and entomopathogenic fungus have shelf life problems. Chemical control of vine weevil is usually directed at the larval stage because several problems are inherent in controlling adults e.g. present over an extended period of the year, only active at night, shelter in inaccessible places and

pesticide resistance. Also available sprays used against adults are generally ineffective and need to be reapplied frequently e.g. *Dursban* (chlorpyrifos). Thus chemical control of vine weevil is normally focused on making the soil toxic to larvae. This may be achieved by application of chemical drenches, but as is the case for adult vine weevil they have proved ineffective and need repeated spray applications to give adequate control.

Until recently vine weevil was also controlled by the incorporation of the persistent organochlorine pesticide aldrin into potting compost. This gave effective and lasting control, but the routine incorporation of aldrin is no longer permitted in the EU and the USA since the 1990's for environmental and toxicological reasons (Cross *et al.*, 1995). Since the ban on aldrin, many other chemicals have been tested for control of larvae in the soil. At present chemical impregnated compost, *Intercept* (imidacloprid) and microencapsulated controlled release pellets *suSCon Green* (chlorpyrifos) have proved to be the most effective alternatives for controlling vine weevil larvae. Both products have shown long residual control of vine weevil larvae. The level of control attained using the above treatments is very good although none are as consistently effective as aldrin, which gave complete control when used. In this thesis the focus will remain on *suSCon Green* pellets, which will be compared to the newly formulated chlorpyrifos loaded halloysite pellets *in vitro* and *in field.*

6.1.1 *suSCon Green* pellets

suSCon Green is a controlled release monolithic (matrix) pellet pesticide product produced by incorporation of active ingredient with pore-producing inert powders in a thermoplastic polymer formulation based on ethylene vinyl acetate and ethylene propylene co-polymer (Hitchcock *et. al.*, 1984). The ingredients are compounded, extruded and palletised into 1 mm x 1 mm spheres (McGuffog *et al.*, 1984). It is used for the control of vine weevil larvae in potting media for hardy ornamental nursery stock and non-edible ornamental grown plants. *suSCon Green* has a release period of at least two years (Buxton *et al.*, 1992). It may be added to growth medium from striking cuttings to final potting and will protect all growth stages of plants up

to final selling. A single application should be sufficient for the control of successive generations of larvae during the growing cycle of plants kept in the same containers for up to two years, with partial control for a third year. The dust free formulation prevents unnecessary particle inhalation by the operator. The dark green colour of the pellets helps monitor even mixing.

6.1.1.1 How suSCon Green technology works

suSCon Green contains 10% chlorpyrifos, a proven active ingredient against vine weevil larvae. The manufacturers claim that the chlorpyrofos is gradually released into the compost by a unique patented process ensuring an even release rate over at least a two year period. Once released, chlorpyrifos gradually degrades but is replenished from the pellets ensuring sufficient chemical is always present to give control over the release period (Figure 1.3e). The release process is triggered by the presence of moisture and under practical growing conditions, temperature fluctuations have no significant effect on the rate of release (Product information leaflet, Consolidated Fertilizers Limited, an Incitec Company). *suSCon Green* should be used at a rate of 0.75 kg per cubic metre (1 m^3 = 1000 L) of compost.

6.1.1.2 Method of control

Chlorpyrifos is a stable but non-persistent pesticide, which does not readily leach out of the compost, therefore thorough even mixing is essential. *suSCon Green* is designed to kill the young larvae shortly after they hatch ensuring damage is kept to a minimum. Vine weevil eggs are laid over a 3 to 4 month period and the unique design of the pellets ensures sufficient chlorpyrifos is always available to kill the larvae (Product information leaflet, Consolidated Fertilizers Limited).

6.1.2 Pellet formulations

Where the pesticide is applied to the soil in a finely divided form, as a spray or dust, it will enter the soil very rapidly. In such a form, it is difficult to prevent the pesticide coming into intimate contact with the soil particles more or less immediately after application. By comparison, pellet formulations consist of

relatively large particles and it is possible to hold the pesticide within the pellets and slow down the rate at which it leaves the formulation and enters the soil.

During recent years there has been an increasing use of pellet formulations for the application of pesticide to soil. Several advantages have been claimed for the use of pellet formulations over the conventional spray or dust formulation other than control of pesticide release; they include the absence of drift away from the target during application, good penetration of obstructing foliage ensuring that the majority of the applied pesticide reaches the soil surface and a reduction in handling hazards through using solid pellets rather than liquid sprays or finely divided powders (Furmidge *et al.*, 1966).

The release of pesticide from a pellet may be influenced under three general headings, climatic, applicational and formulation. In general, the climatic factors such as temperature, quantity and intensity of rainfall cannot be controlled, although in many regions of the world they can be predicted with a reasonable degree of accuracy. The applicational factors are those that govern the effective coverage of the soil surface by the formulations, dosage rate, pellet size, pesticide content and pellet density and these may be used to control pesticide release to a limited extent. The formulation factors, which include variations in the method of preparing the pellets and in the constituents used in their preparation, provide the best means of controlling pesticide release (Furmidge *et al.*, 1966).

Pellets are normally prepared in one of four ways; by impregegnation, by coating, by an agglomeration process or an extrusion method. In the impregnated method, the pesticide is dissolved in a suitable solvent, which is then absorbed into a preformed pellet carrier. Control over release can be achieved by the careful choice of the carrier, the solvent and the use of additives such as surface-active agents or water-proofing agents. The coating method, in which the pesticide is stuck onto the outside of a non-absorbent pellet carrier, does not provide much scope for controlled release except in terms of water-proof pesticide coating. In the agglomeration method, the

pellets are built up from an intimate mixture of powdered ingredients. In the extrusion method the dry ingredients are moistened with a suitable wetting agent and then are passed through an extruder, whereby the mass is forced through a perforated screen, which shapes the material into cylindrical rods. The physical structure of the resulting pellets and hence their release characteristics, may be affected by many factors including the nature of the powdered filler, the quantity and the type of the pesticide incorporated and the inclusion of other materials, which can control the adhesion of the individual particles of the pellets (Furmidge *et al.*, 1966). The extrusion method was used in the formation of *suSCon Green* pellets. The ingredients were mixed together and then extruded as a strand and pelletised into 1 mm pellets. A similar process of extrusion was used for the production of the chlorpyrifos loaded halloysite pellets. However the extrudate in this case was placed into a spheronizer where the extrudate is broken up into smaller fragments and rounded into spherical pellets, with a diameter approximately equal to the diameter of the extrudate.

6.1.3 Aim of this section

The aim of this section was to develop sustained release pellets of chlorpyrifos, which could be used for vine weevil control. This was achieved by using halloysite, which was described in Chapter 1, as having unique sustained release properties due to it's hollow tubular structure. The mineral was loaded with the pesticide, chlorpyrifos under vacuum and also during this loading process ethylene glycol was incorporated as it helped neutralise the 'acid sites' on the halloysite particles, which would result in premature hydrolysis of chlorpyrifos (discussed in more detail in Section 6.2). The pesticide loaded halloysite was then formulated into ~1 mm pellets by extrusion-spheronization. Liquid paraffin was chosen as the granulating liquid for the extrusion-spheronization process because as discussed above chlorpyrifos undergoes hydrolysis and therefore water, which is the most common granulating liquid used for this process was considered inappropriate. Also liquid paraffin would have the added advantage of delaying the release of chlorpyrifos into the soil in the outdoor potted primrose trial. A blue pigment was also incorporated into the pellets

so that they were easily visible to the grower once added to the peat mix, thus aiding confirmation of uniformity of mixing.

The formulated halloysite pellets were compared to the commercially available *suSCon Green* pellets *in vitro* and in an outdoor potted primrose trial for vine weevil control. The *suSCon Green* pellets (microencapsulated pellets) as explained above in detail are composed of a plastic resin that releases chlorpyrifos slowly over a two year period through a micropore structure present in the resin. It was hoped that the newly formulated halloysite pellets would provide sustained release of chlorpyrifos over an appropriate period (i.e. 1 year) and would be equally or preferably more effective for the control of vine weevil. Also the halloysite pellets would offer a cheap method of microencapsulation, ease of production and be environmentally friendly.

6.2 HALLOYSITE/CHLORPYRIFOS INCOMPATIBILITY

6.2.1 Introduction

One of the most important properties to be considered when choosing a mineral carrier for formulating a pesticide product is the compatibility of the pesticide with the carrier. Many of the pesticides available today are inherently unstable to a degree when exposed to long term storage at elevated temperature that may not normally be encountered in the field. Also many pesticide chemicals are hydrolytically unstable to some degree, or have some tendency to suffer catalytic decomposition. Even if the mineral carrier was completely inactive chemically, merely spreading the pesticide over the large surface area of a carrier could increase the rate of inherent degradation of the pesticide. However it has been found that almost all mineral carriers inherently possess one or more attributes, such as free moisture content, acid and base sites, and high surface area properties, which would provide ideal conditions for pesticide degradation. Many attempts have been made to study the roles of some

mineral carrier properties on the degradation of pesticides but these studies have only revealed that mineral carrier/pesticide compatibility is variable and complex.

6.2.2 Theory

The problem of the decomposition of organophosphate pesticides when formulated with mineral carriers was recognised as early as 1950. As discussed in Chapter 1, chlorpyrifos is a member of organophosphate group of pesticides and therefore it would be susceptible to similar decomposition in the presence of a mineral carrier e.g. halloysite. Theories concerning the causes of decomposition will be reviewed and discussed below.

Yost *et al.* (1955) reported that malathion, an organophosphate, which is a reasonably stable compound when properly stored was readily hydrolysed in highly alkaline media, but is quite stable in neutral or even somewhat acidic media. Long term stability tests were carried out using a large number of different malathion wettable powders to determine the effects of moisture, temperature, pH and filler type on the stability of malathion. Fillers were selected to cover a range of pH and moisture content characteristics. Results showed that the presence of moisture accelerated the rates of breakdown of malathion and while pH was also a factor, its effect was least in compositions having low moisture content. However moisture removal by drying the carrier did not eliminate decomposition. High temperature also markedly accelerated the rate of malathion breakdown. In general they concluded that a slightly acidic or neutral reaction pH 4-7 and low moisture content were desirable chacteristics for mineral carriers to be used in the preparation of malathion wettable powders. Also inactivation of catalytic 'hot spots' believed present on the surface of certain mineral carriers, by chemical treatment or physical masking, was necessary. Saltzman *et al.* (1976) and Rosenfield and Van Valkenburg (1965) showed that the type of clay, incubation temperature, saturating cation and degree of clay hydration affected surface-induced hydrolysis rates of organophosphorus esters. McPherson and Johnson (1956) studied the inherent instability of pesticides such as parathion,

methyl parathion, chlorthion and malathion, and reported data on the rate of degradation of these pesticides at various temperatures indicating poor stability.

Rosenfield and Van Valkenburg (1965) studied the influence of various metallic ions and mineral carrier pH. They reported that copper had a pronounced effect on alkaline hydrolysis. These workers tested various mineral carriers for pH and cation exchange capacity and tried correlating these properties with degradation of (O,O-dimethyl-O-2,4,5-trichlorophenyl) phosphorothioate. They reported that both acidic and basic pH minerals with cation exchange capacities (CEC) over 10 meq/100 g caused decomposition, while those with CEG's of 6 meq/100 g gave fair stability. This theory was also supported by Mortland and Raman (1967) who worked with chlorpyrifos and diazinon.

Polen and Sawyer (1962) reported the variability of catalytic activity of two high-sorptive carriers i.e. an attapulgite and a montmorillonite. Comparative data was presented on rates of degradation of malathion on these carriers along with data on a few low-sorptive diluents. The results showed that, of the samples tested, the attapulgite dusts were the least stable with montmorillonite and kaolinite being the most stable. Getzin (1981b) illustrated that clays in his study possessed varying levels of activity, with illite = vermiculite > kaolinite and pure montmorillonite (Panther Creek Bentonite) possessing the lowest chlorpyrifos-degrading activity of all the clays examined. Yost *et al.* (1955) showed that the most promising diluents which gave long term stability of malathion were pyrophyllites such as Pyrax ABB and Pyrolyte, neutral talcs, gypsum and possibly certain calcium carbonates. These findings also were supported by Shell Chemical Corp. (1957) and by using the above diluents no deactivators were needed.

Malina *et al.* (1956) and Fowkes *et al.* (1960) reported that on the surface of mineral carriers there are acid sites, whose strength can be correlated with the decomposition of certain chlorinated organic pesticides. They showed that dieldrin and endrin formulated with neutral diluents, such as talc or calcium carbonate, decomposed

much more slowly than when formulated with acidic diluents such as kaolinite or attapulgite. They explained this outcome in terms of cation exchange capacity where most natural clays have about 1 μmole of cation exchange site for every square metre of surface. Kaolinite with 25 square metres per gram of surface has 25 μmoles of exchangeable cations per gram of clay and attapulgite with 125 square metres per gram of surface has 125 μmoles per gram. Thus the higher cation exchange capacity mineral carriers would accelerate degradation of organophosphates due to the increased presence of catalytic sites on their surface. This acid-site theory was also proposed as the cause of decomposition of some thiophosphate pesticides; namely malathion, parathion and methyl parathion. Oxygen containing compounds, such as glycols, reduce surface activity. However merely neutralising these acid sites by this technique did not completely eliminate the decomposition of malathion impregnated on attapulgite. Polen and Sawyer (1962) showed that the use of 7% of diethylene glycol did not completely eliminate the malathion decomposition, which indicated that either diethylene glycol did not decrease the surface acidity sufficiently to prevent decomposition, or causes other than, or in addition to, surface acidity were responsible. Velsicol Chemical Corp. (1957) suggested the use of glycol-chemicals and recommended approximate concentrations for deactivating various carriers and diluents. Niagara Chemical Division (1965) suggested the use of diethylene glycol or dipropylene glycol for stabilisation of dry formulations of ethion.

6.2.3 Conclusion

From the evidence reviewed above it can be concluded that several factors play an important role in the chemical stability characteristics of organophosphates on mineral carriers. The desirable characteristics to ensure optimum stability of organophosphates would be to have a low moisture and metallic ion content, low temperature, neutral or slightly acidic conditions and also to use a mineral carrier with a low cation exchange capacity.

Halloysite was chosen as the mineral carrier for chlorpyrifos because of its ability to entrap chemicals within it's internal tubular lumen and also less extensively by

chemisorption to the surface. All of the research to-date on stability of organophosphates has involved using other mineral carriers. However kaolin, which is chemically similar to halloysite but differs morphologically was discussed in significant detail. Therefore similarities may be drawn between the two minerals.

Halloysite as discussed in Chapter 1 is capable of existing in a hydrated (endellite) form, which has water bound into the internal lattice of the mineral and a dehydrated (metahalloysite) form. However the halloysite (halloysite Goushan, NZCC) used in this study was present in a dehydrated form, which was confirmed by XRD in Section 4.4. Therefore there was an absence of water in the internal lattice of halloysite used. However a certain amount of water may be present as free water on the surface of the mineral due to exposure to air. This free water was removed by placing the unloaded halloysite powder sample in an oven at 50°C for approx. 48 hr until it reached constant weight. This ensured minimal moisture content in the halloysite sample used, thus ensuring the least possible hydrolytic degradation of chlorpyrifos on exposure to the mineral carrier.

Liquid paraffin was chosen as the granulating liquid for the extrusion-spheronization process because as discussed chlorpyrifos undergoes hydrolysis, therefore water, which is the most common granulating liquid used for this process was inappropriate. Liquid paraffin would therefore avoid this hydrolysis process.

As discussed above in Section 6.2.2, Rosenfield and Van Valkenburg (1965) and Mortland and Raman (1967) reported that metal ions such as nickel, iron, calcium, magnesium, aluminium and zinc had little or no effect on the hydrolysis rate, while copper had a very pronounced effect on chlorinated hydrocarbonates and organophosphates, respectively. Halloysite is principally an aluminosilicate mineral with small amounts of iron, which are substituted for the aluminium ions in the octahedral sheet to alleviate the degree of lateral misfit (Soma *et al.*, 1992). The presence of aluminium and silicate were confirmed by EDX in the halloysite G sample in Appendix 17 (sample provided by New Zealand China Clay). However the

quantitative analysis of the other elements present in the halloysite G sample was confirmed by ICP-MS (Section 4.7 and Appendix 1). Copper was present at a level of 52.01 ppm, while iron was present at a significantly higher level of 1360 ppm. A chemical analysis carried out by New Zealand China Clay Ltd (Technical Information, New Zealand China Clay Ltd, 2001) on a typical premium grade of halloysite also confirmed the above findings with aluminium (35.5%) and silicate (50.4%) present in significant amounts and iron present at a level of 0.25%. Other elements such as sodium, magnesium, barium, potassium and manganese were only present in trace amounts. Therefore from these findings it is unlikely that the copper present in the halloysite Goushan NZCC sample (sample used in the chlorpyrifos pellets, which was also supplied by New Zealand China Clay) would cause significant catalytic destruction of chlorpyrifos and also it is not freely available for substitution in the lattice like aluminium and iron.

Elevated temperatures dramatically increase chlorpyrifos degradation as discussed above in Section 6.2.2. Getzin (1981b) reported that chlorpyrifos degraded rapidly via a temperature-dependent catalytic hydrolysis process on sultan silt loam. Times for 50% degradation at 5, 15, 25, 35 and 45°C were > 20, > 20, 8, 3 and 1 day, respectively. Therefore from these findings it was decided that the chlorpyrifos loaded samples would be allowed to dry at room temperature (20°C) because as previously reported by Kelly (2002) and Levis and Deasy (2003) drug loaded halloysite samples were dried rapidly in an oven at 50°C. This would result in unnecessary degradation of chlorpyrifos.

Chlorpyrifos is relatively stable in neutral or acidic media as explained above. However in the presence of moisture, the compound decomposes rapidly by base induced hydrolysis (Racke *et al.*, 1996b). Therefore chlorpyrifos was dissolved in ethanol, which has an acidic pH and then loaded into the halloysite particles under vacuum, thus preventing base induced degradation of chlorpyrifos. Tomlin (1997) reported that chlorpyrifos does not degrade in aromatic solvents, therefore further enhancing the stability of chlorpyrifos.

Mineral carrier selection is the most important factor in ensuring the long term stability of organophosphates. Halloysite was chosen as the mineral carrier and therefore selection of the most appropriate mineral to reduce organophosphate degradation was not an option in this study. As shown above, certain mineral carriers showed improved organophosphate stability.

Halloysite differs from kaolinite in that it has a higher cation exchange capacity and surface area (Harvey, 1996). As shown in Section 4.6, halloysite Goushan NZCC has a specific surface area of 60.68 m^2/g, therefore it would be regarded as having more catalytic sites for degradation of chlorpyrifos in comparison to kaolinite. Thus to overcome this problem 10% ethylene glycol to chlorpyrifos was added to the halloysite powder during the pesticide loading process to help neutralise the catalytic sites. It may not have caused complete neutralisation of all the catalytic sites on the halloysite sample because as reported by Polen and Sawyer (1962) the use of 7% of diethylene glycol did not completely eliminate the malathion decomposition.

6.3 FACTORIAL EXPERIMENTATION OF PELLETS

The aim of this work was to examine the effect of three variables i.e. liquid paraffin level, spheronization time and spheronization speed on the yield of pellets from a halloysite mix. In order to determine formulation and process variables more closely, it was necessary to conduct a factorial experiment using the above variables in a controlled manner, within one overall experimental design. The above formulation and processing variables were chosen as the most likely candidates to have an effect on pellet formation. An analysis of variance (ANOVA), 3^3 factorial design was used to determine the optimum formulation and process variables for the pellet formation. The information obtained from this factorial experiment was then used to produce the chlorpyrifos loaded halloysite pellets in the optimum size range of between 850 and 1150 μm.

6.3.1 Yield of pellets

The variation in yield of pellets in this experimental design ranged from 42.2% to 86.7% (Table 6.3a). The highest yield of pellets was obtained for a liquid paraffin level of 21%, spheronization speed of 1000 rpm and spheronization time of 5 min. The lowest yield of pellets was obtained for a liquid paraffin level of 23%, spheronization speed of 1500 rpm and spheronization time of 15 min, which was the highest level of all the three factors studied.

Table 6.3a Yield of pellets (%) produced by varying liquid paraffin, spheronization speed and time levels (average of three runs)

Liquid paraffin level	**Sp speed (rpm)**	**Spheronization time**		
		5 min	**10 min**	**15 min**
19% w/w	**1000**	82.7	77.8	71.5
	1250	72.9	72.4	65.6
	1500	65.4	63.7	60.8
21% w/w	**1000**	86.7	84.7	81.43
	1250	79.3	81.6	76.2
	1500	72.7	71.5	68.9
23% w/w	**1000**	74.5	70.2	65.6
	1250	65.2	62.6	57.6
	1500	54.8	48.9	42.2

A general trend of decreasing pellet yield was observed with increasing spheronization speed/time at all liquid paraffin levels with the most significant reduction in pellet yield at the 23% liquid paraffin level. There was an increase in pellet formation on increasing the liquid paraffin level from 19% to 21% at all levels of spheronization speed and time, but there was a significant reduction of pellet formation when the liquid paraffin was increased to 23%. This may be due to the

excessive level of liquid paraffin, which caused the halloysite particles to agglomerate and form oversized pellets, which in effect was further enhanced by excessive spheronization speed and time.

Table 6.3b Analysis of variance for pellets, using 3^3 factorial design

Source	*df*	*SS*	*MS*	*F*	*P*
A	2	4777.43	2388.71	201.56	0.000
B	2	3714.47	2388.71	156.71	0.000
C	2	577.88	1857.24	24.38	0.000
AB	4	205.27	288.94	4.33	0.004
AC	4	99.97	51.32	2.11	0.092
BC	4	23.65	24.99	0.50	0.737
ABC	8	38.07	5.91	0.40	0.915
Error	54	639.96	4.76		
Total	80	10076.70	11.85		

A = liquid paraffin level, **B** = spheronization speed, **C** = spheronization time

The data from the yield of pellets was analysed using ANOVA, which enabled the determination of the main effects and interactions. Table 6.3b shows the ANOVA analysis for the yield of pellets. Table 6.3b and Figure 6.3a shows that all the main effects alone cause definite changes in pellet yield as they have p-values of <0.05, which makes them highly statistically significant. As discussed earlier it can be seen in Figure 6.3a that increasing the spheronization time/speed results in a reduction in pellet yield.

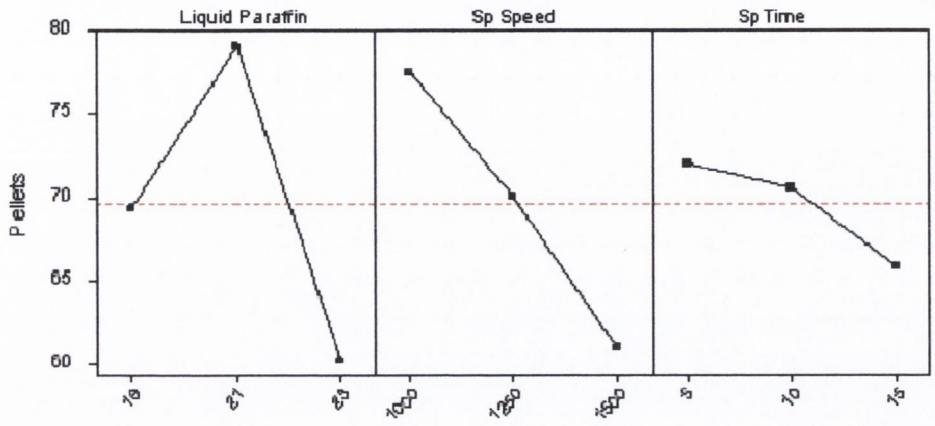


Figure 6.3a Pellet yield main effect plots for liquid paraffin, spheronization speed/time levels.

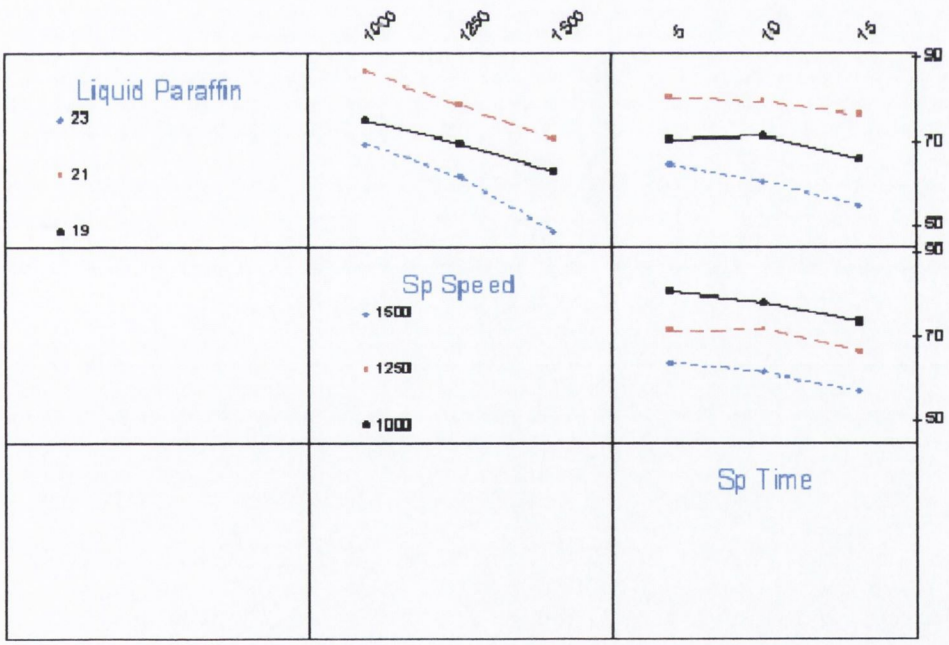


Figure 6.3b Pellet yield interaction plots for liquid paraffin, spheronization speed/time levels.

It is evident from the results in Table 6.3b that the three-factor interaction between liquid paraffin, spheronization time/speed is not statistically significant as the p-value is above 0.05 ($p = 0.915$). The only two-factor interaction that is statistically significant is the liquid paraffin by spheronization speed ($p = 0.004$) as can be seen in Table 6.3b. Figure 6.3b shows the interaction plot for pellet yield. Focusing on the two-factor interaction between liquid paraffin and spheronization speed, it shows that there is a significant reduction in pellet yield as the main effect of spheronization speed is increased from 1000 to 1500 rpm, with the optimum pellet yield at 21% liquid paraffin level. Therefore it can be concluded that the best conditions for optimum pellet yield in the size range 850-1150 μm, is a liquid paraffin level of 21% and a spheronization speed/time of 1000 rpm and 5 min, respectively.

6.3.2 Yield of large pellets

Table 6.3c Yield of large pellets (%) produced by varying liquid paraffin, spheronization speed and time levels (average of three runs)

Liquid paraffin	**Sp speed (rpm)**	**Spheronization time**		
		5 min	**10 min**	**15 min**
19%	1000	6.9	12.2	15.8
	1250	14.5	15.9	18.7
	1500	17.3	17.6	23.7
21%	1000	5.6	7.5	10.8
	1250	12.5	9.8	14.2
	1500	14.8	15.9	17.5
23%	1000	20.3	24.2	29.9
	1250	25.2	31.1	36.2
	1500	29.8	41.7	53.5

The production of large pellets is not desirable in the extrusion-spheronization process as it is associated with a reduction in yield of pellets of the desired size range. The yield of large pellets appeared to be dependent on the liquid paraffin level (Table 6.3c). A general trend can be observed that as the liquid paraffin level was increased, the degree of agglomeration as represented by the yield of large pellets increased. The yield of large pellets formed was found to increase as the spheronization speed/time increased at each liquid paraffin level. The yield of large pellets range from 5.6% to 53.5%. The highest yield occurred when the liquid paraffin level was 23%, and spheronization speed/time was 1500 rpm and 15 min respectively. The production of large pellets is generally due to overwetting of mixes. This leads to a build up of excess liquid paraffin on the surface of pellets, therefore promoting coalescence of the pellets into large or agglomerated pellets.

Table 6.3d shows the ANOVA analysis for the large pellet data, which shows that the three-factor interaction ($p = 0.011$) and the three two-factor interactions are statistically significant (p-values all <0.05). The main effects are also all statistically significant.

Table 6.3d Analysis of variance for large pellets, using 3^3 factorial design

Source	*df*	*SS*	*MS*	*F*	*P*
A	2	3611.92	1805.96	335.04	0.000
B	2	1570.50	785.25	145.68	0.000
C	2	936.21	468.10	86.84	0.000
AB	4	350.02	87.51	16.23	0.000
AC	4	262.23	65.56	12.16	0.000
BC	4	78.48	19.62	3.64	0.011
ABC	8	121.97	15.25	2.83	0.011
Error	54	291.07	5.39		
Total	80	7222.41			

A = liquid paraffin level, **B** = spheronization speed, **C** = spheronization time

6.3.3 Yield of fines

Generally the production of fines during spheronization is due to insufficient wetting of the mix, which is directly opposite to conditions favouring the production of large pellets. Likewise, a high yield of fines is not desirable as it is accompanied by a loss in desirable pellet yield. From the results obtained in Table 6.3e it can be observed that at the lowest liquid paraffin level of 19%, the maximum number of fines are produced because the spheronizing material was more fragile and had greater tendency to shatter. However as the liquid paraffin is increased the yield of fines is reduced because the spheronized material is less susceptible to fragmentation. The production of fines was relatively low ranging from 4.2% to 18.7%. In general as the spheronization speed/time is increased the production of fines is increased at all liquid paraffin levels.

Table 6.3e Yield of fines (%) produced due to varying liquid paraffin, spheronization speed and time levels (average of three runs)

Liquid paraffin	**Sp speed (rpm)**	**Spheronization time**		
		5 min	**10 min**	**15 min**
19%	1000	10.4	10	12.8
	1250	12.6	11.8	15.9
	1500	17.3	18.7	15.5
21%	1000	7.7	7.8	7.8
	1250	8.2	9	9.6
	1500	12.5	12.6	13.6
23%	1000	7.5	6.6	4.6
	1250	9.7	6.3	6.1
	1500	5.36	6.5	4.2

Table 6.3f shows the ANOVA analysis for the fines data. From this Table it can be observed that the three-factor interaction is not statistically significant ($p = 0.396$) and two of the two-factor interactions are statistically significant, which are liquid paraffin by spheronization speed ($p = 0.006$) and liquid paraffin by spheronization time ($p = 0.005$). The main effects of liquid paraffin and spheronization speed are both statistically significant with p-values of 0.00 but the main effect of spheronization time is not statistically significant ($p = 0.363$).

Table 6.3f Analysis of variance for fines, using 3^3 factorial design

Source	*df*	*SS*	*MS*	*F*	*P*
A	2	406.402	203.201	34.24	0.000
B	2	527.276	263.638	44.43	0.000
C	2	12.241	6.120	1.03	0.363
AB	4	97.057	24.264	4.09	0.006
AC	4	99.919	24.980	4.21	0.005
BC	4	59.252	14.813	2.50	0.053
ABC	8	50.901	6.363	1.07	0.396
Error	54	320.453	5.934		
Total	80				

A = liquid paraffin, **B** = spheronization speed, **C** = spheronization time

6.3.4 Conclusion

From the ANOVA study carried out it was hoped to ascertain the best conditions for optimum pellet yield. Liquid paraffin level and spheronization speed/time had a very significant effect on pellet yield with their main effects all showing statistical significance ($p<0.05$). From this study it can be seen that the best conditions for pellet formation was a liquid paraffin level of 21% and a spheronization speed/time of 1000 rpm and 5min, respectively, which gave a high pellet yield of 86.7% (Table 6.3a). For the large pellets the three main factors studied all showed statistical

significance. The largest pellet yield occurred at the highest level of each of the three factors studied (Table 6.3c). As explained earlier in Section 6.3.2, the reason for the increased large pellet yield at the higher levels of each main effect was because the increased liquid paraffin level caused agglomeration of pellets into larger units, which was accentuated further at the higher spheronization speed/time. Therefore the lower levels of the three main factors would be recommended to minimise large pellet production. The highest fines yield occurred at the lowest liquid paraffin level of 19% due to inadequate liquid paraffin levels. Table 6.3f showed that liquid paraffin and spheronization speed proved to have a significant effect on the yield of fines ($p<0.05$), with spheronization time having an insignificant effect ($p = 0.396$).

These studies involving extrusion-spheronization of halloysite were carried out in the absence of chlorpyrifos, which is toxic to handle and hopefully translate to conditions favouring optimum pellet production when the halloysite is pesticide loaded.

6.4 CHLORPYRIFOS CONTAINING PELLETS

The information obtained from the factorial experiment in Section 6.3 was used in the design and manufacture of the chlorpyrifos loaded halloysite pellets. The optimum pellet yield was achieved at a liquid paraffin level of 21% and a spheronization speed/time of 1000 rpm and 5 min, respectively. The final formulation used for the chlorpyrifos loaded halloysite pellets is shown in Table 3.9b. This formulation in addition to the above contained 1% blue pigment and also the halloysite used was halloysite Goushan NZCC rather than halloysite G due to its increased uniform tubular dimensions (halloysite choice discussed in Chapter 4), which was chlorpyrifos/ethylene glycol loaded.

Preliminary work was carried out to ascertain the optimum concentration of chlorpyrifos, which could be loaded into the halloysite Goushan NZCC sample and also the most appropriate solvent to dissolve the pesticide. Chlorpyrifos was

dissolved in an organic solvent, ethanol because the pesticide is only slightly soluble in water i.e. 2 ppm (McEwen and Stephenson, 1979). Ethanol was the organic solvent of choice because on loading the chlorpyrifos solution under vacuum (Section 3.9.2) it remained in contact with the halloysite sample for a sufficient period of time to ensure the dissolved chlorpyrifos was successfully drawn into the halloysite tubules, unlike the other solvents investigated for loading ability (e.g. acetone and dichloromethane), which volatilised off within seconds of applying the vacuum.

At high concentrations of chlorpyrifos in ethanol i.e. > 15% w/v, chlorpyrifos formed a viscous liquid. This proved impossible to load into the small halloysite tubules. A 'shiny' film formed over the halloysite sample, which suggested the chlorpyrifos was not properly loaded into the tubules and remained on the surface instead. Therefore this problem was dealt with by reducing the chlorpyrifos concentration to a lower concentration of 6.5% w/v in ethanol. At this concentration there was a complete absence of this film, thus suggesting that the halloysite tubules were loaded successfully with the chlorpyrifos solution. Also the release studies from the pellet formulation in Section 6.6 showed sustained release of chlorpyrifos. The halloysite sample was double pesticide loaded to ensure enhanced loading of the tubules. 10% ethylene glycol to chlorpyrifos was also added at the first pesticide loading stage as a stabiliser to neutralise the 'acid sites' on the halloysite (Section 6.2.3).

The final formulated pellets were assayed by a HPLC method (Section 3.9.9). The average pesticide content in 4 samples was calculated and the concentration of chlorpyrifos within the pellets was calculated as a % w/w (Table 6.4a). From the results in Table 6.4a it can be seen that the halloysite pellets contain an average of 10.5% w/w chlorpyrifos. The *suSCon Green* pellets contain 10% w/w chlorpyrifos, therefore the halloysite pellets will be applied at a slightly lower rate than the *suSCon Green* pellets (Section 3.10.2). An *in vitro* release study was performed on the two products, which also included unformulated chlorpyrifos to determine the level of sustained release achieved by both products (Section 6.6). However, firstly it was necessary to select an appropriate dissolution medium for the product release studies,

which is explained in Section 6.5. Figure 6.4a shows a photograph of the *suSCon Green* pellets and the chlorpyrifos loaded halloysite pellets.

Table 6.4a Total amount of chlorpyrifos in halloysite pellets (% w/w)

Sample no.	% w/w of chlorpyrifos in halloysite pellets
1	9.84
2	10.76
3	10.39
4	10.90
Average	10.47

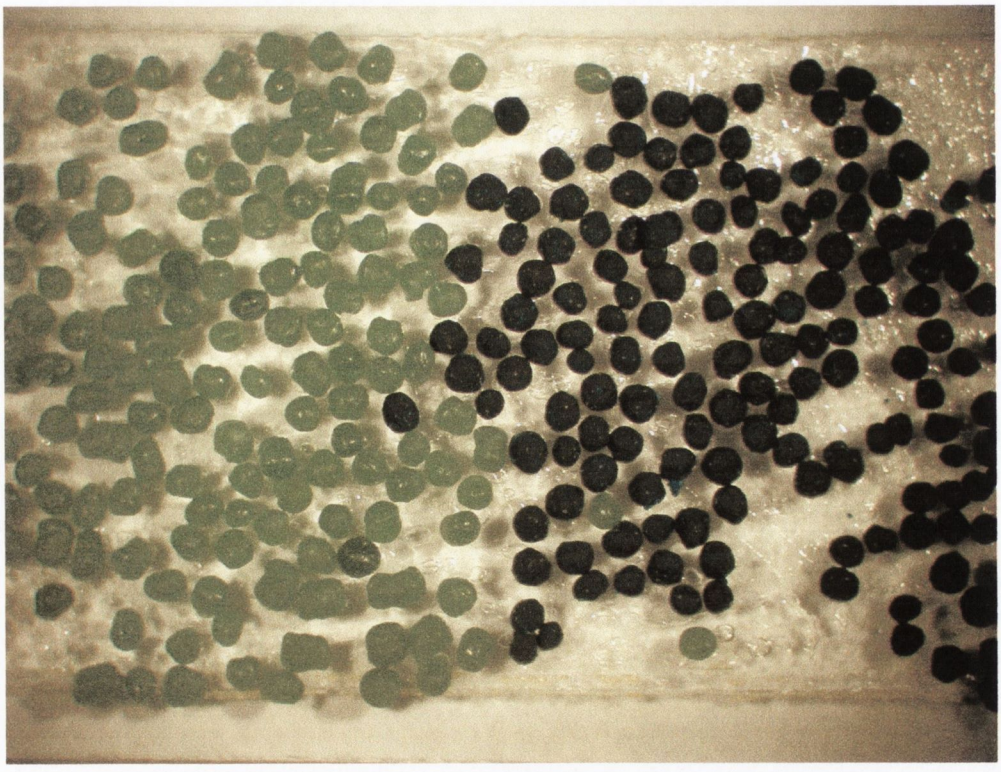


Figure 6.4a Photograph showing *suSCon Green* pellets (green) and chlorpyrifos loaded halloysite pellets (dark blue).

