THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Microencapsulation of Pesticides for Controlling Release from Coatings

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Cover:
Scanning Electron Microscopy (SEM) image of core-shell microcapsules with octadecane oil as core and PMMA as polymer shell. The pesticide BHT is encapsulated within the core-shell microcapsule and the work is described in paper III.

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Abstract

Microencapsulation and controlled release of a pesticide intended for coating application is the central issue of this thesis.

Today forestry industries are facing enormous problems as conifer seedlings are attacked by insect *Hylobius Abietis* which feeds on young cortex. Only in Sweden, the annual loss is 0.5 to 2 billion Swedish kronor. To achieve protection for at least two years, until the plant become strong enough to withstand a pest attack, it necessitates developing an effective pesticide delivery system.

In this work, a pesticide called butylated hydroxytoluene (BHT) has been found effective against the insect. Antifeedant activity bio-assay tests and release studies in aqueous medium revealed that the coating formulation is more efficient in presence of a non-ionic surfactant (alkyl glucoside).

To control the release from coatings microencapsulation technology has been employed. The microcapsules were based on poly(methyl methacrylate) (PMMA). The release of freely dispersed and encapsulated active substances from both oil- and water-based coatings has been studied in an aqueous release medium. Slower release was observed for encapsulated substance compared to freely added substance.

For release of BHT from core-shell microcapsules two different types of core materials, with similar chemical characteristics but different physical states, were studied: dodecane (liquid) and octadecane (solid). The release was faster from liquid-core microcapsules and the state of the core was argued to be the main reason. For both microcapsule types, an initial "burst" release was followed by a slower release. The burst was attributed to accumulation of BHT in the PMMA shell whereas the slowly releasing fraction was attributed to BHT in the microcapsule core. The conclusions were further supported by QCM-D investigations where a PMMA film was used to monitor both

absorption and release. It was noted that the absorption of BHT was much higher than that of dodecane.

In this thesis, it is shown that encapsulated active substances can be effectively formulated into a protective coating. The main advantage using microcapsules is that the release of active ingredients can be better controlled and that the mechanical properties of the coating can stay intact even at high concentrations of the active.

Keywords: microcapsule, microsphere, coating, latex, controlled release, pesticide, BHT, dodecane, octadecane, PMMA, pine weevil.

List of Publications

The thesis is based on the work presented in the following papers:

Paper I

Latex coatings containing antifeedants: Formulation, characterization, and application for protection of conifer seedlings against pine weevil feeding

Liubov Shtykova, Mariam Masuda, Carina Eriksson, Kristina Sjödin, Elisabeth Marling, Fredrik Schlyter, Magnus Nydén.
Progress in Organic Coatings 63 (2008) 160–166

Paper II

Molecular release from painted surfaces: Free and encapsulated biocides

Lars Nordstierna, Atta A. Abdalla, Mariam Masuda, Gunnar Skarnemark, Magnus Nydén.
Progress in Organic Coatings 69 (2010) 45-48

Paper III

Controlled release from microcapsules: Liquid and solid core oil and the effect on release rate

Mariam Masuda, Lars Nordstierna, Alireza Movahedi, Matias Nordin, Magnus Nydén.
Manuscript

Paper IV

New insight into release of active substances from microcapsules by studying release and uptake in thin films with the QCM-D technique

Mariam Masuda, Mattias Berglin, Magnus Nydén, Lars Nordstierna.
Manuscript

Contribution Report

Paper I
Shared responsibility for AFM analysis and for writing.

Paper II
Shared responsibility for experimental work.

Paper III
Shared responsibility for experimental work and major part in writing.

Paper IV
Shared responsibility in experimental plan and major part in writing.

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Chapter 1. Introduction

The pine weevil insect *Hylobius Abietis* is one of the central pests that causes severe damage in conifer forests in Europe, Asia and North America. Forestry-based industries are facing enormous problems when replanting in harvested conifer forests. The main reason is that the pine weevil, which feeds on young cortex, destroys the stems of newly planted seedlings. Thus, high degree of pest attacks results in high costs for forestry-based industries. In Sweden alone, 80% of the pine and spruce seedlings are damaged by the pine weevil to such extent that the plant dies shortly after plantation [1, 2]. An estimated cost arising from this damage is around 0.5 to 2 billion Swedish kronor per year [3]. In the past, the chemical substance permithrin has been applied to minimize the pest attacks. But due to detrimental effects on aquatic organisms, the use of permithrin has been abrogated. Cypermethrin has been proposed as an alternative pesticide. However, it turned out to be even more toxic not only to aquatic organisms but also to terrestrial insects as well as mammals [4, 5]. Since the pesticides that worked against pine weevil were found as broad–range toxics and have detrimental effect on terrestrial and aquatic animals/organisms, it was needed to replace these pesticides with less toxic compounds. In other words, the necessity to develop an effective and more environmentally-friendly pesticide delivery system with proper active ingredients became obvious in order to achieve a long-lasting solution to the pine weevil problem.

There are different kinds of semio-chemicals that either attract or distract pine weevil for different activities [6]. A semio-chemical that attracts is called a “stimulant” or an “attractant” and the one that repels is called an “antifeedant” or a “repellent” [7]. Several antifeedants like the alkanoic acids have showed activity against pine weevil where they act as suppressant/feeding deterrent or as arrestant [7]. But these are rather volatile chemicals, which is an obstacle for maintaining a long action period. A

molecule with smaller volatility, butylated hydroxytoluene (BHT), has been found to be discarded by pine weevil when it is added on stems of pine and spruce seedlings [4, 8, 9]. These results suggest the possibility of using BHT as an antifeedant for the protection of pine and spruce seedlings against pine weevil.

Generally it is considered that an efficient pesticide formulation should not only show an effective biological activity, it must also be user friendly and environmentally friendly. Depending on the criterions, different pesticides delivery system has recently been developed [10-12]. The pesticides are formulated in the form of wettable powders, emulsifiable concentrates, water solutions, powder or granules, aerosols or spray formulations. But such formulations have different degrees of health hazards ranging from respiratory exposure (powder, aerosol, spray) to penetration through the skin (emulsifiable concentrates). Being larger in size, granules do not pose any of the upper mentioned health hazards but the formulation is not suitable enough to deliver in a convenient way for field application. Considering all these facts, perhaps microencapsulation is the most efficient way of delivering pesticides. The versatility of this method leads to the possibility to encapsulate a wide range of pesticides [12].

The delivery system needs to last for a certain period of time to release its content on the treated seedling surface during two or more growing seasons. It is thus essential to add the active substance using a controlled formulated system rather than applying the active as pure substance. Putting the active within a controlled release system helps to release the active slowly and thereby prolonging the protection of the seedling until it is physically strong enough to withstand insect attack. To meet this requirement microparticles, e.g. core-shell particles or microcapsules, could be basis of a system where the active is stored for future release to the surrounding. Besides prolonged action, microencapsulation controls the release to improve residual activity [13-15], to prevent environmental degradation [16, 17], and to reduce the application dose hence aquatic toxicity and volatilization [10, 18]. Microencapsulation of the active provides a possibility to obtain an efficient and economically beneficial pest management [19]. For long-lasting protection purposes, the microcapsules must be adhered to the seedling surface. One way is to disperse

microcapsules in a matrix which is compatible with the microcapsule shell material [20]. Water-based latex paint might be the best choice of use in this respect. This will ensure strong adhesion of microcapsules on the seedling surface [21].

Besides the development of a microencapsulated pesticide delivery system, this thesis also to some extent investigates the development of delivery system for anti-growth agents. Mold and/or algae growth on exterior facades or fouling growth on ship hulls are major problems. The growth is occurring on a painted surface that is applied as a protective coating for the houses and ships. Usually biocides are applied as anti-growth agents within the paint system. A fast release and loss of the biocides renders the paint matrix to be quickly exposed to unwanted growth [22]. By enclosing the biocides in microparticles, the possibility of prolonging the release is also studied in this work.

The purpose of this thesis work is to develop a microencapsulated delivery system to be formulated in protective coatings. By microencapsulation of pesticides/biocides it is possible to prolong the release for a longer period of time and to protect the target objective from attack of insects or from the growth of bio films on the surface.

Chapter 2. Pesticides/biocides and their application

Pesticides or biocides are active substances that destroy, deter, render harmless, or prevent pests or microorganisms in order to protect either any kind of crops or protective coatings. Depending on the target and the place where it would be applied, the distinction between these two substances is made. Usually pesticides are molecules which combat against pest to protect crops and crop products. Biocides, on the other hand, are molecules which combat the living organism in order to prevent or destroy any kind of fouling growth. In this thesis depending on the target of application, active substances are referred to as pesticides or biocides.

2.1 Pesticides in agriculture

Semio-chemicals are compounds that cause behavioural changes among the insects. There are different kinds of semio-chemicals that are named after the action on the insect. Pheromones are semio-chemicals which are secreted from the exocrine glands of insects. These secreted pheromones are used by the insects of same species for finding mates, aggregation, alarm, tracking or trial marking defence [23]. Antifeedants are another kind of semio-chemicals which inhibit food-intake activities. The insect's taste sensitivity is dominated by $GABA_A$ receptors and antifeedants work against one of these receptors [24]. The GABA receptors ($GABA_A$ and $GABA_B$) belong to a class of receptors that respond to the neurotransmitter gamma-aminobutyric acid (GABA). Pheromones and antifeedants have useful potential to control various pests and replace toxic pesticide [6, 25].

Due to excessive use of pesticide chemicals, pest insects have developed resistance towards such chemical substances. The insect development of resis-

tance towards chemicals leads researchers to re-think about the basic underlying tenets of pest control [15, 26-29]. There is a concern of replacing pesticides by other pesticides of natural origin [29]. One group of alternative substances are insect repellents, antifeedants, which are more bio-friendly and often have weak physical effect on insects [30, 31]. Generally, antifeedants are secondary metabolites from natural origin that modify the feeding behaviour of insects [32, 33].

Antifeedants have some advantages over traditional insecticides: faster degradation, less development of insect resistance, less harmful to human and environment [34]. Antifeedants are classified as (a) repellents that repel the insect to get in contact with the plant, (b) arrestants that stop the insect to move towards the plant, (c) suppressants that stop insect to continue the feeding after biting the plant, and (d) feeding deterrents that deter the insect from feeding after it has already bitten the plant once or repeatedly [4, 35, 36].

There are a number of plant-derived carboxylic acids like hexanoic and nonaonic acid, carvone and carvacol as well as esters of 2,4- and 3,5-dimethoxy benjoic acid which are found highly active against pine weevil [5, 32]. Two glucosides, simmondsin and simmondsin 2'-ferulate, isolated from jojoba seed have antifeedant activity against the cotton leaf larvae named *S. Littoralis* an agricultural pest [37]. Flavones are important heterocyclic compounds and some of these compounds are able to affect insects by inhibiting larvae feeding or by acting as feeding deterrent [24]. Terpenoid compounds are the most developed antifeedants that acts on aphids. Since effective antifeedants are often of plant origin and achieved by extraction, they are usually provided in low yield. It is thus necessary to synthesize antifeedants to get more substance [38].

(A)

(B)

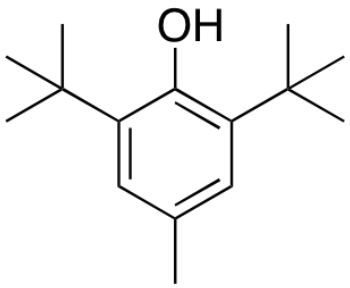


Figure 2.1 (A) A pine weevil, *Hylobius abietis*, that feed on young cortex of seedlings, (Photo: by Fredrik Schlyter). (B) The pesticide and antifeedant, butylated hydroxytoluene (BHT), that has feeding inhibitory effect on pine weevil.

In this work, a synthetic antifeedant 2,6-di(tert-butyl)-4-methylphenol (BHT) is used as an active agent in the development of a pesticide delivery system for pine weevil (see figure 2.1). The outcome of this work is presented in papers **I, III and IV**.

2.2 Biocides in coatings

Biocides are chemical agents that are capable of severely affecting living organisms. Here biocides are referred to as micro-biocides and marine antifouling agents. Micro-biocides are agents that are capable of inhibiting or controlling the growth of microorganisms including fungi, bacteria and algae. Marine antifouling agents include algaecides and molluscicides and their activity includes the elimination and inhibition of growth of marine organisms [39]. The fouling growth of barnacles on a ship hull and a biocide that acts against barnacles are shown in figure 2.2.

