THESIS FOR THE DEGREE OF LICENTIATE OF ENGINEERING

Experimental Design and Evaluation of Biocide Release from Microcapsules

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CHALMERS UNIVERSITY OF TECHNOLOGY

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Cover: Set of experimental methods to study release from microparticles.

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ABSTRACT

To protect a painted house façade from mold or algae, anti-growth agents are usually mixed in the paint. The protection of paint systems used in the past relied on heavy metals for protection and are banned today due to their negative impact on the environment. Today, less harmful heavy metal-free biocides are used and these are mixed directly in the paint. However, contemporary paint systems lose their anti-growth protection long before the end of their intended lifetime. This problem is related to the high diffusivity of the biocide inside the paint matrix. The protection is lost prematurely as the biocides are leached and rinsed from the coating by water at a high rate. A promising improvement can be achieved by encapsulating the biocides and thereby reducing the release rate from the coating. In this thesis the biocide 2-n-octyl-4-isothizolin-3-one (OIT) has been encapsulated in core-shell microcapsules and homogenous polymeric microspheres respectively, using the internal phase separation method.

The main scientific objective of this work is to explore the mechanisms affecting controlled release from microparticles. By understanding how, and to which extent, different parameters influence the release of biocides, a prolonged and controlled surface protection of paint and other coatings can be achieved, which is the general purpose of the work. Moreover, the aim is to design a release methodology in order to evaluate the release of biocides.

The internal phase separation method is a straightforward technique and suitable for the encapsulation of hydrophobic biocides. The formulation has been tuned in order to sustain the release of OIT to a surrounding medium. The effect of two parameters; porosity and surface modification, on the release have been the focus of this thesis. Moreover, the porosity has been subdivided into the *macroscopic porosity* of the coating matrix and the *microscopic porosity* or free volume of the polymer matrix of the microparticles. It was found that the microscopic porosity is highly affected by the evaporation rate of the volatile solvent during the encapsulation. A slow evaporation rate gave a slower release rate of OIT. In addition, it was shown that the macroscopic porosity was significantly dependent on the drying time of the coating. Longer drying times (several weeks) gave a substantial decrease in macroscopic porosity and release rate of OIT. Regarding the surface modification, the assembly of a polyelectrolyte multilayer (PEM) on the surface of the microparticle resulted in a considerable decrease of the release rate of OIT. This is ascribed to the high charge density and hydrophilicity of the PEM barrier in which OIT has a low solubility. The use of these

surface-modified microparticles in coatings rendered the release of OIT more or less independent of the drying of the paint. The PEM was therefore identified as the rate-determining barrier in that system.

Keywords: Microcapsule, microsphere, polyelectrolyte multilayer, microscopic and macroscopic porosity, controlled release, biocide, internal phase separation method

List of publications

This thesis is a summary of the following papers:

I. Charged microcapsules for controlled release of hydrophobic actives Part III: Effect of polyelectrolyte brush- and multilayers on sustained release
Markus Andersson Trojer, Helena Andersson, Ye Li, Jonatan Borg*, Krister Holmberg, Magnus Nydén and Lars Nordstierna
Physical Chemistry Chemical Physics, 2013, 15, 6456-6466

II: Controlled release of microencapsulated 2-n-octyl-4-isothiazolin-3-one from coatings: Effect of microscopic and macroscopic pores
Jonatan Bergek, Markus Andersson Trojer, Alberta Mok and Lars Nordstierna
Colloids and Surfaces A: Physicochemical and engineering aspects, In press

III: Controlled release of microencapsulated 2-n-octyl-4-isothiazolin-3-one from coatings: Polyelectrolyte multilayers as a global and rate-determining barrier
Jonatan Bergek, Markus Andersson Trojer, Hermann Uhr and Lars Nordstierna
Submitted to *Journal of Materials Chemistry B: Materials for biology and medicine*

* I changed surname from Borg to Bergek in July 2013

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INTRODUCTION

Growth of mold and algae at painted façades is a worldwide problem. The esthetic problems are often easy to see; grey or black parts change the appearance undesirably. However, paint is also applied to protect the underlying surface [1] and the way of preserving has changed over time, often due to regulations after discoveries of upcoming environmental issues connected to the selected mode of protection. Toxic metal compounds including tin and mercury have been used before but are now banned [2]. Today, the paint industry relies on metal-free and less hazardous biocides as protection against growth [1, 3, 4]. Almost all biocides are small hydrophobic molecules and they are designed to destroy or repulse organisms as mold and algae via a biological or chemical reaction [5]. In industrial production, the biocides are normally mixed directly in the paint during the formulation. Subsequent to paint application and drying, the small biocides display a relatively fast migration inside the paint matrix, which is both positive and negative from a user's perspective. The biocides protect by destroying growth at the surface of the paint layer, meaning that the biocides have to be able to move easily inside the polymeric paint matrix in order to migrate to the outermost surface [2, 6]. However, the high diffusivity inside the

paint matrix also contributes to fast leakage of biocide, which is rinsed away with the water during rain. This lead to premature loss of surface protection [3, 5, 7].