6.5 RELEASE PROFILES OF UNFORMULATED CHLORPYRIFOS IN DIFFERENT DISSOLUTION MEDIA

The aim of this section was to select a suitable dissolution medium for the release studies of the newly developed chlorpyrifos loaded halloysite pellets in comparison with the *suSCon Green* pellets (Section 6.6). The dissolution medium needed to have a pH of 5 in order to mimic the conditions of the peat mix in the outdoor potted primrose trial and also display sink conditions. Because chlorpyrifos is very poorly soluble in water i.e. 2 ppm (McEwen and Stephenson, 1979), it was necessary to use an organic solvent i.e. acetonitrile with McIlvaine buffer pH 4 (buffered to pH 5 using a 0.1 M NaOH solution) in the dissolution medium to achieve the necessary sink conditions. A number of dissolution media were studied with different volumes of acetonitrile (Table 3.9d) to ensure the optimum system was chosen, which would dissolve the chlorpyrifos at an appropriate rate over a 14 day period. A 14 day period was chosen for the release study because longer periods of time resulted in hydrolysis of chlorpyrifos into it's metabolites (dissolution medium contained 70% water), which proved difficult to quantify using the HPLC assay and also it was a sufficient period of time to show whether the two pellet formulations had sustained release properties in comparison to the unformulated chlorpyrifos.

Figure 6.5a shows the release profiles of unformulated chlorpyrifos using varying volumes of acetonitrile in the dissolution medium over a 14 day period. As expected the 40:60 acetonitrile:McIlvaine buffer dissolution medium dissolved the unformulated chlorpyrifos at the fastest rate with 100% dissolved after day 1, which is due to the high volume of acetonitrile present within the dissolution medium. Also as expected the 10:90 acetonitrile:McIlvaine buffer dissolution medium dissolved the unformulated chlorpyrifos at the slowest rate with only 22% dissolved at day 14, which was due to the low volume of acetonitrile present within the dissolution system. The 20:80 acetonitrile:McIlvaine buffer dissolution medium only dissolved 59% of the unformulated chlorpyrifos after day 14. From this information it can be concluded that the 10:90 and 20:80 acetonitrile:McIlvaine buffer dissolution media

would be unsuitable for use in the release studies of Section 6.6 because sink conditions were probably not achieved and also the chlorpyrifos would probably not be sufficiently released from the two sustained release products within the 14 day period to give appropriate release profiles.

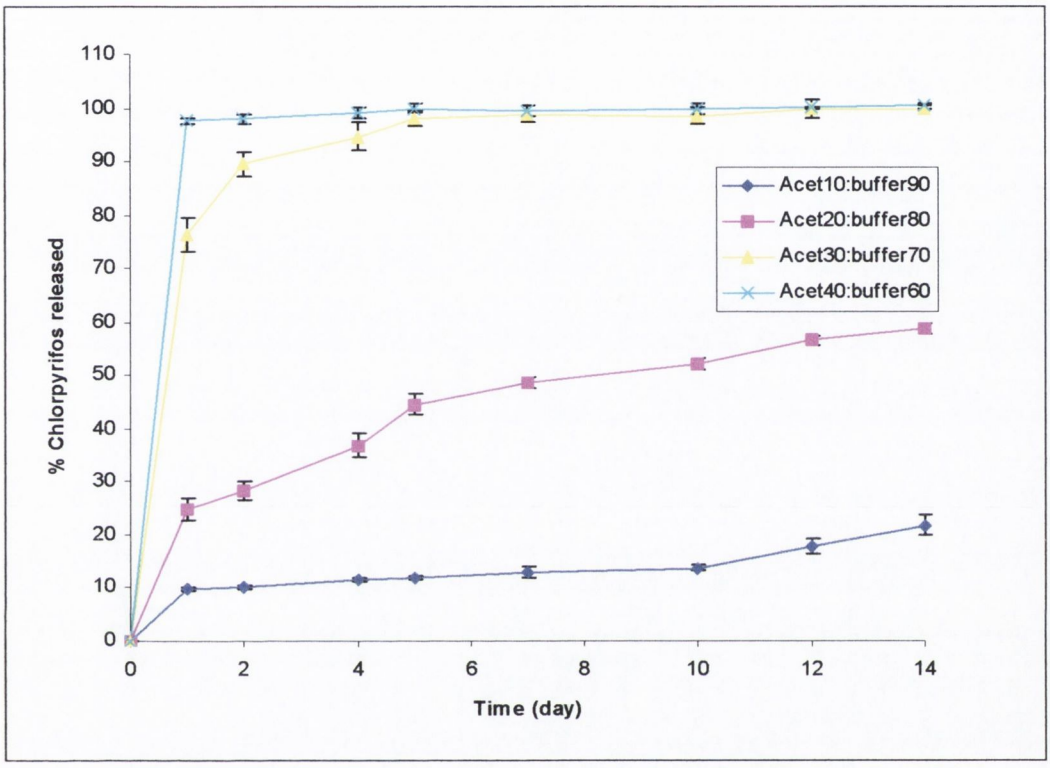


Figure 6.5a Release profiles of unformulated chlorpyrifos using varying compositions of acetonitrile in the dissolution medium (vol. acetonitrile:vol. buffer).

The 40:60 acetonitrile:McIlvaine buffer dissolution medium dissolved the unformulated chlorpyrifos at a fast rate, therefore it would be regarded as being unsuitable for the release studies in Section 6.6 because the chlorpyrifos would be released too quickly and would not give useful release profiles for the two products. However the 30:70 acetonitrile:McIlvaine buffer dissolution medium dissolved the unformulated chlorpyrifos at a slightly slower rate than the 40:60 acetonitrile:McIlvaine buffer dissolution medium with only 100% dissolved after day

5 rather than 100% after day 1. Both these dissolution media gave better sink conditions.

Consequently, from this study it was concluded that the 30:70 acetonitrile:McIlvaine buffer dissolution medium would be the most suitable dissolution medium for using in the release studies of the chlorpyrifos loaded halloysite pellets and the *suSCon Green* pellets as it dissolved the unformulated pesticide at a sufficient rate to enable comparison to be made between the two prolonged release products in a convenient time of 14 days. However, it should be appreciated that because of the presence of the acetonitrile, the dissolution medium is likely to poorly mimic conditions in the peat mixes.

6.6 RELEASE PROFILES OF CHLORPYRIFOS LOADED HALLOYSITE PELLETS, *SUSCON GREEN* PELLETS AND UNFORMULATED CHLORPYRIFOS

It was decided from the information obtained in Section 6.5 that a 30:70 acetonitrile:McIlvaine buffer pH 4 dissolution medium, which was adjusted to pH 5 with a NaOH solution, would be used for the release studies of the two sustained release products because it gave adequate sink conditions and also ensured the unformulated pesticide dissolved completely in 14 days. The two pellet formulations in this study were also compared to unformulated chlorpyrifos to determine whether both pellets provided sustained release of the pesticide. Samples were weighed out so that they contained equivalent quantities of chlorpyrifos (Table 3.9f). This ensured that the release profiles were comparable to each other.

Figure 6.6a illustrates the release profiles of the two products and the unformulated chlorpyrifos. It can be seen from these profiles that there is a significant difference between the three release profiles. The difference between the three release profiles was examined statistically by ANOVA and it was found that they were all

statistically different from each other ($p<0.05$). The *suSCon Green* pellets gave greater sustained release of chlorpyrifos than the halloysite containing pellets. After day 1 there is an initial burst effect of 76.5%, 50% and 23.5% for the unformulated chlorpyrifos, chlorpyrifos loaded halloysite pellets and *suSCon Green* pellets, respectively. The chlorpyrifos loaded halloysite pellets profile shows that by loading chlorpyrifos into the halloysite tubule and formulating it into a pellet, a significant retardation of pesticide release occurs relative to the unformulated pesticide. There is 26.5% burst release difference between the chlorpyrifos loaded halloysite pellets and the unformulated chlorpyrifos. This can be explained by focusing on the halloysite particles within the pellet formulation. They have an ability to entrap drugs within the hollow internal lumen of the tubules and also by chemisorption to the surface (Chapter 1). Kelly (2002) loaded halloysite particles with tetracycline base and sodium fluoride while Levis and Deasy (2003) loaded halloysite particles with diltiazem HCl and propranolol HCl. All these drugs are cationic in nature, which allows them not only to be loaded into the halloysite tubules but also to a lesser extend to bind to the polyanionic surface of the tubules. However the chlorpyrifos pesticide used in this study is neutral in nature and thus will not have the ability to bind onto the anionic outer surface of the halloysite tubules. Despite that Racke, 1993 showed that chlorpyrifos exhibits a strong tendency to be adsorbed by soil and organic matter with average sorption coefficients of $K_d = 173$ ($K_{oc} = 8498$); therefore it will have a high affinity for halloysite particles, which will give the chlorpyrifos further retarded released. Therefore the chlorpyrifos in the halloysite pellets is trapped within the internal lumen of the tubules and also bound to a certain extend to the surface of the halloysite particles. The burst release of 50% chlorpyrifos from these halloysite pellets is probably mainly due to the presence of pesticide between the halloysite particles (interparticulate), which got trapped during the pesticide loading process.

The *suSCon Green* pellets show significant sustained release of chlorpyrifos in comparison to the chlorpyrifos loaded halloysite pellets with an approx. two-fold reduction (26.5%) in the release of chlorpyrifos after day 1. This is explained by the

fact that the chlorpyrifos loaded halloysite pellets disintegrated on addition to the dissolution medium because they were 'soft' pellets, unlike the *suSCon Green* pellets, which were composed of a 'hard' plastic resin that remained fully intact. Consequently the chlorpyrifos in the halloysite pellets is more exposed to the dissolution medium in comparison to the *suSCon Green* pellets. The release rate of the chlorpyrifos from the *suSCon Green* pellets is determined by pore–producing inert powders, which are dispersed throughout the pellet with the active ingredient and dissolve slowly on addition of the dissolution medium.

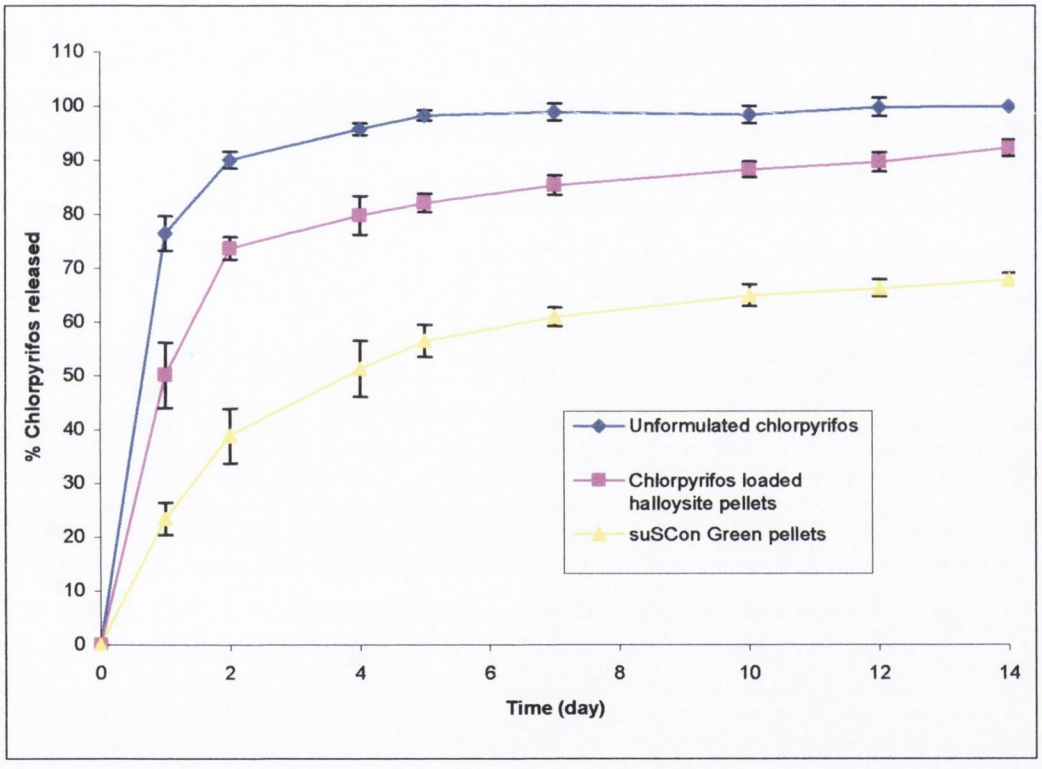


Figure 6.6a Release profiles of unformulated chlorpyrifos, chlorpyrifos loaded halloysite pellets and *suSCon Green* pellets.

After day 2 the unformulated chlorpyrifos is 90% dissolved in the dissolution medium, whereas the halloysite pellets and *suSCon Green* pellets released 73.7% and 39% of chlorpyrifos, respectively. However after day 5 the unformulated

chlorpyrifos is approx. 100% dissolved in the dissolution medium. The halloysite pellets continued to show sustained release of 79.8%, 82%, 85.4%, 88.3%, 89.7% and 92.4% on days 4, 5, 7, 10, 12 and 14 of the release study, respectively, thus showing that after day 2 the chlorpyrifos was slowly being released from the internal lumen of the halloysite tubules. This occurred as the dissolution medium penetrated the open ends of the tubules to allow outward diffusion of the pesticide. The slow release of chlorpyrifos after day 2 may be attributed to the very poor solubility of the pesticide. Levis and Deasy (2003) reported that diltiazem HCl, a highly water soluble drug (>50% w/v at 25°C; Yang and Fassihi, 1997) and propranolol HCl, a less water soluble drug (5% w/v at 25°C; Yang and Fassihi, 1997), were loaded into halloysite using a similar method as described in this thesis. The retardation in release caused by the reduction in drug solubility was very apparent as only 80–90% of the propranolol HCl loading in halloysite was released after 8 hr, compared to approx. 100% in 6 hr for the diltiazem HCl loaded halloysite. As a result, drug solubility has a significant effect on the burst effect and the release profiles of drug entrapped within the mineral. After day 14, 7.6% of the chlorpyrifos remained unreleased from the halloysite pellets. As explained above this may have been due to the very low solubility of the pesticide.

The *suSCon Green* pellets continued to show significant sustained release of chlorpyrifos over the release study period of 14 days. 51.1%, 57.5%, 61.7%, 65.3%, 67% and 68% of the chlorpyrifos was released from the *suSCon Green* pellets after days 4, 5, 7, 10, 12 and 14 of the release study, respectively. Therefore comparing the % release of chlorpyrifos at each of the time points between the two products there is significant difference between both products, which as explained above by ANOVA analysis were statistically different ($p<0.05$). After day 14 there was 32% and 7.6% of the chlorpyrifos remaining unreleased within the *suSCon Green* pellets and the halloysite pellets, respectively. This shows that *in vitro* the *suSCon Green* pellets released equivalent quantities of chlorpyrifos over a longer period of time in comparison to the halloysite pellets. As explained earlier the *suSCon Green* pellets remained intact within the dissolution medium and released the chlorpyrifos as the

pore producing inert powders dissolve and form a micropore structure within the polymer matrix, which allows the chlorpyrifos to be released at a much slower rate in comparison to the halloysite pellets, which disintegrate on addition to the dissolution medium and therefore release the chlorpyrifos at a faster rate.

6.7 OUTDOOR POTTED PRIMROSE TRIAL

6.7.1 Introduction

The chlorpyrifos loaded halloysite pellets formulated above were compared with the commercially available *suSCon Green* pellets *in vitro* using a dissolution medium with a pH of 5, which mimics the conditions of the peat mix used in the outdoor potted primrose trial. This study showed that the *suSCon Green* pellets gave the greatest sustained release of chlorpyrifos. The chlorpyrifos loaded halloysite pellets gave improved sustained release of chlorpyrifos in comparison to the unformulated pesticide. However the drawback to this procedure is that the *in vitro* release studies may bear little relationship to the field conditions. It would be expected that the *in vitro* studies accelerate release but the relationship between *in vitro* release studies and field release is unknown. This section of the work was carried out in an attempt to investigate the pattern of release of chlorpyrifos under typical use conditions. The *in vitro* studies gave no indication of the efficacy of both products in controlling vine weevil.

Consequently an outdoor potted primrose trial was set up to ascertain the release profile of chlorpyrifos and the ability of both products to control vine weevil under typical use conditions. This was determined by carrying out a fully randomised outdoor pot trial at Kinsealy Research Centre, Teagasc, Dublin, Ireland using primroses, which are highly susceptible to vine weevil attack. There were four different treatments (64 pots per treatment) in the trial: *suSCon Green* pellets (10% w/w) (S1), chlorpyrifos loaded halloysite pellets (10.5% w/w) at an equivalent rate (H1) and double (H2) the rate of *suSCon Green* pellets, and an untreated control

(C1). The plants were exposed to natural infestation of vine weevil and supplemented by artificial infestation with adult vine weevil.

The primroses were assessed for foliage vigour in December 2002, and for foliage and root vigour and larvae counts in February 2003. The foliage vigour was assessed by grading from 0 - 10 (0 = dead, 10 = excellent vigour). The root system vigour was assessed in exactly the same manner as the foliage vigour by grading from 0-10 (0 = no root system, 10 = excellent root vigour).

6.7.2 Results and discussion

Table 6.7a Comparative results of chlorpyrifos loaded halloysite pellets and *suSCon Green* pellets on the number of larvae per pot and overall plant development

Treatment	No. larvae (Feb)	Foliage vigour (Feb)	Foliage vigour (Dec)	Root vigour (Feb)
S1	0.6^{a}	6.3^{a}	6.0^{a}	7.4^{a}
H1	0.3^{a}	7.1^{a}	5.9^{a}	5.1^{b}
H2	0.0^{b}	5.0^{b}	6.2^{a}	3.7^{c}
C1	1.5^{c}	7.1^{a}	7.0^{a}	7.2^{a}
p-value	0.00	0.00	0.14	0.00
s.e.	0.21	0.28	0.36	0.33

Values without similar superscripts are statistically different.

Table 6.7a shows the comparative results for the outdoor potted primrose trial. The average number of larvae per pot was recorded for each of the treatments, which is illustrated in Table 6.7a and Figure 6.7a. The untreated pots (C1) gave the highest number of larvae with an average of 1.5 larvae per pot. This would be expected as no treatment was incorporating in these pots at the time of sowing. The *suSCon Green* pellets gave the second highest number of larvae with an average of 0.6 larvae per pot. The halloysite pellets applied at equivalent (H1) and double (H2) the *suSCon*

Green rate gave average larvae numbers per pot of 0.3 and 0.0, respectively, which were lower than both the untreated control (C1) and the *suSCon Green* treatment (S1). Therefore the percentage larvae control from this study for the halloysite pellets applied at equivalent (H1) and double (H2) the *suSCon Green* pellets rate were 100% and 80% respectively, where *suSCon Green* pellets (S1) gave a percentage control level of only 60%.

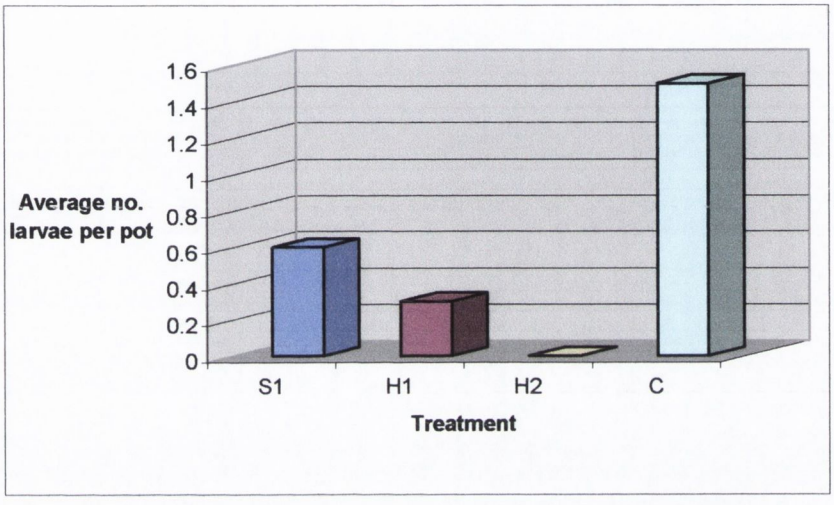


Figure 6.7a Average number of larvae per pot in each treatment of the potted primrose trial.

A Kruskal-Wallis test analysis was performed on the average number of larvae per pot for the four treatments to determine if the treatments were statistically different. An ANOVA test was not suitable because the data series for the larvae counts had not got a normal distribution. The Kruskal-Wallis test revealed that the treatments were statistically different ($p = 0.00$). The Wilcoxon two sample t-test was also used to determine whether the four treatments were statistically different from each other, which revealed that the *suSCon Green* pellets and the chlorpyrifos loaded halloysite pellets at an equivalent rate to (H1) the *suSCon Green* were not statistically different (Table 6.7a – similar superscripts). The untreated control (C1) and the chlorpyrifos loaded halloysite pellets at double (H2) the *suSCon Green* rate were regarded as

statistically different to each other and the other three treatments (Table 6.7a – different superscript).

Several studies have been carried out over the past 20 years with regard to controlling vine weevil using chlorpyrifos. The results of these vine weevil control studies may help in interpreting the results of this study. Blackshaw, (1984a, 1984b) reported that chlorpyrifos pellets 5% (non-controlled release) were applied at 20, 40, 60, 80 and 100 g a.i./m^3 (i.e. 100g of active ingredient in pellet formulation applied to cubic meter of compost) to polyanthus plants cv. *Crescendo Yellow* and gave control levels of 76%, 78%, 81%, 82% and 87%, respectively. Of the three compounds tested (phorate and *B. thuringiensis* were also examined in this study) chlorpyrifos was the only compound sufficiently active to control vine weevil larvae for any useful length of time. However, it is apparent that a compost treatment of chlorpyrifos at 100 g a.i./m^3 provided 16-20 weeks effective protection against vine weevil. This duration is not really adequate for commercial purposes. Blackshaw and O'Neill, 1987 reported that chlorpyrifos pellets (non-controlled release) applied at an application rate of 20, 40, 60, 80 and 100g a.i./m^3 to hardy nursery stock (polyanthus plants) gave total mortality rates of 64%, 68%, 66%, 78% and 87%, respectively. They showed that chlorpyrifos gave good, though not complete, control of larvae.

Buxton *et al.*, 1992 presented results, which showed that controlled release chlorpyrifos (*suSCon Green* pellets) gave excellent control of vine weevil at rates of 75-300 g a.i./m^3 for up to 3 seasons. In year 3 the chlorpyrifos pellets were less effective than the previous year. Cross and Burgess, 1997 reported a series of field experiments, which aimed to identify effective soil treatments for control of vine weevil larvae on field grown strawberries. They showed that the incorporation of controlled-release chlorpyrifos pellets (*suSCon Green* pellets) (104-208 g a.i./m^3) gave significant control of vine weevil larvae in the field (approx. 94-100%).

This outdoor potted primrose trial took place over approx. 32 weeks. It had to be assessed prematurely after this period of time (rather than after 1 year) because the

primroses were starting to die from grey mould i.e. *Botrytis cinerea* (fungal disease causing decay of the plants), which would have resulted in death of the larvae within the pots due to absence of the food source. The grey mould infestation may have been aided by the higher than normal levels of rainfall between October and December 2002 at Kinsealy Research Centre (Figure 6.7b). The excessive rainfall caused significant water logging of the capillary beds where the potted plants were positioned, which resulted in them lying in water for long periods of time. Therefore it is difficult to draw definite conclusions with regard to the efficacy of the newly developed chlorpyrifos loaded pellets over the commercially available *suSCon Green* pellets. However the results from the above study illustrate some interesting findings, which will be discussed in further detail below.

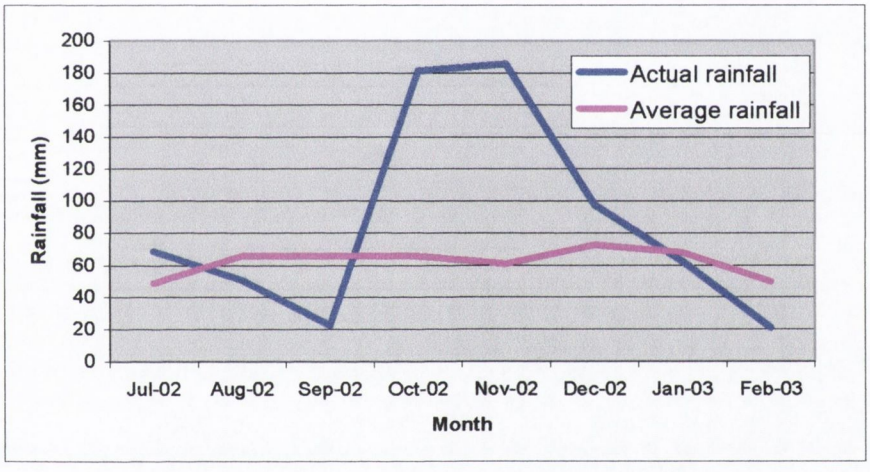


Figure 6.7b Monthly rainfall for time period of outdoor potted primrose trial and average monthly rainfall for the past 30 years at Kinsealy, Research Centre.

As discussed above, the chlorpyrifos loaded halloysite pellets (H1 = 80% and H2 = 100%) gave slightly improved control of vine weevil larvae in comparison to the commercially available *suSCon Green* pellets (60%) over the 32 week time period, but it was not statistically significant taking larvae counts into consideration. The *in vitro* release studies bear some relationship to the outdoor pot trial vine weevil larvae count results. As indicated in these studies, the chlorpyrifos loaded halloysite pellets

(H1 = 75 g a.i./m^3) applied at equivalent rates to the *suSCon Green* pellets (S1 = 75 g a.i./m^3) gave less sustained release of chlorpyrifos in comparison to the *suSCon Green* pellets (discussed in Section 6.6). This is confirmed in the outdoor pot trial as the larvae count for the halloysite pellets was lower (0.3) than for the *suSCon Green* pellets (0.6) because the chlorpyrifos was presumably released at a faster rate from the halloysite pellets (H1), which resulted in a larger initial kill of vine weevil larvae. The halloysite pellets applied at double the *suSCon Green* pellet rate of 150 g a.i./m^3 (H2) gave the best control of vine weevil larvae with no larvae per pot. This is expected, as there was twice the amount of chlorpyrifos available for release from the halloysite pellets 150 g a.i./m^3 (H2), over the 32 week time period in comparison to the halloysite pellets 75 g a.i./m^3 (H1).

Blackshaw (1984a, 1984b) and Blackshaw and O'Neill, 1987 showed that non-controlled release pellets at the higher rate of 100 g a.i./m^3, only gave effective larvae control for 20 weeks. The sustained release halloysite pellets (H1 and H2) developed in this thesis gave very effective control of larvae for 32 weeks. These pellets would probably have been effective for a longer period of time, as they gave very promising results for the 32 weeks i.e. the recommended duration of the outdoor potted trial is 1 year.

The *suSCon Green* pellets (S1) in this outdoor potted trial were less effective at controlling larvae over the 32 week time period in comparison to the halloysite pellets (H1 and H2). However Buxton *et al.*, 1992 and Cross and Burgess, 1997 reported that *suSCon Green* pellets gave good control of vine weevil for up to 3 seasons, providing that the application rate was 75 g a.i./m^3 or more. As explained earlier the *suSCon Green* pellets released chlorpyrifos at a slower rate than the halloysite pellets (Section 6.6) and therefore will probably provide effective vine weevil larvae control over a longer period of time in comparison to the halloysite pellets.

The halloysite pellets show potential for vine weevil larvae control for 1 year. However these pellets would be unlikely to be effective for 2 years (like the *suSCon Green* pellets) because the sustained release halloysite matrix system would more than likely have released the full quantity of chlorpyrifos within the first year in the peat mix and therefore the halloysite pellets could be used for vine weevil larvae control in annual potted plants.

In order for a treatment to be commercially acceptable, a high and consistent standard of preventative control, approaching 100% for at least one season, is required. This is because container grown hardy ornamental plants are valuable, with small numbers of larvae causing considerable losses especially on young plants and often escaping notice buried in the compost until damage is done. The grower may be unaware of the potential threat to the crop. One adult female may lay hundreds of eggs so infesting a large number of plants over an extended period. Besides the undesirability of plant losses or damage, a grower's commercial reputation may suffer if infested plants are marketed and plant health licensing for export/import may be withheld (Cross *et al.*, 1995).

Firstly it is important that the product is effective in controlling the vine weevil larvae. However it is also equally important to take into consideration the effect of a product on plant development. Blackshaw, 1984b stated that the use of any pesticide at relatively high concentrations when the plants are at susceptible stages may cause phytotoxicity. Chlorpyrifos the pesticide present in both products has been reported to cause phytotoxic damage to cauliflower seedlings (Mowat, 1982), but work on 46 cultivars of ornamental plants (Miller *et al.*, 1980) revealed only a single plant cv, *Japanese Red Maple*, showed any symptoms. Blackman and O'Neill, 1987 observed that the use of chlorpyrifos generally resulted in less plant growth than the use of aldrin, which was particularly noticeable for shoot scores and total dry weights of the plant. Anon., 1985 showed that chlorpyrifos was phytotoxic, adversely affecting the growth of a range of hardy ornamental plant species. From these findings it can be

concluded that chlorpyrifos has the potential to cause phytotoxicity, with certain plants being more susceptible.

In this study the primroses were assessed visually but not scored for foliage vigour at the beginning of September 2002. The halloysite pellets (H2) treatment showed signs of phytotoxicity with several plants having died (Figure 6.7c) and others with poor foliage vigour (Figure 6.7d). Table 6.7a shows the comparative results of both products and the untreated control for plant development. The foliage vigour scores for December 2002 assessment and February 2003 assessment and root vigour for February 2003 are shown in Figure 6.7e, 6.7f and 6.7g, respectively.

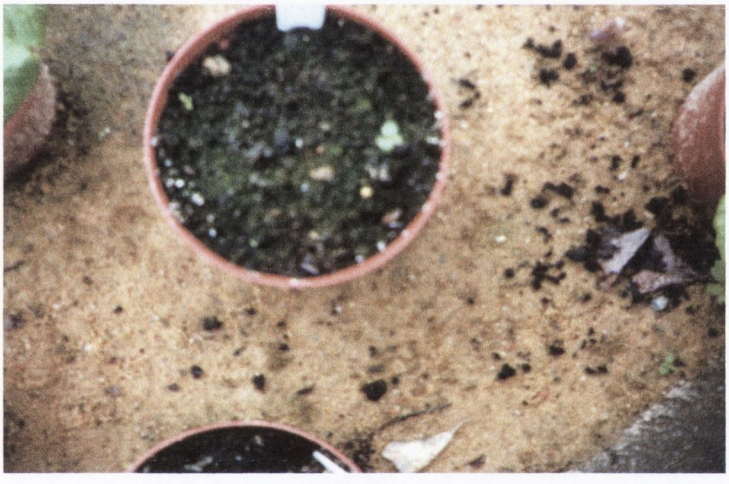


Figure 6.7c Dead primrose treated with halloysite pellets (H2), September 2002.

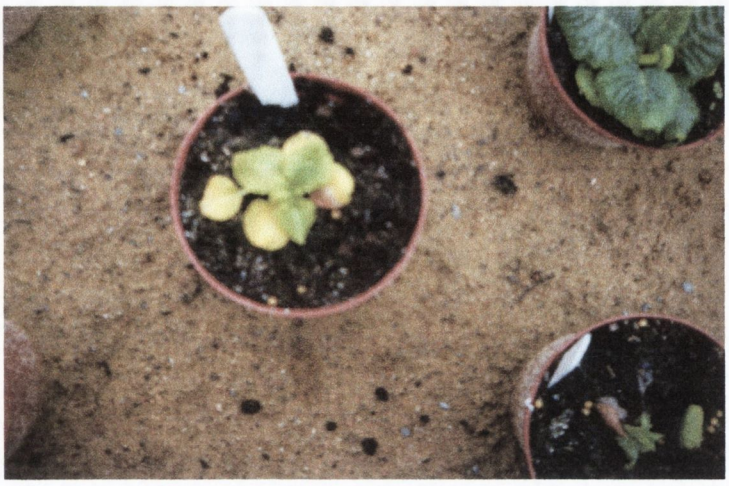


Figure 6.7d Primrose treated with halloysite pellets (H2) showing phytotoxicity, September 2002.

The foliage vigour results in December 2002 show that the untreated control gave the highest foliage vigour score of 7.0 (Table 6.7a and Figure 6.7e). The other three treatments gave similar foliage vigour scores of 6.0, 5.9 and 6.2 for the *suSCon Green* (S1) pellets and the halloysite pellets applied at an equivalent (H1) and double (H2) the rate to the *suSCon Green* pellets (Table 6.7a). The higher foliage vigour score for the untreated control in comparison to the other treatments would be expected, as no pesticide was present within the peat mix to inhibit foliage development of the plant. An ANOVA analysis was performed on the foliage vigour assessment for December 2002 to determine if the treatments were statistically different. However the analysis revealed that the four treatments were not statistically different as the p-value was greater than 0.05 ($p = 0.14$). Therefore it can be concluded that there was no evidence of phytotoxicity in the December 2002 foliage vigour assessment, which was evident in September 2002 for the halloysite pellets (H2).

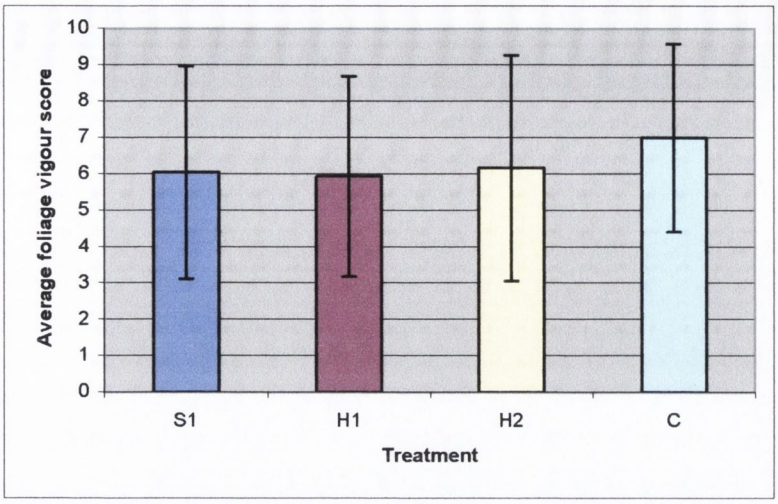


Figure 6.7e Average foliage vigour assessment of primroses for each treatment in December 2002.

A foliage vigour assessment was also performed in February 2003 on completion of the trial. The results are shown in Table 6.7a and Figure 6.7f. The untreated control (C1) and halloysite pellets applied at an equivalent (H1) rate to *suSCon Green* gave the same foliage vigour score of 7.1. The *suSCon Green* pellets gave a lower foliage vigour score of 6.3 where the halloysite pellets applied at double (H2) the rate to the *suSCon Green* pellets only gave a foliage vigour score of 5. An ANOVA analysis was performed on this assessment, which revealed that the four treatments were statistically different ($p = 0.00$) (Table 6.7a) for foliage vigour. However assessing the treatments individually, the ANOVA analysis showed that the *suSCon Green* pellets (S1), halloysite pellets (H1) and the untreated control (C1) were not statistically different (Table 6.7a – similar superscripts). The halloysite pellets applied at double the rate (H2) to the *suSCon Green* pellets were statistically different to the three other treatments (Table 6.7a – different superscripts). Therefore it can be concluded that from the February 2003 foliage vigour assessment, the halloysite pellets (H2) were showing evidence of phytotoxicity with an average score of only 5.

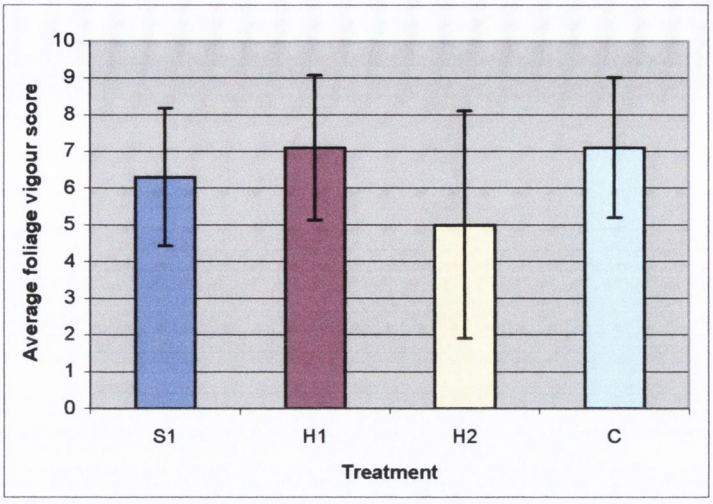


Figure 6.7f Average foliage vigour assessment of primroses for each treatment in February 2003.

The root vigour assessment performed in February 2003 on completion of the trial gave root vigour scores, which are shown in Table 6.7a and Figure 6.7g. The *suSCon Green* pellets and the untreated control gave very similar root vigour scores of 7.4 and 7.2, respectively. However the halloysite pellets applied at an equivalent (H1) and double (H2) the rate to the *suSCon Green* pellets gave much lower root vigour scores of 5.1 and 3.7, respectively. An ANOVA analysis was performed on the root vigour scores, which revealed that the treatments were statistically different ($p = 0.00$) (Table 6.7a). The untreated control (C1) and *suSCon Green* pellets gave root scores, which were not statistically different (Table 6.7a – similar superscripts). The two halloysite treatments (H1 and H2) were statistically different from each other and the two other treatments (Table 6.7a – different superscripts).