A wide range of biocides has been used for the preservation of materials during the last 50 years. Former mercury-based biocides had the potential to meet the requirement for in-can and dry state preservation of paints or coatings against all kind of microorganisms. Tin–based biocides were extensively used in marine paint as antifouling agent. But the toxicity and ecological persistence lead to a phasing-out of such applications. Similarly

PCPs, (pentachlorophenols), PCBs (Polychlorinatedbiphenyls) and formaldehyde are all banned as biocides in the paint application [38-40].

(A) 

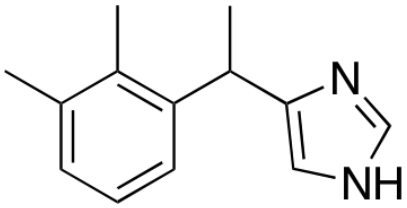
(B) 

Figure 2.2 (A) Barnacle or fouling growth on ship hull, (Photo: Marine Paint programme). (B) An antifouling agent, medetomidine, that works against the fouling growth.

There are a number of products including paint and building materials which are exposed to a number of different microorganisms. On the coating surface, a bio-film of fungus or bacteria grows. The effect of microbial contamination on a product like a coated surface is obvious in the form of visible surface growth, gassing, pH drift, viscosity loss or increase, malodour and discoloration [41, 42]. Fungal growth on outdoor surfaces or even on indoor surfaces is obvious since fungi do not produce their own food and they grow on surfaces that take nutrients from others, may be from the surface itself or from the surrounding environment [42-44]. The surface may be of organic or artificial origin where cellulose or surfactants from water based paints, or resins and oils from solvent based paints supply proper nutrients. Fungi can also penetrate the coating and break down the components of the coating, and thereby allowing moisture penetration which further facilitates growth [41, 42].

Microorganisms cause a considerable economic loss to the paint users and it means excessive use of chemicals. This drives the researchers to develop better paint formulations for surfaces [45]. A prevention of fouling growth can be controlled by incorporating biocides in the coating. Biocides may be used for dual purposes: 1) to protect from biological growth, 2) to be used as in-can preservative while the product is still in wet state.

As biocides are toxic to a certain extent, a legislative pressure has been put to reduce the amount of exposure or leakage. As a molecularly small biocide diffuses through the porous coating, it reaches the surface where it acts against the growing organisms [38]. However, this release of biocide is often rapid and an excessive amount is released very shortly after the paint has been applied. A formulation of microencapsulated biocides rather than freely dispersed biocides can be a way to minimize the amount released to the environment and to prolong the protection. By microencapsulation, a minimum inhibitory concentration at the coating surface can be maintained over an extended period of time while inhibiting aqueous extraction during rainy periods [41, 46].

In this work, a marine biocide has been used as a model compound in a study using water- and solvent–based exterior wall paint. This study is presented in paper **II**. The basic idea was to use free biocide and encapsulated biocide respectively in paint systems and to compare how the release behaviour alters when the biocide is applied in each of the formulations. The incorporation of encapsulated biocides was expected to provide a slower release compared to a freely dispersed biocide in the paint. The reason for working with this biocide is that it was available with a 14C-label which makes release studies much easier and faster.

Chapter 3. Controlled Release Technology

Commercial applications of sustained or prolonged release technology in both pharmaceutical and agricultural industry have existed for decades [47, 48]. The concept of enteric coating was invented in the early 50's and was widely used in pH-sensitive tablets by the pharmaceutical industry. In this process the tablet is designed to be resistant against the low pH of the stomach and instead releases its content in intestine [49]. Thus the action of the drug is maintained and controlled by the specific coating. Other examples of sustained release formulations are encapsulated pellets or beads, sparingly soluble salts, porous insoluble tablets containing a dispersed drug, and other complex systems [50, 51]. In agriculture, sustained release fertilizers of various materials have been used. These can be slowly activated by microbial attack, by complexation of the active agent with an ion exchange resin, or by membrane-regulated formulations [48].

The basic concept of controlled release technology described in this thesis comes from sustained release or prolonged release technology. Such technology provides a release profile which is usually controlled by the design of the system and is less dependent on external factors [52].

Following the concept of sustained release technology, Folkman and Long developed the first membrane diffusion device in 1964 where silicon rubber was used to control the release of anaesthetic and cardiovascular drugs. In the late 1960s, Alex Zaffaroni at the Alza Corporation continued their research with novel controlled release drug delivery systems. The company developed an ophthalmic insert called Ocusert® which releases the anti-glaucoma drug at a constant rate in the eye. Another development called Progestesert® was an intrauterine device (IUD) release contraceptive steroid, progesterone, released at a constant rate in the uterine cavity. This development on controlled release technology inspired the entire pharmaceutical industry.

During the 1970s, the interest of using controlled release technology was extended to agrochemical, cosmetic and food industries [48, 53, 54].

From the first use in 1930, today's agriculture highly depends on synthetic pesticides. The use became widespread after the World War II [55]. From an estimation of the total agrochemicals used in crop protection, only 0.1 % reach the target pest while the rest enters the environment and may cause hazards to non-target organisms including humans [23]. Various applications of controlled release technology in the agricultural area are being used in order to handle the problems. Those can be associated with ground water contamination, degradation, volatilization [55], excessive exposure of chemicals to environment, phyto-toxicity [56] , and other runoff [56, 57].

The purpose of using controlled release technology in both the agriculture and the paint industry is to decrease the excess supply and to prolong the protective use of pesticides, herbicides, agrochemicals or biocides by maintaining an effective concentration over a given interval of time [57, 58]. Additionally, it helps to protect against uncontrolled distribution of xenobiotics in the environment [59].

There are different ways to control the release of an active substance. The release behaviour from a controlled release system can generally be classified into three types: (1) "zero-order release" where the release rate remains constant until the carrier is exhausted of active agent, (2) "first-order release" where the release rate is proportional to the amount of active agent within the reservoir and declines exponentially with time as the reservoir approaches to exhaustion, and (3) "square-root-of-time" release where the release rate is linear with the reciprocal of the square root of time. The release rate remains finite as the carrier advance towards exhaustion [48] (figure 3.1).

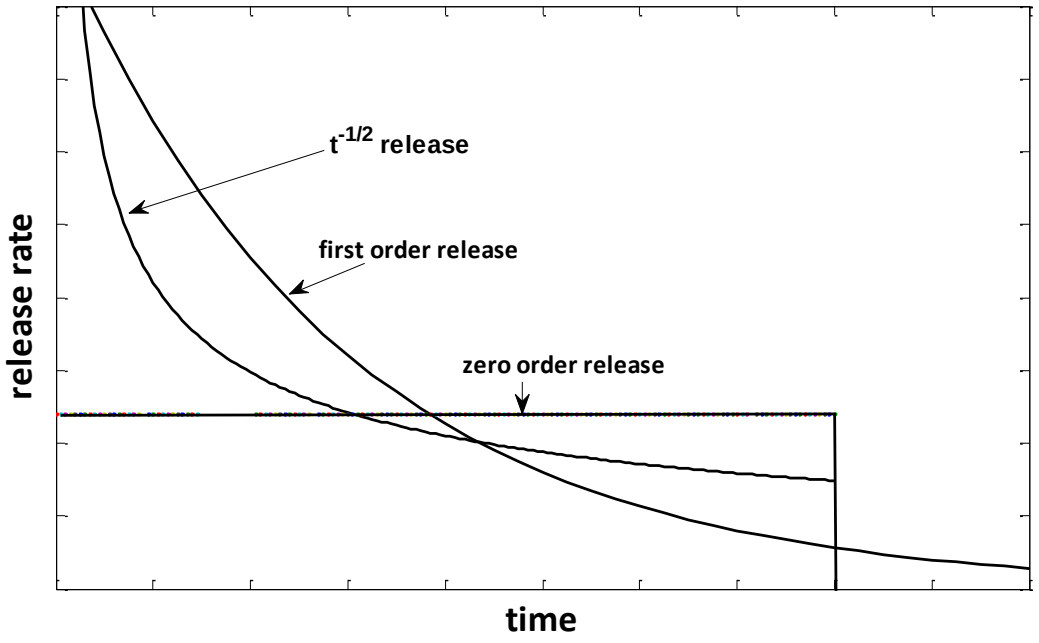


Figure 3.1 Different release mechanisms of actives from micro reservoir delivery device system.

Depending on the application, the mechanism of controlled release device can generally be explained as: (1) chemically–controlled where the release may occur from (a) bio-erodible systems and (b) drug-polymer conjugates, (2) diffusion-controlled where the release may occur from (a) membrane-reservoir systems (solution-diffusion and osmotic pumping) and (b) matrix systems (ex: matrix diffusion, polymer erosion, polymer swelling, geometry and concentration distribution) [60]. The application of controlled release technology can be target specific and the advantage varies from one application to another. By this technology it is possible to deliver effective concentration of an active to the target object without releasing too little or too much. Since excessive release can be suppressed by this method, it is possible to maintain efficient utilization of the active agent. By tuning the internal factors of the carrier system it is possible to make site-specific or target-specific delivery of the agent. Since the release is slowed down by different controlling parameters it is possible to avoid frequent application of the active agent [48, 60, 61].

3.1 Approaches to control pesticide and biocide release

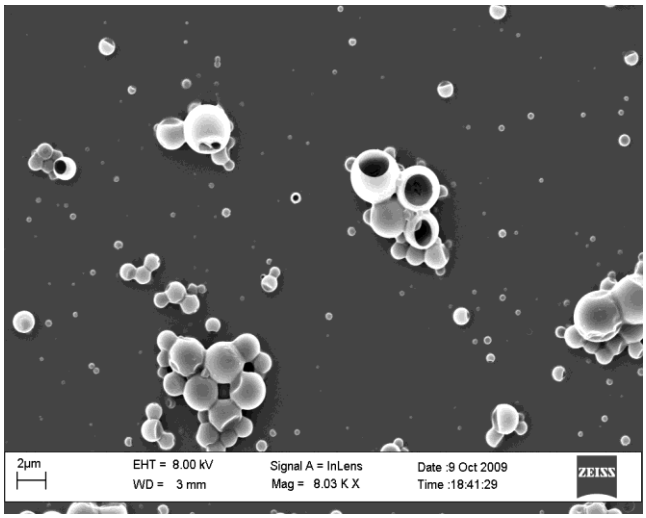
A great challenge in the development of a controlled release delivery system of pesticides lies on the regulation of the length of time during which the effectiveness of the active ingredient is maintained [25]. There are numerous ways to control the release and the final choice depends on the required application. This can be a bio-erodible or drug-polymer conjugate system, membrane-reservoir system, matrix carrier system or an osmotic and mechanical pump system [61]. Hydrogel polymers are extensively used for controlled release applications. The swelling and de-swelling behaviour of a hydrogel makes it useful in biomedical and controlled release pharmaceutical applications [57, 62-65].

Biodegradable microbial polyesters like poly(hydroxyl alkanoates) (PHAs) are used as carrier for pesticides and alginate for controlled release herbicides [18, 59-65]. Temperature sensitive polymers (e.g. Intelimer) are used to encapsulate pesticides and give only slow release below a specific temperature, thereby protecting the active pesticide from unwanted leaching and degradation [55]. Insecticides and biocides can also be applied within granular system [56]. Previously, encapsulation of pesticides could be done by dispersing the pesticides into an aqueous dispersion of gelatinized starch and then cross linking the starch by xanthide or calcium chloride. This technique improves worker safety in the handling of pesticides and also helps to decrease the phyto-toxicity to the crop [66]. In another example, liquid neem oil is dispersed in a matrix where urea-formaldehyde pre-polymer is cross linked with natural polymer such as starch or guar gum [67].

In this thesis work, microparticles (more explicitly microcapsules and microspheres) have been used as controlled release reservoir devices. Membrane-reservoir systems have been used to study the release behaviour of an antifeedant from core-shell microcapsules. Matrix (or monolithic) systems have been used to study the release behaviour of a biocide from microspheres. In both cases the release depends on a diffusion-controlled release mechanism.

For core–shell microcapsule in general, the shell encloses the active agent to be released. In this thesis, the release of the active is shown to initially occur from the shell where the active has partially been dispersed. This release is followed by diffusion from the inner core (figure 3.2). In a membrane-reservoir system, the release rate depends on the thickness, area and permeability of the membrane.