One way to minimize the losses of biocide and to prolong the surface protection against micro-organic growth is by using microparticles. In this thesis, microparticles are solid particles in the micrometer size range and can be both of core-shell structure, so called microcapsules, or homogenous polymer matrices called microspheres [8]. The microparticles are too large to diffuse inside the polymeric paint matrix and they are also easily homogenously mixed in the paint and embedded in the dry coating [9]. These properties give the opportunity to control the release of biocide into the paint matrix, since the biocide first has to diffuse inside the microparticle, then be released from it, thereby diffusing inside the paint matrix, and finally migrate to the outermost surface. A controlled release from microparticles can prolong the protection of the paint since the biocides will stay in the paint matrix for a longer time.

The release rate of a selected biocide can be tuned to a great extent by tailoring several physicochemical properties of the microparticles. Shell thickness, particle size, choice of core and shell material as well as surface charge density are some of the parameters that can be tailored in order to control the release and prolong the protection against biofouler growth. The microparticles can also be modified after encapsulation, so called post-encapsulation, e.g. by building polyelectrolyte multilayers on the surface of the microparticles.

1.1 OBJECTIVES

The main scientific objective of this work is to explore the mechanisms affecting controlled release from microparticles. By understanding how, and to which extent, different parameters influence the release of biocides, a prolonged and controlled surface protection of paint and other coatings can be achieved, which is the general purpose of the work. To analyze both the microparticles and the release from them, several experimental methods have been used. As examples, microscopy gives information of morphology and size distribution of the microparticles and UV/Vis spectrophotometry is used to analyze the release of biocides. There is an eco-efficient aspect to the work since the goal is to develop surface protection systems that are both economically and ecologically sustainable.

A generic method has been designed to study the release of biocides. The goal was to find a setup suitable for different release systems as well as model applications.

In **Paper I** polyelectrolyte multilayers were built on microspheres using the block copolymer poly(methyl methacrylate)-block-poly(sodium methacrylate). A release study using the dye Disperse Red 13 was performed to analyze the effects of a dense

charged surface of the microspheres. The aim of **Paper II** was to investigate and analyze the effects of microscopic pores in microspheres, and macroscopic pores in coatings by release studies using the biocide 2-n-octyl-4-isothizolin-3-one (OIT). Both release of OIT from microspheres in suspension and from a coated surface, both free and encapsulated, were analyzed. The aim of **Paper III** somehow connects **Paper I** and **Paper II**. The study was done to evaluate the effects of using microparticles with polyelectrolyte multilayers as containers for OIT and the release of the biocide. A commercially available paint was used and dried for several different time intervals, to investigate a possibly upcoming macroscopic porous structure, depending on drying time, which would influence the release rate of OIT.

1.2 Outline of the Thesis

This thesis is divided into seven chapters focusing on microencapsulation and the controlled release of biocide from microparticles. After this introduction, Chapter 2 describes microencapsulation, especially using the internal phase separation method, while Chapter 3 is focusing on the used materials. Chapter 4 describes the release measurements for the active substances from suspension and from a coated surface, both freely and encapsulated. In Chapter 5 information about several of the used analysis methods can be found. Chapter 6 discusses the results from the articles while concluding remarks and discussion concerning future work can be found in Chapter 7.

2 MICROENCAPSULATION

Microencapsulation is the technique where small (1-1000 μm) solid particles, liquid droplets, or gas bubbles are coated with a thin layer of coating or shell material [10, 11]. Information about preparation of microcapsules dates back to 1950s when Green and Schleicher produced microencapsulated dyes for the manufacture of carbonless copy paper, which is still used commercially [11-13]. The main reason for encapsulation may vary depending on the aim of the application but it is often to protect and/or control the release of an active substance [11, 14, 15]. Microencapsulation can be found in several different industrial areas [16] and microparticles are for instance very common in the pharmaceutical industry as transportation reservoirs for drugs [17, 18]. Here, the release of a drug has to be direct when addressed, and is often triggered physically or chemically by e.g. pH changes, temperature variations, or light [19-21]. Microencapsulation can also be found in industrial sectors as agriculture [11, 22], food [23-25], textiles [15, 26] and the coating industry [9, 27, 28].