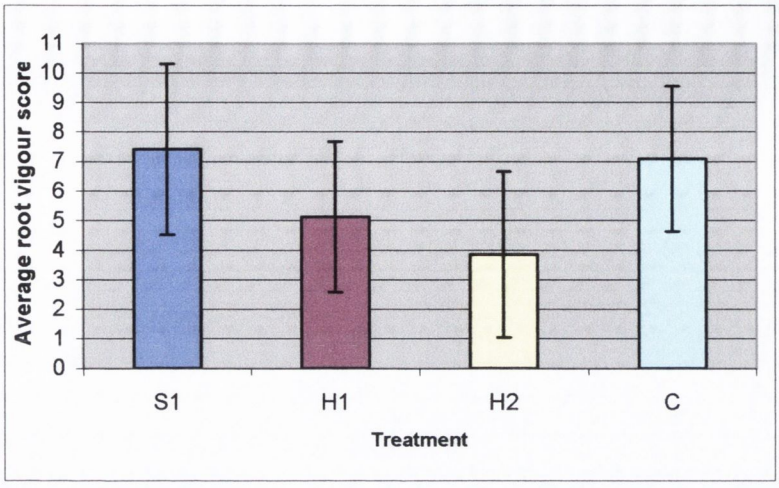


Figure 6.7g Average root vigour assessment of primroses for each treatment in February 2003.

It can be concluded from the root vigour assessment that both halloysite pellet treatments (H1 and H2) were showing signs of phytotoxicity with the halloysite pellets (H2) applied at 150 g a.i./m^3 giving a very low root vigour score of only 3.7. The halloysite pellet treatments gave very poor root scores in comparison to the untreated control and the *suSCon Green* pellet treatment. This may be interpreted by focusing on the release rate of the chlorpyrifos from the halloysite pellets. As explained before, the chlorpyrifos is released at a much faster rate from the halloysite pellets than from the *suSCon Green* pellets, therefore there will be a greater level of pesticide present in the peat mix, which may cause greater damage to the potted plant's root system. The poorer root system was more pronounced for the halloysite pellets (H2) applied at the higher rate of 150 g a.i./m^3, which is evident from the lower root score. A similar explanation may be given for the February 2003 foliage vigour assessment of the halloysite pellets (H2) applied at the higher rate of 150 g a.i./m^3. However in this case the foliage of the primroses exposed to the halloysite pellets at the lower rate of 75 g a.i./m^3 (H1) did not show signs of phytotoxicity.

suSCon Green pellets gave root scores, which were significantly better than the halloysite pellets. In the two foliage vigour assessments the *suSCon Green* pellets gave foliage scores, which did not show evidence of phytotoxicity. As explained above the *suSCon Green* pellets gave greater sustained release of the pesticide in comparison to the halloysite pellets and therefore phytotoxicity would be less likely to occur for the *suSCon Green* pellets.

6.8 CONCLUSION

Chlorpyrifos was successfully formulated into a pellet with sustained release properties. This was achieved by loading the pesticide into the hollow tubules of halloysite particles under vacuum. Ethylene glycol was incorporated in to the pesticide solution during the loading process to neutralise the 'acid sites' on the halloysite powder, so as to prevent premature decomposition of chlorpyrifos on these 'acid sites'. The chlorpyrifos loaded halloysite powder was formulated into pellets with an optimum size range between 850-1150 μm by extrusion-spheronization, using liquid paraffin as the granulating liquid. A 1% blue pigment was incorporated into the halloysite mix to aid uniform mixing during incorporation of pellets into the peat mixes. The halloysite pellets gave improved sustained release of chlorpyrifos in comparison to the unformulated chlorpyrifos *in vitro*, confirming the ability of the hollow internal lumen of the halloysite tubules to give sustained release of the pesticide. The commercially available *suSCon Green* pellets released chlorpyrifos at a slower rate than the halloysite pellets *in vitro*.

From the outdoor primrose pot trial results obtained for the larvae counts, it may be emphasised that the halloysite pellets (H1) gave improved short term control of vine weevil in comparison to the *suSCon Green* pellets (S1) at equivalent rates. For plant development assessments, the halloysite pellets (H1) gave similar foliage scores but significantly lower root scores to the *suSCon Green* pellets (S1). These findings indicated that the chlorpyrifos was released at a much faster rate from the halloysite

pellets (H1), as confirmed by the *in vitro* release studies. The halloysite pellets applied at double the rate (H2) gave significantly lower foliage and root vigour scores compared to the *suSCon Green* pellets (S1), due to the higher levels of pesticide released into the peat mix causing phytotoxicity to the plant. These findings suggest that the halloysite pellets (H2) would be unsuitable for further vine weevil control studies because of the unnecessary high levels of pesticide present in the peat mix causing phytotoxicity to the plant. However, the halloysite pellets (H1) offer definite potential for controlling vine weevil over a shorter period of 1 year, which would make these pellets suitable for use in annual plants.

The halloysite pellets could have been used at a lower rate in the peat mix in comparison to using them at an equivalent rate to the *suSCon Green* pellets (S1), which had a much greater sustained release mechanism and therefore no signs of phytotoxicity. For example the halloysite pellets could have been applied at half the recommended rate of *suSCon Green* pellets, which would probably make these halloysite pellets as effective as the *suSCon Green* pellets over a 1 year period and in doing so would eliminate/reduce phytotoxicity in the plants. Also these pot trials studies using the halloysite pellets (H1) could have been performed with different plants to ascertain which plants were less susceptible to phytotoxicity (in particular the root system) and therefore would be more suitable for using these halloysite pellets for vine weevil control.

The halloysite technology would offer many advantages over the *suSCon Green* technology. Firstly the halloysite present within the pellet formulation is an aluminosilicate mineral that is a natural soil product, which therefore makes the pellet bioerodible and environmentally friendly in comparison to the plastic resin material within the *suSCon Green* pellets, which is non-bioerodible. This property of the halloysite pellets would offer a definite advantage over the *suSCon Green* pellets because of the strict laws governing environmental issues. The halloysite tubules offer a cheap sustained release microencapsulation method in comparison to the more expensive plastic resin microencapsulating method used in the *suSCon Green* pellets.

Also the halloysite pellets would be relatively easy and cheap to produce. The 'soft' halloysite pellets disintegrate on contact with moisture into their initial powder formulation and therefore this may result in more uniform and effective mixing of the pesticide because the individual halloysite particles are widely dispersed throughout the peat mix resulting in an improved mix. The *suSCon Green* pellets are present in small numbers and are non-disintegrating pellets, which therefore will result in a more heterogeneous mix, with pellets that are not very evenly dispersed. The less successful mixing of these pellets may result in a lower kill of the vine weevil larvae because the pesticide is not evenly dispersed throughout the peat mix in order to increase the probability of the larvae coming in contact with the released pesticide.

Chapter 7

Formulation of cypermethrin loaded halloysite suspension concentrate for aphid control

7.1 INTRODUCTION

Aphids, are small soft-bodied insects, which are common pests of nearly all indoor and outdoor ornamental plants, as well as vegetables, field crops and fruit trees. There are hundreds of different species of aphids, some of which attack only one host plant, while others attack numerous hosts (Day, 2001). Aphids have piercing-sucking mouthparts and cause damage by sucking the plant juices, which results in curled and distorted leaves and therefore stunted plants. However, their ability to act as vectors for plant virus diseases (e.g. BYDV) may be more harmful than any direct feeding damage (Herbert *et al.*, 2003). BYDV is a very widespread virus disease of cereals. BYDV is tentatively diagnosed from the presence of aphid vectors and the occurrence of yellowed, stunted cereal plants grouped singly or in small patches among normal cereal plants. Leaf discolouration in shades of yellow, red or purple from the tip down and from the margin to midrib is typical in cereal plants infected with BYDV. In years with high aphid activity, the patches of BYDV-infected plants can become quite large in some cereal fields (Watkins, 2002).

Early detection is the key to reducing aphid infestation. If colonies can be found on about 5% or more of foliage tips of a plant or planting, then a control measure should be considered (Townsend, 2000). Cultural and biological control measures are not sufficient to ensure large cereal crops remain protected from aphid attack. Therefore in commercially grown cereal crops it is necessary to use pesticides to control the aphid numbers effectively e.g. pyrethroids. Pyrethroids are widely used in Western Europe to control aphid vectors and thus reduce the incidence of BYDV (Gibson and

Campbell, 1986). Cypermethrin and deltamethrin are both examples of pyrethroids, which are commonly used in Ireland to control aphids in cereal crops. However the drawback in using these pesticides is that they are only available commercially as an emulsion concentrate version, which needs to be reapplied every few weeks to ensure effective control of aphid numbers is maintained.

In this Chapter a commercially available emulsion concentrate of cypermethrin (*Cyperkill*), which is widely used in Ireland on cereal crops for aphid control, will be compared to a newly formulated suspension concentrate of cypermethrin. Both products will be compared to each other *in vitro* and in a field trial to control aphids in a winter and spring barley crop. It is hoped that the newly developed suspension concentrate of cypermethrin will give sustained release of the pesticide and remain on the cereal leaves for a longer period of time in comparison to the emulsion concentrate version of the pesticide. Therefore it should be unnecessary to respray the cereal crop for aphid control, which is the case for the emulsion concentrate version of the pesticide. As discussed in Chapter 1, the sustained release technology of the suspension concentrate will be achieved by loading the pesticide into the centre of the tubular halloysite under vacuum. The halloysite will allow the pesticide to be released over a long period of time.

7.1.1 Dispersions and disperse systems

Agrochemical formulations cover a wide range of systems that are prepared to suit a specific application (Tadros, 1990). These formulations need to satisfy a number of criteria such as optimum biological efficacy, ease of application with minimum hazard to the operator, convenient and cheap production methods and a suitable procedure for pack disposal (Tadros, 1994). These criteria put some constraints on the use of the early formulations such as wettable powders and emulsifiable concentrates, both of which may cause problems on application such as dustiness and harmful volatile solvents, respectively. For these reasons, suspension concentrates and emulsion concentrates were introduced as alternatives (Tadros, 1994).

7.1.1.1 Suspension concentrates

Suspension concentrates are an important formulation type in the design of crop protection products and are one of the vehicles through which an active ingredient is applied to its target crop by the farmer in a stable, safe and biologically active form (Faers and Kneebone, 1999). Suspension concentrates are solid in liquid dispersions, in which the particle size is usually set (roughly) between 1 nm and 1 μm. Solid in liquid dispersions within the colloid range are usually referred to as colloidal suspensions. Coarse colloid suspension concentrates are distinguished from colloidal suspensions in the sense that on standing the particles of the former settle to the bottom of the container (as a result of gravitational field on the particles) whereas with colloidal dispersions the very mild mixing produced by ambient thermal fluctuation and/or Brownian motion can keep the particles uniformily dispersed in the continuous medium (Tadros, 1980). The formulation of solid in liquid suspension concentrates of drugs and pesticides, and the maintenance of their physical stability over long periods of time under various conditions (e.g. temperature variation, transport) still remains a challenging problem to the formulation chemist.

Two main requirements are needed for formulation of a suspension concentrate, namely a good deflocculating agent and a suitable suspending agent. The deflocculating agent should satisfy the following main criteria: (a) aid wetting of the powder and (b) enable the breaking of aggregates and agglomerates into smaller units (Tadros, 1980). These deflocculating agents may use a combination of steric and electrostatic stabilization. They must strongly adsorb onto the particle surface and cover them, ensuring the stability of the dispersion in the colloid sense. In other words, they should prevent particle aggregation during dispersion and also during storage.

Sedimentation can be particularly acute in agricultural suspension concentrates where a balance of properties is required. Therefore a suspension needs to be sufficiently viscous to prevent sedimentation and yet also be sufficiently fluid to allow it to be readily emptied and rinsed from its pack. This is achieved by the addition of

suspending agents, which form gels that are sufficiently strong to overcome gravitational stresses and prevent sedimentation. However, upon application of a slightly greater stress, such as encountered when inverting the pack or pouring out the contents, the 'gel' must be sufficiently weak such that it breaks down and allows the suspension to flow. Rheologically this corresponds to a 'solid' on standing, which breaks down to a 'fluid' on application of a stress or with shearing i.e. pseudoplastic with thixotropy. It is this high degree of shear-thinning, which is required to satisfy both requirements simultaneously (Faers and Kneebone, 1999).

7.1.1.2 Emulsion concentrates

Emulsion concentrates are not as well understood as suspension concentrates. An emulsion may be defined as two immisicible liquids, one of which is finely subdivided and uniformily distributed as droplets throughout the other (Aulton, 2002). These systems are firstly formulated by emulsification of the oil (which may be the active ingredient or its solution with an oil such as liquid paraffin or an aromatic solvent) in aqueous solution of a surfactant (referred to as the emulsifier). For this purpose, a high speed mixer or homogenizer may be used. The emulsifier should satisfy a number of roles. Firstly, it should reduce the oil/water interfacial tension to reduce the energy required for emulsification and produce small droplets. Secondly, it should provide an interfacial tension gradient to reduce coalescence during emulsification. Thirdly, the surfactant should aid disruption of the liquid into small droplets (Tadros, 1994).

The factors responsible for emulsion instability will not be discussed for emulsion concentrates. This Chapter is concerned with the formulation of a stable suspension concentrate. The emulsion concentrate i.e. *Cyperkill* used in this Chapter is a commercial product for comparative purposes, which has already been stabilised by the manufacturer.

7.2 DEFLOCCULATION STUDIES

The formulation of aqueous suspension concentrates of a pesticide requires the use of powerful deflocculating agents, which keep the particles in a deflocculated state. This is particularly the case when high volume fractions (>0.4) of the solid are required. Flocculation produces a rapid increase in viscosity thus causing problems during preparation. Moreover, strong flocculation is undesirable since it may cause severe thickening on storage and reduces the spontaneity of dispersion on dilution of the suspension (Heath *et al.*, 1983).

The control of stability against irreversible flocculation (where the particles aggregate together into 'non-redispersible' flocs) is usually achieved by the use of powerful deflocculating agents e.g. surface active agents of ionic or non-ionic type, macromoloecules or polyelectrolytes. With ionic surfactants, irreversible flocculation is prevented by repulsive forces generated from the presence of an electrical double layer at the particle/solution interface (Tadros, 1980). However these are seldom suitable for producing concentrated suspensions since double layer overlap leads to an increase in viscosity at moderately high volume fractions. Moreover, these systems are sensitive to electrolytes and the surfactant may not be strongly adsorbed on the particle surfaces (Heath *et al.*, 1983). With non-ionic surfactants and macromolecules, repulsion between the particles is ensured by steric interaction of the adsorbed layers on the particle surfaces (Tadros, 1980). Provided the chains are well solvated, strong steric repulsion enables one to obtain deflocculated systems. However, these systems may suffer from desorption of the chains, which are in many cases only weakly adsorbed on the particle surfaces (Heath *et al.*, 1983). With polyelectrolytes, both electrostatic and steric repulsion exist (Tadros, 1980).

As explained in Section 5.3 halloysite particles form a flocculated system in suspension because of the negative and positive charges of the halloysite tubule 'faces' and 'edges' 'clumping' together. However a flocculated system is

undesirable for the cypermethrin loaded halloysite suspension concentrate because the halloysite particles form agglomerates due to the difference in charge distribution of the tubules, which may result in blockage of the spray nozzle and inaccurate rates of pesticide delivered to the barley crop. Therefore in an agrochemical application it is necessary that the halloysite particles are present as individual units, which is the case for a deflocculated system, to prevent the above occurring.

In Section 5.3 a halloysite sample from Baliksir was deflocculated successfully using sodium citrate (dissociates in water to form negatively charged trivalent citrate ions), which is an anionic deflocculating agent that caused neutralisation of the positive 'edge' charges of the halloysite particles. This resulted in each halloysite particle being completely or nearly completely negatively charged. This caused electrostatic repulsion between the particles because of their similar negative charges, which resulted in particles being present as individual units (deflocculated system). However cetrimide, a cationic deflocculating agent did not deflocculate the halloysite sample. An explanation for this is that cetrimide contains a long hydrocarbon tail (14 carbon atoms), which may have associated together to form 'complexes' and therefore resulted in a flocculated system. It was hoped cetrimide would have caused charge neutralisation mainly of the negative 'face' charges of the tubules, similar in mechanistic action to sodium citrate. The information obtained from the use of these two deflocculating agents in Section 5.3 is transferable to this deflocculation study.

Two deflocculating agents were used in this deflocculation study, which were *Dispex N40* (sodium polyacrylate) and *Atlox 4913* (methylmethacrylate graft copolymer). *Dispex N40* is a low molecular weight sodium polacrylate polyelectrolyte, supplied as a non foaming liquid with excellent solubility (Product information leaflet, Ciba Speciality Chemicals). The linear anionic polymer is composed of monomer acrylic acid, $CH^2{=}CHCOO^-H^+$ (Figure 7.2a). The acrylic acid groups are evenly spaced along the chain and acrylic acid polymer neutralised with NaOH is sodium polyacrylate. Low molecular weight sodium polyacrylates are widely used as clay deflocculants (Schlumberger Oilfield Glossary, 2002).

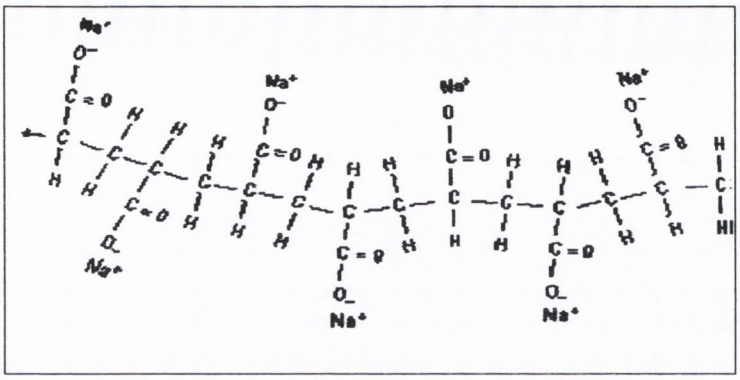


Figure 7.2a Sodium polyacrylate with linear anionic polymer composed of monomer acrylic acid, $CH^2{=}CHCOO^-H^+$ neutralised with NaOH (Schlumberger Oilfield Glossary, 2002).

Anionic polymers are widely used for control of colloidal stability and rheological properties of kaolinite suspensions (Jarnstrom and Stenius, 1990). Kaolin is widely used as a pigment for paper coating. The clay is used as a high-solids, deflocculated slurry with a low molecular weight grade of sodium polyacrylate present as a dispersant (Husband, 1998). Adsorption takes place on two types of sites on the kaolin particle, where the energetically more favourable sites appear to be the edges and other sites are the basal planes, which are positively and negatively charged, respectively (Jarnstrom and Stenius, 1990). As discussed in Section 5.3, most of the work to-date on deflocculation of clays involves using kaolin, which is chemically similar to halloysite. Therefore the literature results obtained for deflocculation of kaolin may be applicable to halloysite.

Atlox polymeric surfactants are medium molecular weight non-ionic materials, which use steric mechanisms to stabilise emulsions and dispersions. The hydrophilic portion is polyethylene oxide and the hydrophobic portion is polyoxysteric acid or alkyd resin. The mechanism of stabilisation involves the solvated pendant side chains extending far into the continuous medium with the polymeric backbone adsorbing to the solid or liquid internal phase. The *Atlox* polymerics fall into 3

categories: random, block and graft copolymers. *Atlox 4913* used in this deflocculation study is a hydrophilic methyl methacrylate graft copolymer with an HLB of around 11-12. It is useful as a deflocculating agent for organic or inorganic active ingredients in water (Product Information Leaflet, Uniqema).

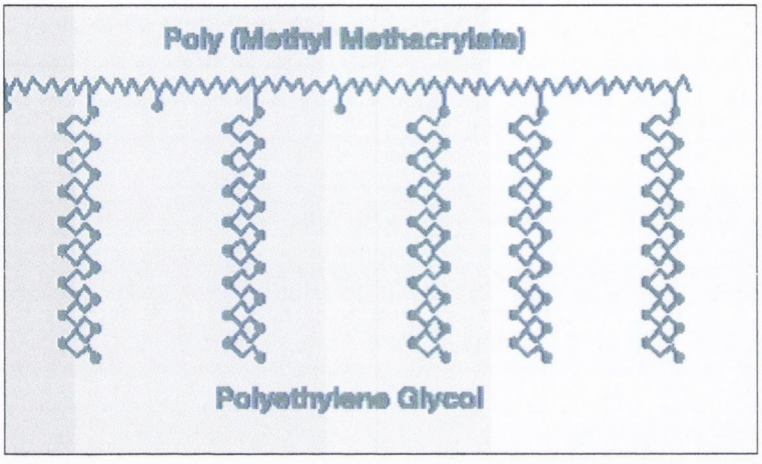


Figure 7.2b Schematic representation of graft copolymer structure, *Atlox 4913* (Product Information Leaflet, Uniqema).

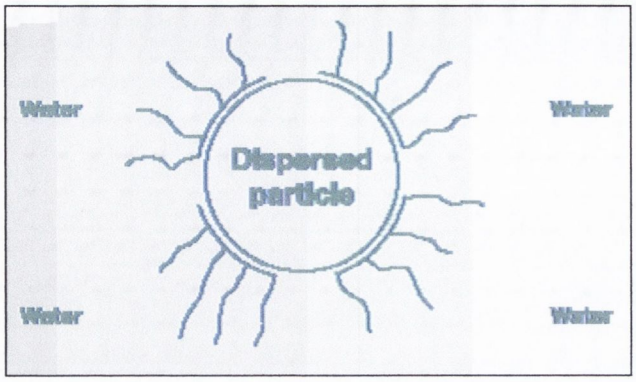


Figure 7.2c Schematic representation of graft copolymer in position with dispersed particle (Product Information Leaflet, Uniqema).

Atlox 4913 (Figure 7.2b) consists of a backbone of methylmethacrylate (the 'anchoring' part of the chain that is strongly adsorbed on the particle surface) and several chains of poly(ethylene oxide) (PEO), which provide the stabilising components (Figure 7.2c). Since PEO is strongly solvated with the medium (water), when two particles covered with these chains approach to a separation distance that is smaller than twice the adsorbed layer thickness, overlap of the chains is prohibited as a result of the increase in osmotic pressure in the overlap region. This results in strong repulsion between the particles. Since the PEO chains are not very long (few nm), highly concentrated suspensions (up to a volume of ~0.6) can be obtained without increase in the viscosity of the suspension (Heath *et al.*, 1983). It is relatively insensitive to the presence of salts or solubilised organic material in the aqueous phase (Product Information Leaflet, Uniqema).

7.2.1 Phase 1: preliminary deflocculation studies involving halloysite G

Dispex N40 and *Atlox 4913* are 40% w/w and 30% w/w solid solutions, respectively, which were preliminarily added to halloysite G suspensions (20% w/v) because the halloysite Goushan NZCC was in short supply, had a much higher tubular content, and therefore it was needed for the final product. 2% w/w of the solid deflocculating material to 20% w/v of halloysite was used as a starting point, which was equivalent

to 0.4 g in 1 g and 1.33g of *Dispex N40* and *Atlox 4913* solution, respectively. Table 3.11a and 3.11b show the quantities of *Dispex N40* and *Atlox 4913* weighed out into each jar containing 10 g of halloysite G in 50 ml deionised water and then transferred to graduated cylinders after mixing.

Figure 7.2d illustrates the flocculated/deflocculated systems achieved on addition of varying concentrations of *Dispex N40* deflocculating agent to the halloysite suspensions in the concentration range 2 to 16% w/w. Halloysite forms a flocculated suspension (graduated cylinder number 5) on addition to water, which is present for comparative purposes. As illustrated in Figure 5.3a, a flocculated system forms a completely clear supernatant, which is the case for halloysite suspension without a deflocculating agent (graduated cylinder number 5). The halloysite suspensions containing 2 and 4% w/w *Dispex N40* (graduated cylinders number 1 and 2) form deflocculated systems. Also as illustrated in Figure 5.3a a deflocculated system forms a cloudy supernatant due to fine particles suspended indefinitely because of Browian motion. The halloysite suspension containing 8% w/w *Dispex N40* (graduated cylinder number 3) forms a flocculated/deflocculated system because it contains a large 'fluffy' sediment and also the supernatant is only slightly cloudy, which is characteristic of a flocculated and deflocculated system, respectively. However the halloysite suspension containing 16% w/w *Dispex N40* (graduated cylinder number 4) formed a flocculated system, which was evident by the presence of a clear supernatant. Therefore from this preliminary study on halloysite G it is seen that a deflocculated system was achieved using *Dispex N40* at the lowest level of 2% w/w. Therefore concentrations below this level needed to be used in the next phase of experiments to ascertain the lowest possible concentration of the *Dispex N40* deflocculating agent necessary to achieve a controlled deflocculated system.

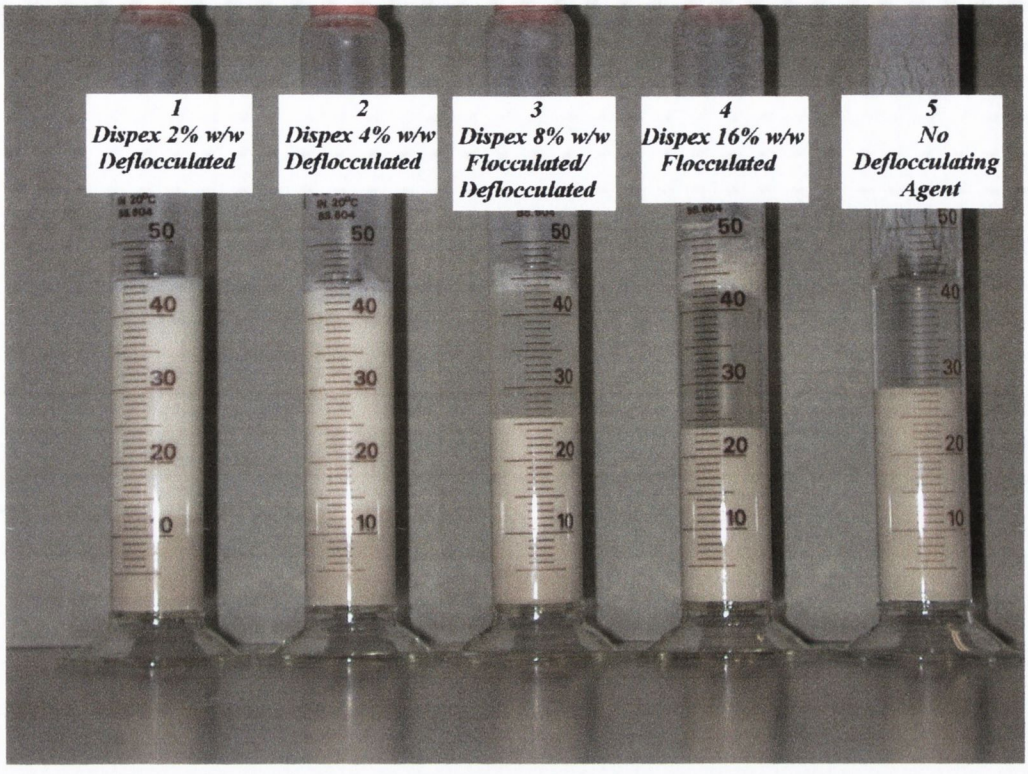


Figure 7.2d Photograph of halloysite G suspensions with varying concentrations of *Dispex N40* deflocculating agent (2 to 16% w/w) after 18 hr at room temp. (~20°C).

As discussed above, sodium polyacrylate is widely used as a deflocculating agent in the paper industry to deflocculate kaolin (Husband, 1998). Therefore it is possible that sodium polyacrylate may be used as a deflocculating agent for halloysite, which is similarly charged to kaolin. From the results obtained above it can be seen that sodium polyacrylate has the ability to act as a strong deflocculating agent for halloysite in suspension.

Sodium polyacrylate works by a similar mechanism to sodium citrate used in Section 5.3, by neutralising the positive 'edge' charges of the halloysite particles and thus causing deflocculation of the tubules. The polymer is composed of monomers of acrylic acid (Figure 7.3a) that are neutralised by sodium hydroxide. However in

water the polymer dissociates to form negatively charged acrylic acid groups and sodium ions. The negatively charged acrylic acid polymers will associate with the positive 'edge' charges of the halloysite particles. From this there will be little or no edge-to-face association of the halloysite particles, which results in flocculation of halloysite suspensions. The sodium polyacrylate has an added advantage over sodium citrate in that it also gives steric stabilisation. The hydrophobic carbon chain part of the polymer allows for a steric component to play a role in further stabilising the deflocculated halloysite system by preventing any particle aggregation. In Section 5.3, while using cetrimide as a deflocculating agent the long hydrocarbon chains may have resulted in failure to deflocculate the halloysite suspension because of 'complex' association of these chains. However in this case the sodium polyacrylate is composed of much shorter carbon chain lengths in comparison to cetrimide, therefore resulting in little or no association of these chains.

The halloysite suspension containing 16% w/w *Dispex N40* (graduated cylinder number 4) formed a flocculated system, which illustrates that above a certain concentration the *Dispex N40* no longer works successfully as a deflocculating agent. An explanation for this may be that the positively charged sodium ions present in solution after dissociation from the polyacrylate chain, start adsorbing on to the negatively charged halloysite tubules. This may result in reversal of effects of the deflocculating agent, with association of the halloysite particles occurring. This will cause the halloysite particles to 'clump' together and form agglomerates, which will ultimately result in flocculation of the system. As explained above the excess presence of hydrocarbon chains (after the tubule 'edges' have been neutralised) may also result in flocculation of the system. They may become entangled in one another and cause agglomeration of the halloysite particles and also become trapped within the hydrocarbon chain network.

Figure 7.2e illustrates the flocculated systems achieved on addition of *Atlox 4913* deflocculating agent to the halloysite suspensions in the concentration range 2 to 16% w/w (graduated cylinders numbers 1 to 4). Therefore this deflocculating agent

proved to be unsuccessful in deflocculating the halloysite suspensions and was not used for subsequent deflocculation studies. Also this deflocculating agent caused excessive foaming, while being dispersed in water using the Ultra-Turax high shear mixer, even though the jars (which the suspensions were mixed in) were placed in beakers of ice. This foaming problem did not occur with *Dispex N40* deflocculating agent.

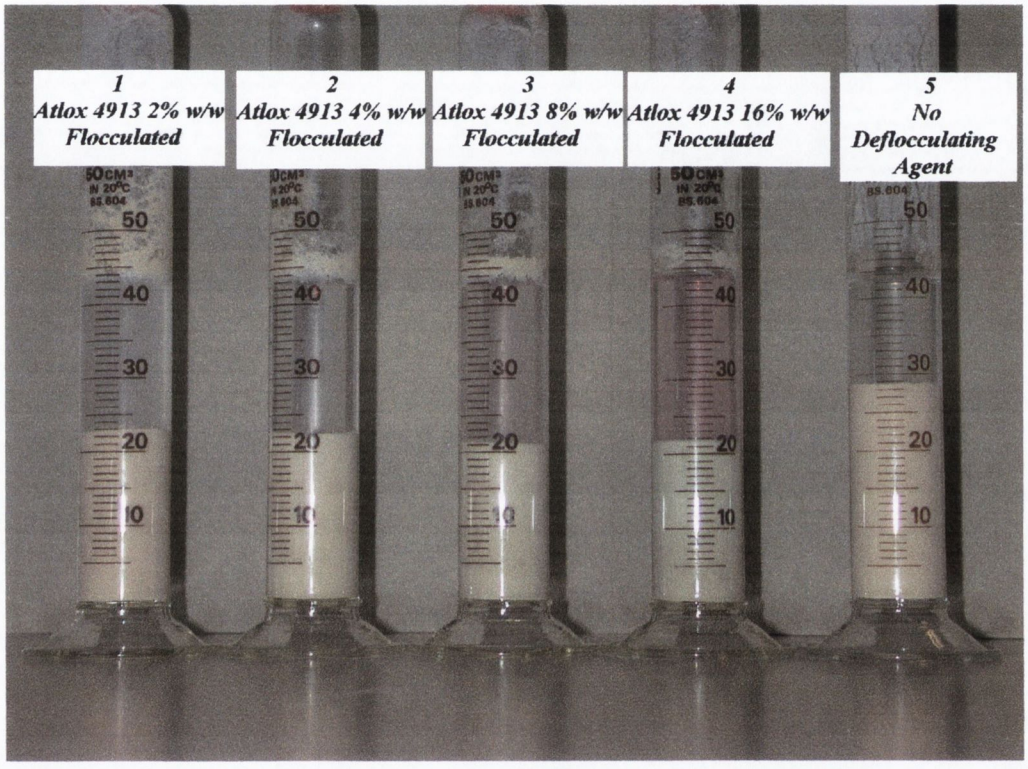


Figure 7.2e Photograph of halloysite G suspensions with varying concentrations of *Atlox 4913* deflocculating agent (2 to 16% w/w) after 18 hr at room temp. (~20°C).

An explanation for the unsuccessful use of *Atlox* 4913 as a deflocculating agent may be that as indicated above this graft copolymer is composed of two main groups A and B, where A has a strong affinity to the particle surface (i.e. strongly and irreversibly adsorbed and being insoluble in the continuous medium) and B is a group that provides the steric stabilisation, being highly solvated by the continuous medium

and of sufficient length to provide a steric barrier (Heath *et al.*, 1983). Halloysite particles are highly electrostatic and therefore are largely hydrophilic in nature. However as seen in Figure 7.2a and 7.2b the A group (polymethymethacrylate), which is attracted to the particle surface in the case of the *Atlox 4913* deflocculating agent has a strong affinity to hydrophobic particles e.g. pesticide particles such as ethirimol, a fungicide (Heath *et al.*, 1983). Therefore the A group will not be strongly adsorbed onto the surface of the halloysite particles because of their strong electrostatic charge. This may result in desorption/weak adsorption of group A from the halloysite particle surfaces. Thus as a result group B (polyethylene glycol chains), which are hydrophilic, will not be able to align appropriately in the liquid medium to give steric stability (Figure 7.2b). The insufficient adsorption onto the tubules may cause the hydrophobic group A to 'clump' together to avoid exposure to the hydrophilic continuous medium and along with the unsuccessful stabilisation of the halloysite particles will ultimately lead to flocculation of the suspension.

7.2.2 Phase 2: deflocculation studies involving halloysite G

Dispex N40 was the only deflocculating agent examined in phase 2, which was used at a lower concentration range (0.031 to 1.5% w/w of solid deflocculating material) than in phase 1 to ascertain the minimum concentration of deflocculating agent necessary to achieve a controlled deflocculated system. Table 3.11c shows the required amount of *Dispex N40* to be weighed out.

Figure 7.2f illustrates the flocculated/deflocculated systems achieved on addition of *Dispex N40* deflocculating agent to halloysite suspensions in the concentration range of 0.5 to 2% w/w. It can be seen that within the 0.5 to 2% w/w concentration range of *Dispex N40* the halloysite suspensions are in a deflocculated state because of the very cloudy supernatants (graduated cylinders number 1 to 4). Therefore it is necessary to use lower concentrations of *Dispex N40* to achieve the minimum concentration of deflocculating agent necessary to obtain a controlled deflocculated system.

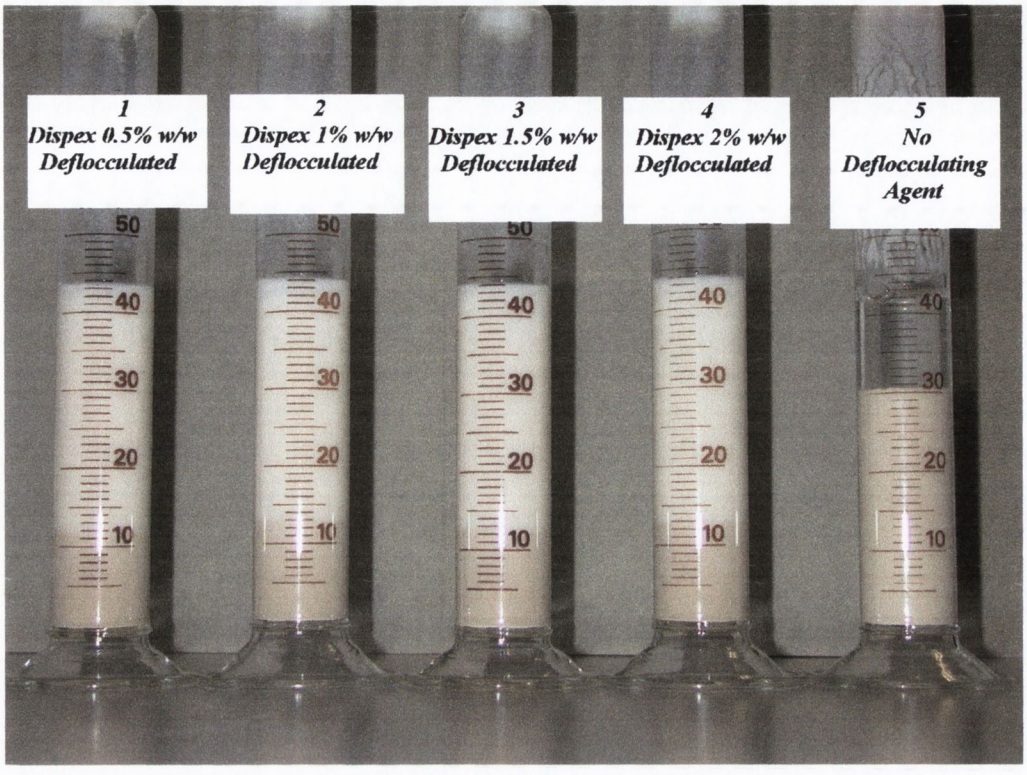


Figure 7.2f Photograph of halloysite G suspensions with varying concentrations of *Dispex N40* deflocculating agent (0.5 to 2% w/w) after 18 hr at room temp. (~20°C).

Figure 7.2g illustrates the flocculated/deflocculated systems achieved on addition of *Dispex N40* deflocculating agent to halloysite suspensions in the concentration range 0.031 to 0.25% w/w. The halloysite suspensions containing 0.031 and 0.062% w/w *Dispex N40* are in a flocculated state (graduated cylinders number 1 and 2). This illustrates that below a certain concentration (in this case 0.062% w/w) the deflocculating agent is not present in a sufficient concentration to successfully deflocculate the halloysite suspension. This may be explained by the fact that insufficient acrylic acid polymer is present to neutralise all the positive 'edge' charges of the halloysite particles.