(A)



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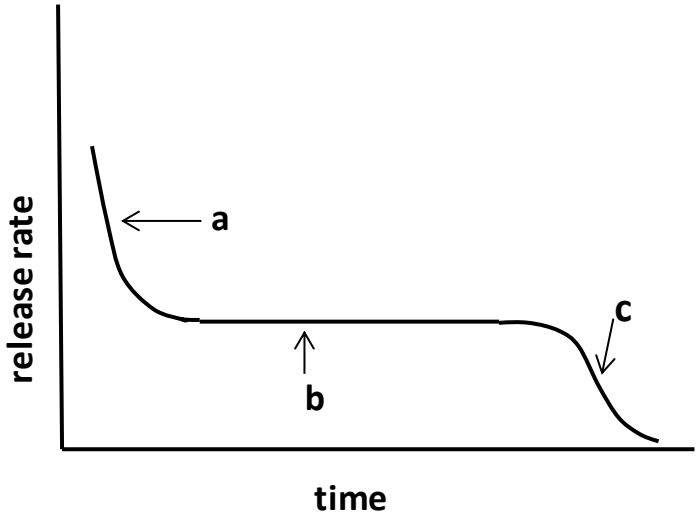

Figure 3.2 (A) SEM image of microcapsules where the core is surrounded by a PMMA shell and active is reserved in the oil core. (B) Typical release behaviour of the active from core-shell microcapsules, (a) initial fast release from the membrane**,** (b) constant release rate as long as a constant concentration is maintained, (c) rapid declination of release when device approaches zero concentration.

In a microsphere, the active agent is dispersed or dissolved in a rate-controlling polymer matrix. In a matrix system, the release of active depends on the nature of active and polymer and the geometry of the matrix device. In general, the release of active starts from the surface layer followed by the next layer. The release rate also depends on the loaded amount of active within the polymer matrix. When the loading is low, a slower release can be observed since the diffusional path is more restricted due to extensive polymer network within the matrix. A microsphere containing homogeneously or heterogeneously dispersed active, and the release behaviour of active from microspheres are shown in figure 3.3.

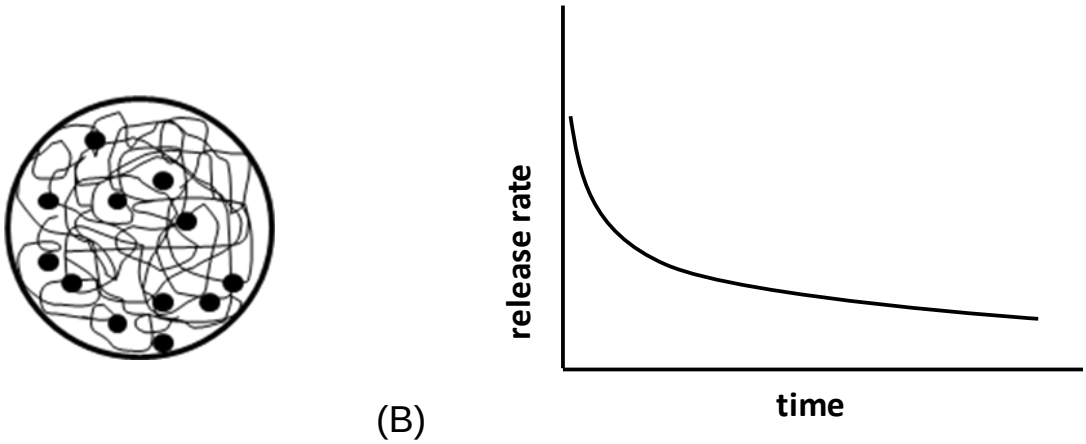

Figure 3.3 (A) Microsphere or polymer matrix where the active is homogeneously or heterogeneously dispersed. (B) Typical release behaviour of active from a microsphere.

3.1.1 Microparticles

There are a number of controlled release devices. These include microcapsules, microspheres, coated granules and granular matrices (see examples in figure 3.4) among others. During the last 20 years much of the research in industry and academia has been devoted to specialization and utilization of microcapsules in particular [68].

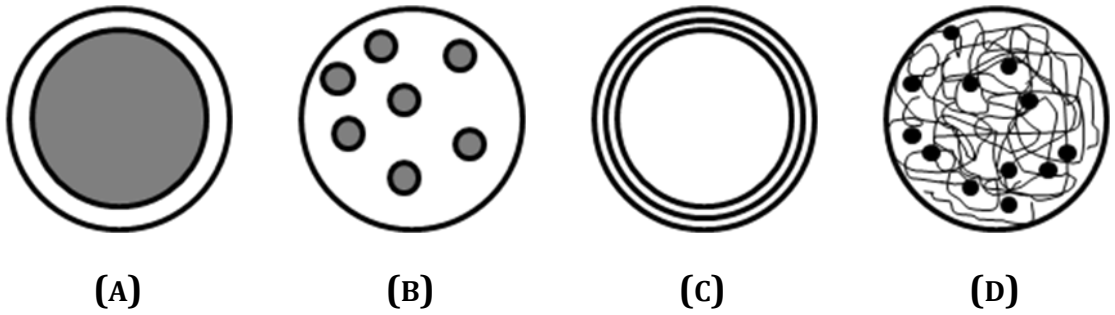

Figure 3.4 Morphologies of microparticles: (A) Mononuclear core and homogeneous shell microcapsule, also called core-shell microcapsule. (B) Poly-nuclear core and homogeneous shell microcapsule. (C) Mononuclear core and multi-shell microcapsule. (D) Polymer matrix, also called microsphere, where active is homogeneously or heterogeneously dispersed.

A microcapsule is a reservoir system where the active chemical is contained within the core and is surrounded by shell or membrane. There may be one or several cores and one or several shells. The inner core can be solid, liquid, gaseous, or a combination of any of these. The protective matrix may be an organic or an inorganic polymer or even a metal oxide [69]. The microcapsule wall material protects the active ingredient from adverse reactions, volatilization, and restrict a direct exposure to outside environment. Microcapsules can also be utilized as micro-reactors where the membrane is used to separate as well as help to perform chemical reactions [70-72]. Microencapsulation has a wide range of applications including pharmaceuticals, dyes, perfume, agriculture, printing, adhesives, cosmetics, and food products [73, 74]. Techniques involved in microencapsulation include spray-drying, spray-cooling, extrusion, freeze-drying, co-crystallization, emulsification, photo-polymerization [30, 75, 76].

A microsphere is a monolithic system where the active agent is dissolved or dispersed in a polymer matrix [51]. Microspheres are spherical or irregularly shaped particles in the size ranging from 20 nm to 2000 μm and are composed of one or more polymers.

Different properties and performances of microcapsules and microspheres can be achieved by variation at the molecular level. By property-performance morphology and choice of the core material, it is possible to achieve a desired release of the active [75, 77]. For instance, enhanced release of a highly

hydrophobic compound can be obtained by increasing the surface area of the microcapsule. This can be achieved by decreasing the size of the particle [78]. If it is an organic polymer employed in the microcapsule or microsphere, it is biodegradable or non-biodegradable. The naturally occurring polymers mostly used are polysaccharides including cellulose, agarose, dextran, alginates, carrageenans, starch, chitosan [79-85] and proteins including gelatin and albumin [86, 87]. Among synthetic polymers the most frequently used are polystyrene, polyacrylamide, polymethylacrylates, polyamides, polyesters, polyanhydrides, polyurethanes, amino resins and polycyanoacrylates [69, 71, 88]. Inorganic materials for microsphere preparations include silica, zeolites, inorganic oxides as well as glass beads and ceramics [69, 71, 82-84, 87, 88].

In this work, poly(methyl methacrylate) or PMMA is used as protective membrane for core-shell microcapsules and matrix material for microspheres. Alkane oil of various chain length (C12 and C18) are used as core material within the active is dissolved.

3.1.2 Coatings

A latex coating is a polymeric coating which can be used to control the release of active agents [89, 90]. Latex can either be an aqueous or non-aqueous based polymer particle with a co-polymer of different glass transition temperature or Tg is present. These are often submicron-sized particles [90-92]. Depending on the ratio of low Tg and high Tg polymer in the dispersion , latex are called soft latex and hard latex [93]. In latex coatings, the polymer is used as a binder which is capable of coalescing to form a water-permeable coating upon curing [92, 94, 95]. The presence of low Tg polymer in the dispersion allows the particles to deform under surface tension and capillary forces to results in a relatively void-free film. High Tg polymer on the other hand, ensures a high mechanical strength of the coating while retaining the elasticity of the film [96-98]. Also, the polymer chains in latex particles are mobile enough to inter-diffuse between adjacent particles in order to form a homogeneous film. The formation of a latex film occurs in a series of steps. Initially, evaporation of water or another solvent allows the particles to form a

close-packed structure. With the progress of water evaporation, deformation of particles takes place without void formation. This finally leads to inter-diffusion of polymer chains across the particle boundaries and the formation of a continuous film (figure 3.5) [99-102].

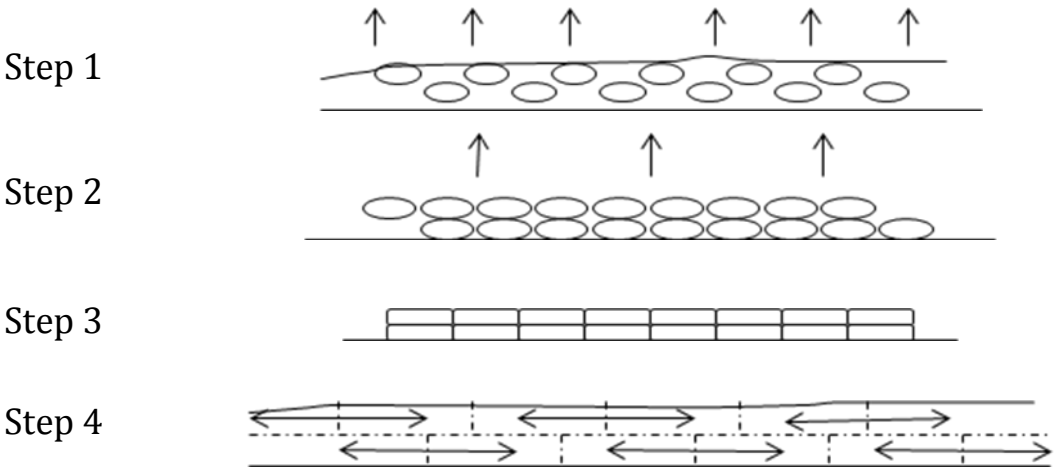

Figure 3.5 The mechanism of latex film formation: **Step 1**, evaporation of water until the latex particles come into contact to each other. **Step 2**, more evaporation of water increase particle connectivity. **Step 3**, deformation of particles. **Step 4**, complete particle deformation and inter-diffusion of polymer chains.

In this work, a 30 % latex dispersion in water, called Eudragit NE 30 D, was used. The co-polymer is produced by an emulsion polymerization of ethyl acrylate and methyl methacrylate in a ratio of 2:1. To obtain an elastic film with high mechanical strength, additional additives are used in the mixture. Alkyl glucoside, a non-ionic surfactant, was added to improve the surface properties of the coating. A hydrophobically modified EHEC (ethyl hydroxyl ethyl cellulose), a non-ionic cellulose ether, was used as a thickener to improve the rheological properties of the latex paint.

In this work, it was expected that the application of encapsulated BHT within the latex coating should allow for gradual release of the active. Another advantage would be a reduction of environmental hazard risks [103].

Chapter 4. Analytical methods

4.1 UV-visible spectroscopy

One of the most common applications of UV-visible spectroscopy is to determine the concentration of an analyte in a solution. Molecules that absorb light in the UV-visible region (200-800 nm) have pi-electron functions [104]. When the molecules are exposed to light, the light absorbing groups absorbs energy from the radiation beam and promotes a pi electron to a higher molecular orbital. Thus, the intensity of the radiation beam decreases once a molecule is excited. The wavelength at which the light is absorbed is recorded by an optical spectrophotometer along with the intensity of absorption. The instrument compares the intensity before and after passing through the sample and the resulting spectrum is presented with absorbance (A) as a function of radiation wavelength (λ).

At a specific wavelength for a given molecule, there is a relationship between the absorbance and the number of absorbing molecules. The relation is explained by Beer's law which says that the amount of absorbed light is proportional to the number or concentration of absorbing molecules.

$$A = \log I_0/I = \varepsilon_\lambda b\, c \qquad [1]$$

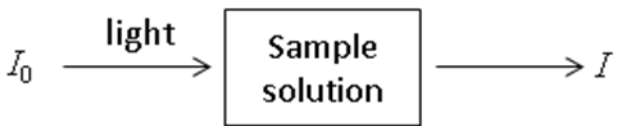

Figure 4.1 Absorption of light by the sample solution, I_0, the incident light and I, the transmitted light that passes through the sample solution.