2.1 MICROENCAPSULATION TECHNIQUES

There are many different techniques used to synthesize microcapsules. The methods can be divided into physical, chemical or mechanical routes, or often combinations of these [10, 14, 16]. Some of the common methods are listed in Table 1.

Table 1. Common methods for microcapsule preparation.

Chemical processes	**Physical processes (Physico-chemical)**	**Mechanical processes (Physico-mechanical)**
- Chain growth or Step growth (polycondensation) polymerization	- Coacervation/Phase separation	- Spray-drying
- Interfacial polymerization	- Self-assembly	- Fluid-bed coating
- Emulsion polymerization	- Sol-gel encapsulation	- Pan coating
- Suspension polymerization	- Polyelectrolyte layer-by-layer assembly	- Electrostatic encapsulation

The techniques based on coacervation, or phase separation, are the broadest class of encapsulation methods [11, 29]. There are three main methods of coacervation if long term controlled release is intended; internal phase separation (see Section 2.1.1), multiple emulsion route and interfacial polymerization [29].

2.1.1 Internal Phase Separation

The internal phase separation, induced by solvent evaporation, was described by Loxley and Vincent 1998 [30] and is the chosen microencapsulation technique in this thesis. It is a physicochemical versatile, straightforward and industrially applicable method. Also, the internal phase separation method gives an almost full encapsulation yield of the active substance [14]. The route, described in Figure 1, starts with the formation of an oil/water emulsion where the continuous phase consists of water and a dispersant, often a water-soluble and surface-active polymer. The oil phase includes a volatile solvent, ingredients for the microcapsule core and shell, and an active substance. The volatile solvent has to have low miscibility in water, be able to dissolve the core oil and be a good solvent for both the shell-forming polymer and the active substance. After the emulsion step, a phase separation of the polymer within the droplets occurs due to evaporation of the volatile solvent. The polymer-rich phase migrates to the interface starting to create a shell. When all volatile solvent has evaporated the microcapsule has got its final structure. If producing microspheres, the setup is identical except no core-material is used.

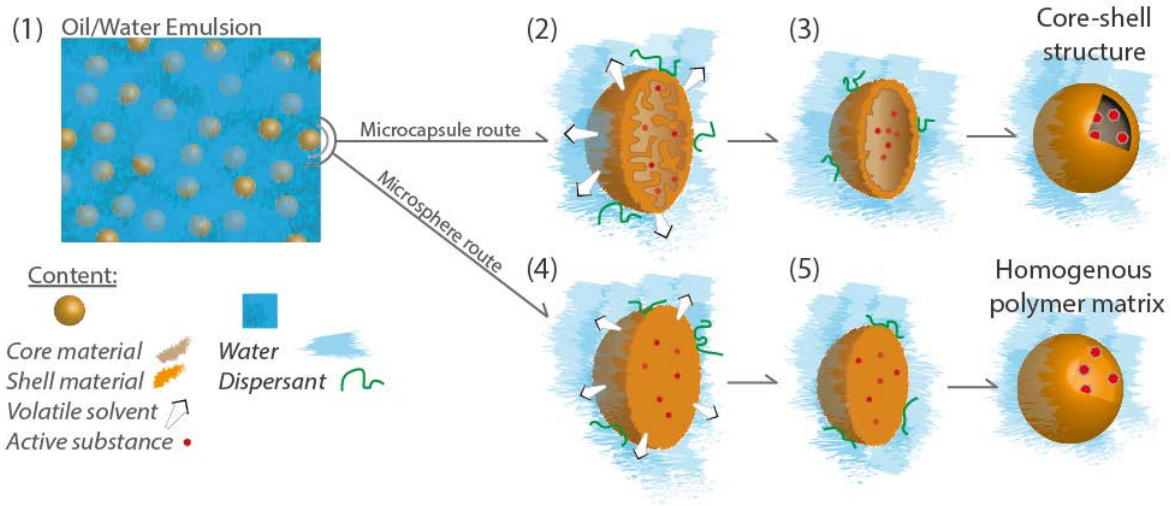


Figure 1. The microencapsulation process via the internal phase separation method. (1) The encapsulation starts with an O/W emulsion where the dispersed oil phase contains the core material, the shell material and the biocide dissolved in a volatile solvent. (2) As the volatile solvent evaporates, phase separation of the polymer within the droplets occurs and (3) the polymer-rich phase migrates to the interface. When all volatile solvent has evaporated, the microcapsule is formed. (4) When the volatile solvent evaporates a homogenous polymeric sphere starts to get its shape. (5) The final microsphere is formed when all volatile solvent has evaporated.