The halloysite suspension containing 0.125% w/w *Dispex N40* is in a flocculated/deflocculated state because as explained in Section 7.2.1 it has a large

'fluffy' sediment and a slightly cloudy supernatant, which are characteristic of a flocculated and deflocculated system, respectively. However the halloysite suspension containing 0.25% w/w *Dispex N40* is in a deflocculated state because of the very cloudy supernatant. Therefore from phase 2 experiments it can be concluded that a very low concentration of *Dispex N40* is sufficient to deflocculate the halloysite G suspension successfully i.e. 0.25% w/w *Dispex N40*.

Figure 7.2g Photograph of halloysite G suspensions with varying concentrations of *Dispex N40* deflocculating agent (0.031 to 0.25% w/w) after 18 hr at room temp. (20°C~).

7.2.3 Phase 3: deflocculation studies involving halloysite Goushan NZCC

It was evident from Section 7.2.2 that 0.25% w/w *Dispex N40* (solid deflocculating material) was necessary to obtain a controlled deflocculated system of halloysite G suspension. Therefore in phase 3 of the experiment, a concentration range between 0.125 and 1% w/w of *Dispex N40* was used to ensure a controlled deflocculated system of the halloysite Goushan NZCC was also achieved. Table 3.11c shows the required quantity of *Dispex N40* to be weighed out. Figure 7.2h illustrates the flocculated/deflocculated systems achieved on addition of *Dispex N40* deflocculating agent to halloysite Goushan NZCC suspensions in the concentration range of 0.125 to 1% w/w. The halloysite suspension containing 0.125% *Dispex N40* (graduated cylinder number 1) is in a flocculated/deflocculated system similar to the halloysite G system obtained for the same concentration in Figure 7.2g. The halloysite suspensions containing 0.25 to 1% w/w *Dispex N40* (graduated cylinders number 2 to 4) are all in a deflocculated state because of the very cloudy supernatant. Therefore from phase 3 of the experiments it can be concluded that 0.25% w/w *Dispex N40* deflocculating agent is necessary to achieve a controlled deflocculated system of halloysite Goushan NZCC. This is exactly the same concentration of *Dispex N40* deflocculating agent that was required to achieve a controlled deflocculation system of halloysite G (Figure 7.2g).

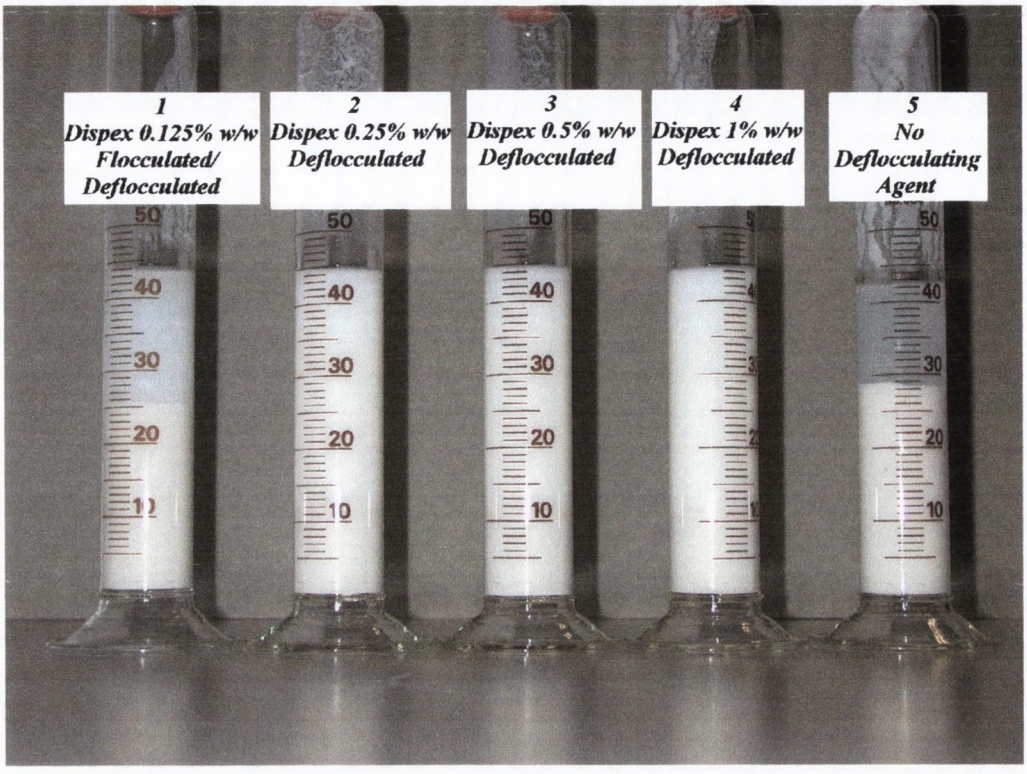


Figure 7.2h Photograph of halloysite Goushan NZCC suspensions with varying concentrations of *Dispex N40* deflocculating agent (0.125 to 1% w/w) after 18 hr at room temp. (~20°C).

7.2.4 Phase 4: deflocculation studies involving cypermethrin loaded halloysite Goushan NZCC

As discussed in Section 7.2.3, 0.25% w/w *Dispex N40* deflocculating agent was necessary to achieve a controlled deflocculated system of halloysite Goushan NZCC. However in the final suspension concentrate product the halloysite Goushan NZCC is double loaded with cypermethrin 5% w/v from ethanol under vacuum (Section 3.11.2). Therefore it was necessary to ensure that the cypermethrin did not alter the concentration of *Dispex N40* deflocculating agent required to achieve a controlled deflocculated system. In phase 4 of the experiment *Dispex N40* in the concentration range 0.125 and 0.25% w/w was repeated for the cypermethrin loaded halloysite

Goushan NZCC suspension. Table 3.11c shows the required amount of *Dispex N40* to be weighed out.

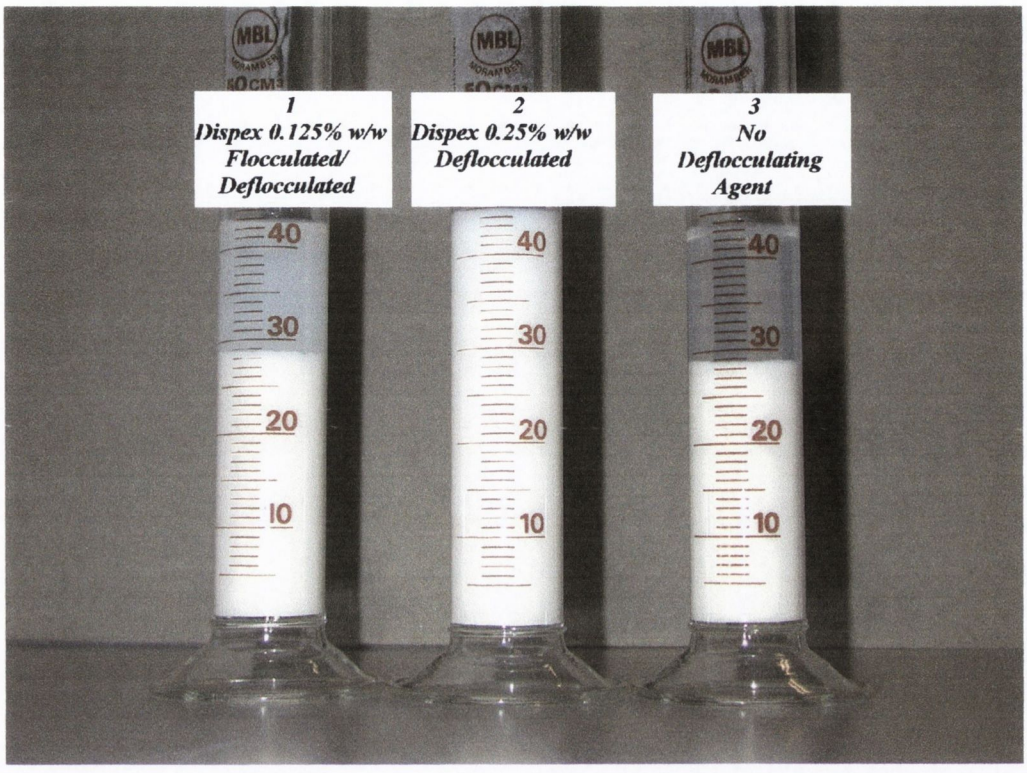


Figure 7.2i Photograph of cypermethrin loaded halloysite Goushan NZCC suspensions with varying concentrations of *Dispex N40* deflocculating agent (0.125 to 0.25% w/w) after 18 hr at room temp. (~20°C).

Figure 7.2i illustrates the flocculated/deflocculated systems achieved on addition of *Dispex N40* deflocculating agent to cypermethrin loaded halloysite Goushan NZCC suspensions in the concentration range 0.125 to 0.25% w/w. The halloysite suspension containing 0.125% w/w *Dispex N40* deflocculating agent (graduated cylinder number 1) was in a flocculated/deflocculated state. The halloysite suspension containing 0.25% w/w *Dispex N40* deflocculating agent (graduated cylinder number 2) was in a deflocculated state. These findings are the same as those obtained for the halloysite suspension without the cypermethrin (Figure 7.2h).

Therefore this confirms that the presence of cypermethrin did not alter the concentration of *Dispex N40* deflocculating agent necessary to give a deflocculated system. From this final study it can be concluded that 0.25% w/w *Dispex N40* deflocculating agent (solid deflocculating material) was required in the cypermethrin loaded halloysite Goushan NZCC suspension to achieve a controlled deflocculated system.

7.3 RAINFASTNESS STUDIES

Rainfastness studies were performed to determine the quantity of adhesive agent needed within the suspension concentrate to ensure the pesticide loaded halloysite particles within the diluted product remained adhered to the barley leaves for a prolonged period of time in comparison to the commercially available emulsion concentrate, *Cyperkill*. A simulated rainfall experiment was designed, which involved using a garden sprinkler, Hozelock Lawn Queen Sprayer®. The *Phormium tenax* cv. yellow wave plant was used in this study because its waxy leaves were similar to the barley plant leaves. As discussed in Section 3.11.6.2, the recommended quantity of adhesive (*Agrimer AL30*) to be used in the suspension concentrate (1:200 diluted spray solution) ranged between 0.02–0.4 g (verbal correspondence with Dr. Narayanan, International Specialist Products, USA). Therefore the required quantity weighed into each undiluted suspension concentrate (i.e. 100 ml volume) was 2 g, 10 g, 20 g and 40 g.

The adhesive agent chosen for use in the suspension concentrate was 20% *Agrimer AL 30*, which is manufactured by International Specialist Products. It is used specifically in agricultural formulations because it claims to offer outstanding properties, which include ability to enhance formulation properties and biological efficacy on a cost effective basis. The product is biodegradable, of low toxicity and low fire hazard thus meeting the most demanding environmental and safety requirements of the agricultural industry (Ag Chem Reference Guide for the Agricultural Industry, 1999).

Agrimer AL (alkylated vinylpyrrolidone polymers) is a family of graft polymer products having widely diverse properties that vary with molecular weight of the product, the length of the alkyl group and degree of alkylation of the polymer. The *Agrimer AL* copolymer series vary in properties and solubility (Table 7.3a). They are used in liquid formulations to increase/decrease viscosity, solid matrices as binders or protective coatings, film formers to act as moisture resistant films and stabilisers in emulsions and suspensions (Ag Chem Reference Guide for the Agricultural Industry, 1999).

Table 7.3a *Agrimer AL* copolymer series (Ag Chem Reference Guide for the Agricultural Industry, 1999)

Product	Supplied as	Soluble in	R group	% alkylation
AL10	100% solid	water, alcohol	C4	10
AL10LC	100% solid	water, alcohol	C4	10
AL25	55% in isopropranol	alcohol, aromatic petroleum	C16	50
AL22	viscous liquid	aromatic petroleum, vegetable oil	C16	80
AL30	waxy solid	aromatic petroleum	C20	80

Adhesiveness is an important attribute to consider when selecting an adhesive agent, while secondary attributes, such as resistance to washoff, must also be kept in mind. Screening tests on many polymers and copolymers revealed that the adhesiveness of the *Agrimer AL* was up to five times greater than that of any other product tested. While the low water solubility of most of the *Agrimer AL* polymers results in some limitations, ISP (International Specialists Products) has developed new technology, which allows the hydrophobic *Agrimer AL25* or *AL30* to be used in aqueous systems for superior performance (discussed below). ISP has shown that adhesiveness is maximised when the polymer contains:

1. About 50% of the more hydrophobic pyrrolidone moiety.
2. A longer alkyl group than a shorter one for a given percentage of alkylation.

As shown in Table 7.3a, *Agrimer AL 30*, having 80% pyrrolidone and an alkyl group of C20, is a very effective commercially available adhesive agent. Adhesiveness is related to rainfastness and is a primary consideration in a sticker adjuvant. Studies carried out by ISP revealed that rainfastness (1 inch of simulated rain) can be more than doubled by simple addition of *Agrimer AL25* or *AL30* polymers to the commercial formulation of pendimethalin (*Agrimer AL* Product Information, International Specialist Products).

As can be seen from Table 7.3a, *Agrimer AL30* is present as a waxy solid, which is only soluble in aromatic petroleum. 20% *Agrimer AL30* (new technology recommended by Dr. Narayanan, International Specialist Products, USA) was used as the adhesive agent in the suspension concentrate, which was dissolved in aromatic petroleum and then dispersed in *Reax 85A* (lignosulphonic acid, sodium salt). A broad spectrum biocide was also incorporated in the 20% *Agrimer AL30*, to act as a preservative, which was *Proxel GXL* (1,2-benzisothiazolin-3-one). The suspension of 20% *Agrimer AL30* allowed it to be easily incorporated into the water based cypermethrin loaded halloysite suspension concentrate. Lignosulphonates are of a high molecular weight and they contain ionic groups on the chains (polyelectrolyte type) (Tadros, 1994). They provide stability in this instance (of the *Agrimer AL30* suspension) by a combination of double layer repulsion (due to the presence of charges) and steric repulsion (due to dangling chains at the solid/liquid interface). The presence of the lignosulphonate within the final suspension may also help in stabilising the system.

Table 7.3b Average % of cypermethrin deposits per cm^2 leaf surface remaining after 20 mm of simulated rain for different cypermethrin systems

Cypermethrin system	Average % cypermethrin deposits per cm^2 leaf surface
Initial (No rain)	100^{a}
No adhesive (S/C)	27.2^{b}
2% w/v adhesive (S/C)	43.2^{c}
10% w/v adhesive (S/C)	51.8^{cd}
20% w/v adhesive (S/C)	61.4^{d}
40% w/v adhesive (S/C)	78.1^{e}
E/C	27.5^{b}

Values without similar superscripts are statistically different.

S/C : suspension concentrate, E/C : emulsion concentrate

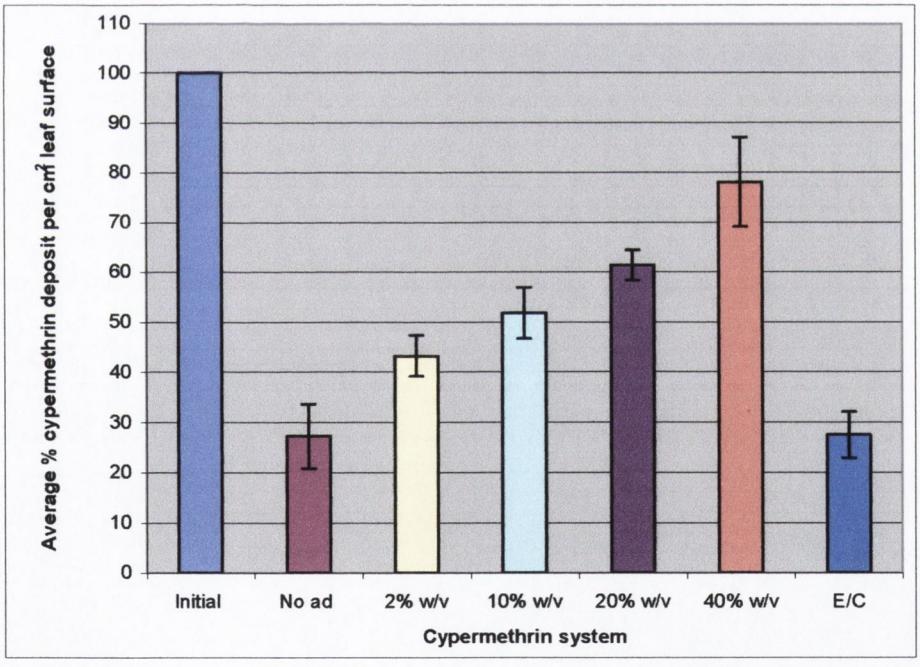


Figure 7.3a Graph illustrating average % cypermethrin deposits per cm^2 leaf surface after 20 mm of simulated rain with different cypermethrin systems.

Table 7.3b and Figure 7.3a illustrates the average % cypermethrin deposits per cm^2 leaf surface remaining after 20 mm of rain was applied. The volume of simulated rainfall was decided as 20 mm because studies carried out by Pick *et al.* (1984) revealed that 10 mm of rainfall was sufficient to show effective rainfastness of a pesticidal product over a period of time. Also, after spraying the leaves with pesticide, 1 hr was allowed to lapse before application of simulated rain.

The five suspension concentrates formulated, contained varying levels of adhesive agent as described above, to ascertain the optimum level required within the system for rainfastness. The suspension concentrate containing no adhesive gave the lowest quantity of cypermethrin remaining on the leaves after the simulated rain was applied (27.2%/cm^2). This would be expected, as no adhesive was present within the suspension concentrate formulation to ensure the cypermethrin loaded halloysite particles remained attached to the leaves. The suspension concentrates containing 2% w/v, 10% w/v, 20% w/v and 40% w/v adhesive gave cypermethrin quantities of 43.2%/cm^2, 51.8%/cm^2, 61.4%/cm^2 and 78.1%/cm^2 remaining on the leaves after the simulated rainfall, respectively (Table 7.3b and Figure 7.3a). A general trend of increasing quantities of cypermethrin remaining on the leaves after simulated rainfall is observed as the level of adhesive agent is increased. The suspension concentrate containing the highest concentration of adhesive agent (40% w/v) gave the highest quantity of cypermethrin remaining after the simulated rainfall was applied. The commercially available emulsion concentrate retained a very low quantity of cypermethrin on the leaves of only 27.5% /cm^2, after the simulated rainfall. This was similar to the value obtained for the suspension concentrate containing no adhesive agent. This value revealed the inability of the emulsion concentrate to retain the cypermethrin on the leaves for an appropriate time period in comparison to the other suspension concentrate systems containing adhesive agent.

An ANOVA analysis was performed on the data obtained from the rainfastness study, which revealed that they were all statistically different ($p = 0.00$). However as seen in Table 7.3b some of the systems are not statistically different from one

another. The initial system (100%/cm^2), which was not exposed to simulated rainfall, was statistically different from all the other systems (different superscript). This would be expected because no rainfall was used to wash off the cypermethrin on the leaves. As discussed above, the suspension concentrate containing no adhesive agent (27.2%/cm^2) gave a similar value to the emulsion concentrate (27.5%/cm^2). These values were not statistically different from each other (same superscript). The suspension concentrate systems containing 2% w/v (43.2%/cm^2) and 10% w/v (51.8%/cm^2) were not statistically different (same superscripts). Also the suspension concentrate systems containing 10% w/v (51.8%/cm^2) and 20% w/v (61.4%/cm^2) were not statistically different (same superscripts). However, the suspension concentrate containing 40% w/v (78.1%/cm^2) adhesive agent was statistically different from all the above systems (different superscript).

From this rainfastness study it can be concluded that the suspension concentrate containing 40% w/v adhesive agent gave the highest quantity of cypermethrin remaining on the leaves after 20 mm of simulated rainfall. It only resulted in a pesticide loss of 22%/cm^2 from the leaves within this study. However the commercially available emulsion concentrate, *Cyperkill* resulted in a very significant pesticide loss of 72.5%/cm^2, which was comparable with the suspension concentrate containing no adhesive (72.8%/cm^2). This study revealed that the suspension concentrate containing 40% w/v adhesive agent was the optimum system for ensuring the pesticide remained on the leaves for an extended period of time. It showed improved efficacy with regard to prolonged contact of pesticide over the commercially available emulsion concentrate. Thus from this study an adhesive concentration of 40% w/v was chosen to be used within the newly developed suspension concentrate. A higher level of adhesive agent was not incorporated within the suspension concentrate because of excessive viscosity of the system, therefore resulting in difficulties in shaking the container and ensuring the product was uniformly dispersed by the user.

7.4 SUSPENSION STABILITY STUDY

A suspending agent is present to provide a kind of "gel network" in the continuous phase. This is essentially for the prevention of "caking" or "claying", which is the result of settling of suspension as a consequence of the density difference between the particles and the medium. Since the particle size is outside the colloid range (usually greater than 1 μm), the very weak thermal motion is insufficient to overcome the gravitational force. As a result of the repulsive force between the particles (which is essential for maintaining colloid stability) in a sediment, these particles are able to move past each other forming a hard sediment (which in rheological terms is dilatant). As a result of small spacing between these particles such sediments are difficult to redisperse and therefore they must be avoided (Tadros, 1990).

The most commonly used suspending agents in suspension concentrates are polysaccharides (mostly xanthan gum), swellable clays (such as sodium montmorillonite), finely divided silica or mixtures of these. These 'gels' have a very high viscosity at low shear rates, but a low viscosity at relatively high shear rates. The high viscosity at low shear can reach very high values thus preventing sedimentation of the particles. As the shear rate is increased the viscosity decreases rapidly and it reaches reasonably low values in the high shear limit. This is precisely what is required for a suspension concentrate i.e. the system should have very high viscosity at low shear to prevent sedimentation, but once the shear rate is increased by shaking and pouring the formulation, the viscosity drops rapidly allowing easy flow and dispersion in the spray tank. The 'gel' in the continuous phase should have enough 'elasticity' to prevent separation of the formulation on storage. Many suspension concentrates separate on storage leaving a clear liquid layer at the top. Although technically this may not be a problem, since the suspension can be redispersed by gently shaking the container, it is not acceptable to the customer who prefers to have a homogenous system (Tadros, 1994).

Xanthan, a polysaccharide produced by the micro-organism *Xanthomonas campestris*, (which was used as the suspending agent in this study) is commonly used in suspension concentrates in the agrochemical industry on account of the highly elastic nature of its aqueous solutions on standing and their high degree of shear thinning under applied stresses (Symes, 1980). The molecule consists of a backbone identical to that of cellulose, with side chains attached to alternate glucose residues. These consist of two α-D-mannose and one β-D-glucuronate unit (Figure 7.4a) (Product Information, Kelco International).

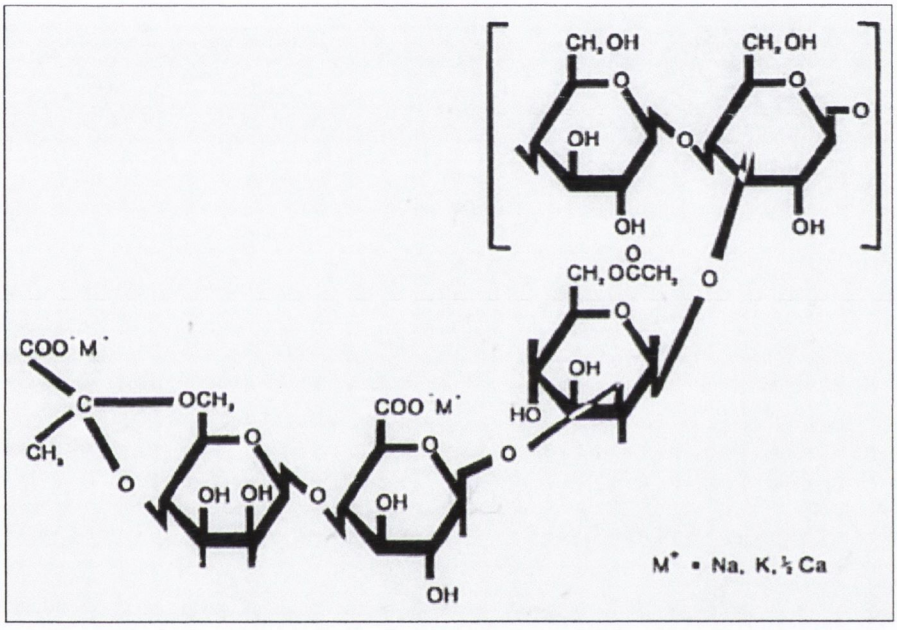


Figure 7.4a Repeating unit of xanthan gum polymer (Product Information, Kelco International).

In solution, the xanthan gum molecule adopts a rigid rod-like conformation and owing to the side chains, has a high hydrodynamic volume. At rest, the molecules associate into complex aggregates stabilised by hydrogen bonding. When shear is applied, the aggregates are disrupted. The polymer molecules become aligned in the direction of shear with a resulting decrease in viscosity. However, when the shear is removed, aggregate formation occurs rapidly resulting in a return to the original high

viscosity. Thus xanthan gum solutions have a quasi-gel character (Product Information, Kelco International).

In this suspension stability study, xanthan gum ranging from 0.05-0.2% w/v (Recommended range, Rhodia) was added to the suspension concentrates in graduated cylinders (Section 3.11.7.2), which were sealed and left undisturbed for six months at room temp. (~20°C). The suspension concentrates containing varying amounts of xanthan gum were observed over the six month period to ascertain if sedimentation/separation of the system occurred. Ease of redispersion of the systems was also assessed after six months.

In this study it is also necessary to take into consideration not only the suspending agent but also the adhesive agent (*Agrimer AL30*), which is incorporated into the final suspension concentrate at a very high level of 40% w/v (largest constituent within the suspension concentrate). It is a viscous solution of alkylated vinylpyrrolidone polymers dispersed in a lignosulphonate (polyelectrolyte), which therefore contributes proportionally in preventing sedimentation of the cypermethrin loaded halloysite particles in the suspension concentrate. The adhesive agent level was already optimised in Section 7.3 at 40% w/v to ensure the halloysite particles remained attached to the leaves of the barley crop, therefore this level was not adjusted in the sedimentation studies. The xanthan gum was added to the suspension concentrate to aid the adhesive agent in preventing sedimentation of the halloysite particles over a prolonged period of time because of it's 'gel' like structure (explained above) aiding thixotropy within the system.

Table 7.4a shows the results from the sedimentation volume study, which was determined at regular intervals over a 6 month period. It shows that the xanthan gum over the studied range of 0.05-0.2% w/v (in conjunction with the adhesive agent) ensured that the cypermethrin loaded halloysite particles remained suspended for the required time period of the study (6 month) because all the sedimentation volume ratios are 1.00. This confirmed that the suspension concentrates remained stable over

this period and did not show signs of sedimentation, which ensured long term physical stability of the systems. The sedimentation volumes of 1.00 were further confirmed by photographic evidence as shown in Figure 7.4a and 7.4b, after 1 month and 6 month, respectively. The photographs show that the suspension concentrates containing the different concentrations of xanthan gum are identical in appearance with no evidence of sedimentation. The suspension concentrate containing the least amount of xanthan gum, (0.05% w/v) gave the same sedimentation volumes as the suspension concentrate containing the greatest amount of xanthan gum (0.2% w/v). This indicated that the level of xanthan gum did not contribute significantly in preventing sedimentation of the halloysite particles because there was no evidence of sedimentation throughout the concentration range studied. Therefore the adhesive agent alone was predominantly functioning as the anti-settling agent within the formulation. However it was felt necessary that the xanthan gum at the lowest concentration may have ensured long term stability and also it could ensure appropriate thixotropy of the final suspension concentrate, which will be investigated further in Section 7.5.

Table 7.4a Sedimentation volumes (Hu/Ho) of 2% w/v cypermethrin loaded halloysite suspension concentrate containing varying amounts of xanthan gum (0.05-0.2% w/v) at room temp. (~20°C)

Time	0.05% w/v	0.1% w/v	0.15% w/v	0.2% w/v
7 days	1.00	1.00	1.00	1.00
14 days	1.00	1.00	1.00	1.00
1 month	1.00	1.00	1.00	1.00
2 month	1.00	1.00	1.00	1.00
3 month	1.00	1.00	1.00	1.00
4 month	1.00	1.00	1.00	1.00
5 month	1.00	1.00	1.00	1.00
6 month	1.00	1.00	1.00	1.00

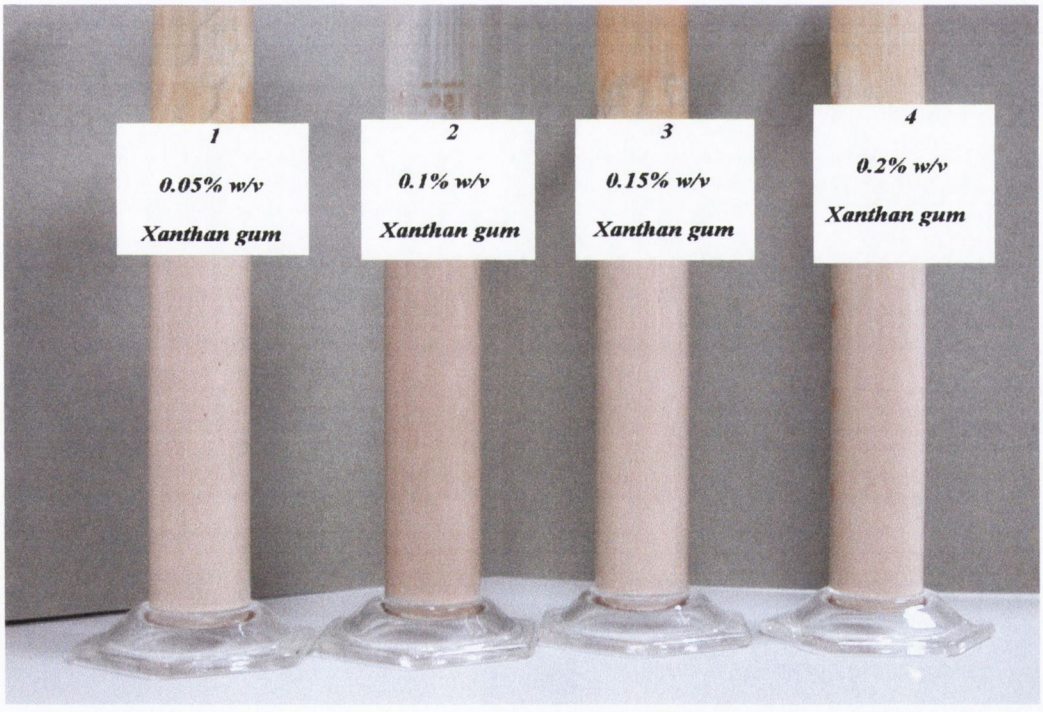


Figure 7.4a Photograph of suspension concentrates with varying amounts of xanthan gum stored at room temp. (~20°C) after 1 month.

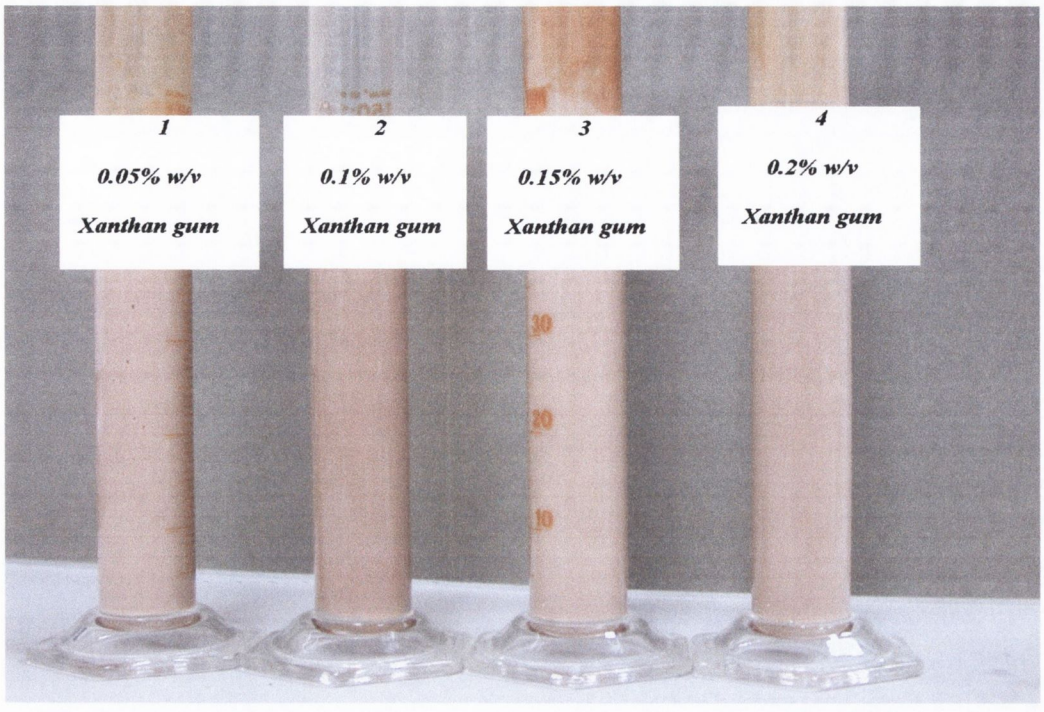


Figure 7.4b Photograph of suspension concentrates with varying amounts of xanthan gum stored at room temp. (20°C) after 6 months.

It is very important to ensure the cypermethrin loaded halloysite particles remained suspended within the suspension concentrate for a sufficiently long period of time to ensure physical stability i.e. high viscosity at low shear. However it is equally important that the suspension concentrate is easily redispersed on shaking i.e. low viscosity at high shear. This is necessary to ensure the product flows easily from the container and also that uniform doses of diluted pesticide are sprayed over the crop.

Table 7.4b Ease of redispersion of suspension concentrates with varying amounts of xanthan gum (0.05-0.2% w/v) stored at room temp. (~20°C) after 6 months.

Xanthan gum (% w/v)	**Ease of redispersion (number of inversions)**
0.05	5
0.1	5
0.15	6
0.2	8

Table 7.4b shows the results for redispersibility of the suspension concentrates containing varying concentrations of xanthan gum (0.05-0.2% w/v). As explained above it must be noted that the *Agrimer AL30* present in the suspension concentrate was present at a very high level (40% w/v) and because of its viscous nature and adhesive properties, after 6 months of undisturbed contact in the graduated cylinders, proved to be quite difficult to redisperse. However it is important to take into consideration from the results above (Table 7.4a and Figurer 7.4b) that there was no sedimentation present within the different systems with varying concentrations of xanthan gum. Thus the higher than expected number of inversions necessary to redisperse the suspension concentrates was due to the viscous nature of the *Agrimer AL30* rather than sedimentation of the halloysite particles within the systems. The redispersion studies showed that using xanthan gum at the lower concentration levels of 0.05% w/v and 0.1% w/v required 5 inversions to ensure the system was redispersed. However as the concentration of xanthan gum increased to 0.15% w/v and 0.2% w/v and the suspension concentrates became progressively more viscous, 6 and 8 inversions were necessary to ensure the systems were appropriately redispersed, respectively. Therefore from these findings in the redispersion study it would be inappropriate to use the higher concentrations of xanthan gum in the suspension concentration (0.15% w/v and 0.2% w/v) because of the greater number of inversions needed. This number of inversions would prove more difficult for the farmer to redisperse in practice. The suspension concentrates with the lower

concentration of xanthan gum (0.05% w/v and 0.1% w/v) required less inversions (5 inversions) to redisperse the systems. Therefore from this study 0.05% w/v xanthan gum was chosen as the optimum quantity of suspending agent necessary to allow easy redispersibility of the final suspension concentrate. This was further confirmed by the sedimentation volume study, which also chose the 0.05% w/v xanthan gum concentration because a higher concentration of suspending agent was not necessary as there was no sedimentation within any of the systems (sedimentation volumes of 1.00), with the adhesive agent contributing proportionately as an anti-settling agent.

7.5 RHEOLOGY

Rheology deals with the deformation of matter including flow (Whorlow, 1980). This is by far the best quantitative method to obtain information on the properties of suspension concentrates. The control of physical stability of suspension concentrates requires a build up of "structure" in the system, either by the control of flocculation (Section 7.2), or addition of a suspending agent (Section 7.4). However in the case of this suspension concentrate it is important to take into consideration the adhesive agent, as it also plays a role in the structure of the system because of its viscous nature. The degree of structure formation can be investigated using rheological measurements (Tadros, 1980).

It is common to describe the flow behaviour of any system in terms of the relationship between shear stress and the shear rate. As shown in Figure 7.5a four different types of flow curves can be distinguished. Newtonian flow is only exhibited by liquids and solutions and perhaps by very dilute suspensions, in which the particles do not interact with each other. Intermediate and highly concentrated dispersions always show non-Newtonian flow, the most common being pseudoplastic, which becomes more marked as the systems sometimes show time dependent flow behaviour, usually referred to as thixotropy. In this case the viscosity decreases with the duration of the applied stress because the structure is destroyed.

When the stress is removed, the structure gradually reforms, raising the viscosity. Dilatant systems, where the viscosity shows an increase with increase of shear rate, may also show some time dependence and these systems are referred to as rheopectic. In this case the system shows reversible thickening with time, at constant shear rate (Tadros, 1980).

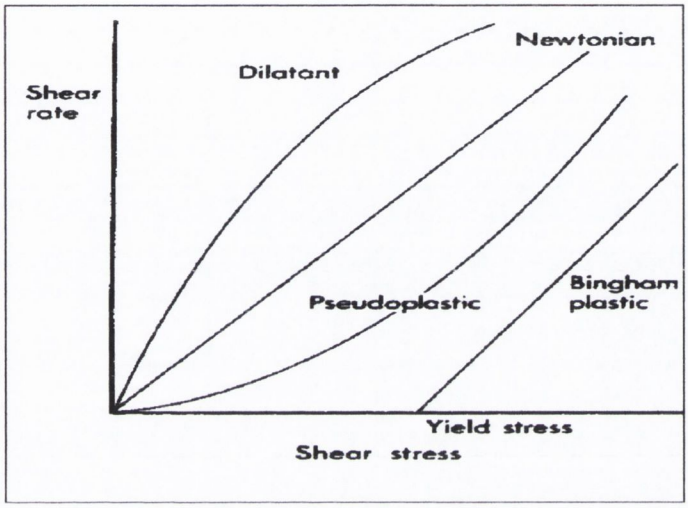


Figure 7.5a Various flow behaviour (Tadros, 1980).