The absorption and transmission of light by the sample solution has been presented in figure 4.1. Here, I_0 and I are the intensity of incident light and

transmitted light respectively, A is the absorbance, c is the concentration, b is the sample cell path length and ε_λ is the molar absorptivity at the wavelength λ. If $I < I_0$, then $A > 0$ and it indicates that molecule has absorbed energy. If $I = I_0$, then $A = 0$ which indicates that the molecule has not absorbed energy. Absorbance is usually measured in the range of 0-1 [104].

In a UV-visible spectrometer the sample solution is often placed in a quartz cuvette. Two lamps are used; one is a hydrogen or deuterium lamp for the ultraviolet region and the other a tungsten lamp for the visible region. In this way, radiation across the whole wavelength range is scanned by one spectrometer [105].

In this work, a UV-visible spectroscopy (GBC UV-Vis 920 spectrophotometer, Australia) is used for a complementary quantitative analysis of the active compound BHT released from coating in an aqueous SDS solution.

4.2 Light microscopy

In light microscopy, visible light is transmitted through or reflected from the sample through a single or multiple lenses in order to give a magnified view of the sample. The magnified image of an object is generated by the objective lens and this image is further magnified by a second lens system the ocular or eyepiece for viewing. The final magnification of the objective is the result of magnifying power of the objective times the magnifying power of the ocular. The resulting image can be directly detected by the eye and is often recorded by a digital charge-coupled device or CCD camera. The maximum resolution with light microscopy is 0.2 micrometers due to the wavelength region of visible light [106].

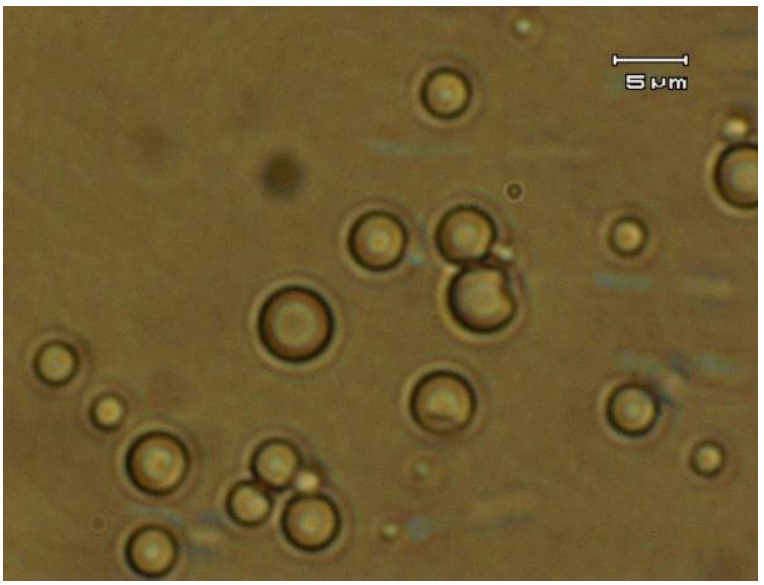

Figure 4.2 Optic micrographs of core-shell microcapsules containing octadecane oil as core and PMMA polymer as shell.

In this work, an optical light microscope (Olympus BH-2) equipped with a digital camera (Olympus DP1) has been used to analyze the morphology and to determine the size distribution of microcapsules. A light microscopy image of oil-core and polymer shell microcapsules has been shown in figure 4.2.

4.3 Scanning electron microscopy (SEM)

In scanning electron microscopy (SEM), an electron beam is used to get information about the sample. Electrons do not pass through the sample as the acceleration voltage is considerably low (1-50 kV). A beam of electrons (primary) is focused onto a sample which gives rise to emission of different kinds of electrons from the sample. These emitted electrons are collected to form an image. Depending on the intensity of the electrons, these might be low-energy secondary electrons (SE) or high-energy backscattered electrons (BSE). SE that is emitted from the sample surface gives rise to a topographical image from a depth down to 5 nm below the surface. BSE which is emitted from 0.5 µm below the surface give more qualitative information about the phases in the material rather than topography. For a conventional type of SEM, resolutions of few nm to 1 nm

can typically be obtained and it depends strongly on the nature of the sample. In SEM analysis, solid and conducting samples are most appropriate to analyze. However, non-conducting sample can also be analyzed but in this case, samples are coated with a thin conducting layer of e.g. gold. From SEM very local information of the sample can be obtained [107-110].

In this work, topography of the coating and morphology of microcapsules have been analyzed by a Leo Ultra 55 FEG SEM (**paper II and paper III)**. A SEM image of dry coating with and without microcapsule has been presented in figure 4.3. Before analysis, the samples are spinned on glass plates using a spin coater. The glass plate is then placed on a so-called SEM stub and coated by gold film of 105 Å thickness under reduced pressure using a JEOL JFC-1100 E Ion Sputter.

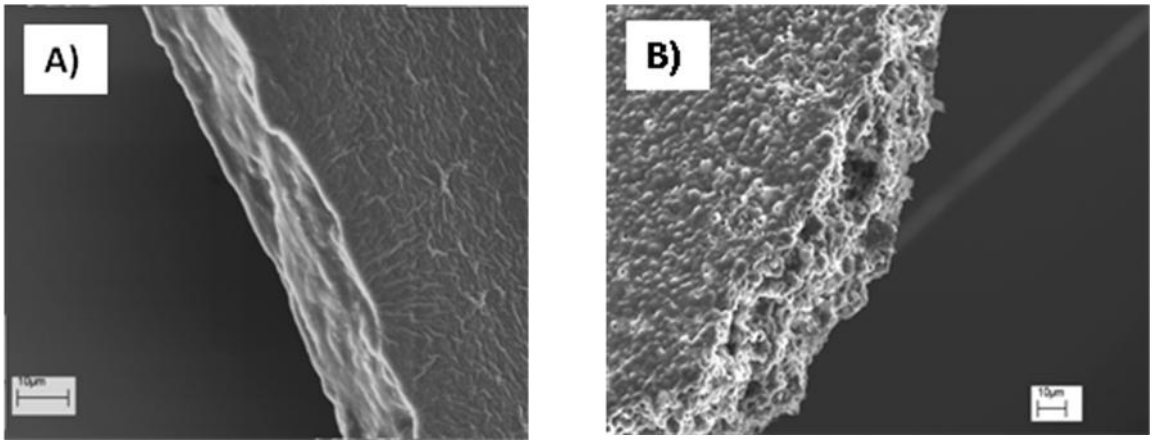

Figure 4.3 Scanning electron micrographs (SEM) of (A) a dry coating. (B) a dry coating containing microcapsules.

4.4 Nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy is a powerful tool for determination of molecular structure and dynamics. NMR signals provide a quantitative measure of the analyte concentration [108]. The principle of NMR is based on the atom nuclei that possess magnetic properties. These properties can be utilized to yield chemical information [108]. The spectral position is called the chemical shift and it gives valuable information

regarding the structure of the molecule. Different atoms within a molecule have different electronic environments. Differences in the electronic environment or variations in electronic density will cause shielding or de-shielding effects on the local magnetic field around the atom. As an example, a proton in close proximity to an electron-withdrawing group will cause the resonance signal to appear downfield in the spectrum due to the experienced de-shielding effect. The opposite is found when the proton is near to an electron-donating group [111-114]. The integral area of an NMR signal is directly proportional to the number of nuclei. It is thus possible to quantify the population of one compound relative to another [111].

In this work quantification of the sample with respect to different molecules was determined by integration of signals in the spectrum against an internal standard substance. A JEOL NMR spectrometer, with 1H resonance at 400 MHz, has been used for this purpose.

4.5 Scintillation Counter

Difference in atomic mass due to the presence of less or more neutrons in the atomic nucleus makes an element to exist as different isotopes. Usually isotopes are stable but some isotopes possess too few or too many neutrons in order to be stable. These are so-called radioactive isotopes and they rearrange their nuclei by emitting electromagnetic radiation or particles. Different types of radioactive emission are alpha particles (α), beta particles (β) and gamma radiation (γ). To quantify the radioactive compound in scintillation counter, a scintillation cocktail is used which is mixed with the sample. The scintillation cocktail absorbs the energy emitted by sample isotopes during radioactive decays and re-emit it as flashes of light. The resulting flashes of fluoresce are quantified by the scintillation counter. A scintillation cocktail contains two basic components, the solvent and the phosphor(s). The solvent absorbs energy and the phosphor within the solvent convert the absorbed energy into light. Quantification of radioactive decay is done by measuring the intensity of radiation emitted and it is often expressed as disintegrations

per minute or DPM. The SI unit of radiation is Becquerel or Bq which is equal to one disintegration per second. The curie or Ci, which is another measure, is equal to $3.7 \cdot 10^{10}$ Bq [115].

In this work, a liquid scintillation counter is used to determine the solubility of radio-labelled BHT in water and to quantify the released amount of (radio-labelled) active. A scintillation cocktail Ultima GoldTM and a liquid scintillation counter (PerkinElmer Wallac Guardian 1414) has been used to measure the radioactivity.

4.6 Quartz Crystal Microbalance with Dissipation Monitoring (QCM-D)

The quartz crystal microbalance with dissipation monitoring (QCM-D) is an ultra-sensitive weighing device that simultaneously measures the frequency and amplitude of an oscillating quartz crystal covered with gold electrodes. It utilizes the mechanical resonance of piezoelectric single-crystalline quartz. A thin quartz disc placed between two electrodes (typically gold electrodes) is subjected to an AC voltage across the electrodes. The quartz crystal starts to oscillate at a specific frequency which is directly proportional to the total mass of the crystal. Absorption of any species on the crystal surface results in an increase of total mass and change in oscillation frequency (Δf) [116, 117].

When the absorbed mass is less than the weight of the crystal, rigidly absorbed and evenly distributed over the active area of the crystal, the mass, Δm (g cm^{-2}) , can be calculated by the Sauerbrey equation [118, 119].

$$-\frac{C_f}{n_r}\Delta f = \rho_f \delta_f = \Delta m_{QCM-D} \qquad [2]$$

Here C_f is the mass sensitive constant (17.7 10^{-9} g cm^{-2} Hz^{-1}), n_r is the shear wave number, ρ and δ are the density and volume of the film, respectively.

However, the adsorbed films do not obey the Sauerbrey relationship if the surface is non-rigid and flexible. The combined effect of hydration

water, water trapped between adsorbed species, and the non-rigid character of many polymers/biomolecules induces frictional (viscous) losses and thus a dampening of the crystal's oscillation [117].

The quartz crystal microbalance with dissipation monitoring (QCM-D) used in this study was a Q-Sense D300 (Q-Sense AB, Sweden) with a temperature controlled fluid cell.

Chapter 5. Results and discussion

Papers I and II presented in this thesis are related to the designing of pesticide and biocide delivery systems which are applicable in the protective coating for plants, ship hulls, or building facades and many other situations. In **paper I**, pesticide is freely dispersed in a latex-based coating and the release behaviour and antifeedant activity are evaluated with respect to pesticide concentration and coating properties such as elasticity, wet ability and surface morphology. In **paper II**, encapsulated biocides and free biocides respectively are dispersed in both water- and organic solvent-based exterior wall paints. The release behaviours are compared in order to understand the controlled delivery system with respect to release retardation imposed by the encapsulation. In **paper III**, alkane oils of different melting points are employed as microcapsule core material from where the release behaviour of the pesticide to an aqueous SDS solution has been studied. This work helps to understand the effect of liquid and solid oil core on the diffusion of pesticide through the polymer shell. In **paper IV,** a study regarding the uptake and release to and from the microcapsule shell has been performed by QCM-D. The results help to understand the reasons behind the initial burst release out from the microcapsule.

5.1 Surface and release properties of coatings containing antifeedant

Understanding of surface properties like porosity and wettability are crucial since these properties have direct effect on the release behaviour of active from the coating [120]. The coating properties depend critically on the composition, i.e. the coating formulation. In the coatings studied in

paper I, water-based latex containing Eudragit copolymer (ethyl acrylate-co-methyl methacrylate, Eudragit NE 30 D) was used as binder. Such polymer forms a highly flexible, elastic coating. A hydrophobically modified cellulose polymer (HM-EHEC) was used as thickener to enhance the viscosity in order to facilitate the application procedure. Moreover, a non-ionic surfactant, alkylglucoside and the pesticide BHT (2, 6-butylated hydroxytoluene) were added to the coating. The variation of surfactant and pesticide concentration in the coating was done to check their effects of coating surface properties and ultimately on the release. Microscopic roughness and wettability of the coatings were analyzed by atomic force microscopy (AFM) and contact angle measurement, respectively.