The above measurements are usually referred to as high deformation measurements since the experiments are carried out under conditions where the structure of the system is broken down during the flow. Although these measurements give valuable information on the behaviour of the system under application, such as redispersion, they do not give enough information on the system on standing (storage). For the latter purpose, low deformation (i.e. under conditions whereby the structure of the system is maintained) measurements are required. Two main types of such measurements are available, namely, constant stress (creep) and dynamic (oscillatory) measurements. However in this study, dynamic (oscillatory) measurements will only be performed on the suspension concentrates.

In dynamic measurements, one applies a sinusoidal strain (stress) with a frequency ν in Hz or ω in rad s^{-1} ($\omega = 2\pi\nu$) on the system. The strain and stress are simultaneously measured to obtain their amplitudes and the time shift, Δt, between the sine waves of strain and stress. The latter shift occurs for a viscoelastic material as is the case with suspension concentrates. The product of Δt and ω gives the phase angle shift δ in radians. For a purely elastic system $\delta = 0$, for a purely viscous system $\delta = 90^{\circ}$ and for a viscoelastic system $0 < \delta < 90^{\circ}$. The ratio of the stress amplitude, τ_o, to the strain amplitude, γ_o, gives the complex modulus. The complex modulus is formed from a storage component G' (which is a measure of the elastic energy stored in a cycle) and a loss component G" (which is a measure of the energy dissipated as viscous flow in a cycle) (Tadros, 1995). The ratio of G" to G' is known as tan δ and is a good indicator of how viscoelastic a system is, as it measures the lag in sine response after an oscillating mechanical field has been applied to a sample. A viscoelastic system is one that shows frequency dependence i.e. it responds differently to changing frequencies.

In this study continuous shear (destructive test) and dynamic oscillatory measurements (non destructive test) were performed on the suspension concentrates. Continuous shear was performed on the final product (Table 3.11f) at time 0, 30, 90 and 180 min to ascertain the level of thixotropy (reversible shear breakdown) and irreversible shear breakdown of the suspension concentrate. Continuous shear (Table 3.11g) and dynamic oscillatory (Table 3.11h) measurements were performed on suspension concentrates containing varying quantities of *Agrimer AL30* and xanthan gum. The adhesive agent was regarded as the main ingredient that could vary the rheological properties of the suspension concentrate, with the quantity of cypermethrin loaded halloysite particles remaining constant within the system. However the level of xanthan gum (0.05-0.2% w/v) used within the suspension concentrate stability study was also studied in this Section, to confirm its contribution of thixotropy properties to the system.

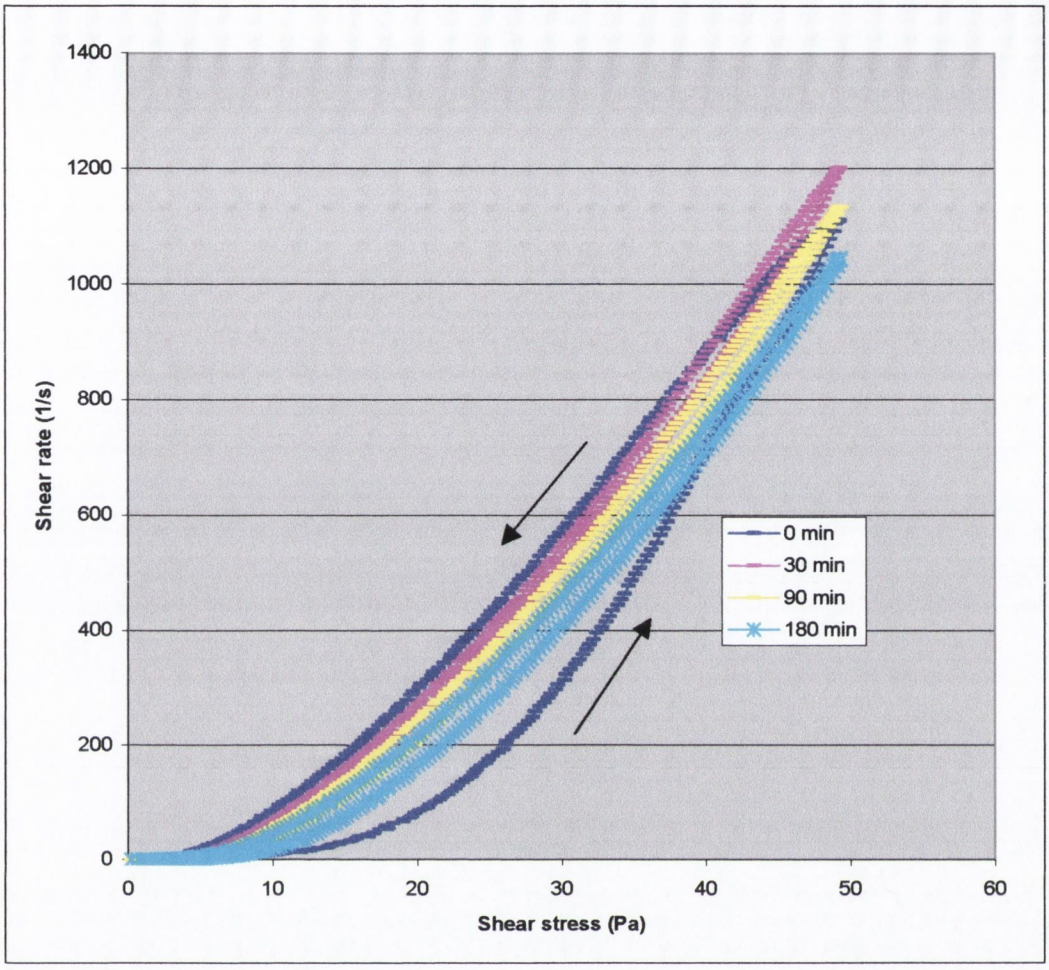


Figure 7.5b Flow curves indicating thixotropy and irreversible shear breakdown in final cypermethrin loaded halloysite suspension concentrate at t = 0, 30, 90 and 180 min.

Figure 7.5b illustrates the flow curves of the final suspension concentrate. The initial flow curve (t = 0) (ascending curve) shows that the suspension concentrate is pseudoplastic in nature i.e. the slope of the curve and therefore the viscosity is high initially and decreases with increasing shear stress (as shown in Figure 7.5a), which is representative of most agricultural suspension concentrates (Tadros, 1980). The descending curve (t = 0) of the initial flow curve is displaced to the left of the ascending curve and exhibits a hysteresis loop. The presence of the hysteresis loop indicates that a breakdown in structure has occurred and the area within the loop may

be used as an index of the degree of breakdown (Aulton, 2002). As discussed above the term used to describe such a behaviour is thixotropy. However, it has become common to describe as thixotropic any material that exhibits a reversible time-dependent decrease in apparent viscosity (Aulton, 2002).

Thixotropic systems are usually composed of asymmetric particles or macromolecules, which are capable of interacting by numerous secondary bonds to produce a loose three-dimensional structure, such that the flowing elements become aligned and the viscosity falls, so that the material is gel-like when unsheared. The energy imparted during shearing disrupts these bonds, so that the flowing elements become aligned and the viscosity falls, as a gel-sol transformation has occurred. When the shear stress is eventually removed the structure will tend to reform, although the process is not immediate and will increase with time as the molecules return to the original state under the influence of Brownian motion. In some cases the structure that has been destroyed is never recovered, no matter how long the system is left unsheared, which is referred to as 'shear destruction' (Aulton, 2002).

Using the same sample of suspension concentrate, successive flow curves were obtained at 30, 90 and 180 min after commencement of the experiment (Figure 7.5b). This experiment will help distinguish between thixotropy and irreversible shear breakdown by testing for recovery of structure on resting. The flow curves at all these time points are displaced to the left of the initial flow curve (t = 0), with the displaced loops showing statistically (ANOVA analysis performed, $p<0.05$) less hysteresis than the original loop i.e. area in hysteresis loops significantly smaller at t = 30, 90, 180 min in comparison to t = 0 (Table 7.5a). However it can also be observed that as the resting time is increased from 30 min to 60 min and then 90 min for the t = 30, 90, 180 min flow curves, respectively they gradually show increased area within the hysteresis loops (Table 7.5a). This reveals that the suspension concentrate exhibits thixotropy, in that the system continued to recover towards its initial ground state structure over the time period of the experiment. For example as seen in Table 7.5a, the sample recovery increased from 16.9% to 23% after a resting

time of 60 min and 90 min (t = 90 and 180 min, respectively). However the sample did not recover completely to its initial ground state during the time period of the experiment, with the sample only 23% recovered after 180 min (Table 7.5a). The experiment could not be extended for a longer time period because of problems of solvent evaporation from the sample between the cone and plate test geometry. It is possible that the suspension concentrate sample could have returned to its ground state if the experimental time was extended because it continued to recover over the 180 min time period (Table 7.5a). Therefore from this experiment it is difficult to distinguish between the reversible (thixotropy) and irreversible shear breakdown of structure. Using the data shown in Table 7.5a it is possible that the suspension concentrate sample could have recovered to its ground state in 5-6 hr approx. However, as discussed above there is no guarantee that the suspension concentrate sample would have returned to ground state.

Table 7.5a Area within hysteresis loop after different resting times of final cypermethrin loaded halloysite suspension concentrate sample

Time (min)	**Area within hysteresis loop ($Pa.s^{-1}$)**	**Recovery of sample (%)**
0	7455.3	100
30	1071.3	14.4
90	1258.7	16.9
180	1714.7	23

In practice a shear thinning system is desirable with as little time dependence (thixotropy) as necessary. In addition the limiting high shear viscosity reached should not be too high (usually less than 50-100 mPa), otherwise redispersion for dilution becomes difficult. However, the limiting low shear viscosity should be made as high as possible to overcome settling and claying (Tadros, 1995).

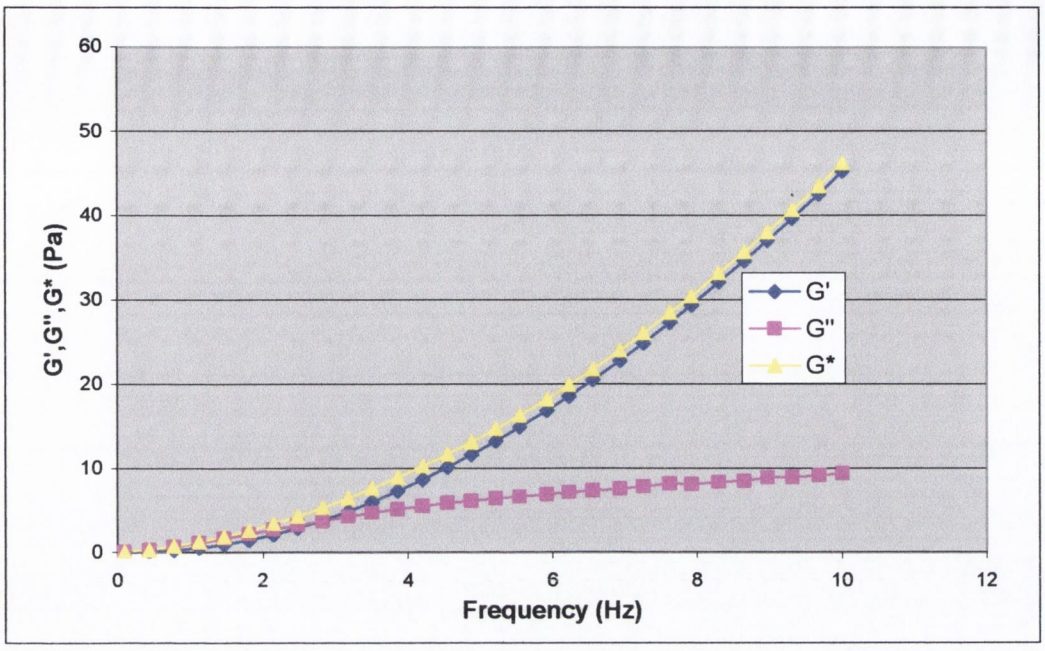


Figure 7.5c Variation of storage modulus (G'), loss modulus (G") and complex modulus (G*) with frequency for the final cypermethrin loaded halloysite suspension concentrate containing 40% w/v *Agrimer AL30* and 0.05% w/v xanthan gum.

As discussed above continuous shear gives information on the sedimentation and ease of dispersibility of a suspension concentrate. However dynamic oscillatory measurements can be used to give information on the long term physical stability of a suspension concentrate. Figure 7.5c illustrates the dynamic oscillatory measurements of the final suspension concentrate. The trend obtained, which depends on the system, can be used to determine information on the "structure" of the suspension concentrate. According to Tadros (1995), many viscoelastic structured suspension concentrates show the behaviour illustrated in Figure 7.5c. In the low frequency range (<2 Hz), the system is more viscous than elastic i.e. G" greater than G'. In this low frequency region (which corresponds to long time), the system can dissipate energy as viscous flow (Tadros, 1995). In contrast, in the high frequency region (>3 Hz), G'>G", since in this region most of the energy is stored elastically. At high frequency (>8 Hz) G' approaches G* and G" tends toward zero. Also many structured suspensions show small dependence of G' on frequency (gel network

structure) in high frequency region (Tadros, 1995), which is shown in Figure 7.5c. The phase angle shift (Table 7.5b) confirms the above findings in that at a low frequency (0.1 Hz) and a high frequency (10 Hz), the phase angle shift was 88.47° and 11.74°, respectively, which is representative of a more viscous and elastic system, respectively. However overall the system is viscoelastic in nature ($0 < \delta < 90°$). According to Tadros, (1995) one usually aims for a system that shows a long viscoelastic region, which was confirmed in this study as the suspension concentrate remained linear viscoelastic between 10 and 2000 μNm torque during the oscillatory torque sweep. This implies that the "gel" structure of the suspension concentrate is sufficiently coherent to prevent extensive separation. These above measurements show that the final suspension concentrate is viscoelastic in nature with appropriate physical stability. The rheological findings for dynamic oscillatory measurements (Figure 7.5c) are further confirmed by the suspension stability study in Section 7.4. The final suspension concentrate remained uniformily dispersed after 6 months, with no visible signs of sedimentation (Table 7.4a and Figure 7.4b).

The rainfastness studies and suspension stability studies have previously optimised the concentrations of adhesive agent (Section 7.3) and suspending agent (Section 7.4) required in the suspension concentrate, respectively. A 40% w/v *Agrimer AL30* and a 0.05% w/v xanthan gum were used in the final suspension concentrate product (Table 3.11f). However part of this rheological study was carried out to ascertain the effect of varying concentrations of adhesive agent and suspending agent on the rheology of the suspension concentrate.

Figure 7.5d illustrates the flow curves of suspension concentrates with 20% w/v, 40% w/v (final product) and 60% w/v *Agrimer AL30*. The flow curves of the suspension concentrates containing 20% w/v and 40% w/v *Agrimer AL30* are similar in shape, illustrating pseudoplastic behaviour. However the suspension concentrate containing the highest concentration of *Agrimer AL30* (60% w/v) displays plastic behaviour, with a minimum shear stress of 4.55 Pa necessary to ensure the sample flows initially.

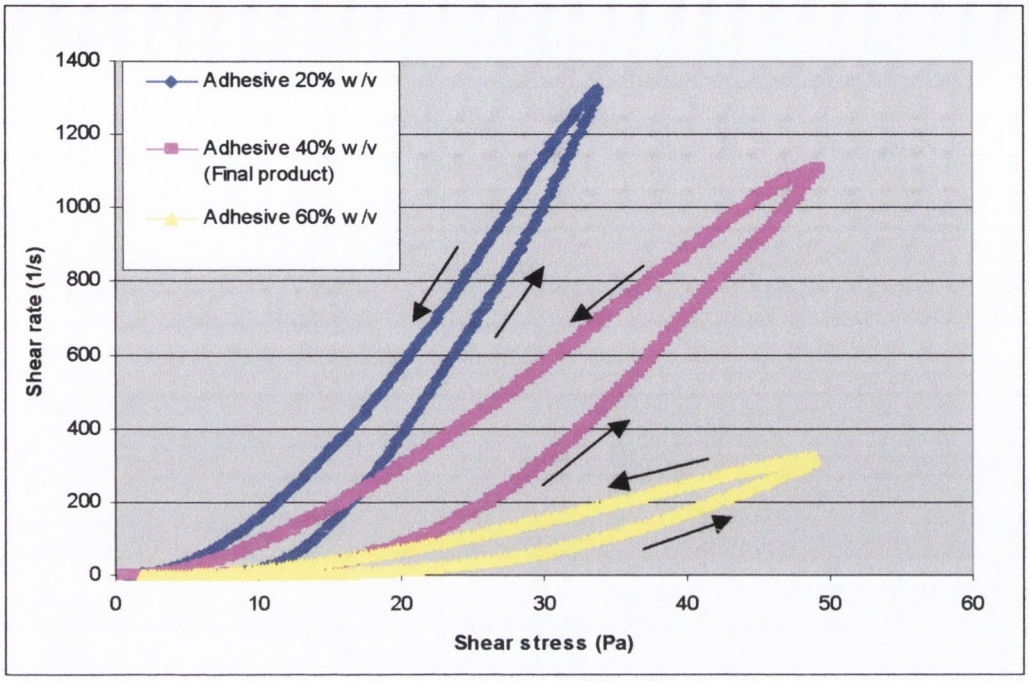


Figure 7.5d Flow curves indicating the effect of varying concentrations of adhesive agent (*Agrimer AL30*) on the suspension concentrate.

As the concentration of *Agrimer AL30* is increased from 20% w/v to 60% w/v, the flow curves are displaced towards the x-axis. This confirms that increased levels of shear stress are needed to allow the suspension concentrates with increased concentrations of *Agrimer AL30* to flow. This is a direct indication that an increase in apparent viscosity of the suspension concentrates occurs with increasing concentrations of *Agrimer AL30*. For example at the highest concentration of *Agrimer AL30* (60% w/v), increased shear stress is needed to allow appropriate flow of the sample compared to samples with lower concentrations of *Agrimer AL30*. This is expected as the suspension concentrate is more structured as the continuous phase becomes more viscous due to the higher concentration of *Agrimer AL30* present. The suspension concentrate containing 20% w/v adhesive agent could only withstand shear stresses up to 30 Pa, because above this point the shear rates could not be measured on the rheometer used. In practical terms the suspension concentrate with

the highest concentration of adhesive agent (60% w/v) was considered too difficult for an operator to redisperse effectively to ensure uniform pesticide dosing. However as discussed earlier, the adhesive concentration (40% w/v) had been optimised in the rainfastness studies (Section 7.3).

Dynamic oscillatory measurements were also performed on the suspension concentrates containing 20% w/v (Figure 7.5e), 40% w/v (Figure 7.5c) and 60% w/v *Agrimer AL30* (Figure 7.5f). Table 7.5b illustrates representative values of suspension concentrates with these varying concentrations of *Agrimer AL30*, which show similar trends. However as the concentration of *Agrimer AL30* increases the values for G' (storage modulus), G" (loss modulus), G* (complex modulus) and δ (phase angle shift) increase at the different frequency levels (Table 7.5b). The dynamic oscillatory graphs for the varying concentrations of *Agrimer AL30* further confirm these similar trends, which were discussed above in detail for the final suspension concentrate (Figure 7.5c). The suspension concentrates are all referred to as viscoelastic structured systems. As the concentration of *Agrimer AL30* is increased, the cross-over point between the G' (storage modulus) and G" (loss modulus) becomes more pronounced. For the suspension concentrates containing 20% w/v, 40 % w/v and 60 % w/v *Agrimer AL30*, the cross-over points are 0.7 Hz, 2.5 Hz and 4.6 Hz, respectively. At a frequency of 10 Hz (Table 7.5b) the G' (storage modulus) increases from 38.85 Pa to 53.32 Pa for the suspension concentrate containing 20 % w/v and 60 % w/v *Agrimer AL30*, respectively. An explanation for this is that the system becomes more structured as the *Agrimer AL30* concentration increases, indicating a clear tendency for a more solid-like behaviour at higher frequencies. The findings of the dynamic oscillatory study are in agreement with the continuous shear study of varying *Agrimer AL30* concentrations because as the concentration of *Agrimer AL30* increases the viscosity of the systems increase and therefore they become more structured in nature.

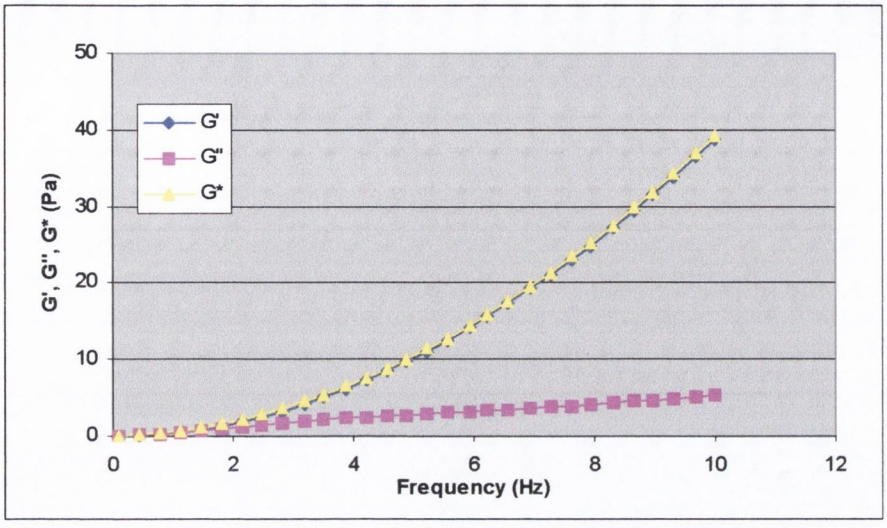


Figure 7.5e Variation of storage modulus (G'), loss modulus (G") and complex modulus (G*) with frequency for suspension concentrates with 20% w/v *Agrimer AL30.*

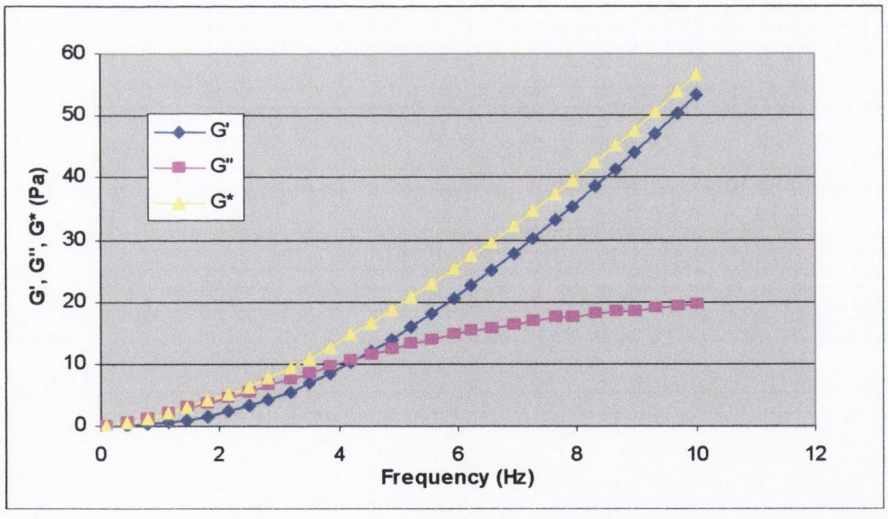


Figure 7.5f Variation of storage modulus (G'), loss modulus (G") and complex modulus (G*) with frequency for suspension concentrates with 60% w/v *Agrimer AL30.*

Table 7.5b Representative values of suspension concentrates with varying concentrations of *Agrimer AL30*

	G' (Pa)	G" (Pa)	G* (Pa)	δ
20% *Agrimer AL30*				
0.1 Hz	0	0.02	0.02	82.26
1 Hz	0.48	0.55	0.72	49.28
10 Hz	38.85	5.32	39.11	7.81
40% *Agrimer AL30* Final product				
0.1 Hz	0	0.57	0.58	88.47
1 Hz	0.52	1.16	1.28	65.61
10 Hz	45.26	9.41	46.22	11.74
60% *Agrimer AL30*				
0.1 Hz	0	0.13	0.13	90.98
1 Hz	0.59	2.06	2.14	74.06
10 Hz	53.32	19.63	56.83	20.21

(G' = elastic modulus, G"= loss modulus, G* = complex modulus, δ = phase angle shift)

The same rheological studies were performed on suspension concentrates with varying levels of xanthan gum (suspending agent). Figure 7.5g illustrates the flow curves for the suspension concentrates containing varying concentrations of xanthan gum (0.05% w/v to 0.2% w/v). The flow curve of the suspension concentrate containing 0.05% w/v xanthan gum is for the final suspension concentrate product, which was discussed previously (Figure 7.5b). Again all the flow curves show pseudoplastic behaviour. The flow curves are displaced towards the x-axis as the concentration of xanthan gum is increased, which also occurred for increasing concentrations of *Agrimer AL30*. This results in increased apparent viscosities of the suspension concentrates. These observations can be compared with the finding of the stability study (Section 7.4) on ease or redispersibility of the suspension concentrates

(Table 7.4b) using varying concentrations of xanthan gum. The suspension concentrate containing the highest (0.2% w/v) and lowest (0.05% w/v) concentration of xanthan gum required 8 and 5 inversions to ensure redispersion, respectively (Table 7.4b). This is evident in the continuous shear flow curves in Figure 7.5g with the suspension concentrates containing 0.2% w/v and 0.05% w/v xanthan gum requiring higher and lower shear stresses, respectively to ensure appropriate flow of the systems. For example the increased shear stress necessary to ensure appropriate flow of the suspension concentrate containing 0.2% w/v xanthan gum is a result of the increased viscosity due to the increased concentration of xanthan gum within the system.

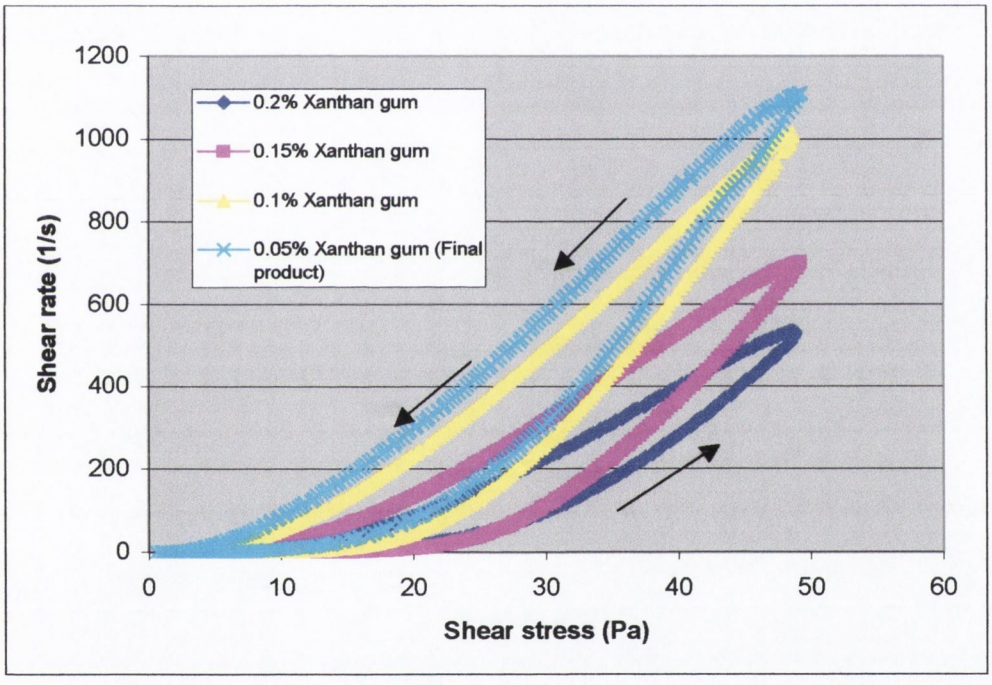


Figure 7.5g Flow curves indicating the effect of varying concentrations of suspending agent (0.05% w/v to 0.2% w/v) on the suspension concentrate.

Dynamic oscillatory measurements were also performed on the suspension concentrates containing 0.05% w/v (Figure 7.5c), 0.1% w/v (Figure 7.5h), 0.15% w/v (Figure 7.5i) and 0.2% w/v (Figure 7.5j) xanthan gum. Table 7.5c shows

representative values of suspension concentrates with these varying concentrations of xanthan gum, which all show similar trends. The trends observed for this study of varying suspending agent are similar to the varying concentrations of adhesive agent study. The cross-over point for the G' and G" becomes slightly more pronounced as the concentration of xanthan gum increases, with the suspension concentrate containing 0.05% w/v (final product), 0.1% w/v, 0.15% w/v and 0.2% w/v xanthan gum having a cross-over point of 2.5 Hz, 2.5 Hz, 2.8 Hz and 3.2 Hz, respectively. However the difference in cross-over of the G' and G" is not as pronounced for variations in concentration of xanthan gum as it is for the variations in concentration of *Agrimer AL30*. In this study, varying concentrations of xanthan gum resulted in less structural change in the suspension concentrate, as is evident by the smaller differences in cross-over of the G' and G" between the highest and lowest xanthan gum concentrations i.e 3.2 and 2.5 Hz, respectively. This is not the case for the suspension concentrates with the highest and lowest concentrations of adhesive agent, having cross-over points of 4.6 Hz and 0.7 Hz, respectively. This is also illustrated from the flow curves of the highest and lowest concentrations of xanthan gum (Figure 7.5g) in comparison to the flow curves of the highest and lowest concentrations of adhesive agent (Figure 7.5d), with a smaller magnitude of difference in flow for the suspension concentrates with varying xanthan gum concentrations. An explanation for this is that the concentrations of *Agrimer AL30* added to the suspension concentrate are much larger than the xanthan gum concentrations, with half and double the quantities of the adhesive agent giving a much larger difference in the consistency of the suspension concentrate. Therefore suspension concentrates with varying concentrations of xanthan gum are less likely to give pronounced rheological changes.

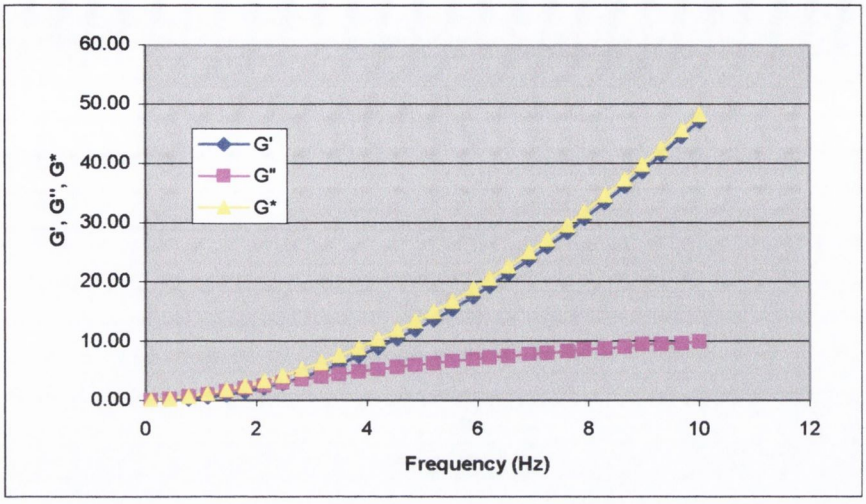


Figure 7.5h Variation of storage modulus (G'), loss modulus (G") and complex modulus (G*) with frequency for suspension concentrates with 0.1% w/v xanthan gum.

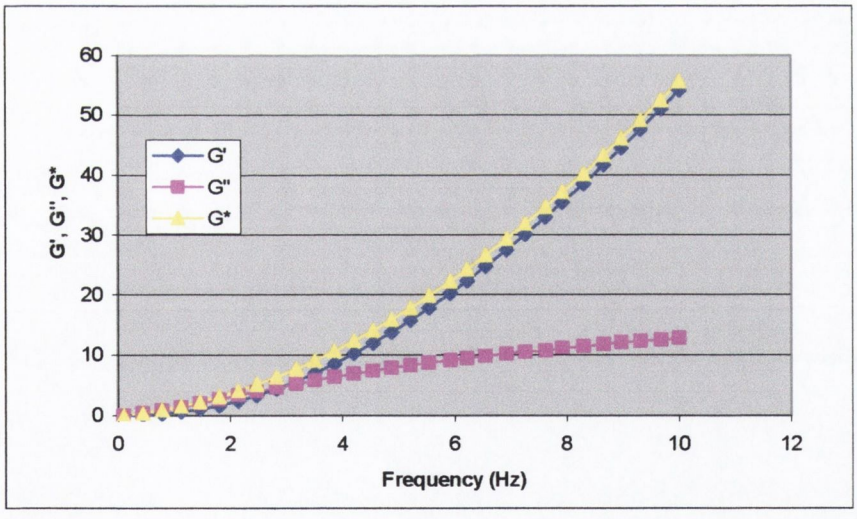


Figure 7.5i Variation of storage modulus (G'), loss modulus (G") and complex modulus (G*) with frequency for suspension concentrates with 0.15% w/v xanthan gum.

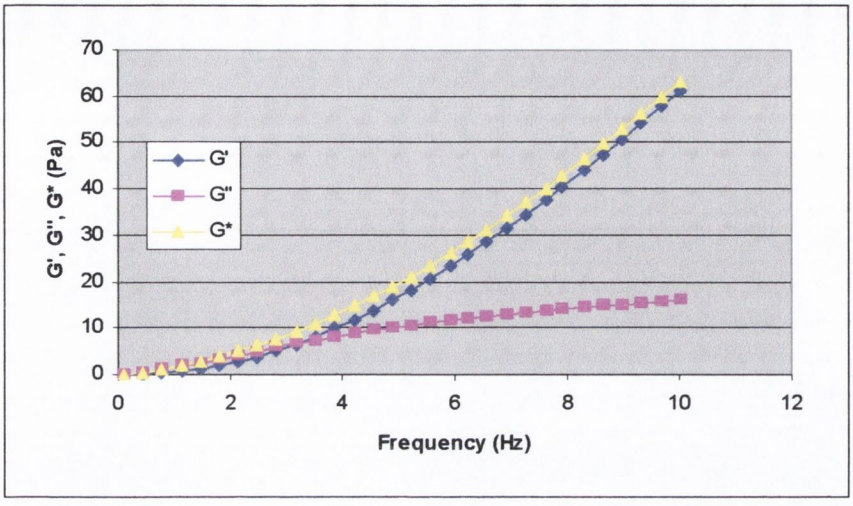


Figure 7.5j Variation of storage modulus (G'), loss modulus (G") and complex modulus (G*) with frequency for suspension concentrates with 0.2% w/v xanthan gum.

Table 7.5c Representative values of suspension concentrates with varying concentrations of xanthan gum (0.05% w/v to 0.2% w/v)

	G' (Pa)	G" (Pa)	G* (Pa)	δ
Xanthan gum (0.05% w/v) Final product				
0.1 Hz	0	0.57	0.58	88.47
1 Hz	0.52	1.16	1.28	65.61
10 Hz	45.26	9.41	46.22	11.74
Xanthan gum (0.1% w/v)				
0.1 Hz	0	0.05	0.05	87.78
1 Hz	0.54	1.08	1.28	63.66
10 Hz	47.2	9.99	48.23	11.95
Xanthan gum (0.15% w/v)				
0.1 Hz	0	0.07	0.07	88.67
1 Hz	0.58	1.42	1.54	67.65
10 Hz	54.17	12.91	55.69	13.41
Xanthan gum (0.2% w/v)				
0.1 Hz	0	0.1	0.1	91.39
1 Hz	0.66	1.84	1.96	70.37
10 Hz	61.11	16.19	63.2	14.84

(G' = loss modulus, G"= elastic modulus, G* = complex modulus, δ = phase angle shift)

By carrying out rheological measurements as a function of strain amplitude and frequency, one is able to obtain correlations between the various rheological parameters and the long term physical stability of the suspension concentrate. These

correlations should be established during the formulation of the suspension concentrate and they could be used for further development of the system. In addition, these correlations may be used as guidelines for the formulation of any future suspension concentrate. However, the formulation chemist should be careful in the interpretation of the results since the rheological results depend on several parameters of the system that may simultaneously change during the formulation. The results obtained in a small scale laboratory preparation must be correlated with the properties of the large scale preparation before the optimum rheological parameters are established. This requires more research before it can be used as a quality assurance method. At present dynamic measurements can be used by the formulation chemist to establish the optimum composition of the antisettling system and therefore predict the long term physical stability of the suspension concentrate (Tadros, 1995).

7.6 CYPERMETHRIN CONTENT OF HALLOYSITE POWDER

The cypermethrin pesticide was loaded into the halloysite powder (halloysite Goushan NZCC) under vacuum, in a similar manner as the chlorpyrifos pesticide used in Section 6.4. However the cypermethrin pesticide was loaded into the halloysite at 5% w/v in ethanol. As explained in Section 6.4, a low concentration of pesticide was used because at elevated concentrations the pesticide formed a shiny 'film' on the surface of the halloysite powder, indicating unsuccessful loading of the halloysite tubules.

The double cypermethrin loaded halloysite powder samples were assayed by HPLC method (Section 3.11.11). The average pesticide content in three samples was calculated and the concentration of cypermethrin within the powder samples was expressed as % w/w (Table 7.6a).

Table 7.6a Total amount of cypermethrin in halloysite powder samples (% w/w)

Sample no.	% w/w of cypermethrin in halloysite powder samples
1	10.21
2	9.97
3	9.68
Average	9.95

From the results in Table 7.6a it can be seen that the halloysite powder samples contained an average of 10% w/w cypermethrin. The final suspension concentrate contained 20% w/v halloysite powder. Therefore the suspension concentrate contained 2% w/v cypermethrin. The commercially available emulsion concentrate, *Cyperkill* contains 10% w/v cypermethrin. An *in vitro* release study was performed on the two products, which was used to determine the level of sustained release achieved by the suspension concentrate in comparison to the emulsion concentrate of cypermethrin.