5.1.1 Pesticide effect on the coating surface

The surface morphological changes of latex coating in presence or absence of antifeedant have been analyzed at nanometer length scale by atomic force microscopy (AFM). From the AFM analysis it is observed that the addition of BHT alters the dried coating properties. With BHT present in the dry coating, the surface becomes rougher. The difference in coating structure at the nanometre length was apparent and is shown in figure 5.1 and figure 5.2. This can be explained by the hydrophobic nature of BHT. The solubility of BHT in water is 0.2 to 1.0 ppm [16]. Being a rather hydrophilic latex there is a possibility of lower solubility of BHT in the coating and the surplus of BHT might form crystals upon drying. This is apparent as the coatings get dried and the roughness of the coating is enhanced [121]. In dynamic angle tests, almost a constant contact angle θ = 60° was observed when the BHT concentration was increased from 0% to 12 %. The hydrophobicity of BHT might enhance the wetting resistance of the coating which was confirmed by the higher contact angle in DAT analysis (figure 5.3B).

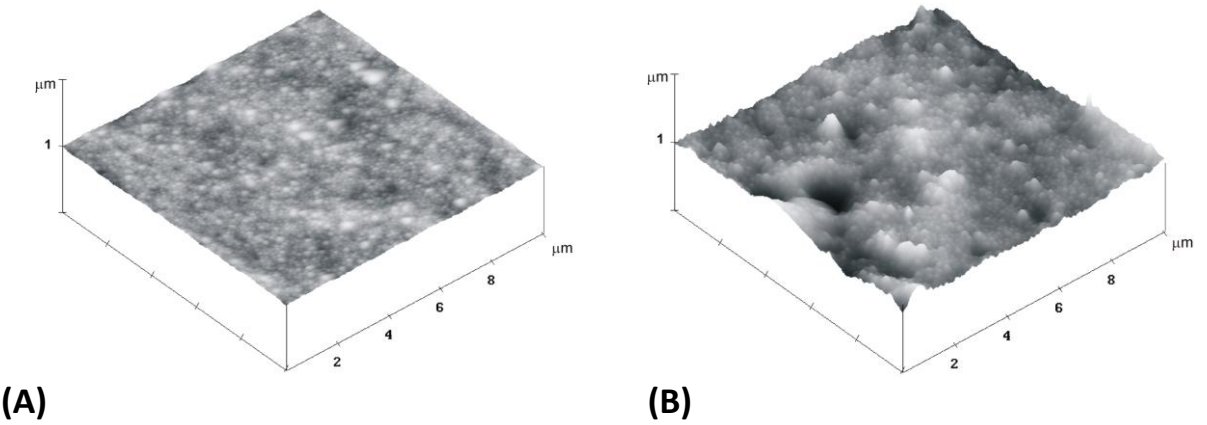

Figure 5.1 Atomic force microscopy image (AFM) of (A) Eudragit co-polymer coating surface. (B) Eudragit co-polymer + BHT (antifeedant) coating surface.

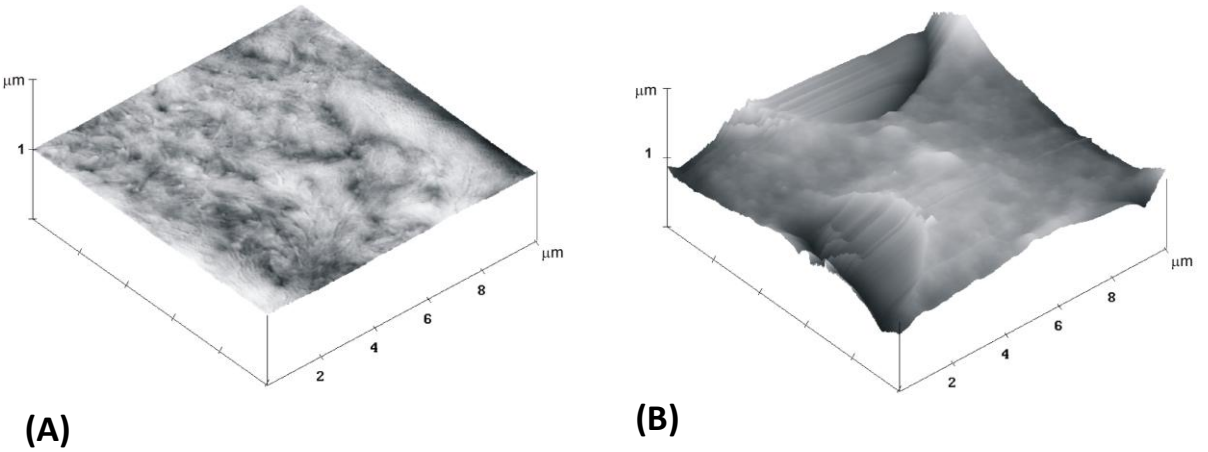

Figure 5.2 Atomic force microscopy image (AFM) of (A) Eudragit co-polymer + surfactant coating surface. (B) Eudragit co-polymer +surfactant+ BHT (antifeedant) coating surface.

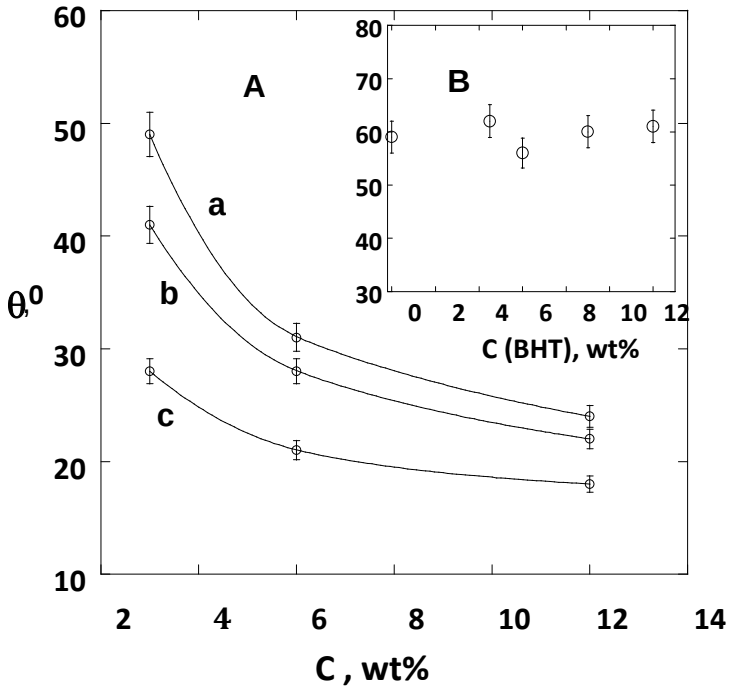

Figure 5.3 Contact angle of water on dry coating containing (A) Eudragit + BHT + surfactant at varying surfactant concentration and BHT concentration of (a) 4.5%, (b) 6%, and (c) 9%. (B) Eudragit + BHT at varying BHT content. Data points are means ± 1SD (n= 3 per data point).

5.1.2 Surfactant effect on the coating surface

The addition of alkylglucoside, revealed an effect on the surface properties of the coating. From AFM analysis, insignificant difference between latex coating and latex + surfactant coating was observed (figure 5.1A and figure 5.2A) although by a simple ocular observation indicated smoother coating in presence of surfactant. A plausible explanation may be that the surfactant renders the surface smoother by enhancing the plasticizing effect of the coating. Another explanation is that the surfactant phase separates from the latex medium and migrates towards the coating-air interface or redistributes in the coating matrix [122]. The former explanation was partly supported the observation of a decrease in contact angle (figure 5.3A). The water contact angle of the latex coating decreased with the increase of surfactant indicating that the wetting resistance of the coatings decreased at higher surfactant content. This signifies the immense effect of surfactant on the increased wettability of the coating.

On the other hand, presence of BHT makes the coating rough with significant protrusions of the surface even in the presence of surfactant (figure 5.1B and figure 5.2B). The roughness varies between ca. 7 to 70 nm. This could be due to a significant difference in latex content in the various coating formulations. To attain a fixed concentration of BHT (33%), the total latex content in EC+B+S and EC+B formulation was decreased by more than one third compared to pure EC+S and EC formulations where the latex content was 93.2% and 99.2%, respectively. With the decrease of latex and the addition of pesticide the smoothness of the coating decreases which appeared as an enhanced roughness of the coating surface.

5.1.3 Surfactant effect on coating release properties

In this work it has been critical to set up methods that quantify the release of pesticides from coatings. In **paper I and paper III**, an aqueous SDS solution was used as a release medium for BHT. The choice to include SDS was by pragmatic laboratory means in order to provide a decent time-scale for the experiments. Naturally, this does not provide the future applied situation where the pesticide is released from a plant coating in outdoor environment. The hydrophobicity of BHT made it difficult to study the release to pure water medium. In **paper I and paper III**, 0.01 and 0.125 M SDS respectively, were used as release media that (i) speed up the release of BHT from the coating and (ii) enable a water reservoir large enough to avoid influence of BHT water saturation.

To study the release behaviour of BHT, two different coating formulations, with EC+BHT and EC+BHT+S (berol) respectively, were studied. For each system, the BHT concentration was varied.

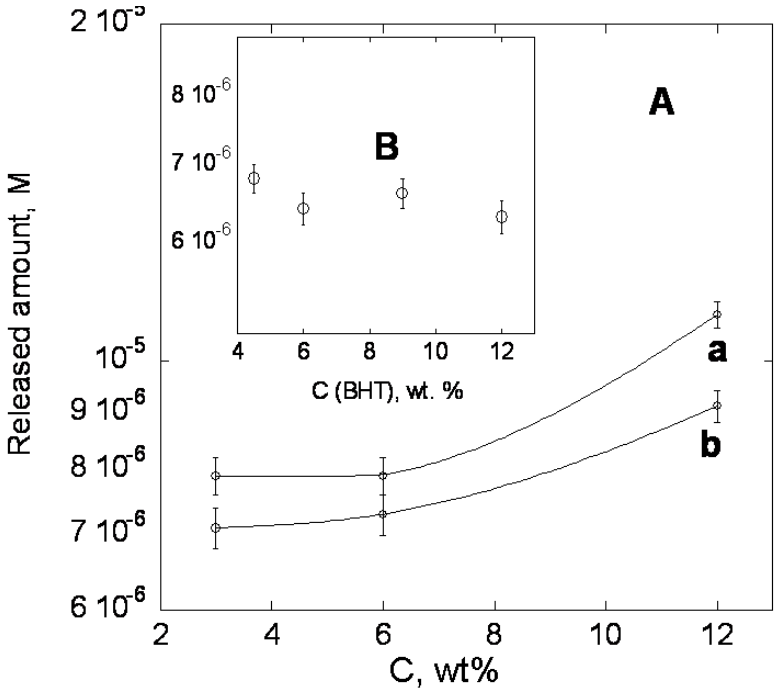

Figure 5.4 Concentration of BHT in a 0.01 M SDS aqueous solution after the release from following coating compositions: (A) Eudragit + BHT + S at (a) 9% of BHT (b) 4.5% of BHT. (B) Eudragit + BHT where BHT concentration has been varied. Experiments carried out in triplicates (n= 3 per data point).

In the Eudragit + BHT coating, the concentration of BHT ranged 4%-12 %. Surprisingly and within experimental accuracy, from all coating formulations, a similar amount of released BHT was found (figure 5.4 B). A higher release from coatings with higher concentration of BHT was expected. The result from this study indicates that the increase of BHT content does not lead to higher BHT release. During the coating drying process, BHT other than the molecularly dispersed fractions forms crystalline phases (figure 5.5). Such effect could explain the similar released amount of BHT despite various concentrations of BHT in the coating formulation. The dispersed fraction of BHT first enters into the release medium. The following crystalline fraction most probably releases as a very slow process.

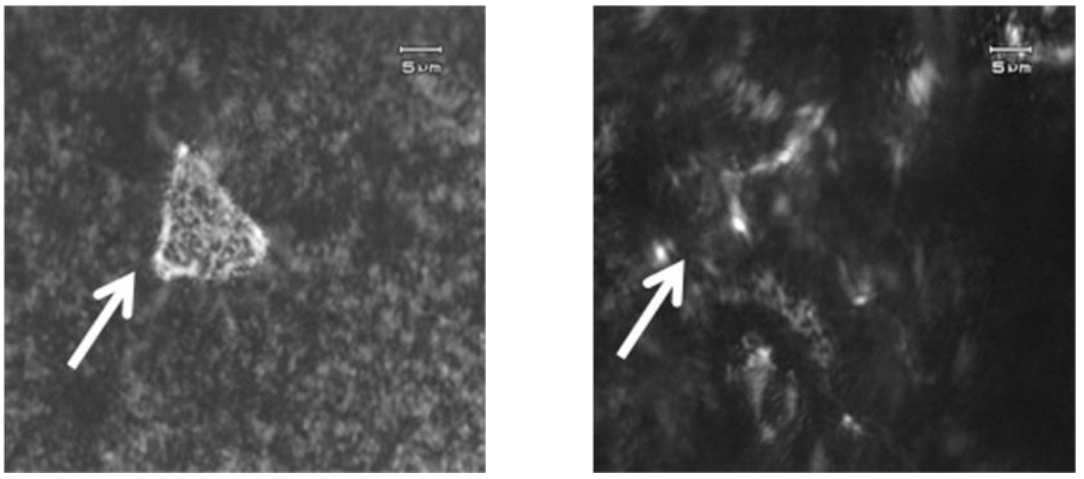

Figure 5.5 Optical micrograph under cross polarizer where free BHT crystals were observed in the dry Eudragit coating.

For the Eudragit + BHT + surfactant formulation, two different concentrations of BHT were loaded, 4.5 % and 9 % (dry coating weight). For each formulation, the concentration of surfactant was varied from 3% - 12 %. The released amount of BHT from these coatings were similar within the experimental accuracy (figure 5.4 A). In other words, we see a similar behaviour as shown in figure 5.4B and described above. On the other hand, with the increasing concentration of surfactant an increasing amount of released BHT was observed. The latter result signifies the effect of surfactant on the release behaviour of BHT from the coating. The surfactant may increase the solubility of BHT in the wet coating and provides a molecular dispersion in dry coating. It may also increase the porosity as well as hydrophilicity of the coating during the film forming process, at the final stage by segregation of the surfactant at the surface of the coating [122-125].

From the surface morphology and release studies, it can be assumed that the surfactant is one key additive in the coating formulation and governs the release of BHT from the coating.

5.1.4 Antifeedant activity bioassays

The antifeedant activity of a test compound is generally demonstrated through laboratory bioassays and/or field tests. In the antifeedant activity tests, the insects are introduced to an antifeedant compound. The activity of the test compound is then quantified on the basis of value from antifeedant index (AFI). A unity value of antifeedant index, AFI=1.00, corresponds to a complete inhibition of feeding, half effect at a value of 0.50 and no effect at zero AFI. Negative values indicate (< 0) feeding stimuli.

In this work, the pine twigs were coated with coating formulation containing varying concentration of BHT and surfactant. The antifeedant activity of BHT at various concentrations in coating formulation is presented in figure 5.6.

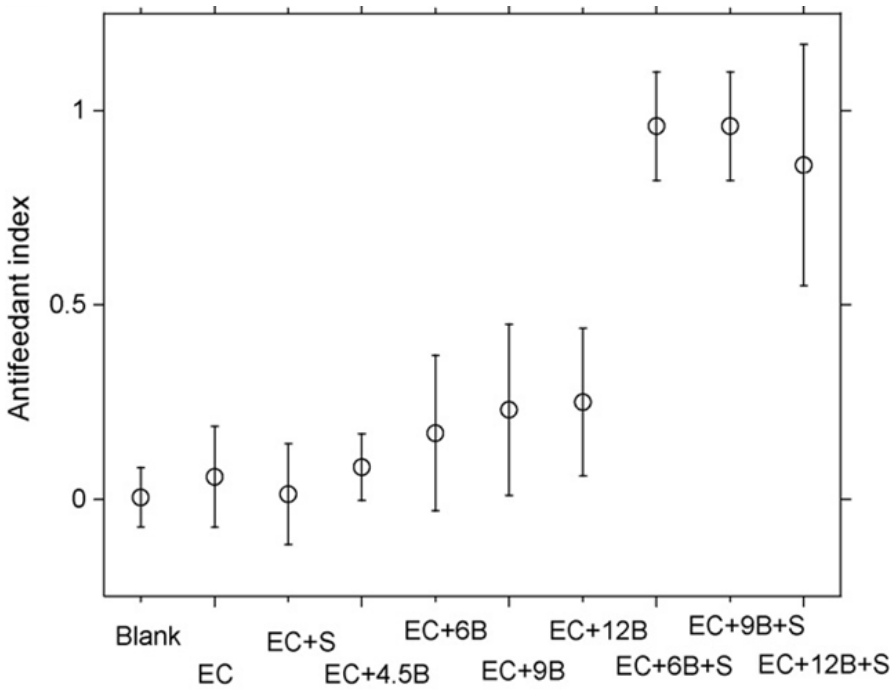

Figure 5.6 Feeding inhibition expressed as antifeedant index for coatings with laboratory no-choice test on pine twig sections (*n* = 10). The bark on conifer twig sections and seedlings was uncoated (Blank) or coated by formulations. Abbreviations: Eudragit copolymer (EC), BHT antifeedant (B), surfactant (S). Numbers next to the abbreviations below the x-axis denote the wt % of BHT in the dry coating. The y-bars denote a 95% confidence interval.

In figure 5.6, the antifeedant activity of BHT with increasing doses and with (EC+S, EC+6B+S, EC+9B+S, EC+12B+S) or without surfactant (EC, EC+4.5B, EC+6B, EC+9B, EC+12B) are compared. No antifeedant activity was obtained for the control samples (blank, EC, EC+S) since these coatings did not contain any antifeedants. The EC+BHT formulations showed moderate antifeedant activity (0-0.5). The highest antifeedant activity was obtained from the Eudragit copolymer + BHT+ surfactant formulations giving an AFI close to 1. The results from these two series of formulations indicate the strong effect of surfactant on BHT release. Surfactant seems to enhance antifeedant activity of BHT and impart specific properties of the coating, allowing it to inhibit feeding of pine twigs.

5.2 Gravimetric analysis of free and encapsulated pesticide

A simple gravimetric analysis was performed to monitor the evaporation of BHT in free and encapsulated form and thereby to follow the effect of encapsulation. The encapsulation can restrict the diffusion of BHT and this is presented in figure 5.7. A specific amount of free BHT and encapsulated BHT (18 % of the microcapsule) has been placed on glass slides. The declining weight of BHT over time from both systems has been measured by weight measurements. From figure 5.7, faster decrease in weight is observed from pure BHT compared to the encapsulated form. The considerable difference in weight loss indicated the restricted evaporation of BHT from microcapsules compared to air-exposed free BHT.

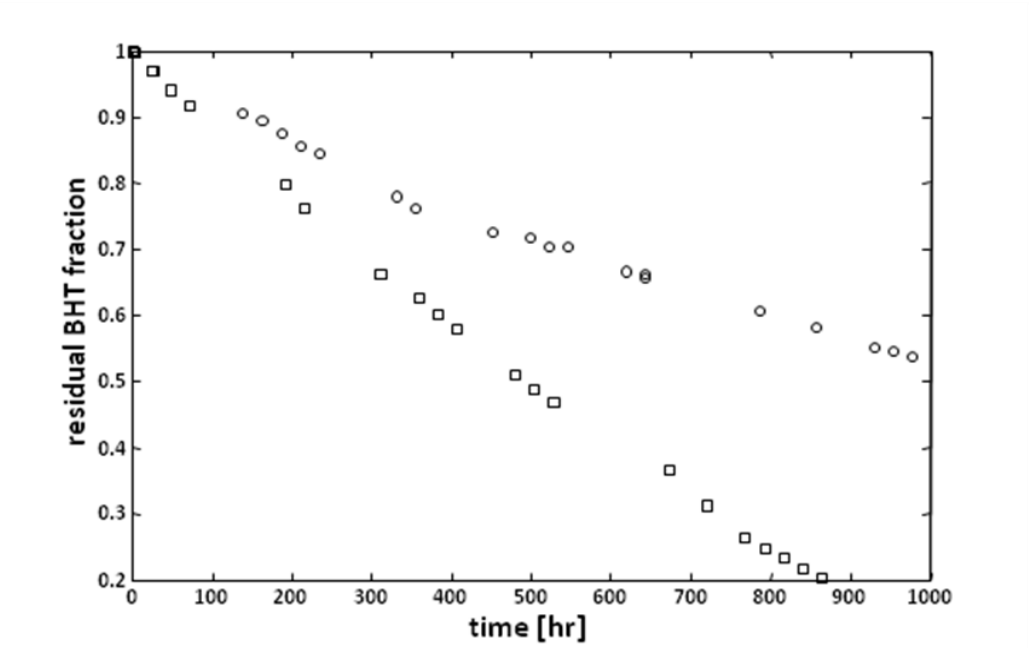

Figure 5.7 Gravimetric analysis of BHT evaporation placed on glass slides: (□) free BHT and (○) encapsulated BHT.

5.3 Release behaviour of free and encapsulated biocide containing coatings

In **paper II**, the encapsulation and its effect on the release behaviour of a biocide from a coating system have been presented. PMMA polymer-based microspheres have been formulated rather similar to the procedure described by Loxley and Vincent [126]. The biocide medetomidine, which is used in marine applications, was used here as a model compound and dispersed within the microsphere polymer matrix. It has already been discussed in section 5.2 that by encapsulating the active in a polymer network it is possible to restrict the diffusion of molecules into the outer release medium (e.g. coating, air or water). The main objective of **paper II** is to investigate the use of microspheres in coating systems for controlled release of biocides ultimately for prolonging biofouling protection. In this part of work, both water- and organic solvent-based exterior wall paints were used and the study compared the release behaviour of free and encapsulated biocide into water release medium. The release profiles are presented in figure 5.8.

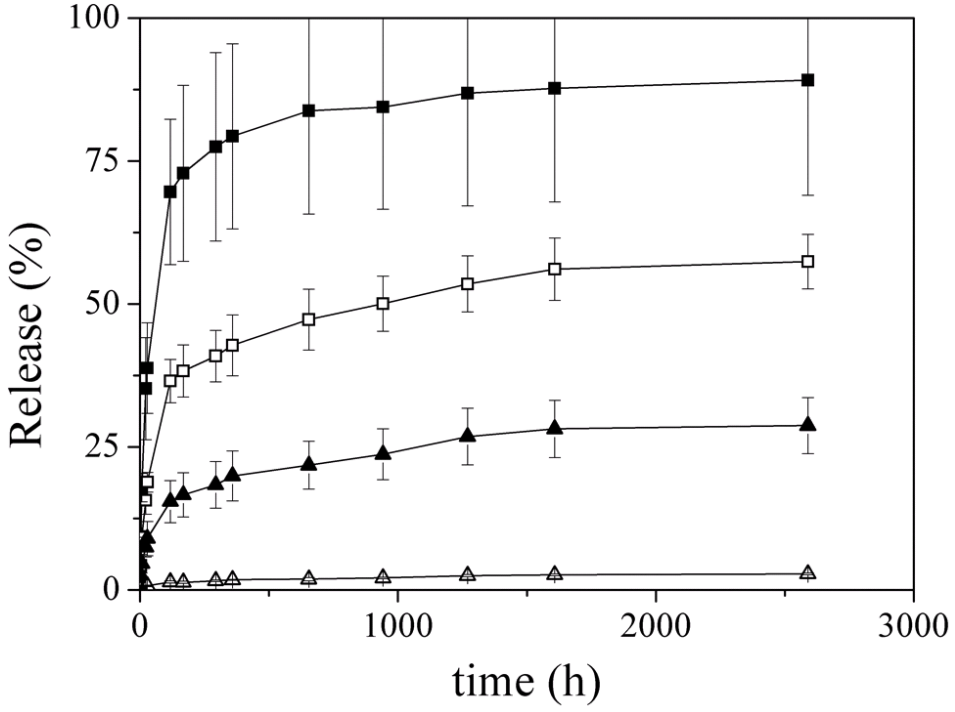

Figure 5.8 The release of biocides, from (■) water-based paint with free biocide, (□) water-based paint with encapsulated biocide, (▲) solvent-based paint with frbiocide, and (△) solvent-based paint with encapsulated biocide.