7.7 RELEASE PROFILES OF CYPERMETHRIN LOADED HALLOYSITE SUSPENSION CONCENTRATE AND CYPERMETHRIN EMULSION CONCENTRATE, *CYPERKILL*

As explained in Section 7.6, the cypermethrin loaded halloysite suspension was compared to the commercially available emulsion concentrate, *Cyperkill in vitro*. This was achieved by using a dissolution medium containing 30:70 methanol:McIlvaine buffer pH 4 over a 21 day period, which ensured the cypermethrin was released over an appropriate period of time. A higher volume of methanol was not used in this release study because it would be less likely to mimic the natural conditions within the field. Samples of the two products were measured out so that they contained equivalent quantities of cypermethrin (Table 3.11j). The

release study for the emulsion concentrate, *Cyperkill* was repeated using 100 ml of dissolution medium and an extra 7 ml of deionised water to take into account the difference in volume and concentration gradient between the emulsion concentrate and the suspension concentrate. This ensured the release profiles of the two products were more comparable to each other. Dialysis tubing with a molecular weight cut off in the range 12,000 to 14,000 was used to hold the liquid samples, which is permeable to water and allows low molecular weight compounds to diffuse, while refusing passage to higher molecular weight materials (Technical Information, BDH). Therefore the tubing allowed the cypermethrin to be released slowly into the dissolution medium and prevented the halloysite particles in the suspension concentrate from entering the external dissolution medium.

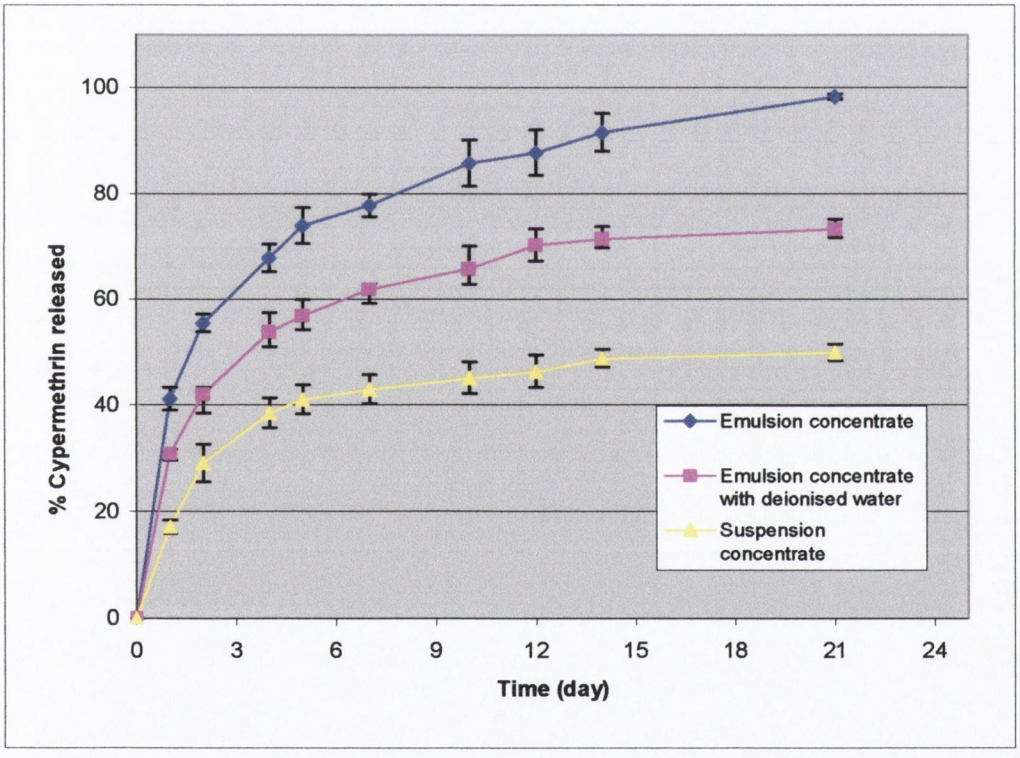


Figure 7.7a Release profiles of cypermethrin loaded halloysite suspension concentrate and cypermethrin emulsion concentrate with/without deionised water.

Figure 7.7a shows the release profiles of the cypermethrin loaded halloysite suspension concentrate and the commercial cypermethrin emulsion concentrate (*Cyperkill*) with and without the added deionised water in the dissolution medium over a 21 day period. An ANOVA analysis was performed on the three release profiles, which showed that they were all statistically different from each other at all the time points ($p<0.05$). The newly formulated halloysite suspension concentrate gave the greatest sustained release of cypermethrin. After day 1, the burst release was 17.1%, 30.8% and 41.19% for the halloysite suspension concentrate and the emulsion concentrate with/without the deionised water added to the dissolution medium, respectively. The burst release of pesticide from the halloysite suspension concentrate is probably mainly due to the cypermethrin that was not successfully loaded into the halloysite tubules (interparticulate) and therefore remained dispersed within the suspension concentrate. This pesticide would probably easily diffuse out into the dissolution medium because of the difference in concentration gradient.

Figure 7.7a shows that there is a considerable difference in release of pesticide between the emulsion concentrate with/without the extra deionised water added to the dissolution medium. The cypermethrin is released at a much slower rate from the emulsion concentrate in the dissolution medium containing the deionised water because there is a smaller difference in concentration gradient between the emulsion concentrate liquid sample and the dissolution medium. Consequently the release profile for the emulsion concentrate in the presence of the extra deionised water within the dissolution medium is probably more comparable to the release profile of the suspension concentrate. The burst release for the emulsion concentrates is also higher than for the halloysite suspension concentrate because the pesticide is formulated within an aromatic xylene based emulsion, which ensures that the cypermethrin is more readily soluble and therefore will diffuse more efficiently outwards across the dialysis membrane into the dissolution medium.

The halloysite suspension concentrate shows the greatest sustained release of cypermethrin because as explained in Chapter 1 the halloysite particles have a unique

ability to entrap active agents within their hollow internal lumens and also by adsorption to the outer polyanionic surfaces of the tubules. Price *et al.*, 2001; Kelly, 2002 and Levis and Deasy, 2003 utilised this technology to give sustained release of a number of active agents for marine and pharmaceutical applications over different lengths of time. Also in Section 6.6, the pesticide chlorpyrifos, which similar to cypermethrin is highly insoluble in water, was successfully loaded into the halloysite tubules and formulated into pellets to give effective sustained released. Therefore the sustained release achieved by the suspension concentrate can be attributed to the halloysite particles, which were loaded with the pesticide under vacuum and released the cypermethrin slowly from the tubules over the 21 day period. After day 2 the halloysite suspension concentrate had released 29.1% cypermethrin, whereas the emulsion concentrates had released 42% and 55.5% (for the dissolution medium with/without deionised water) into the dissolution medium. The halloysite suspension concentrate continued to show sustained release of 38.5%, 41.1%, 43%, 45%, 46.4%, 48.8% and 49.9% after days 4, 5, 7, 10, 12, 14 and 21 of the release study, respectively. These results indicate that after day 2 the cypermethrin is primarily being released from the ends of the tubules by diffusion (Price *et al.*, 2001). This occurs as the dissolution medium penetrates the tubule and allows slow release of the pesticide.

As discussed above the difference in release profiles between the emulsion concentrate with/without deionised water in the dissolution medium is the concentration gradient difference between the two systems. The emulsion concentrate (with deionised water in dissolution medium) released 53.8%, 61.9%, 65.8%, 70.3%, 71.4% and 73.2% after days 4, 5, 7, 10, 12, 14 and 21 of the release profile, respectively. The emulsion concentrate (without deionised water in dissolution medium) released 67.8%, 73.9%, 77.8%, 85.7%, 87.7%, 91.5%, 98.3% after days 4, 5, 7, 10, 12, 14 and 21 of the release profile, respectively. These release results show that the cypermethrin was released at a much faster rate from the latter system. The emulsion concentrate release profile (without deionised water in dissolution medium) continued to show an upward trend in release of pesticide to the

final day of the release profile and was nearly completely released after 21 days (98.3%). However the emulsion concentrate release profile (with deionised water in dissolution medium) showed a levelling off of pesticide release after day 10, with only 73.2% released after 21 days, which indicated that this system may have been at equilibrium i.e. no difference in concentration between liquid sample in dialysis tubing and dissolution medium. The halloysite suspension concentrate also showed this levelling off of pesticide release after day 10, but in this case it was more pronounced, with only 49.9% released after 21 days. The cypermethrin is released at a slower rate from this system because as discussed above the pesticide is entrapped within the halloysite tubules and also the suspension concentrate is aqueous based, which gives a diffusion barrier that slows the pesticide release.

From this release study it can be concluded that the halloysite suspension concentrate gives significant sustained release of cypermethrin *in vitro* in comparison to the emulsion concentrate over a long period of time, which is also further evident by the repeated release study of the emulsion concentrate in the presence of extra deionised water in the dissolution medium. The cypermethrin should be released from the two products at a much slower rate *in field* into an aqueous medium in comparison to the *in vitro* dissolution medium (contained 30% methanol). However the suspension concentrate would be expected to give greater retarded release of cypermethrin.

7.8 WINTER AND SPRING BARLEY FIELD TRIALS

7.8.1 Introduction

The newly formulated cypermethrin loaded halloysite suspension concentrate was compared to the commercially available cypermethrin emulsion concentrate, *Cyperkill in vitro*. This study revealed that the suspension concentrate gave improved sustained release of cypermethrin in comparison to the emulsion concentrate, which should ensure prolonged protection of the cereal crop from aphid

infestation. However as discussed in Section 6.7.1 *in vitro* release studies may bear little relationship to field conditions.

Consequently, field trials were carried out to determine the ability of the two products to control aphids under natural conditions. This was achieved by carrying out fully randomised field trials on winter and spring barley crops at the University College Dublin farm at Lyons Research Farm, Newcastle, Co. Dublin. There were eight different treatments in the winter and spring barley trials (Table 3.12b). The barley crops were exposed to natural infestation of aphids. They were regularly assessed for aphid numbers after pesticide spraying of the crops (Table 3.12h). The winter barley crop was also assessed for BYDV. After harvesting the barley crops were assessed for yield and grain quality.

7.8.2 Winter barley crop results and discussion

The winter barley crop was sown on the 8th October 2002 and first sprayed for aphid control on the 19th December 2002 (Table 3.12b). The crop was not assessed for aphid numbers during the winter period because of the very low numbers or complete absence of aphids due to the very high levels of rainfall that occurred (Figure 7.8a). The temperature during the period of the field trials was also recorded because low temperatures have a significant influence on aphid reproduction, with numbers increasing with an increase in temperature. However as seen in Figure 7.8b, there is very little difference in temperature between the actual and average temperatures recorded during the winter barley field trial, with the actual temperature slightly higher than average during the winter months.

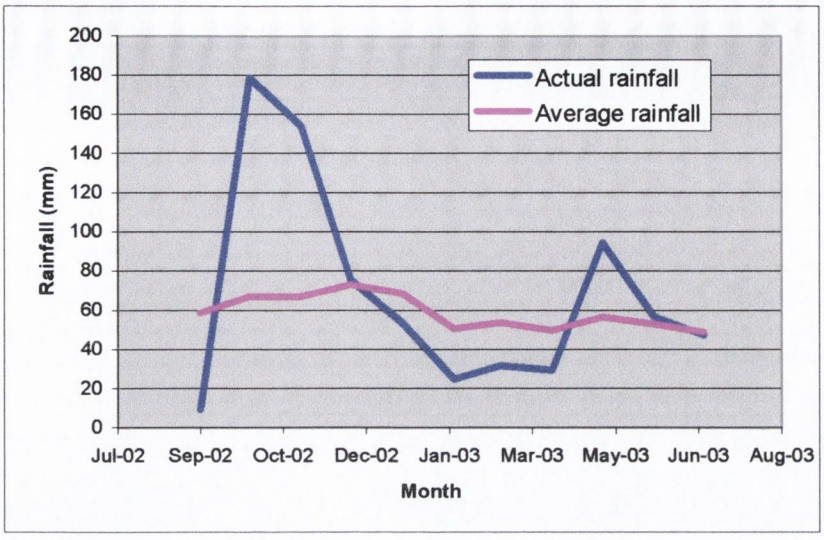


Figure 7.8a Monthly rainfall for time period of winter and spring barley field trials and average monthly rainfall for the past 30 years at Lyons Research Farm.

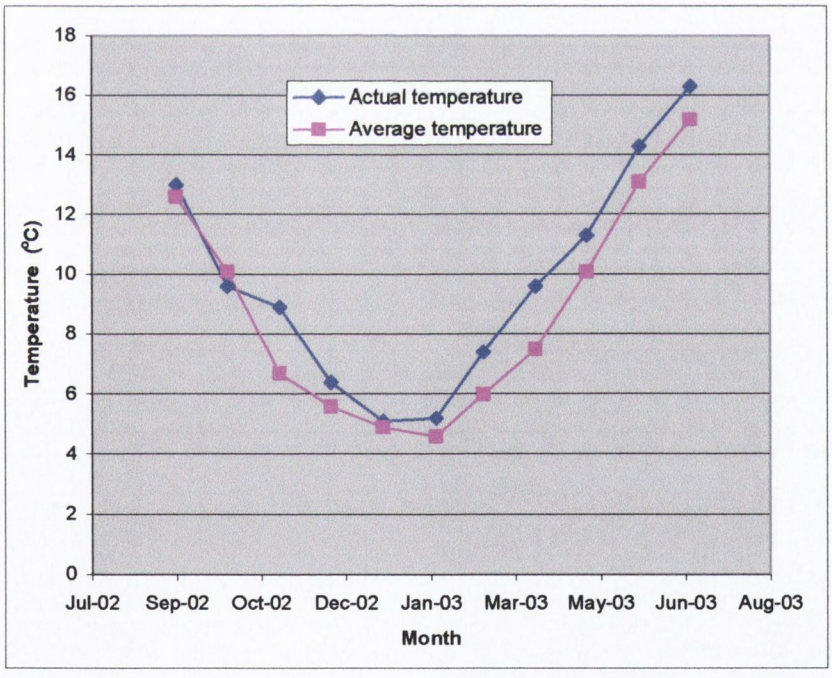


Figure 7.8b Monthly temperatures for time period of winter and spring barley field trials and average monthly temperature for the past 30 years at Lyons Research Farm.

On the 28th May 2003 the aphid numbers per treatment were counted for the first time (Table 7.8a). An ANOVA analysis was performed on the results obtained, which revealed that there was no statistical difference between the different treatments for aphid control ($p = 0.24$). A non-statistically different p-value would be expected for the cypermethrin suspension and emulsion concentrate treatments because neither product was likely to remain attached to the barley leaves for 160 days after spraying, to continue to provide effective aphid control (Table 3.12h).

Table 7.8a Average aphid numbers per treatment on winter barley crop on the 28th May 2003 after 1st pesticide spraying

Treatment no.	**Average aphid numbers per treatment**
1	5.58
2	1.25
3	0.49
4	3.40
5	2.20
6	0.72
7	2.40
8	5.25
L.S.D. at 5%	n.s.
C.V. (%)	102.74

n.s. = not statistically different

L.S.D = least significant difference

C.V. = coefficient of variance

The winter barley crop was also assessed for BYDV on the 28th May 2003 by determining the average % BYDV infection per treatment i.e. dwarfing and yellowing of plants (Table 7.8b). An ANOVA analysis was performed on the results, which showed that the treatments were statistically different ($p = 0.007$). The

untreated control gave statistically different results in comparison to the other seven treatments (different superscripts). However there was no statistical difference between the other seven treatments (same superscript). Therefore these results suggest that the suspension and emulsion concentrate were equally effective at reducing the % BYDV infection of the winter barley crop. Despite this result, the disease is spread solely by aphids and therefore control of the disease greatly depends on the extent to which populations of virulent aphids are controlled. As seen in Table 7.8a, the aphid numbers for the different treatments did not show statistical difference. Therefore there is a high probability that the increased % BYDV infection on the untreated control in comparison to the other treatments is a random occurrence.

Table 7.8b Average % BYDV per treatment in winter barley crop on the 28th May 2003 after 1st pesticide spraying

Treatment no.	Average % BYDV per treatment
1	9.56^{a}
2	1.12^{b}
3	1.81^{b}
4	2.06^{b}
5	3.25^{b}
6	3.12^{b}
7	1.38^{b}
8	5.12^{b}
L.S.D. at 5%	4.19
C.V. (%)	86.22

Values with different superscripts are statistically different.

The winter barley crop was pesticide resprayed for aphid control on the 9th June 2003 and was first assessed on the 17th June 2003 for aphid numbers (Table 7.8c). An

ANOVA analysis was performed on the results obtained, which revealed that the eight treatments were not statistically different from each other ($p = 0.41$).

Table7.8c Average aphid numbers per treatment on winter barley crop on the 17th June 2003 after 2nd pesticide spraying (1st assessment)

Treatment no.	**Average aphid numbers per treatment**
1	5.15
2	3.90
3	2.55
4	3.95
5	5.00
6	5.90
7	6.40
8	4.00
L.S.D. at 5%	n.s.
C.V. (%)	52.0

The winter barley was assessed a second time for aphid numbers on the 26th June 2003 (Table 7.8d). However yet again the ANOVA analysis showed that there was no statistical difference between the eight treatments ($p = 0.16$). This p-value result showed that there was no reduction in aphid numbers between the untreated plots and the treated plots, which suggests that neither product was effective at reducing aphid numbers in the winter barley crop. The aphid counts were not repeated for a third or subsequent times because the crop had ripened considerably with the complete absence of green foliage and therefore it would not support aphid feeding at this growth stage.

Table 7.8d Average aphid numbers per treatment on winter barley crop on the 26th June 2003 after 2nd pesticide spraying (2nd assessment)

Treatment no.	Average aphid numbers per treatment
1	6.80
2	4.60
3	3.30
4	5.15
5	6.90
6	5.50
7	5.72
8	7.55
L.S.D. at 5%	n.s.
C.V. (%)	36.80

The information obtained from the aphid counts was inconclusive, showing no statistical difference between the eight treatments before harvesting. However yield and grain quality analysis was performed on the harvested grain from each of the plots, to ascertain if there was statistical difference in this regard between the different treatments within the trial.

The yield of grain and thousand grain weight per treatment were two measurements, which were carried out on the post-harvested winter barley crop. The yield of grain can give a good indication of whether aphid infestation (BYDV vector) has occurred extensively within a crop because it will result in a suppression of plant growth and therefore ultimately a reduction in yield of grain. The suppression of plant growth within a crop infested with aphids will directly result in a reduction in grain size and therefore the weight of a thousand grains will be less in comparison to grains within a healthy well developed crop. In this study, the average grain yield for the winter barley crop for each treatment is shown in Table 7.8e. An ANOVA analysis was

performed on the results obtained, which showed that there was no statistical difference between the eight different treatments ($p = 0.16$). The thousand grain weight per treatment (Table 7.8f) also showed no statistical difference between the eight treatments ($p = 0.30$).

Table 7.8e Average grain yield per treatment for winter barley crop

Treatment no.	**Average grain yield per treatment (t/ha)**
1	7.71
2	8.85
3	7.97
4	8.64
5	8.78
6	8.52
7	8.38
8	8.22
L.S.D. at 5%	n.s.
C.V. (%)	7.25

Table 7.8f Average thousand grain weight per treatment for winter barley crop

Treatment no.	Average thousand grain weight per treatment (g)
1	51.68
2	54.30
3	55.69
4	55.25
5	54.02
6	53.89
7	55.12
8	54.96
L.S.D. at 5%	n.s.
C.V. (%)	4.73

Grain quality analysis was also carried out on the post-harvested winter barley crop, which included determining grain size and hectolitre weight. Grain size is closely correlated with yield and a thousand grain weight because a reduction in grain size would result in reduction in these two measurements. As explained above an increase in aphid infestation inhibits plant development and therefore growth of grains within the crop. The hectolitre weight is also related to yield and thousand grain weight measurements because a reduction in these measurements will result in a reduction in hectolitre weight.

Table 7.8g shows the average percentage grain <2.0 mm and <2.25 mm per treatment for the winter barley crop. An ANOVA analysis was performed on the results obtained and revealed that the <2.0 mm ($p = 0.25$) and <2.25 mm ($p = 0.23$) grain fractions were not statistically different. Table 7.8h shows the average hectolitre weight per treatment for winter barley crop, which also showed that there was no statistical difference between the eight treatments ($p = 0.40$).

Table 7.8g Average percentage grain <2.0 mm and <2.25 mm per treatment for winter barley crop

Treatment no.	% grain <2.0 mm	% grain <2.25 mm
1	1.21	2.99
2	0.38	1.315
3	0.24	0.85
4	0.40	0.99
5	0.62	1.60
6	0.61	1.65
7	0.33	1.04
8	0.45	1.32
L.S.D. at 5%	n.s.	n.s.
C.V. (%)	96.64	75.88

Table 7.8h Average hectolitre weight per treatment for winter barley crop

Treatment no.	Hectolitre weight per treatment (kg/hl)
1	68.37
2	69.27
3	70.15
4	69.12
5	68.65
6	67.55
7	69.37
8	68.6
L.S.D. at 5%	n.s.
C.V. (%)	2.16

The four post-harvest measurements show that there was no difference between the eight different treatments i.e. p-values <0.05 and therefore no further information was

obtained from these post-harvest measurements to determine whether the suspension concentrate was superior to the commercially available emulsion concentrate version of cypermethrin and also to the untreated control.

The combined results of the aphid counts and the preharvest yield and quality assessments did not show whether the suspension concentrate was efficacious over the emulsion concentrate at controlling aphid numbers. As explained before a reason for this may have been that the very high levels of rainfall in comparison to the average rainfall levels prevented/inhibited the spread of aphids and therefore presence of pesticide was not absolutely necessary. Also field trials of this nature are normally performed for three consecutive seasons, therefore the results from one season would not be sufficient to draw definitive conclusions on efficacy of products.

7.8.3 Spring barley crop results and discussion

The winter barley trial was not effective in determining which product showed improved efficacy for aphid control and therefore a spring barley trial was carried out. The spring barley crop was sown on the 10th April 2003, which was pesticide sprayed on the 27th of May for aphid control using the same treatments as for the winter barley crop (Table 3.12b).

Table 7.8h Average aphid numbers per treatment on spring barley crop on the 6th June 2003 (1st assessment)

Treatment no.	Average aphid numbers per treatment
1	1.95^{a}
2	0.15^{b}
3	0.15^{b}
4	0.35^{b}
5	0.05^{b}
6	0.20^{b}
7	0.35^{b}
8	0.50^{b}
L.S.D. at 5%	0.476^{b}
C.V. (%)	70.06

Values with different superscripts are statistically different

Aphid numbers were counted on the 6th June 2003 (Table 7.8h). An ANOVA analysis was performed on the results, which revealed that they were statistically different ($p = 0.00$). The untreated control plots had a statistically higher number of aphids in comparison to the treated plots (different superscript), which revealed that the suspension and emulsion concentrates of cypermethrin were effective at reducing aphid numbers after 10 days from spraying. However there was no statistical difference between the different treated plots. This confirmed that there was no difference between the two products in controlling aphid numbers. The aphid numbers were counted for the second time on the 17th June 2003, which was 21 days after spraying (Table 7.8i). An ANOVA analysis was performed, which showed that there was no statistical difference between the eight different treatments ($p = 0.23$).

Table 7.8i Average aphid numbers per treatment on spring barley crop on the 17th June 2003 (2nd assessment)

Treatment no.	Average aphid numbers per treatment
1	0.45
2	1.30
3	0.60
4	1.90
5	0.35
6	0.95
7	0.45
8	1.85
L.S.D. at 5%	n.s.
C.V. (%)	106.27

The third aphid count assessment (Table 7.8j) was carried out on the 26th June 2003 (30 days after pesticide spraying) and an ANOVA analysis was performed which revealed that there was statistical difference between the eight treatments (p=0.00). This assessment for aphid numbers gave the same results, as the first assessment on the 6th June 2003 i.e. the untreated control gave statistically higher numbers of aphids to the seven other treatments (different superscripts). Therefore these results show that the two products were reducing aphid numbers after 30 days from pesticide spraying. However there was no statistical difference between the two products for aphid control.

Table 7.8j Average aphid numbers per treatment on spring barley crop on the 26th June 2003 (3rd assessment)

Treatment no.	Average aphid numbers per treatment
1	2.40^{a}
2	0.90^{b}
3	0.75^{b}
4	0.85^{b}
5	0.70^{b}
6	0.95^{b}
7	1.05^{b}
8	1.00^{b}
L.S.D. at 5%	0.603^{b}
C.V. (%)	38.08

Values with different superscripts are statistically different

The fourth (Table 7.8k) and fifth (Table 7.8l) assessment for aphid numbers were performed on the 3rd July 2003 and 11th July 2003 and an ANOVA analysis revealed that there was no statistical difference between the eight treatments after 37 days ($p = 0.68$) and 45 days ($p = 0.31$) from pesticide spraying, respectively. The final assessment (Table 7.8m) was carried out on the 27th July 2003 (after 61 days from pesticide spraying) and an ANOVA analysis showed that there was no statistical difference between the eight different treatments ($p = 0.21$).

Table 7.8k Average aphid numbers per treatment on spring barley crop on the 3rd July 2003 (4th assessment)

Treatment no.	Average aphid numbers per treatment
1	0.75
2	0.10
3	0.20
4	0.40
5	0.85
6	0.25
7	0.30
8	0.75
L.S.D. at 5%	N.S.
C.V. (%)	139.12

Table 7.8l Average aphid numbers per treatment on spring barley crop on the 11th July 2003 (5th assessment)

Treatment no.	Average aphid numbers per treatment
1	0.55
2	0.25
3	0.40
4	0.75
5	0.10
6	0.65
7	0.70
8	0.70
L.S.D. at 5%	n.s.
C.V. (%)	110.50

Table 7.8m Average aphid numbers per treatment on spring barley crop on the 27th July 2003 (6th assessment)

Treatment no.	Average aphid numbers per treatment
1	0.85
2	0.52
3	0.62
4	0.60
5	0.85
6	0.72
7	0.55
8	0.92
L.S.D. at 5%	n.s.
C.V. (%)	71.73

Therefore the spring barley trial using the aphid counts assessment was inconclusive in determining if the suspension concentrate was more efficacious at reducing aphid numbers in comparison to the commercially available emulsion concentrate of cypermethrin because there was no statistical difference between the two products. Despite that, after the first assessment (10 days after spraying) the untreated control plots gave statistically increased number of aphids in comparison to the treated plots using the two products. The third assessment (21 days after spraying) showed the same findings as the first assessment. However the second, fourth, fifth and sixth assessment of aphid numbers did not repeat these findings. Therefore there is a high probability that the findings of the third assessment for aphid numbers were a chance occurrence. The aphid counts assessment in the spring barley crop did not give definitive findings on the ability of either product to reduce aphid numbers. However the aphid numbers on this crop were very low, which would make it difficult to draw definite conclusion on the efficacy of the two products over each other and the untreated control.

The yield and grain quality analysis were also repeated on the post-harvested spring barley crop. The average grain yield per treatment results are shown in Table 7.8n. An ANOVA analysis was performed on the results, which showed that there was no statistical difference in grain yield for the eight different treatments ($p = 0.10$). The average thousand grain weights per treatment are shown in Table 7.8o and ANOVA analysis revealed that there was no statistical difference between the different treatments ($p = 0.16$).

The grain quality analysis results, which include average percentage grain <2.0 mm and <2.25 mm per treatment and average hectolitre weight per treatment are shown in Table 7.8p and 7.8q, respectively, for the spring barley crop. An ANOVA analysis was performed on the latter results obtained and revealed that the <2.0 mm ($p = 0.18$) and <2.25 mm ($p = 0.15$) grain fractions were not statistically different. An ANOVA analysis was also performed on the average hectolitre weight per treatment for spring

barley crop, which showed that there was no statistical difference between the eight different treatments ($p = 0.26$).

Table 7.8n Average grain yield per treatment for spring barley crop

Treatment no.	**Average grain yield per treatment (t/ha)**
1	9.05
2	9.00
3	9.05
4	8.92
5	9.05
6	9.14
7	8.86
8	9.06
L.S.D. at 5%	n.s.
C.V. (%)	2.23

Table 7.8o Average thousand grain weight per treatment for spring barley crop

Treatment no.	Average thousand grain weight per treatment (g)
1	51.82
2	52.49
3	51.19
4	51.00
5	52.19
6	53.67
7	49.87
8	51.10
L.S.D. at 5%	n.s.
C.V. (%)	3.38

Table 7.8p Average percentage grain <2.0 mm and >2.25mm per treatment for spring barley crop

Treatment no.	% grain <2.0 mm	% grain >2.25 mm
1	0.21	0.66
2	0.24	0.72
3	0.24	0.88
4	0.21	0.73
5	0.24	0.67
6	0.21	0.74
7	0.26	0.84
8	0.36	0.88
L.S.D. at 5%	n.s.	n.s.
C.V. (%)	44.35	29.33

Table 7.8q Average hectolitre weight per treatment for spring barley crop

Treatment no.	Average hectolitre weight per treatment (kg/hl)
1	67.40
2	67.60
3	66.85
4	66.25
5	68.12
6	67.05
7	65.52
8	66.87
L.S.D. at 5%	n.s.
C.V. (%)	2.04

Yet again there was no statistical difference between the two products, therefore there was no definite results to determine whether the suspension concentrate was more effective at controlling aphids in comparison to the commercially available emulsion concentrate. The high levels of rainfall, which occurred during the winter barley growing period, did not occur to the same extent during the spring barley growing period (Figure 7.8a). Also the temperatures during the summer months were higher than the average temperatures recorded, therefore it would be presumed that there would be heavy infestation of the spring barley crop by aphids (Figure 7.8b). However the aphid numbers during the aphid counting assessments in the summer months were very low. Therefore it is very difficult to draw conclusions on the results obtained from the field trial. As discussed it would be necessary to repeat the field trial again over a minimum of three years.

7.9 CONCLUSION

Cypermethrin was successfully formulated into a stable suspension concentration with considerable sustained release properties. This was achieved by firstly loading the pesticide into the halloysite tubules under vaccum, which gave a cheap method to microencapsulate the pesticide. The cypermethrin loaded halloysite was formulated into a suspension concentrate by firstly ensuring the halloysite particles were deflocculated. Halloysite forms a flocculated system in suspension because of the high electrostatic charges of the individual particles causing agglomeration to occur, which would ultimately result in blockage of the sprayer and also uneven dosing of diluted pesticide onto the cereal crop. *Dispex N40* (sodium polyacrylate), an anionic polyelectrolyte (0.25% w/w solid material) successfully deflocculated the halloysite particles by neutralising the positive edge charges on the halloysite particles, therefore preventing agglomeration occurring (flocculation). *Agrimer AL30* ((poly(vinylpyrrolidone/1-eicosene)), an adhesive agent was then incorporated into the suspension concentrate to ensure that when the diluted product was sprayed onto the barley crop the halloysite particles loaded with pesticide, remained attached to the barley leaves for a sufficient long period of time. The *in vitro* rainfastness study revealed that a concentration of 40% w/v *Agrimer AL30* was necessary to ensure that a large quantity of cypermethrin remained attached to the leaves. A suspending agent, xanthan gum was incorporated to prevent sedimentation of the halloysite particles, to aid thixotropy of the system and ultimately to ensure long term stability of the system. Despite that, it was found from these studies that the high concentration of adhesive agent, which was viscous in nature predominantly contributed to preventing sedimentation in the system because no sedimentation occurred over the xanthan gum range studied (0.05-0.2% w/v). However the lower level of xanthan gum (0.05% w/v) was incorporated to aid thixotropy because of it's gel network structure within the final formulation. Also the lower concentration of xanthan was more suitable to use because only 5 inversions of the graduated cylinders were necessary to ensure the product was uniformly redispersed, unlike the higher concentration (0.2% w/v), which needed 8 inversions to ensure redispersion.

If the number of inversions were too high, which was the case for the latter system, the system would prove impractical to mix for the operator and may result in incorrect dosing. The final suspension concentrate was compared to the commercially available emulsion concentrate, *Cyperkill*, *in vitro*, which showed that the suspension concentrate gave significantly improved sustained release of cypermethrin in comparison to the emulsion concentrate.

Because *in vitro* release studies bear little resemblance to *in field* conditions, the final suspension concentrate was also compared to the emulsion concentrate, *Cyperkill* in a winter and spring barley field trial, for aphid control. However the findings for both trials were inconclusive in determining whether the suspension concentrate was advantageous over the emulsion concentrate in controlling aphids. The aphid numbers were very low for both the winter and spring barley crops, which may be due to the high levels of rainfall preventing the aphids from reproducing/increasing in number. The low aphid numbers would not result in noticeable damage of the crops with BYDV infection and therefore the grain yield and quality between the untreated and treated plots would be undifferentiated making it difficult to draw conclusions of efficacy of products. The above trials needed to be repeated over at least three seasons to give useful results which definitive conclusions could be determined.

Chapter 8

General Discussion

The work of this thesis was to develop two sustained release products for use in agrochemical applications, utilising a naturally occurring mineral, halloysite. Halloysite is an aluminosilicate mineral, chemically similar to kaolin. However morphologically, it has a hollow tubular structure, unlike kaolin, which has a stacked-plate structure. This hollow cylindrical structure has the ability to entrap active agents within the internal lumen and also less extensively by chemisorption to the surface, which are gradually released, offering a sustained release mechanism. As discussed in Chapter 1, halloysite has shown sustained release in marine (Price and Gaber, 1997) and pharmaceutical applications (Price *et al.*, 2001; Levis and Deasy, 2003 and Kelly, 2003) but not as yet in an agrochemical application. This research investigated the ability of halloysite to give sustained release of two pesticides in plant protection.

Because halloysite's primary use function at present is within the ceramics industry where quality criteria do not necessarily specify which mineral morphology is present, commercial sources tend to produce mineral grades, which are poorly uniform in terms of average particle size and tubular content and also appear to contain large quantities of aggregated debris material in conjunction with intact tubules as illustrated in Section 4.3 by SEM examination. From a sustained release point of view, since we intended principally to examine the ability of halloysite to entrap and retain pesticides within it's tubular structure, thereby delaying release from this inherent tubular matrix at a controlled rate, we were interested in acquiring a highly tubular sample of the mineral. A number of different samples of the mineral, differing in terms of their tubule dimensions were sourced from various regions of the world. Small samples of halloysite were obtained from the Balikesir

and Konya region, Central Anatolia, Turkey and the Goushan region of China (Halloysite Balikesir, halloysite Konya and halloysite Goushan, respectively). The two primary sources of halloysite were provided by New Zealand China Clay Ltd, which is the world's largest current supplier of halloysite (halloysite G and halloysite Goushan NZCC).

Therefore the first section of this thesis was concerned with the physicochemical characterisation of halloysite samples from different regions of the world to ascertain which sample would be selected for utilisation in the agrochemical products. The five different halloysite samples were characterised using a number of techniques such as SEM, x-ray diffraction, helium pycnometry, surface area analysis and inductive coupled plasma-mass spectrometry. The physical strength of a number of halloysite samples was also determined.

The microscopic study by SEM showed that there was considerable variability in tubular dimensions between the five different halloysite samples. However the halloysite Goushan NZCC sample (supplied by New Zealand China Clay Ltd) had the highest tubular content with average tubular dimensions of 1.8 x 0.2 μm, which are seen by SEM in Figure 4.3c. The XRD analysis revealed that the five different halloysite samples were all present in a dehydrated state (7 Å-form). However the XRD plot of halloysite Balikesir sample differed in comparison to the other four samples in that it had two other peaks, which showed that the sample also contained traces of sepiolite. The skeletal density of halloysite Balikesir sample (2.6029 g/cm^3) also differed in comparison to the other four samples, which showed similar skeletal density measurements ranging between 2.4593 and 2.4978 g/cm^3. The surface area analysis study illustrated that the five different halloysite samples varied considerably from 15.34 m^2/g to 65.51 m^2/g. Thus the higher the specific surface per gram of halloysite sample the higher the content of smaller tubules. The level of trace elements was determined using the inductive coupled plasma-mass spectrometry, which showed there was high levels of iron present within the halloysite G sample. However this would be expected because it is widely known that iron is substituted

for aluminium within the gibbsite sheet of halloysite. Other elements present in large amounts were sodium, magnesium, barium, potassium and manganese. A number of different mechanical processes typical of a production setting were employed to determine the physical strength of the different halloysite samples. The halloysite Balikesir sample proved to be extremely robust with tubules remaining fully intact after both an autogenous grinding and milling process. However the halloysite Konya sample (with tubular dimensions of 2.4 x 0.2 μm) was unable to withstand the high impact processes, with tubule lengths reduced to <0.5 μm. The halloysite G tubules remained unaltered after exposure to a mortar and pestle and a ball mill at a vibration setting of 60%. However there was a reduction in tubule size on exposure of the halloysite sample to a ball mill at 90% vibration, rotar mill at a higher speed of 18000 rpm using a 0.5 mm blade and at 14000 and 18000 rpm speed settings using a 0.2 mm blade. An Alpine air jet sieve proved to be an effective technique at removing debris and unwanted agglomerates. Therefore, from these preliminary physicochemical characterisation studies, the halloysite Goushan NZCC sample was chosen for use in the agrochemical studies due to it's apparent uniform high tubular content.

The second section of this thesis was concerned with preliminary studies of halloysite powder and pellets for pharmaceutical and agrochemical applications. It was hoped that the information gained from this section could be used in subsequent sections for development of the agrochemical products and also to determine whether halloysite could be used in applications other than those explained in Chapter 1.