From figure 5.8, it is seen that the biocide release is affected by both release medium [71] as well as by the choice of paint system. Using an aqueous release medium it is expected that the water-based coating has higher wettability than the solvent-based coating and indeed a faster release was observed from the water-based paint. Water penetration into the coating swells the paint polymer network and facilitates the biocide leakage. More importantly, the release of free biocide in both water- and solvent-based coatings was faster compared to coatings with encapsulated biocides. This result significantly implies the effect of restriction imposed by the microsphere PMMA matrix on the diffusion of the biocide. Figure 5.9 confirms the consistency of the embedded microsphere and equivalent SEM images were detected for both types of coating systems.

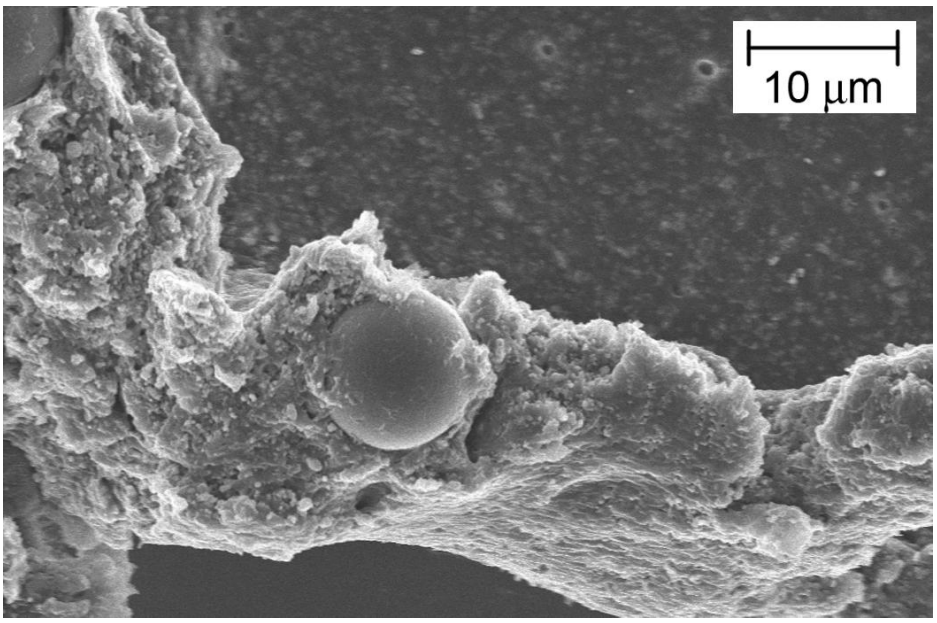

Figure 5.9 Electron micrograph of a dry paint matrix (scratched by a needle) of water-based paint with encapsulated biocide.

From figure 5.8, it is noticeable that the initial release is faster which might be interpreted as the primary swelling of the coating which facilitates the dynamic activity of biocide within the coating.

The results of this study provide a conclusion that a coating containing microencapsulated biocide prolonged the release compared to a coating with non-encapsulated biocide.

5.4 Controlled release from liquid and solid core microcapsules and the effect on release rate

In **paper III**, the pesticide BHT, is encapsulated in an oil-core PMMA-shell microcapsule. In the previous work presented in **paper I**, it is described that BHT acts as a pesticide, an antifeedant, against the pine weevil *Hylobius abietis.* In **paper I**, BHT was freely dispersed in the latex coating. Being dispersed in this simple way it may deplete too rapidly from the coating which mean that the protective nature of the coating will be too short [127]. A more long-lasting protection is here proposed to be achieved if the BHT is encapsulated before added to the latex. In **paper III**, BHT was encapsulated in solid and liquid oil-core PMMA-shell

microcapsules, respectively. The ultimate aim was to prolong the life-time of the coating by controlling/slowing down the release of actives and keep the antifeedant activity of the coating over an extended period of time. The core medium can be an effective parameter for controlling the release of organic substances from microcapsule systems [128]. In **paper III**, two alkane oils of different melting temperature, dodecane (-9°C) and octadecane (28-30°C), were used and BHT release studies were performed with dispersed microcapsules in a 0.125 M SDS aqueous solution at room temperature. The size distributions of microcapsules were investigated by optical light microscopy images and are presented in figure 5.10. Subsequent to the release studies, the experimental data were compared to analytical release models that describe diffusive release out from core-shell systems. The release profiles are presented in figure 5.11**.**

5.4.1 Size distributions of microcapsules

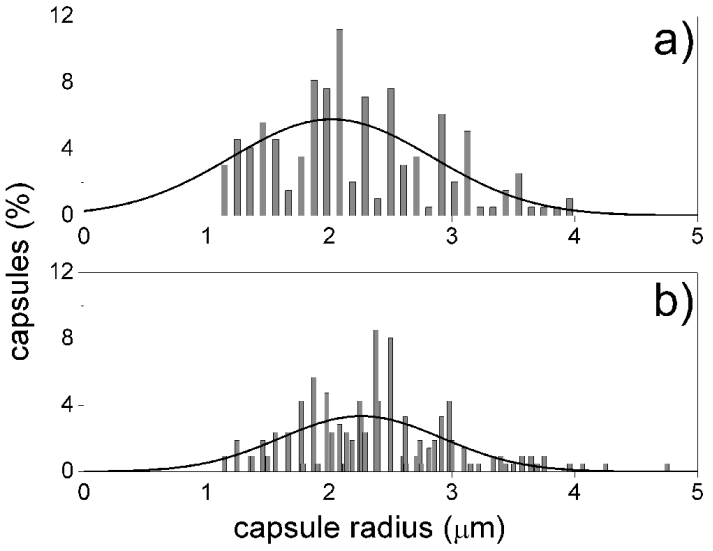

Figure 5.10 Size distributions of microcapsules with (a) dodecane and (b) octadecane as core medium. Data collected from optic micrographs and are fitted according to a normal Gaussian function.

The size distribution of microcapsules was obtained from 200 particles from light microscopy images. The experimental data (bars) and the results from fitting a normal Gaussian distribution model (lines) to the dodecane and octadecane microcapsule systems are presented in figure 5.10 .The mean radius was 2.0±0.8 μm and 2.3±0.7 μm for dodecane and octadecane oil-core microcapsules, respectively.

5.4.2 Release studies from microcapsules

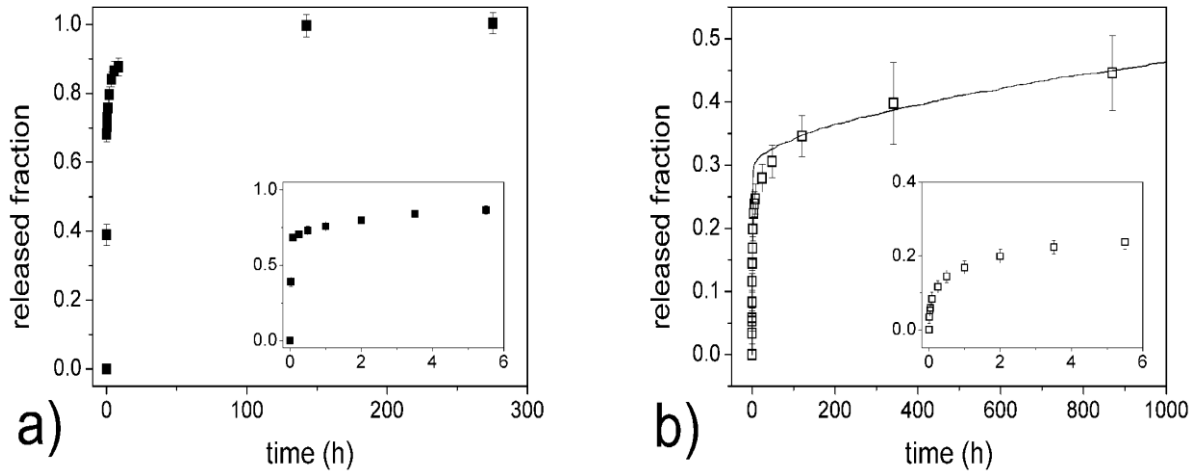

Figure 5.11 Release of BHT from microcapsules with (a) dodecane and (b) octadecane as core medium. Solid line represents the release from octadecane capsules calculated by computer simulation of Brownian motion. Note the different axis scales.

The release profiles (figure 5.11) obtained from dodecane and octadecane microcapsules are both characterized by an initial burst release followed by a slow release over an extended period of time. The very high initial release rate might be due to accumulation of BHT in PMMA shell during the formulation procedure [48]. A major fraction of BHT is thus released from the microcapsule very rapidly leaving a minor fraction to release slowly. This behaviour was more pronounced in the dodecane microcapsule system. The fraction released at short times most probably originated from the shell whereas the fraction that released at long times originates from the core of the microcapsule which rendered the active molecule to diffuse over a longer distance to reach to the aqueous phase. Also, the BHT in the core was thermodynamically prone to enter the shell only when the concentration of the molecule in the shell was low sufficiently. In both the dodecane and octadecane microcapsules, BHT was initially present in unsaturated concentration. This ultimately leads to the continuous declining of BHT concentration as well as decreased release rate over time [48].

The initial release rate as well as the total released yield of BHT from the liquid dodecane microcapsule (figure 5.11a) is higher than from the solid octadecane (figure 5.11b) microcapsule. This result is considered to origin from a combination of several factors. The movement of the liquid dodecane phase allow BHT (by diffusion) to come into contact with the inner surface of the shell, an effect which speeds up the overall flux [48]. When BHT was dispersed in the octadecane core the release was slower as the diffusion coefficient is several orders of magnitude lower in a solid alkane compared to a corresponding liquid. The plasticizing effect of oil in the shell (PMMA) might also increases the permeability of the polymer material. Being a liquid with lower molecular weight, dodecane exerts more plasticizing effect on PMMA shell than octadecane. The presence of dodecane in PMMA thus increases the flexibility of the PMMA chain as well as the free volume of the polymer [129]. Thereby the permeability of the shell increases which means that the diffusion rate of BHT increases and the release is faster [130-132]. Also the solubility of dodecane in water is higher compared to octadecane oil which in turn adds to faster diffusion of BHT from the microcapsule [133, 134]

5.4.3 Computer Simulations

The release profile of BHT from core-shell microcapsules was calculated from Brownian dynamics simulations and the result that best compared to the experimental data is presented in figure 5.11b. Here, a BHT molecule is allowed to "jump" a certain distance chosen from Gaussian probability distribution function. The jump length during one time step was set to 0.1% of the domain length L (where $L = 1.6 \cdot 10^{-6}$ m, i.e. L is set to the radius of the core). The polymer shell thickness was set to $0.4 \cdot 10^{-6}$ m. A particle (BHT molecule) is considered to diffuse in the predefined domain length (within the core and the shell) with two different diffusion constants – one in the core and the other in the shell. The diffusion coefficient of BHT in the octadecane core was set to $D_{core} = 1 \cdot 10^{-20}$ m^2s^{-1} which is a typical value for diffusion in a solid material. The diffusion coefficient of BHT in the polymer shell and the initial partition coefficient between core and shell were fitted to experimental data and the results were $D_{shell} = 1 \cdot 10^{-17}$ m^2s^{-1} and $p = 0.5$, respectively. This clearly indicated the presence of a significant

initial fraction of BHT both in the core and in the shell. The result obtained for the dodecane microcapsule (i.e. a liquid core) was not in good agreement with experimental result, an effect that we attribute to the burst release of BHT.

The result of this study indicated that the material in the core of the microcapsule has a strong influence on the diffusion of active substances from core-shell microcapsules. In an octadecane microcapsule the core is solid at room temperature resulting in slow release compared to the case when the core consists of liquid dodecane. By using solid or liquid oils as core material it is possible to control the release rates from microcapsule systems.

5.5 Kinetics of uptake and release from a model microcapsule shell

In **paper III**, oils of different melting temperatures were used in the core of the microcapsules. The active substance was BHT and PMMA was the shell material of the microcapsules. The release behaviour of BHT from microcapsules of different physical states was then studied. One interesting finding was the initial "burst" release followed by a much slower release. The results formed the basis of the investigation performed in **paper IV.** The aim was to further enhance our basic understanding of the release mechanisms of BHT from microcapsule systems.