Deflocculation studies were carried out on halloysite suspensions (halloysite Balikesir), which form flocculated systems. Sodium citrate and cetrimide, an anionic and cationic deflocculating agent, respectively were studied as they are both widely used in the pharmaceutical industry to aid deflocculation. pH was also used as another method to deflocculate the halloysite suspensions. The use of sodium citrate as a deflocculating agent was also used as a method to determine whether the unwanted debris could be separated from the tubules in the deflocculated halloysite

suspensions. Sodium citrate successfully deflocculated the 0.25% halloysite suspension at a concentration of 100 μM. The anionic deflocculating agent dissolved in water to form both citrate and sodium ions. The trivalent citrate ions (negatively charged) worked by neutralising the positive 'edge' charges of the halloysite particles and in doing so caused the particles to be completely or nearly completely negatively charged. This prevented the halloysite particles from associating, which normally form an edge-to-face arrangement because of the electrical attraction of one tubule for another caused by the difference in the charge of the 'edges' and 'faces' of the particles. Sodium citrate also proved effective at separating the unwanted debris from the larger tubules. In the deflocculated halloysite suspensions using this deflocculating agent, the mainly unwanted debris remained indefinitely suspended in the supernatant, which could be discarded off, therefore proving that the deflocculation method had the potential to clean up halloysite samples containing unwanted debris.

The cationic deflocculating agent, cetrimide did not prove to be as successful at deflocculating halloysite suspensions. It was hoped that deflocculation of the halloysite suspensions would also be possible via neutralisation of the more extensive negative 'face' charges of the tubules. However, from the results obtained the halloysite suspensions remained flocculated, even after using an elevated cetrimide concentration of 300 mM. A reason for this was that the long hydrocarbon tail with the positive end charge may have caused complex formation and therefore ultimately flocculation of the halloysite suspension, rather than charge neutralisation of the negative 'face' charges, which would have given a deflocculated system. However, it was also noted that charge neutralisation of the extensive negatively charged 'faces' would prove more difficult than charge neutralisation of the positively charged smaller 'edges'.

Variations in pH successfully resulted in deflocculation of the halloysite suspensions. According to Theng and Wells (1995a) the net charge on the crystal edges, which is essentially composed of gibbsite (alumina), could be either negative or positive with

changes in pH due to the amphoteric nature of alumina. In acidic conditions, the halloysite particles associate together in an edge-to-face arrangement since the edges and faces are positively and negatively charged, respectively, causing flocculation. However in alkaline conditions, the halloysite particles tend to repel each other, since the charge on both the edge and the face surface was negative, causing deflocculation. Therefore an alkaline pH proved an effective method in deflocculating the halloysite suspensions.

These studies showed that halloysite could be successfully deflocculated using an anionic deflocculating agent (sodium citrate) and pH changes. Also these studies confirmed that halloysite tubules could be separated from the unwanted debris using the anionic deflocculating agent. The information gained here was successful in helping to select an appropriate deflocculating agent for the development of the cypermethrin loaded suspension concentrate in Chapter 7.

Isolated films containing halloysite were studied to determine whether halloysite could be used as an alternative filler type in films, using talc for comparative purposes. A permeation study was performed, which revealed that the addition of fillers resulted in a significant decrease in permeability of the films. However, halloysite gave less retardation of diltiazem HCl in comparison to talc, which may have been explained by focusing on particle morphology and alignment of the particles. Mechanical measurements such as tensile strength, elastic modulus and elongation at break were also carried out on the different films. Filler loading had a detrimental effect on tensile strength, with the addition of halloysite significantly reducing tensile strength in comparison to the addition of talc, which may also be explained by focusing on particle morphology and filler-polymer interactions. The addition of fillers resulted in a reduction in elastic modulus measurements, but they were not statistically different ($p>0.05$). There was a slight increase in elastic modulus between the talc and halloysite filled films, which may have been due to improved filler-polymer interaction and therefore increased rigidity of the polymer. There was a decrease in elongation at break with the addition of fillers to the films,

where the halloysite filled films gave slightly lower measurements, but they were not statistically different ($p>0.05$). The isolated film study showed that halloysite films gave similar results to kaolin films. However, the halloysite films gave slightly lower results. Therefore halloysite showed potential as an alternative to kaolin for use as a filler in film coatings.

A tabletting study was performed to determine the role of halloysite as a tablet excipient in comparison to kaolin, the widely used pharmaceutical excipient. The results revealed that the halloysite mix gave significantly improved flow properties in comparison to the kaolin mix, which was due to the similarly sized and shaped particles of the halloysite mix. This improved flowability of the halloysite mix lead to the production of superior tablets with improved uniformity of weight and thickness, which resulted in increased tablet hardness because of improved packing efficiency of the similarly shaped particles. There was a reduction in disintegration of the halloysite tablets in comparison to the talc tablets because of the mechanical interlocking of the halloysite particles, which inhibited water from penetrating the tablets. The dissolution study revealed similar findings, with an initial reduction in release of paracetamol from the halloysite tablets. From these studies halloysite showed potential as an alternative tablet excipient to talc with improved flow and binding properties and therefore the production of harder and superior tablets.

Mercury porosimetry was performed on halloysite G pellets, which were unheated and heated to 100°C, 200°C, 300°C and 1200°C. The pellets heated to 1200°C gave a significantly lower mercury intrusion volume and porosity in comparison to the other pellets, which was due to the elevated temperatures causing fusion of the halloysite particles into large masses with a virtual loss of intraparticulate voids within the halloysite tubules. Therefore these pellets were unsuitable for pesticide loading because of the reduced internal space within the pellets. The pellets heated above 200°C remained intact on addition to water, with only a slight reduction in porosity in comparison to the unheated pellets, therefore these pellets proved to be the most effective pellets for use in pesticide loading. The mercury porosimetry studies

revealed that increasing the temperature exposure of pellets, resulted in a reduction in the porosity of the pellets and therefore a reduction in pesticide loading capacity.

The third section of this thesis involved developing an agrochemical product, which was a pellet formulation used for vine weevil (*Otiorhynchus sulcatus*) control. Vine weevil attack a wide range of plants, including small fruits and ornamental crops. Damage is caused primarily by the larvae, whose feeding on the plant roots reduces vigour and, in heavy infestations, kills the plants. The adults feed on leaves, which is usually of no economic consequence, except where it detracts from the appearance of ornamentals (Garth and Shanks, 1978).

Changes in cultural practices and in the range of pesticides available have led to an increase in the occurrence and intensity of crop damage caused by this pest. However, the main reason for the increased prevalence of vine weevil is the current lack of effective and easily applied soil pesticides. Until the 1970s, the pest was controlled easily and effectively with persistent organochlorine pesticides, especially aldrin and DDT, incorporated in the soil before planting. It has long been known that the neonate and young larvae are much more susceptible to pesticides than are more mature larvae. The pre-planting incorporation of these low-cost, persistent pesticides into the entire rooting medium of the plant ensured that neonate larvae came immediately into contact with the pesticide. However, such pesticides were withdrawn from use in the UK in 1989 and equivalent alternatives have not been available since (Cross and Burgess, 1997). Adults have also proved difficult to control with currently available organophosphate and pyrethroid pesticides, even by repeated foliar applications at night.

Biological control provides an alternative option. Several species of entomopathogenic nematode (*Steinernema* and *Heterorhabditis* spp.) have been developed commercially for control of vine weevil larvae, mainly on protected ornamental plants. However, they are costly and not well adapted to the lower soil temperatures that occur in the field. The entomopathogenic fungus *Metarhizium*

anisopliae has also been shown to be effective on hardy ornamental nursery stock (Moorehouse *et al.*, 1993). However this technology has not been exploited for biological reasons.

There is, thus, a current need for effective treatments for control of this pest. In view of the successful control achieved previously by soil incorporation of persistent organochlorine pesticides, an option worthy of exploration is the use of granular or controlled release formulations of less persistent pesticides that are acceptable environmentally, with a view to increasing greatly their effective persistent (Cross and Burgess, 1997). This strategy has proved successful on containerised grown hardy nursery stock, where previous research (Buxton *et al.*, 1992 and Cross *et al.*, 1995) identified a controlled release pellet formulation of chlorpyrifos (*suSCon Green*) to be effective. Incorporation of the pellets into compost has now become standard practice in commercial hardy ornamental nursery stock production in the UK.

Consequently, it was decided to develop a sustained release pellet, which was environmentally friendly, cost effective, easy to produce and gave efficacious control of vine weevil for at least a 1 year period in comparison to the commercially available, expensive *suSCon Green* technology (microencapsulated plastic resin pellets). The pesticide chosen for use in the pellet formulation was chlorpyrifos because it is one of the leading pesticides available at present for control of vine weevil and also it is the pesticide used in the *suSCon Green* pellets, which therefore allowed both products to be directly compared *in vitro* and *in field*. The sustained release aspect of the pesticide was considered first. *suSCon Green* pellets have a sustained release period of chlorpyrifos for 2 years in peat mixes (Buxton *et al.*, 1992). In order to achieve this sustained release of chlorpyrifos in the newly developed pellets, the novel excipient halloysite was used. Halloysite has only recently been looked at for use in sustained delivery of active agents, but it has been shown to give effective sustained release of a variety of drugs (explained in detail in Chapter 1). It has the ability to give significantly improved sustained release of

poorly soluble active agents (Levis and Deasy, 2003). Because it is a naturally occurring material it offers a cheap method of microencapsulating active agents in order to provide sustained release. As discussed above the halloysite sample supplied by New Zealand China Clay Ltd (halloysite Goushan NZCC) was chosen for use in this agrochemical application because it had the maximum tubular content.

Therefore, the microtubular material was loaded with a chlorpyrifos solution (chlorpyrifos, 6.5% w/v in ethanol) using a vacuum, which displaced the air within the mineral tubules for pesticide. This loading process was repeated to ensure the halloysite tubules were optimally loaded with pesticide. Ethylene glycol was also incorporated within the pesticide solution for the initial pesticide loading process to neutralise the catalytic sites on the halloysite particles, therefore preventing premature decomposition of the chlorpyrifos.

The pesticide loaded halloysite was not a suitable formulation for direct delivery to the peat mixes because the dust created would have been hazardous to the operator and also there would have been difficulty in ensuring uniform mixing of the product. As a result, a pellet formulation had to be developed to prevent the above occurring, which had a blue pigment incorporated to ensure uniform mixing could be monitored. The chlorpyrifos loaded halloysite pellets were produced by extrusion-spheronisation and liquid paraffin was chosen as the granulating liquid because chlorpyrifos undergoes hydrolysis. The liquid paraffin further improved the sustained release properties of the chlorpyrifos loaded halloysite particles by ensuring the pellets remained fully intact for a prolonged period of time in the peat mix because it increased the hydrophobic nature of the pellets, therefore protecting the pellets from heavy rainfall. An ANOVA analysis (3^3 factorial design) was carried out to determine the correct liquid paraffin level, spheronization time and spheronization speed to give the optimum yield of pellets in the 850-1150 μm size range. From this study it was revealed that the best conditions for optimum pellet yield, was a liquid paraffin level of 21% and a spheronisation speed/time of 1000 rpm and 5 min, respectively. Therefore the final pellet formulation was composed of 78%

chlorpyrifos loaded halloysite, 21% liquid paraffin, 1% brilliant blue lake pigment, and contained 10.5% w/w chlorpyrifos.

An *in vitro* release study was performed on the chlorpyrifos loaded halloysite pellets (10.5% w/w), the *suSCon Green* pellets (10% w/w) and unformulated chlorpyrifos to determine the level of sustained release achieved by both products. A dissolution medium with 30:70, acetonitrile:McIlvaine buffer pH 4 (adjusted to pH 5) was selected to achieve sink conditions and also to mimic the pH 5 conditions of the peat mix. The *suSCon Green* pellets gave the greatest sustained release of chlorpyrifos. This could be explained by the fact that the *suSCon Green* pellets, which were composed of a 'hard' plastic resin that remain fully intact, where the halloysite pellets, which were 'soft', disintegrate on addition to the dissolution medium. However, the halloysite pellets gave improved sustained release of chlorpyrifos in comparison to the unformulated chlorpyrifos. This was explained by focusing on the halloysite particles within the pellet formulation. The chlorpyrifos was entrapped within the hollow internal lumen of the tubules and less extensively adsorbed to the surface.

An outdoor primrose pot trial was carried out at Kinsealy Research Centre, Teagasc, Dublin because *in vitro* release studies were not comparable with field conditions and gave no indication of the efficacy of the two products in controlling vine weevil. There were four different treatments (64 pots per treatment) within the trial: *suSCon Green* pellets (S1), chlorpyrifos loaded halloysite pellets at equivalent (H1) and double (H2) the rate to *suSCon Green* pellets, and an untreated control (C1). The primroses were assessed for foliage vigour, root vigour and larvae counts.

For larvae counts, the halloysite pellets (H1 + H2) gave the lowest larvae numbers per pot of 0.3 and 0.0, respectively, where the *suSCon Green* pellets gave an average of 0.6 larvae per pot. The untreated plants (C1) gave the highest number of larvae, with an average of 1.5 larvae per pot, which was expected as no pesticide was incorporated in the pots. Therefore the percentage larvae control for the halloysite

pellets (H1 and H2) was 80% and 100% respectively, where *suSCon Green* pellets (S1) only gave a percentage larvae control of 60%.

It is important that the product is effective in controlling the vine weevil larvae. However, it is also equally important to take into consideration the effect of a product on plant development. Therefore, in this study, the primroses were assessed for foliage and root vigour. The halloysite pellets (H2) gave significantly lower scores for root and foliage vigour compared to the other treatments, therefore indicating a certain level of phytotoxicity. The *suSCon Green* pellets gave root vigour scores, which were significantly better than the halloysite pellets. Thus, from these findings, it was concluded that the halloysite pellets (H1) showed definite improvements in controlling vine weevil in comparison to the commercially available *suSCon Green* pellets at equivalent rates. However it must be noted that the halloysite pellets (H1) showed definite signs of phytotoxicity of the root system.

This outdoor primrose pot trial only took place over a 32 week period rather than the 1 year planned period because the primroses were dying from *Botrytis cinerea* (fungal disease causing decay of the plants). Therefore it was difficult to draw definitive conclusions with regard to the efficacy of the newly developed chlorpyrifos loaded pellets over the commercially available *suSCon Green* pellets. The halloysite pellets (H1) showed potential for controlling vine weevil for 1 year because the pellets released the pesticide at a faster rate in comparison to the *suSCon Green* pellets, as indicated by the *in vitro* and *in field* results. Despite that, these halloysite pellets (H1) would be unlike to give sustained release of pesticide for 2 years like the *suSCon Green* pellets as it was expected that the pesticide would be completely released from the pellets within the first year. Thus the halloysite pellets (H1) showed potential for use in vine weevil larvae control in annual plants. However as discussed in Chapter 6, it may have been necessary to reduce the application rate of halloysite pellets (H1), instead of using equivalent rates to *suSCon Green* pellets (S1), half the application rate of halloysite pellets could have been adequate to give

similar vine weevil control to the *suSCon Green* pellets and avoid phytotoxicity of the root system.

There are a number of areas where future research could be carried out on this pellet formulation. Firstly, in relation to the sustained release properties of halloysite, these could have been improved by using cationic coating polymers (e.g chitosan). Chitosan is a naturally occurring material found in structural components of shellfish, insects and the walls of bacteria and mushrooms. It has been shown to be biocompatible and biodegradable (Hirano *et al.*, 1988 and Orienti *et al.*, 1996). This technology was utilised by Kelly, 2002 and Levis and Deasy, 2003 by binding the chitosan onto the anionic sites of halloysite to give an improved sustained release system (Chapter 1). Coating the chlorpyrifos loaded halloysite particles with this chitosan, would be a cheap biodegradable coating technique that could have made the halloysite pellets comparable with the commercially available *suSCon Green* pellets in terms of extending release of the pesticide over a longer period of time and avoiding unnecessary phytotoxicity.

The level of ethylene glycol added to the pesticide solution during the loading process of the halloysite tubules was not optimised to determine the exact quantity that was needed to completely neutralise the 'acid sites' on the halloysite particles to prevent hydrolysis of chlorpyrifos occurring. Therefore studies could be performed on the different samples of halloysite to ascertain the precise quantity of ethylene glycol that was needed to prevent premature hydrolysis of chlorpyrifos. Temperature studies could also have been performed on the chlorpyrifos loaded halloysite samples to determine the maximum temperature and the length of time the samples could be exposed to these temperatures, before significant hydrolysis of chlorpyrifos occurred. Thus, the halloysite pellets could have been dried at temperatures above room temperature (20°C), which would have resulted in 'harder' pellets that could have stayed intact for a longer period of time, similar to the plastic resin *suSCon Green* pellets. This could have further extended the sustained release properties of the chlorpyrifos loaded halloysite pellets.

There are also a number of improvements, which could be considered for the outdoor primrose pot trial. The experimental method used in this trial had a number of shortfalls, which lead to a large number of the pots in the untreated control having no larvae, thus giving results for the treatments that were not statistically different. Therefore artificial augmentation of the pots with a known number of eggs would ensure that each potted plant in the untreated control had a high level of larvae infestation compared to the other treatments. However the method used in the July 2002 pot trial had the advantage of closely simulating normal conditions, but the interval between treatment and infestation was difficult to precisely control and plots were unlikely to be evenly dosed with eggs. The artificial augmentation method used would be more precise in this respect, where a known number of eggs would be added to each pot.

The pot trial was assessed prematurely because the plants were exposed to fungal diseases such as grey mould (*Botrytis cinerea*) due to excessive levels of rainfall. Therefore a future plan could be to perform the trial in an indoor setting e.g. glasshouse. The warmer indoor conditions would result in better survival of eggs and therefore larger numbers of larvae being recovered from untreated controls. An outdoor setting exposes eggs to high levels of rainfall and low temperature, therefore more likely to result in poor egg survival. These improvements should ensure that there would be higher numbers of larvae present within the untreated control and therefore the trial will be more likely to result in statistically significant results for the different treatments.

The fourth and final section of this thesis involved developing an agrochemical product, which was a suspension concentrate formulation used for aphid control in cereal crops. Aphids feed by piercing plant tissues with needle-like mouth parts, then suck plant juices, which causes stunted and distorted (twisted, curled or swollen) growth of flowers, shrubs and trees. Occasionally, aphids may actually kill leaves or small shoots. Since aphids can move from one plant to another, they may transmit plant diseases or pathogens, which contaminate the sucking mouthparts and are

passed onto the plant during feeding. Perhaps the biggest aphid-related problem of cereal crops is the potential for transmission of barley yellow dwarf virus (BYDV). BYDV causes barley to turn a yellowish colour and infected plants become stunted and non-productive. Yield losses due to BYDV can be severe with considerable economic loss to the grower. With the continuing intensification of cereal crops, the incidence and importance of BYDV has become a major consideration among arable farmers. A number of cultural (Section 1.4.6.1) and biological (Section 1.4.6.2) control methods can be used to curtail the spread of aphids on a small scale (e.g. within the garden), but this is not possible in large cereal crops. Therefore at present the use of pesticides is the only option available to prevent widespread infestation of aphids within cereal crops.

Synthetic pyrethroids are very effective treatments for controlling aphid vectors and thus reduce the incidence of BYDV in cereal crops (Brain and Hewson, 1984). The most widely used pyrethroids in Ireland for aphid control are cypermethrin and deltamethrin. However, the pyrethroids are only available commercially as emulsion concentrates for aphid control on cereal crops. The emulsion concentrate versions of these pesticides are prone to photolysis and also will only remain attached to the cereal leaves for a short period of time, due to wash off by rainfall, which makes respraying of the crop necesssary. Therefore it was decided to develop a suspension concentrate of these pyrethroids i.e. cypermethrin, which remained attached to the cereal leaves for a prolonged period of time, gave sustained release of the pesticide and was protected from photolysis in comparison to the commercially available emulsion concentrate, *Cyperkill*.

Halloysite was yet again utilised to give sustained release of the pesticide. Cypermethrin (5% w/v in ethanol solution) was loaded into the halloysite tubules using a similar technique as for the chlorpyrifos pesticide. The halloysite was double loaded to ensure maximum loading of the tubules with pesticide. It was not possible to apply this cypermethrin loaded halloysite to the barley crops, which was subsequently formulated into a suspension concentrate.

It was firstly necessary to ensure the cypermethrin loaded halloysite particles could be deflocculated in suspension to prevent blockage of the sprayer, which may have resulted in incorrect dosing of the pesticide. Two deflocculating agents were chosen to deflocculate the halloysite suspension, which were *Dispex N40* (sodium polyacrylate) and *Atlox 4913* (methylmethacrylate graft copolymer), a polyelectrolyte and a graft copolymer, respectively. The cypermethrin loaded halloysite suspension was successfully deflocculated using 0.25% w/w *Dispex N40* deflocculating agent, which was equivalent to 0.062 g of the deflocculating solution in 50 ml deionised water (containing 10 g cypermethrin loaded halloysite). This deflocculating agent worked by neutralising the positive 'edge' charges of the halloysite particles, therefore preventing edge-to-face arrangement of the tubules and causing deflocculation of the tubules. However, *Atlox 4913* proved to be unsuccessful in deflocculating the cypermethrin loaded halloysite suspension. *Atlox* 4913 is composed of two main groups A and B, where A has a strong affinity to the particle surface (hydrophobic surfaces) and B is a group that provides the steric stabilisation, being highly solvated by the continuous medium. The halloysite tubules are strongly electrostatically charged, therefore the A group did not strongly adsorb onto their surfaces, thus as a result group B (polyethylene glycol chains) was not able to align properly to give steric stability, which ultimately resulted in flocculation of the halloysite suspension.

Secondly, an adhesive agent (*Agrimer AL30*) needed to be added to the suspension concentrate, which ensured the cypermethrin loaded halloysite particles when diluted remained on the barley leaves for a prolonged period of time in comparison to the commercially available emulsion concentrate, *Cyperkill*. Rainfastness studies were performed using varying quantities of adhesive agent to ensure the optimum level was chosen for the final suspension concentrate formulation. The suspension concentrate containing the highest concentration of adhesive agent (40% w/v) gave the highest quantity of cypermethrin remaining on the leaves (78.1%/cm^2), while the suspension concentrate containing no adhesive gave the lowest quantity of

cypermethrin remaining on the leaves after the simulated rain was applied (27.2%/cm^2). The commercially available emulsion concentrate only had 27.5%/cm^2 cypermethrin remaining on the leaves, which indicated the inability of the commercially available emulsion concentrate to retain the cypermethrin on the leaves for a period of time in the presence of rain compared to the suspension concentrate systems. Consequently, from this rainfastness study, 40% w/v *Agrimer AL30* was added to the final suspension concentrate. A higher quantity of adhesive agent was not used because of difficulty in effectively shaking the container and uniformity of mixing.

Thirdly, it was necessary to add a suspending agent to the suspension concentrate to preventing sedimentation of the halloysite particles in suspension. Xanthan gum, which is commonly used in the agrochemical industry, was chosen as the suspending agent of choice in this suspension concentrate. Xanthan gum ranging from 0.05-0.2% w/v was added to the suspension concentrates in graduated cylinders, which were observed over a six month period to determine if sedimentation/separation of the systems occurred. All the suspension concentrates remained uniformly suspended after 6 months, with sedimentation volume ratios of 1.00. Ease of redispersion of the suspension concentrates was also assessed after six months, which showed that the suspension concentrate containing 0.05% w/v was the most easily redispersed, with only 5 inversions of the graduated cylinder necessary to ensure uniform redispersion. Therefore from this study, 0.05% w/v of xanthan gum was added to the final suspension concentrate. Despite that the high concentration of *Agrimer AL30* (40% w/v) within the suspension concentrate had a major role to play in preventing sedimentation of the halloysite particles because from these studies it was seen that no sedimentation occurred over the concentration range of xanthan gum used. However the lowest level of xanthan gum was used in the suspension concentrate to aid the adhesive agent in ensuring the system had appropriate thixotropy and therefore long term stability and ease of use.

Rheological measurements were performed to gain information on the properties of the suspension concentrates, which included continuous shear (destructive test) and dynamic oscillatory measurements (non destructive test). Continuous shear and dynamic oscillatory measurements were performed on suspension concentrates containing varying concentrations of *Agrimer AL30* and xanthan gum. The flow curve (t = 0) of the final suspension concentrate (contained 40% w/v *Agrimer AL30* and 0.05 % w/v xanthan gum) showed the suspension concentrate was pseudoplastic in nature. There was a hysteresis loop present, which indicated that breakdown of structure occurred within the suspension concentrate on application of shear stress. Using the same sample of final suspension concentrate, successive flow curves were obtained at 30, 90 and 180 min after commencement of the experiment, which helped determine whether thixotropy or irreversible shear breakdown of the sample occurred. The area within the hysteresis loops of these flow curves increased as the time for recovery increased, therefore indicating that the suspension was recovering to its initial ground state (t = 0). However, the final suspension concentrate did not completely return to initial ground state within the time period of the experiment. Therefore it was uncertain whether the system would completely return to ground state over an extended period of time.

The flow curve of suspension concentrate with 20% w/v *Agrimer AL30* was similar in shape to the final suspension concentrate, displaying pseudoplastic behaviour. However the suspension concentrate containing 60% w/v *Agrimer AL30* displayed plastic behaviour. It was noted that as the concentration of *Agrimer AL30* was increased the flow curves were displaced to the x-axis, therefore indicating that an increase in apparent viscosity of the suspension concentrates occurs with increasing concentrations of *Agrimer AL30*. Dynamic oscillatory measurements were also performed on the suspension concentrates containing 20% w/v and 60% w/v *Agrimer AL30*, which showed similar trends to the final suspension concentrate (viscoelastic structured systems). However as the concentration of *Agrimer AL30* increased the values for G' (storage modulus), G" (loss modulus), G* (complex modulus) and δ

(phase angle shift) increased, which indicated that the suspension concentrates were becoming more structured in nature.

The flow curves for the suspension concentrates containing varying concentrations of xanthan gum (0.1% w/v to 0.2% w/v) all showed pseudoplastic behaviour. Yet again as shown with varying concentrations of adhesive agent, the flow curves were displaced to the x-axis as the concentration of xanthan gum was increased. Dynamic oscillatory measurements were also performed on the suspension concentrates containing varying concentrations of xanthan gum, which showed similar trends to the varying concentrations of adhesive agent study, but the differences were not as pronounced for G', G", G* and δ because of the very low levels of xanthan gum used in comparison to the levels of *Agrimer AL30* used.

The final suspension concentrate contained 2% w/v cypermethrin, whereas the commercially available emulsion concentrate contained 10% w/v. An *in vitro* release study was performed on the two products, using a dissolution medium containing 30:70 methanol:McIlvaine buffer pH 4 over a 21 day period, to determine the level of sustained release achieved by the suspension concentrate in comparison to the emulsion concentrate of cypermethrin. The release study for the emulsion concentrate, was repeated using 100 ml of dissolution medium and an extra 7 ml of deionised water to take into account the difference in volume and concentration gradient between the emulsion concentrate and the suspension concentrate. The halloysite suspension concentrate showed the greatest sustained release of cypermethrin because the pesticide was entrapped within the hollow internal lumen of the halloysite particles. The slower release of pesticide from the emulsion concentrate with the extra deionised water in comparison to the emulsion concentrate without the deionised water within the dissolution medium was caused by the decrease in concentration gradient between the two phases.

Winter and spring barley field trials were carried out at the University College Dublin farm at Lyons Research Farm, Newcastle, Co. Dublin to determine the ability of the

two products to control aphids under natural conditions. There were eight different treatments in the winter and spring barley trials, which were fully randomised. The crops were assessed for aphid numbers, BYDV, yield and grain quality. The winter barley crop showed no statistical difference between the eight treatments for aphid counts ($p>0.05$). The winter barley was also assessed for % BYDV, which showed that the untreated control gave statistically different results in comparison to the other seven treatments ($p<0.05$). However, there was a high probability that the increased % BYDV infection on the untreated control in comparison to the other treatments was a random occurrence because the aphid counts were not statistically significant for the different treatments (BYDV is spread by virulent aphids). The yield (yield of grain and thousand grain weight) and grain quality (grain size and hectolitre weight) analysis were performed on the harvested grain from each of the plots within the treatments, which were not statistically different ($p>0.05$). Therefore the winter barley field trial did not ascertain whether the suspension concentrate was advantageous in comparison to the commercially available emulsion concentrate for aphid control.

A spring barley field trial was also carried out to determine whether differences between the two products could be observed. Aphid numbers were counted for the first time after 10 days after the crop was pesticide sprayed, which showed that there was statistical difference between the untreated control and the seven different treatments ($p<0.05$). However there was no statistical difference between the different treated plots, thus indicating that there was no difference between the two products in controlling aphid numbers. The aphid numbers were counted for the second time after 21 days post pesticide spraying, which showed that there was no statistical difference between the eight different treatments ($p>0.05$). The third aphid count was carried out 30 days after pesticide spraying, which revealed that there was statistical difference between the eight treatments ($p = 0.00$). The untreated control gave statistically higher numbers of aphids to the other seven treatments, but there was no statistical difference between the seven different treatments. The fourth, fifth and sixth aphid count assessments were performed after 37 days, 45 days and 61

days, respectively, but there was no statistical difference between the eight treatments ($p>0.05$). The yield and grain quality analysis was performed on the different treatments, but there was also not statistically difference ($p>0.05$). Yet again there was no indication whether the suspension concentrate was superior to the commercially available emulsion concentrate for aphid control.

Other areas of research that would be worthwhile would be to avoid using xanthan gum in the final suspension concentrate formulation to prevent sedimentation. There was a high probability that because of the very low concentration of xanthan gum used in the formulation (0.05% w/v) and the complete absence of sedimentation after 6 months that the presence of xanthan gum was unnecessary within the final formulation. The high level of *Agimer AL30* (40% w/v), which had a very viscous consistency, would have probably prevented sedimentation completely without the presence of xanthan gum.

When carrying out aphid field trials, it must be taken into consideration that aphids are winged insects, which can cause damage to crops in random locations, so therefore results from these trials proved difficult to analyse. Future work in this area would be to repeat the above field trials for a number of years in different locations around the country. For example the field trials could be repeated at a coastal region, because aphids are found in larger numbers near the coast. A shortfall with these trials was that only small numbers of aphids were found on the winter and spring barley crops, which made it very difficult to draw definitive conclusions with regard to the efficacy of the suspension concentrate over the commercially available emulsion concentrate.

Overall the two agrochemical formulations developed showed advantages over the commercial products available on the market. The chlorpyrifos loaded halloysite pellets showed improved ability to control vine weevil larvae in potted primroses in comparison to the *suSCon Green* pellets. The cypermethrin loaded halloysite suspension concentrate showed improved sustained release and rainfastness of

cypermethrin *in vitro* in comparison to the cypermethrin emulsion concentrate *(Cyperkill)*, but *in field* (winter and spring barley trials) there was no conclusive evidence to suggest whether the newly developed suspension concentrate was superior to the commercially available emulsion concentrate at controlling aphids. The barley trials needed to be repeated for a number of years to ascertain efficacy of the new product in comparison to the older developed product.

Halloysite showed definite potential in giving sustained release of pesticides in two agrochemical applications. Collectively the two novel agrochemical products developed gave improved sustained release in comparison to the commercially available products. Therefore, in the future, the halloysite technology could be utilised in a number of different agrochemical applications, other than those mentioned above. From this thesis in can be concluded that halloysite not only has potential for use in marine and pharmaceutical application, but also in agrochemical applications for sustained delivery of active agents.

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Appendices

Appendix 1 Quantitative analysis of halloysite G using inductive coupled plasma mass spectrometry (ICP-MS)

Element	Quantity (ppm)
Iron	1360
Sodium	469.40
Magnesium	391.40
Barium	384.80
Potassium	119.20
Manganese	76.31
Cerium	59.91
Copper	52.01
Lead	48.69
Gallium	35.56
Gold	28.66
Chromium	22.07
Iridium	21.24
Cobalt	20.08
Scandium	18.55
Tungsten	17.56
Molybdenium	17.07
Neodymium	15.67
Nickel	15.39
Lanthanum	14.43
Strontium	13.94
Praseodymium	13.77
Samarium	13.59
Zirconium	13.08
Yttrium	12.97
Europium	12.94
Gadolinium	12.77
Titanium	12.29
Terbium	12.17
Dysprosium	12.06

Element	Quantity (ppm)
Holmium	11.96
Erbium	11.84
Thulium	11.80
Lutetium	11.76
Ytterbium	11.61
Hafnium	9.34
Ruthenium	8.43
Platinum	7.57
Silver	6.53
Niobium	6.00
Uranium	4.89
Selenium	4.23
Zinc	3.88
Thorium	3.69
Vanadium	3.52
Arsenic	3.46
Lithium	2.45
Boron	2.25
Palladium	1.93
Rhenium	1.79
Tin	<1.95
Selenium	1.50
Tellerium	1.03

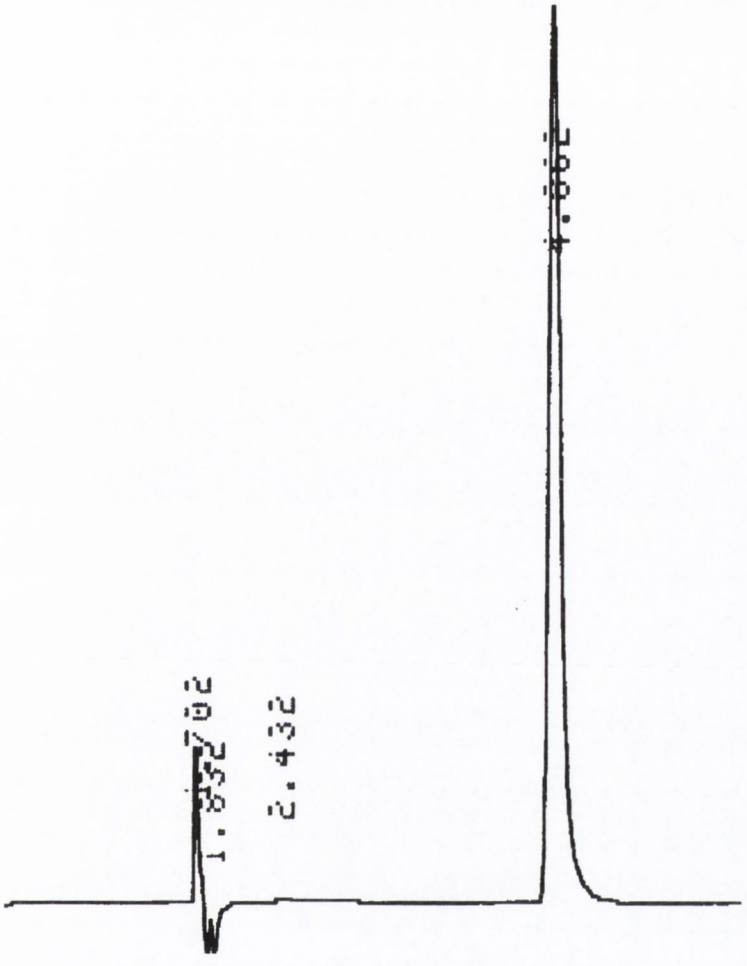
Element	Quantity (%)
Calcium	0.80

Element	Quantity (ppb)
Tantalum	850
Germanium	735.9
Cadmium	217.3
Rhodium	145.2
Berylium	98.53
Mercury	87.61

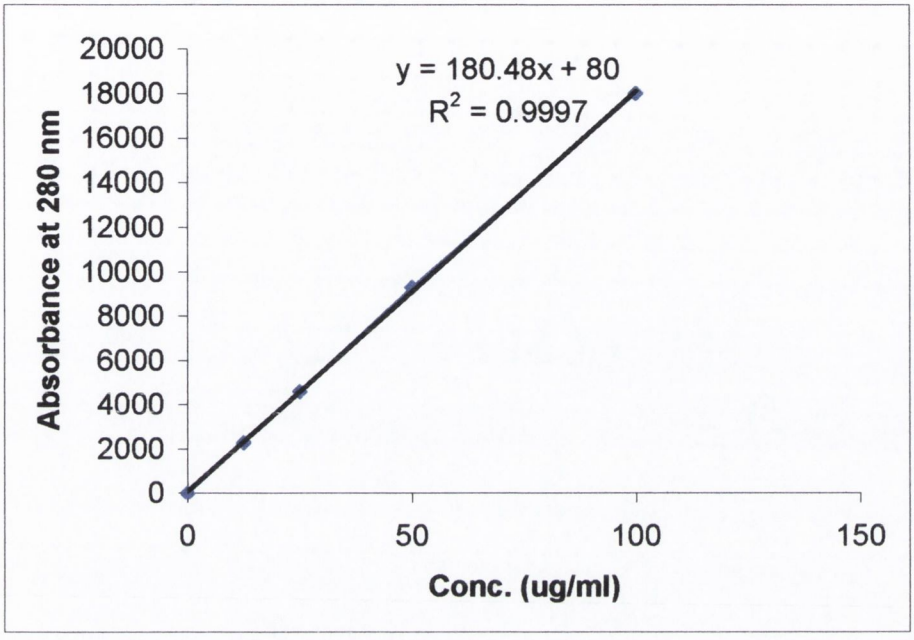
Appendix 2 Pressure Table used with PoreSizer 9320

Point	Pressure (Psia)	Point	Pressure (Psia)	Point	Pressure (Psia)	Point	Pressure (Psia)
1	10	45	560	89	1440	133	6500
2	15	46	580	90	1460	134	7000
3	20	47	600	91	1480	135	7500
4	25	48	620	92	1500	136	8000
5	30	49	640	93	1520	137	8500
6	35	50	660	94	1540	138	9000
7	40	51	680	95	1560	139	9500
8	45	52	700	96	1580	140	10000
9	50	53	720	97	1600	141	11000
10	55	54	740	98	1620	142	12000
11	60	55	760	99	1640	143	13000
12	65	56	780	100	1660	144	14000
13	70	57	800	101	1680	145	15000
14	75	58	820	102	1700	146	16000
15	80	59	840	103	1720	147	18000
16	85	60	860	104	1740	148	19000
17	90	61	880	105	1760	149	20000
18	95	62	900	106	1780	150	22000
19	100	63	920	107	1800	151	24000
20	110	64	940	108	1820	152	26000
21	120	65	960	109	1840	153	28000
22	130	66	980	110	1860	154	30000
23	140	67	1000	111	1880	155	29000
24	150	68	1020	112	2000	156	27000
25	160	69	1040	113	2050	157	25000
26	180	70	1060	114	2100	158	23000
27	200	71	1080	115	2150	159	21000
28	220	72	1100	116	2200	160	19000
29	240	73	1120	117	2250	161	17000
30	260	74	1140	118	2300	162	15000
31	280	75	1160	119	2400	163	13000
32	300	76	1180	120	2500	164	11000
33	320	77	1200	121	2700	165	9000
34	340	78	1220	122	2900	166	7000
35	360	79	1240	123	3100	167	5000
36	380	80	1260	124	3300	168	3000
37	400	81	1280	125	3500	169	1000
38	420	82	1300	126	3700	170	500
39	440	83	1320	127	4000	171	250
40	460	84	1340	128	4300	172	100
41	480	85	1360	129	4600	173	50
42	500	86	1380	130	5000	174	30
43	520	87	1400	131	5500	175	15
44	540	88	1420	132	6000		

Appendix 3 Sample chromatogram of chlorpyrifos, obtained using UV detection



Appendix 4 Sample HPLC calibration curve for chlorpyrifos using UV detection



Appendix 5 Randomised layout for outdoor primrose pot trial

Row	T	T	T	T	T	T	T	T
1	4	4	1	2	2	4	2	1
2	3	3	2	4	4	1	2	3
3	2	2	4	3	3	4	2	1
4	3	3	1	2	2	1	2	4
5	3	3	2	4	4	3	4	1
6	2	2	4	3	3	4	1	3
7	1	1	3	2	2	1	2	3
8	3	3	2	1	1	1	3	2
9	3	3	2	4	4	2	3	1
10	1	1	2	4	4	1	3	4
11	1	1	3	2	2	3	1	4
12	3	3	4	2	2	2	1	4
13	1	1	4	2	2	3	4	1
14	2	2	3	4	4	2	1	4
15	2	2	3	4	4	1	3	2
16	3	3	1	2	2	4	2	1
17	4	4	1	2	2	3	2	1
18	3	3	1	2	2	3	1	2
19	3	3	1	2	2	1	3	4
20	3	3	1	2	2	3	2	4
21	2	2	1	4	4	2	1	4
22	3	3	2	1	1	2	1	3
23	4	4	1	2	2	3	1	2
24	4	4	1	2	2	3	2	1
25	2	2	4	3	3	1	3	4
26	2	2	4	1	1	3	2	1
27	1	1	3	4	4	1	2	4
28	2	2	1	4	4	1	2	4
29	1	1	4	3	3	3	1	4
30	4	4	3	1	1	2	4	3
31	2	2	1	4	4	2	3	4
32	1	1	4	3	3	3	4	2

T = Treatment No.