In this investigation, a 25 nm thick PMMA film was prepared on a gold surface by spin coating from a dichloromethane solution. The film was then used as a model of the microcapsule shell. The idea was that the film behaves in a similar manner, with respect to BHT uptake and release, as the PMMA shell of the microcapsule. The properties under investigation were the presence of BHT and alkane oil in PMMA by monitoring the time-dependent uptake and release of PMMA by the QCM-D technique. Upon uptake of BHT in the PMMA film a change in QCM-D resonance frequency was noted. The basics of the experiment are shown in figure 5.13.

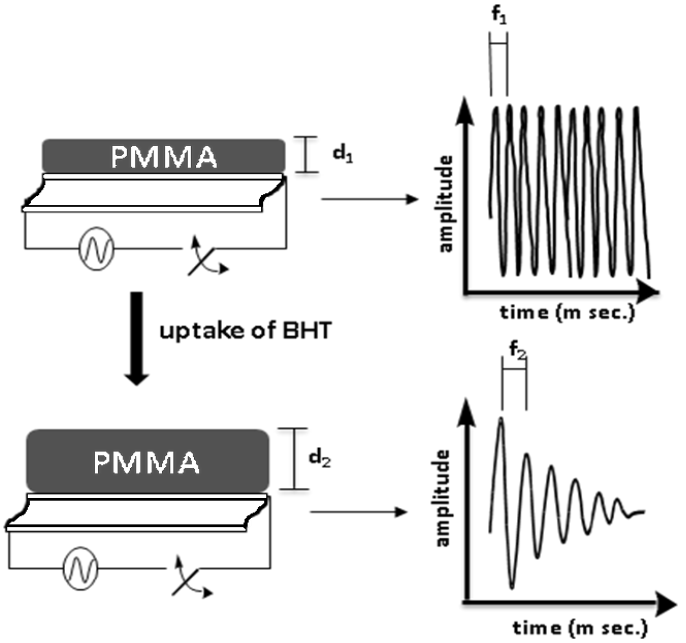

Figure 5.13 A schematic diagram of a model PMMA shell showing the frequency change with BHT uptake.

The uptake or mass absorption of dodecane and BHT in the film as well as release of the above-mentioned compounds into milli-Q water and aqueous SDS solution were monitored and are presented in figure 5.14 and figure 5.15, respectively.

5.5.1 Kinetics of dodecane and BHT uptake on PMMA film

The uptake of BHT into the PMMA film was monitored at 22˚ C. A thin film (25 nm thickness) was prepared on a QCM-D sensor surface and 300 µl dodecane was applied on top of the film. The frequency and dissipation changes were monitored as a function of time until a stable baseline was obtained. After stabilization, 300 µl BHT/dodecane solution (5 mg/ml) was applied on the dodecane-soaked PMMA film in order to monitor the uptake of BHT. Following the uptake, dodecane was again applied on the film to flush the surface of excess BHT. The release was monitored at the same time. The result is presented in figure 5.14.

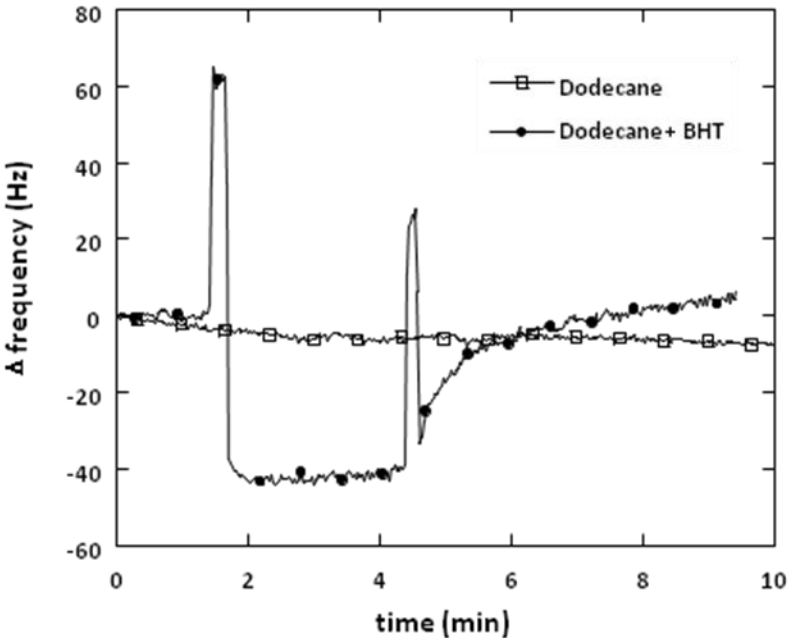

Figure 5.14 Kinetics of dodecane and BHT uptake in PMMA film: (□) dodecane, (●) dodecane + BHT.

As noted in figure 5.14, a negative frequency shift was observed following dodecane/BHT solution addition, indicating that absorption into the PMMA film had taken place. The quantification of frequency data into mass was done by the Sauerbrey equation. This equation relates the change in frequency to change in mass of the film according to equation 3 [118, 119],

$$-\frac{C_f}{n_r}\Delta f = \rho_{polymer} \quad \delta_{polymer} \quad = m_{polymer} \qquad [3]$$

Here, $\rho_{polymer}$ and δ_{polymer} are the density and volume of the film, respectively.

It is obvious that BHT was the reason for change in frequency and not the dodecane. The change in frequency for dodecane was only -8Hz after 10 minutes whereas a rapid drop to -40 Hz was observed for BHT. Flushing the film with pure dodecane resulted in a slow increase in frequency indicating that the desorption was much slower than the absorption process of BHT [135].

5.5.2 Release studies of dodecane and BHT from PMMA film

To measure the release of BHT and dodecane from the PMMA film, 0.125 M aqueous sodium dodecyl sulfate (SDS) solution was used as release medium. As a reference, the same experiment was carried out with milli-Q water as release medium. The release profiles of BHT and dodecane from the film are presented in figure 5.15.

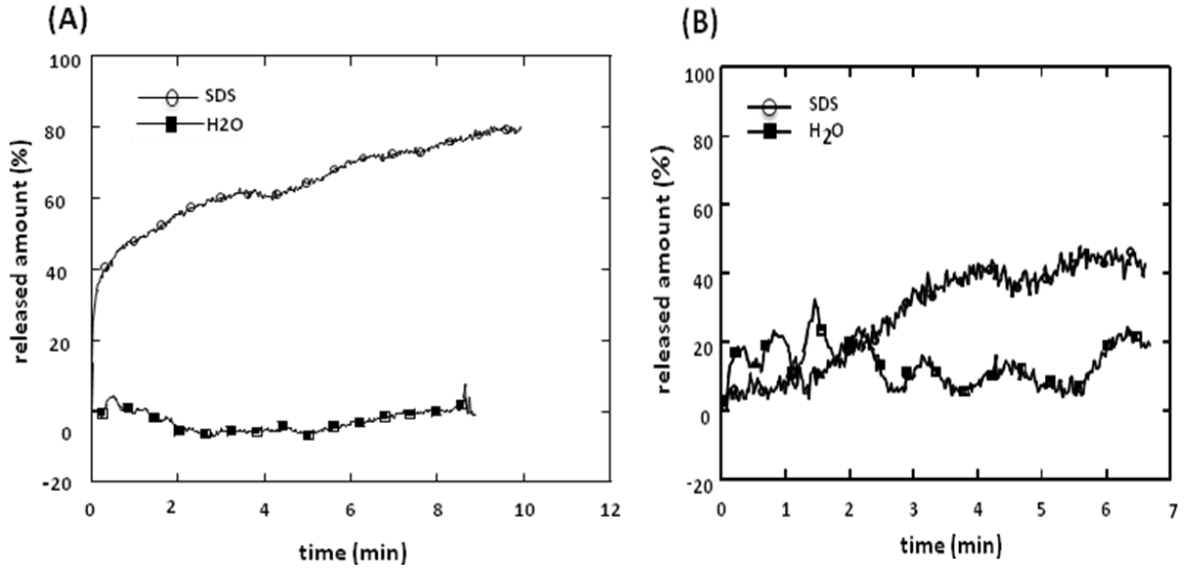

Figure 5.15 Release studies of (A) BHT from BHT+ dodecane PMMA film, (B) dodecane from dodecane PMMA film in (○) aqueous SDS solution and in (■) milli-Q water.

Upon rinsing with SDS solution a positive frequency shift was observed. The positive shift was an indication of mass loss from the film. The amount desorbed was again quantified by Sauerbrey equation and presented as released amount. The loss of BHT was relatively high for the BHT/dodecane-treated film. For dodecane, from dodecane-treated film, it was already observed in previous section that dodecane absorbs to a small amount compared to BHT. The small absolute amount of absorbed and released dodecane is the most likely reason for the signal being dominated by noise and noted change is likely to be originating from drift in the measurements. With milli-Q water as release medium, a negligible amount of BHT was desorbed most likely due to the very low solubility of BHT (0.2-1.0 ppm) in milli-Q water [16].

The result of this study showed that the extent of BHT uptake (7.7% of the total mass of PMMA) in the PMMA film was much higher than that of dodecane. This indicated that the PMMA shell in microcapsules is likely to contain significant concentrations of BHT. We therefore suggest that this fraction is the reason for the initial “burst" release. The release of BHT was strongly facilitated by SDS solution. Taken together, these results strongly indicate that the PMMA film contains relatively large amounts of BHT but rather insignificant amounts of dodecane.

Chapter 6. Conclusion

The goal of this work was to better understand how to control the release of pesticides from microparticles formulated into protective coatings. In the first part of the thesis, release studies of molecularly dispersed and encapsulated pesticides/biocides from coatings have been performed. In the second part, the effect of different oils present in the microparticles was studied.

The release of molecularly dispersed pesticide in a latex coating into an aqueous release medium was studied with respect to coating properties such as elasticity, wettability and surface morphology **(paper I).** It was found that the release of butylated hydroxytoluene (BHT) is higher when an alkyl glucoside surfactant is present in the coating. The surfactant likely increases the dispersibility of BHT as well as the porosity and wettability of the coating. Bio-assay tests were also performed and showed that the antifeedant activity is higher in the presence of surfactant.

In order to control the release and thereby to prolong the protection of coatings, biocides were encapsulated and formulated into coating systems and characterized in terms of release. The results are presented in **paper II**. The release of free and encapsulated biocide from water-based and solvent-based exterior wall paint was studied. A slower release was found in systems where the biocide was encapsulated. It was observed that encapsulation of biocide significantly restricts the release and the physical properties, e.g. swelling, of the coating may affect the release behaviour.

To further study the release behaviour from microcapsule systems, two oils with different melting temperatures (solid and liquid at room temperature, respectively) were used as microcapsule core materials. The

release behaviour of the BHT pesticide from these capsules is presented in **paper III**. For both the oils an initial burst release was followed by a slower release pattern. An accumulation of BHT in the shell during microcapsule formulation was likely the reason for the burst release. Once the BHT concentration in shell became small enough a much slower release mechanism, from the microcapsule core, took over. The release rate from dodecane (liquid at 25°C) microcapsules was higher than that of octadecane (solid at 25°C) microcapsules. The release data were modeled by computer simulations assuming two diffusion coefficients, one for the core and the other for the shell, respectively. In addition, the initial core/shell partition of BHT was varied in the simulations. The result obtained for the octadecane microcapsule was in good agreement with the experimental results. The diffusion constant of BHT in the core was set to 10^{-20} m^2s^{-1}. The diffusion constant of BHT in the polymer shell and the core-shell partition coefficient were calculated to be 10^{-17} m^2s^{-1} and 0.5, respectively.

To increase our understanding concerning the shell impact on the burst release, noted from core-shell microcapsule systems, the distribution of oil and BHT in a PMMA film was studied by Quartz Crystal Microbalance with Dissipation monitoring (QCM-D) in **paper IV**. The total mass uptake and release of dodecane and BHT into and from the film was quantified by the crystal frequency shift. The absorption of BHT was 7.7 wt% with respect to PMMA, a value that was much higher than for dodecane absorption. The results supports the results of **paper III** where the initial burst release was described as an accumulation of BHT within the shell during microcapsule formulation.

From the studies in this thesis it can be concluded that the formulation of a coating plays a major role on the release behaviour of pesticides and biocides. The release of active compounds can be restricted by encapsulation within a reservoir system. In future studies, it would be highly significant to investigate the compatibility of microparticles in various coating formulations and to tune the chemistry of core-shell materials to be able to control release behaviour. It would also be highly interesting to investigate these systems in real-life situations.

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