1 = *suSCon Green* pellets
2 = Halloysite pellets
3 = Halloysite pellets (double rate)
4 = Untreated Control (no pellets)

Appendix 6 Assessment of the foliage vigour of each individual plant in December, 2002

Row	T	FV	T	FV	T	FV	T	FV	T	FV	T	FV	T	FV	T	FV
1	**3**	7	**4**	8	**1**	7	**2**	0	**3**	0	**4**	8	**2**	5	**1**	6
2	**1**	9	**3**	8	**2**	8	**4**	7	**4**	6	**1**	5	**2**	9	**3**	0
3	**1**	8	**2**	8	**4**	7	**3**	7	**3**	1	**4**	6	**2**	8	**1**	7
4	**4**	6	**3**	5	**1**	7	**2**	7	**3**	8	**1**	7	**2**	7	**4**	5
5	**1**	7	**3**	1	**2**	7	**4**	8	**2**	9	**3**	8	**4**	5	**1**	4
6	**1**	8	**2**	8	**4**	8	**3**	6	**2**	6	**4**	7	**1**	6	**3**	2
7	**4**	7	**1**	6	**3**	6	**2**	7	**4**	8	**1**	7	**2**	7	**3**	5
8	**4**	8	**3**	8	**2**	8	**1**	8	**4**	8	**1**	7	**3**	4	**2**	7
9	**1**	8	**3**	6	**2**	8	**4**	5	**4**	6	**2**	3	**3**	2	**1**	5
10	**3**	7	**1**	6	**2**	6	**4**	7	**2**	6	**1**	5	**3**	5	**4**	5
11	**4**	0	**1**	6	**3**	3	**2**	7	**2**	8	**3**	5	**1**	8	**4**	8
12	**1**	8	**3**	0	**4**	8	**2**	6	**3**	0	**2**	8	**1**	2	**4**	7
13	**3**	9	**1**	7	**4**	8	**2**	7	**2**	7	**3**	7	**4**	6	**1**	6
14	**1**	9	**2**	7	**3**	2	**4**	7	**3**	9	**2**	8	**1**	0	**4**	4
15	**1**	8	**2**	8	**3**	7	**4**	9	**4**	9	**1**	7	**3**	0	**2**	5
16	**4**	9	**3**	9	**1**	8	**2**	8	**3**	5	**4**	5	**2**	8	**1**	7
17	**3**	4	**4**	8	**1**	5	**2**	7	**4**	7	**3**	5	**2**	8	**1**	6
18	**4**	8	**3**	4	**1**	9	**2**	8	**4**	8	**3**	7	**1**	7	**2**	9
19	**4**	9	**3**	2	**1**	4	**2**	3	**2**	6	**1**	7	**3**	8	**4**	8
20	**4**	9	**3**	6	**1**	6	**2**	4	**1**	4	**3**	9	**2**	7	**4**	7
21	**3**	3	**2**	5	**1**	4	**4**	8	**3**	8	**2**	9	**1**	5	**4**	9
22	**4**	6	**3**	1	**2**	6	**1**	4	**4**	6	**2**	8	**1**	8	**3**	7
23	**3**	5	**4**	8	**1**	7	**2**	8	**4**	6	**3**	9	**1**	8	**2**	9
24	**3**	0	**4**	7	**1**	7	**2**	8	**4**	5	**3**	3	**2**	4	**1**	2
25	**1**	6	**2**	7	**4**	9	**3**	3	**2**	8	**1**	6	**3**	5	**4**	9
26	**3**	10	**2**	8	**4**	7	**1**	6	**4**	9	**3**	9	**2**	9	**1**	9
27	**2**	9	**1**	5	**3**	2	**4**	4	**3**	9	**1**	7	**2**	7	**4**	9
28	**3**	0	**2**	8	**1**	7	**4**	8	**3**	10	**1**	7	**2**	10	**4**	5
29	**2**	10	**1**	7	**4**	8	**3**	7	**2**	10	**3**	5	**1**	6	**4**	9
30	**2**	6	**4**	6	**3**	7	**1**	9	**1**	4	**2**	1	**4**	9	**3**	0
31	**3**	6	**2**	6	**1**	7	**4**	8	**1**	1	**2**	10	**3**	2	**4**	10
32	**2**	9	**1**	5	**4**	10	**3**	10	**1**	6	**3**	5	**4**	10	**2**	3

FV = Foliage vigour, graded from 0 to 10 (0 = dead, 10 = excellent vigour)

T = Treatment No. **1** = *suSCon Green* pellets
2 = Halloysite pellets
3 = Halloysite pellets (double rate)
4 = Untreated control

Appendix 7 Assessment of the foliage vigor of each individual plant in February, 2003

Row	T	FV	T	FV	T	FV	T	FV	T	FV	T	FV	T	FV	T	FV
1	**3**	3	**4**	6	**1**	7	**2**	0	**3**	0	**4**	7	**2**	5	**1**	7
2	**1**	7	**3**	4	**2**	7	**4**	7	**4**	7	**1**	8	**2**	7	**3**	0
3	**1**	3	**2**	5	**4**	8	**3**	5	**3**	0	**4**	6	**2**	5	**1**	5
4	**4**	1	**3**	2	**1**	0	**2**	6	**3**	5	**1**	8	**2**	3	**4**	4
5	**1**	10	**3**	1	**2**	5	**4**	9	**2**	4	**3**	4	**4**	5	**1**	5
6	**1**	7	**2**	3	**4**	7	**3**	5	**2**	4	**4**	9	**1**	1	**3**	5
7	**4**	7	**1**	10	**3**	7	**2**	5	**4**	8	**1**	9	**2**	3	**3**	6
8	**4**	4	**3**	3	**2**	6	**1**	8	**4**	8	**1**	9	**3**	3	**2**	1
9	**1**	8	**3**	2	**2**	9	**4**	5	**4**	2	**2**	2	**3**	1	**1**	8
10	**3**	7	**1**	7	**2**	5	**4**	8	**2**	8	**1**	10	**3**	1	**4**	8
11	**4**	3	**1**	10	**3**	3	**2**	3	**2**	2	**3**	7	**1**	10	**4**	10
12	**1**	9	**3**	0	**4**	8	**2**	8	**3**	0	**2**	5	**1**	0	**4**	7
13	**3**	8	**1**	10	**4**	9	**2**	5	**2**	8	**3**	7	**4**	7	**1**	9
14	**1**	10	**2**	7	**3**	2	**4**	4	**3**	3	**2**	8	**1**	0	**4**	2
15	**1**	8	**2**	7	**3**	4	**4**	8	**4**	10	**1**	7	**3**	1	**2**	5
16	**4**	10	**3**	6	**1**	10	**2**	6	**3**	2	**4**	4	**2**	8	**1**	8
17	**3**	4	**4**	7	**1**	6	**2**	4	**4**	10	**3**	4	**2**	5	**1**	10
18	**4**	9	**3**	4	**1**	10	**2**	10	**4**	9	**3**	6	**1**	10	**2**	10
19	**4**	8	**1**	1	**1**	9	**2**	6	**2**	1	**1**	10	**3**	8	**4**	9
20	**4**	9	**8**	8	**1**	8	**2**	3	**1**	9	**3**	7	**2**	5	**4**	2
21	**3**	3	**7**	7	**1**	10	**4**	7	**3**	6	**2**	7	**1**	2	**4**	10
22	**4**	5	**1**	1	**2**	10	**1**	8	**4**	9	**2**	8	**1**	6	**3**	3
23	**3**	1	**7**	7	**1**	2	**2**	6	**4**	5	**3**	10	**1**	5	**2**	10
24	**3**	0	**10**	10	**1**	9	**2**	5	**4**	10	**3**	7	**2**	3	**1**	2
25	**1**	10	**5**	5	**4**	9	**3**	3	**2**	6	**1**	10	**3**	1	**4**	10
26	**3**	6	**6**	6	**4**	7	**1**	10	**4**	10	**3**	2	**2**	7	**1**	10
27	**2**	9	**10**	10	**3**	1	**4**	8	**3**	10	**1**	9	**2**	3	**4**	5
28	**3**	0	**4**	4	**1**	7	**4**	6	**3**	3	**1**	8	**2**	4	**4**	7
29	**2**	4	**5**	5	**4**	8	**3**	2	**2**	2	**3**	4	**1**	3	**4**	6
30	**2**	1	**3**	3	**3**	10	**1**	10	**1**	9	**2**	0	**4**	8	**3**	0
31	**3**	3	**4**	4	**1**	7	**4**	7	**1**	1	**2**	2	**3**	6	**4**	8
32	**2**	1	**8**	8	**4**	10	**3**	7	**1**	8	**3**	2	**4**	1	**2**	5

RV = Root vigour, graded from 0 to 10 (0 = dead, 10 = excellent vigour)

T = Treatment No. **1** = *suSCon Green* pellets
2 = Halloysite pellets
3 = Halloysite pellets (double rate)
4 = Untreated control

Appendix 8 Assessment of the root vigour of each individual plant in February, 2003

Row	T	RV	T	RV	T	RV	T	RV	T	RV	T	RV	T	RV	T	RV
1	**3**	8	**4**	7	**1**	4	**2**	0	**3**	0	**4**	8	**2**	5	**1**	7
2	**1**	8	**3**	7	**2**	7	**4**	8	**4**	4	**1**	7	**2**	9	**3**	0
3	**1**	4	**2**	5	**4**	5	**3**	9	**3**	2	**4**	8	**2**	1	**1**	3
4	**4**	3	**3**	5	**1**	0	**2**	4	**3**	5	**1**	4	**2**	3	**4**	4
5	**1**	9	**3**	2	**2**	6	**4**	9	**2**	10	**3**	10	**4**	4	**1**	5
6	**1**	8	**2**	5	**4**	9	**3**	9	**2**	2	**4**	9	**1**	5	**3**	6
7	**4**	6	**1**	8	**3**	9	**2**	5	**4**	9	**1**	7	**2**	4	**3**	8
8	**4**	7	**3**	8	**2**	5	**1**	5	**4**	9	**1**	9	**3**	8	**2**	1
9	**1**	9	**3**	4	**2**	9	**4**	2	**4**	5	**2**	8	**3**	4	**1**	9
10	**3**	8	**1**	0	**2**	7	**4**	10	**2**	8	**1**	7	**3**	4	**4**	8
11	**4**	2	**1**	9	**3**	7	**2**	7	**2**	5	**3**	10	**1**	9	**4**	8
12	**1**	9	**3**	1	**4**	7	**2**	9	**3**	0	**2**	7	**1**	0	**4**	7
13	**3**	8	**1**	9	**4**	10	**2**	5	**2**	3	**3**	8	**4**	6	**1**	6
14	**1**	10	**2**	9	**3**	2	**4**	7	**3**	5	**2**	8	**1**	0	**4**	2
15	**1**	8	**2**	7	**3**	8	**4**	9	**4**	9	**1**	7	**3**	2	**2**	6
16	**4**	10	**3**	9	**1**	6	**2**	9	**3**	9	**4**	4	**2**	9	**1**	7
17	**3**	8	**4**	9	**1**	7	**2**	7	**4**	10	**3**	8	**2**	4	**1**	8
18	**4**	10	**3**	8	**1**	10	**2**	9	**4**	10	**3**	9	**1**	10	**2**	9
19	**4**	9	**1**	4	**1**	8	**2**	9	**2**	2	**1**	8	**3**	10	**4**	9
20	**4**	7	**8**	8	**1**	7	**2**	4	**1**	5	**3**	10	**2**	7	**4**	6
21	**3**	7	**7**	6	**1**	6	**4**	8	**3**	10	**2**	8	**1**	7	**4**	8
22	**4**	7	**1**	5	**2**	10	**1**	8	**4**	10	**2**	9	**1**	8	**3**	7
23	**3**	6	**7**	9	**1**	0	**2**	8	**4**	6	**3**	5	**1**	7	**2**	9
24	**3**	0	**10**	9	**1**	9	**2**	8	**4**	10	**3**	9	**2**	7	**1**	2
25	**1**	5	**5**	8	**4**	10	**3**	9	**2**	9	**1**	8	**3**	8	**4**	10
26	**3**	9	**6**	7	**4**	8	**1**	6	**4**	10	**3**	9	**2**	6	**1**	6
27	**2**	8	**10**	8	**3**	6	**4**	6	**3**	9	**1**	9	**2**	4	**4**	2
28	**3**	0	**4**	6	**1**	8	**4**	7	**3**	7	**1**	6	**2**	4	**4**	2
29	**2**	4	**5**	3	**4**	9	**3**	4	**2**	5	**3**	7	**1**	1	**4**	5
30	**2**	0	**3**	3	**3**	7	**1**	6	**1**	5	**2**	0	**4**	4	**3**	0
31	**3**	5	**4**	5	**1**	1	**4**	4	**1**	0	**2**	0	**3**	0	**4**	7
32	**2**	1	**8**	5	**4**	7	**3**	9	**1**	1	**3**	5	**4**	1	**2**	7

RV = Root vigour, graded from 0 to 10 (0 = dead, 10 = excellent vigour)

T = Treatment No.
1 = *suSCon Green* pellets
2 = Halloysite pellets
3 = Halloysite pellets (double rate)
4 = Untreated control

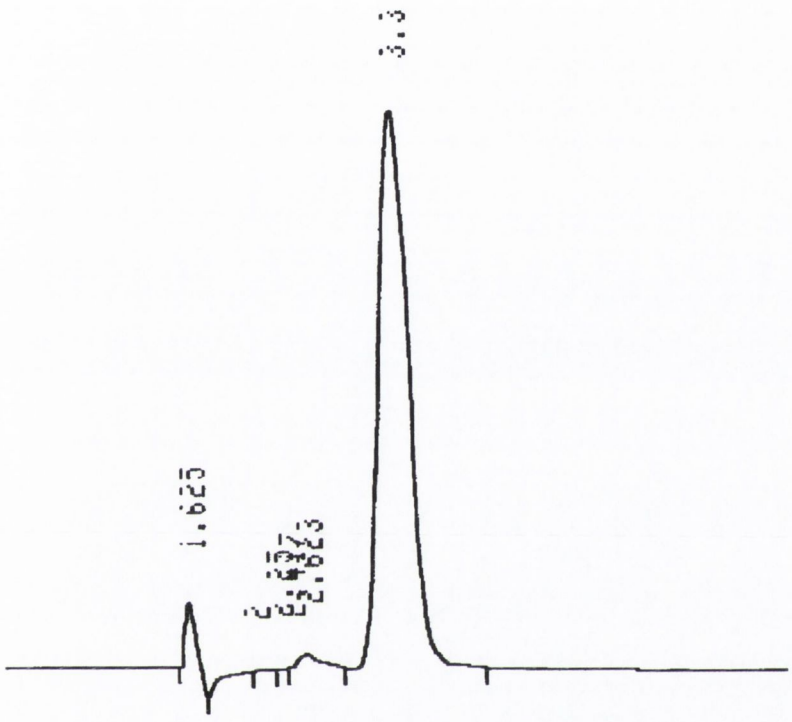
Appendix 9 Number of larvae counted in February, 2003

Row	T	NL	T	NL	T	NL	T	NL	T	NL	T	NL	T	NL	T	NL
1	**3**	0	**4**	0	**1**	0	**2**	0	**3**	0	**4**	0	**2**	0	**1**	7
2	**1**	0	**3**	0	**2**	0	**4**	0	**4**	0	**1**	0	**2**	0	**3**	0
3	**1**	0	**2**	0	**4**	1	**3**	0	**3**	0	**4**	1	**2**	0	**1**	0
4	**4**	2	**3**	0	**1**	2	**2**	0	**3**	0	**1**	0	**2**	0	**4**	1
5	**1**	1	**3**	0	**2**	0	**4**	1	**2**	0	**3**	0	**4**	1	**1**	1
6	**1**	0	**2**	0	**4**	4	**3**	0	**2**	0	**4**	1	**1**	0	**3**	6
7	**4**	1	**1**	0	**3**	0	**2**	0	**4**	1	**1**	0	**2**	0	**3**	0
8	**4**	1	**3**	0	**2**	0	**1**	1	**4**	10	**1**	1	**3**	0	**2**	1
9	**1**	0	**3**	0	**2**	0	**4**	22	**4**	1	**2**	1	**3**	0	**1**	0
10	**3**	0	**1**	0	**2**	0	**4**	0	**2**	1	**1**	1	**3**	0	**4**	0
11	**4**	6	**1**	0	**3**	0	**2**	0	**2**	1	**3**	0	**1**	1	**4**	1
12	**1**	2	**3**	0	**4**	0	**2**	0	**3**	0	**2**	0	**1**	0	**4**	1
13	**3**	0	**1**	0	**4**	0	**2**	0	**2**	1	**3**	0	**4**	0	**1**	0
14	**1**	0	**2**	0	**3**	0	**4**	1	**3**	0	**2**	1	**1**	0	**4**	1
15	**1**	0	**2**	0	**3**	0	**4**	1	**4**	1	**1**	0	**3**	0	**2**	0
16	**4**	1	**3**	0	**1**	0	**2**	0	**3**	0	**4**	0	**2**	0	**1**	0
17	**3**	0	**4**	2	**1**	1	**2**	0	**4**	1	**3**	0	**2**	0	**1**	0
18	**4**	1	**3**	0	**1**	0	**2**	0	**4**	0	**3**	0	**1**	0	**2**	1
19	**4**	0	**3**	0	**1**	0	**2**	0	**2**	1	**1**	0	**3**	0	**4**	0
20	**4**	0	**3**	0	**1**	1	**2**	0	**1**	0	**3**	0	**2**	0	**4**	1
21	**3**	0	**2**	0	**1**	1	**4**	2	**3**	0	**2**	1	**1**	0	**4**	0
22	**4**	3	**3**	0	**2**	0	**1**	2	**4**	1	**2**	1	**1**	0	**3**	0
23	**3**	0	**4**	1	**1**	1	**2**	0	**4**	0	**3**	0	**1**	1	**2**	0
24	**3**	0	**4**	2	**1**	2	**2**	0	**4**	1	**3**	0	**2**	0	**1**	0
25	**1**	3	**2**	1	**4**	4	**3**	0	**2**	1	**1**	0	**3**	0	**4**	0
26	**3**	0	**2**	1	**4**	6	**1**	1	**4**	0	**3**	0	**2**	1	**1**	0
27	**2**	0	**1**	1	**3**	0	**4**	3	**3**	0	**1**	0	**2**	3	**4**	4
28	**3**	0	**2**	0	**1**	0	**4**	0	**3**	0	**1**	0	**2**	0	**4**	0
29	**2**	0	**1**	0	**4**	0	**3**	0	**2**	0	**3**	0	**1**	6	**4**	0
30	**2**	1	**4**	0	**3**	0	**1**	0	**1**	0	**2**	0	**4**	0	**3**	0
31	**3**	0	**2**	0	**1**	0	**4**	0	**1**	0	**2**	0	**3**	0	**4**	0
32	**2**	0	**1**	0	**4**	0	**3**	0	**1**	0	**3**	0	**4**	0	**2**	0

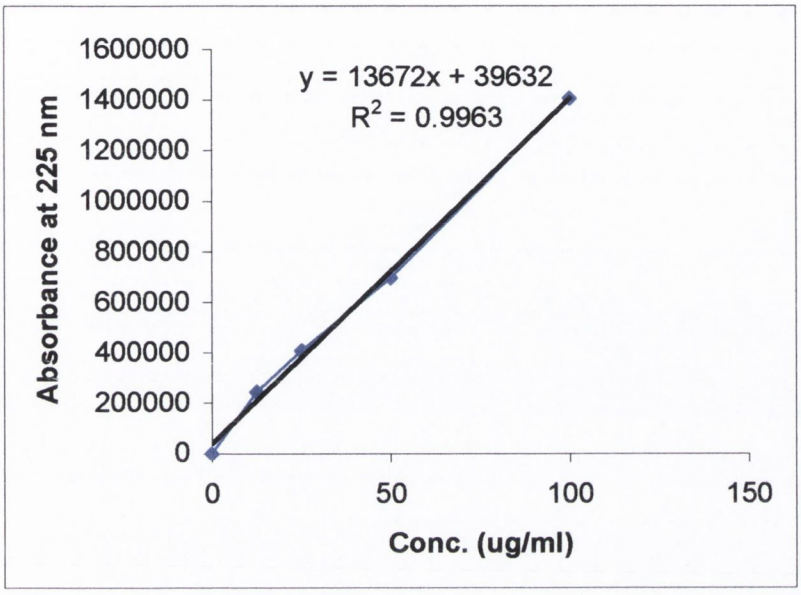
NL = Number of larvae

T = Treatment No. **1** = *suSCon Green* pellets
2 = Halloysite pellets
3 = Halloysite pellets (double rate)
4 = Untreated control (no pellets)

Appendix 10 Sample chromatogram of cypermethrin, obtained using UV detection (mobile phase consisting of 90% methanol and 10% deionised water)

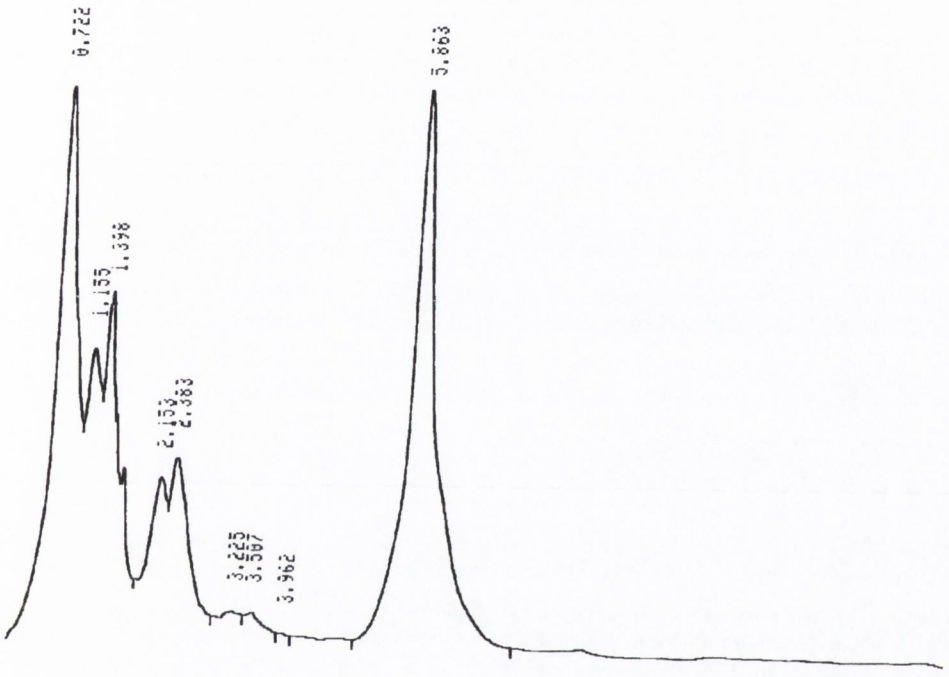


Appendix 11 Sample HPLC calibration curve for cypermethrin using UV detection (mobile phase consisting of 90% methanol and 10% deionised water)

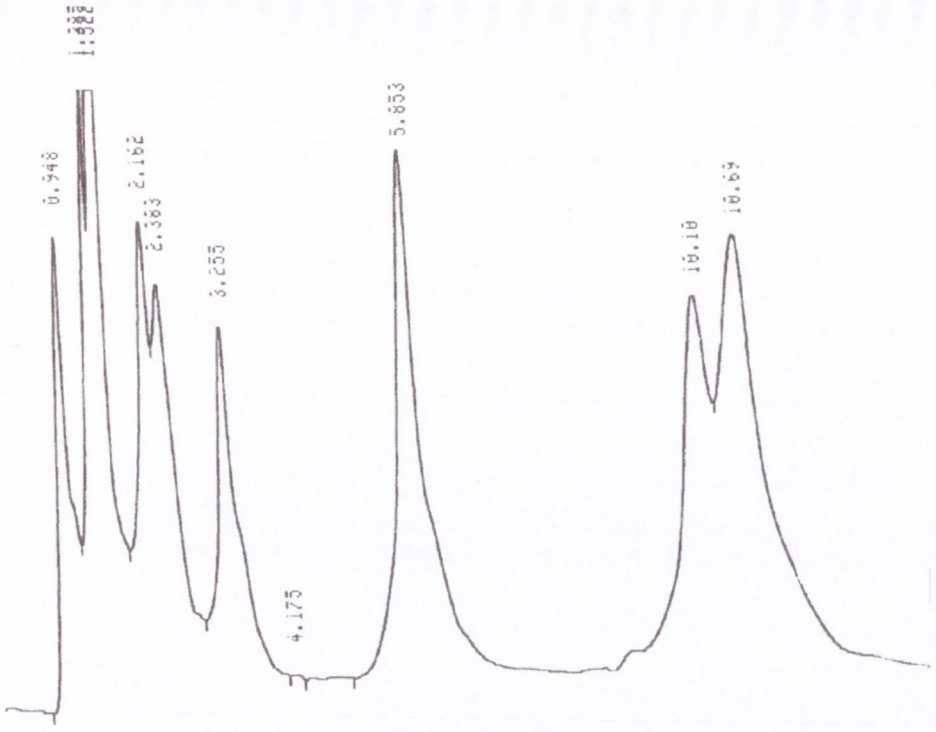


Appendix 12 Sample chromatogram of cypermethrin, obtained using UV detection (mobile phase consisting of 75% acetonitrile and 25% deionised water) in rainfastness study

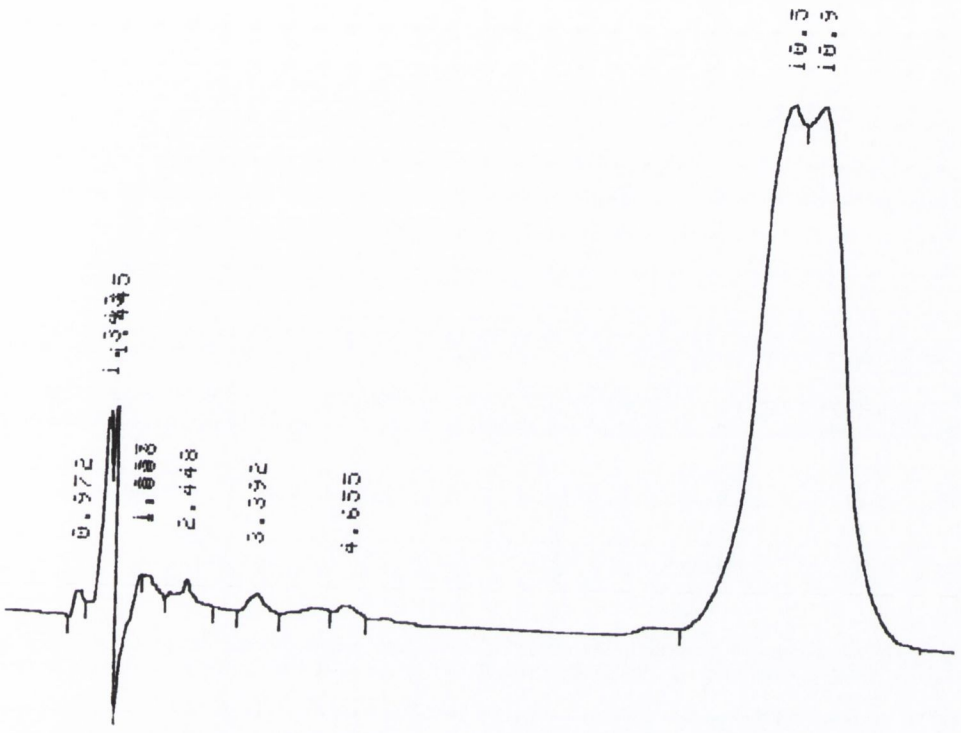
(a) Sample chromatogram of leaf without cypemethrin in rainfastness study



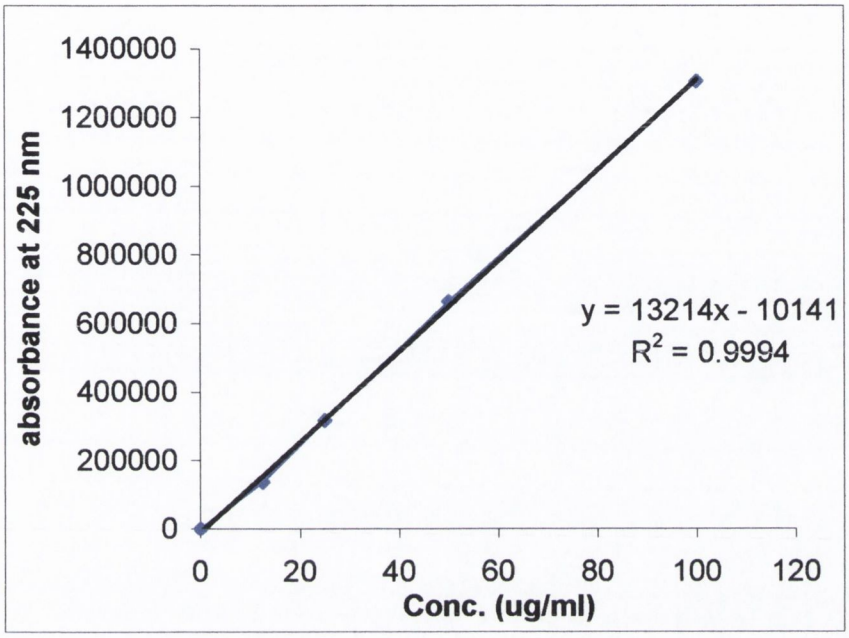
(b) Sample chromatogram of leaf with cypemethrin in rainfastness study



Appendix 13 Sample chromatogram of cypermethrin, obtained using UV detection (mobile phase consisting of 75% acetonitrile and 25% deionised water) for dissolution studies



Appendix 14 Sample HPLC calibration curve for cypermethrin using UV detection (mobile phase consisting of 75% acetonitrile and 25% deionised water)



Appendix 15 Randomised layout of field trial for winter and spring barley crops

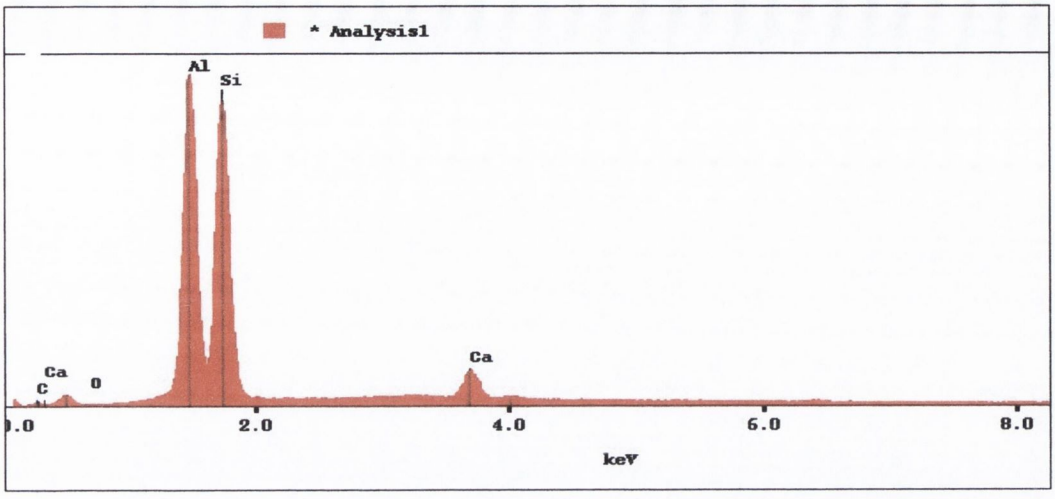
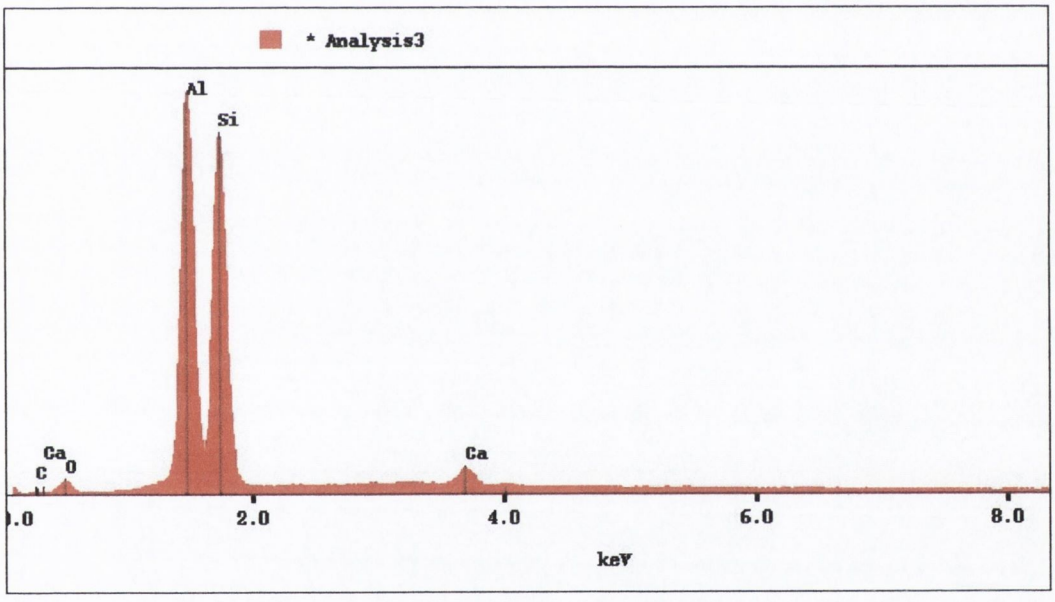
2	**7**	**1**	**5**	**6**	**3**	**8**	**4**
4	**8**	**3**	**7**	**2**	**6**	**5**	**1**
3	**5**	**7**	**6**	**1**	**8**	**4**	**2**
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**

Appendix 16 Cereal growth stage identification key using a decimal code system

Germination		**Stem elongation**	
00	Dry seed	30	Ear at 1 cm
03	Imbibition complete	31	1st node detectable
05	Radicle emerged from caryopsis	32	2nd node detectabe
07	Coleoptile emerged from caryopsis	33	3rd node detectabe
09	Leaf at coleoptile tip	34	4th node detectabe
Seedling growth		35	5th node detectabe
10	First leaf through coleoptile	36	6th node detectabe
11	First leaf unfolded	37	Flag leaf just visible
12	2 leaves unfolded	39	Flag leaf ligule/collar just visible
13	3 leaves unfolded	**Booting**	
14	4 leaves unfolded	41	Flag leaf sheath extending
15	5 leaves unfolded	43	Boots just visibly swollen
16	6 leaves unfolded	45	Boots swollen
17	7 leaves unfolded	47	Flag leaf sheath opening
18	8 leaves unfolded	49	First awns visible
19	9 or more leaves unfolded	**Inflorescence**	
Tillering		51	First spikelet of inflorescence just visible
20	Main shoot only	52	¼ of inflorescence visible
21	Main shoot and 1 tiller	55	½ of inflorescence visible
22	Main shoot and 2 tiller	57	¾ of inflorescence visible
23	Main shoot and 3 tiller	**Anthesis**	
24	Main shoot and 4 tiller	60	Beginning of anthesis
25	Main shoot and 5 tiller	61	Beginning of anthesis
26	Main shoot and 6 tiller	64	Anthesis half-way
27	Main shoot and 7 tiller	65	Anthesis half-way
28	Main shoot and 8 tiller	68	Anthesis complete
29	Main shoot and 9 or more tiller	69	Anthesis complete

Milk development		**Ripening**	
71	Caryopsis watery ripe	91	Caryopsis hard (difficult to divide by thumb-nail)
73	Early milk	92	Caryopsis hard (can no longer be dented by thumb-nail)
75	Medium milk	93	Caryopsis loosening in daytime
77	Late milk		
Dough development			
83	Early dough		
85	Soft dough		
87	Hard dough		

Appendix 17 Energy dispersive analysis plot of unheated halloysite G pellets



Energy dispersive analysis of halloysite G pellets heated to 1200°C