2.2 Surface Chemistry

This thesis will focus on core-shell particles, also called microcapsules, and microspheres which are homogenous polymer matrices. The spectrum of sizes, shapes and morphology of microparticles is broad [10, 11, 14] and examples of microcapsule outcome can be found in Figure 2.

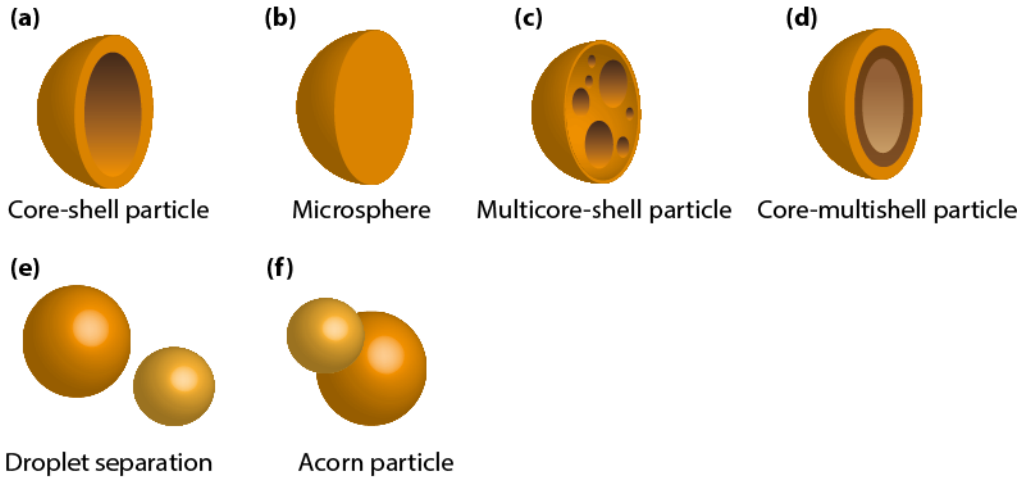


Figure 2. Possible outcomes of the morphologies of microcapsules.

To be able to form capsules with core-shell morphology, the polymer (index *p*) needs to wet the oil (index *o*) and aqueous phase (index *w*), i.e. spreading between the oil and water. The definition of the spreading coefficient S_p is found in Equation 1:

$$S_p = \Delta G_p^c - \Delta G_p^a = \gamma_{ow} - \left(\gamma_{pw} + \gamma_{op}\right) \tag{1}$$

where ΔG^c_p is the free energy for cohesion, ΔG^a_p the free energy for adsorption and γ_{ij} the interfacial tension between phases *i* and *j* (described above). A derivation has been made by Torza and Mason [31] to be able to predict the morphology of droplets of three immiscible liquids in terms of their spreading coefficients. Loxley and Vincent developed the theory to also include solid polymers [30]. With the precondition $\gamma_{ow} > \gamma_{op}$, there are three possible spreading conditions [30, 31]:

$$S_o < 0; \quad S_w < 0; \quad S_p > 0 \tag{2}$$

$$S_o < 0; \quad S_w < 0; \quad S_p < 0 \tag{3}$$

$$S_o < 0; \quad S_w > 0; \quad S_p > 0 \tag{4}$$

The microparticle will obtain core-shell morphology if the conditions in Equation 2 are fulfilled while Equation 3 generates so-called acorn particles, and Equation 4 results in separate oil and polymer droplets. In addition, Trongsatitkul and Budhlall [32] have suggested that multicore-shell particles may form if $S_p >> 0$. If aiming for producing microspheres, the experimental procedure is equivalent to that of microcapsules but the spreading coefficient discussed above is not applicable as the only interface is that between polymer and water phase.

2.3 Polyelectrolytes and Polyelectrolyte Multilayers

The adsorption of polyelectrolytes at a surface, for instance the surface of a microcapsule, is governed mainly by electrostatic interactions and the outcome is therefore very dependent on the salt concentration [33, 34]. At low salt concentrations and if the surface has the same sign of the charge as the polyelectrolyte, only small amounts, if any, will adsorb [35, 36]. Also, if the surface has no charge, the result will be only week adsorption. If we instead consider high salt concentrations, the adsorption of polyelectrolytes of opposite charge as compared to that of the surfaces is very strong if there is also a non-electrostatic interaction between the two. This is due to entropy gain from the release of surface bound counter ions [37]. The outcomes of the adsorption depending on salt concentration, polymer and surface charge can be

found in Figure 3. As can be noticed, the adsorption of the polyelectrolyte at a charged surface is much more affected at low salt concentrations than at high.

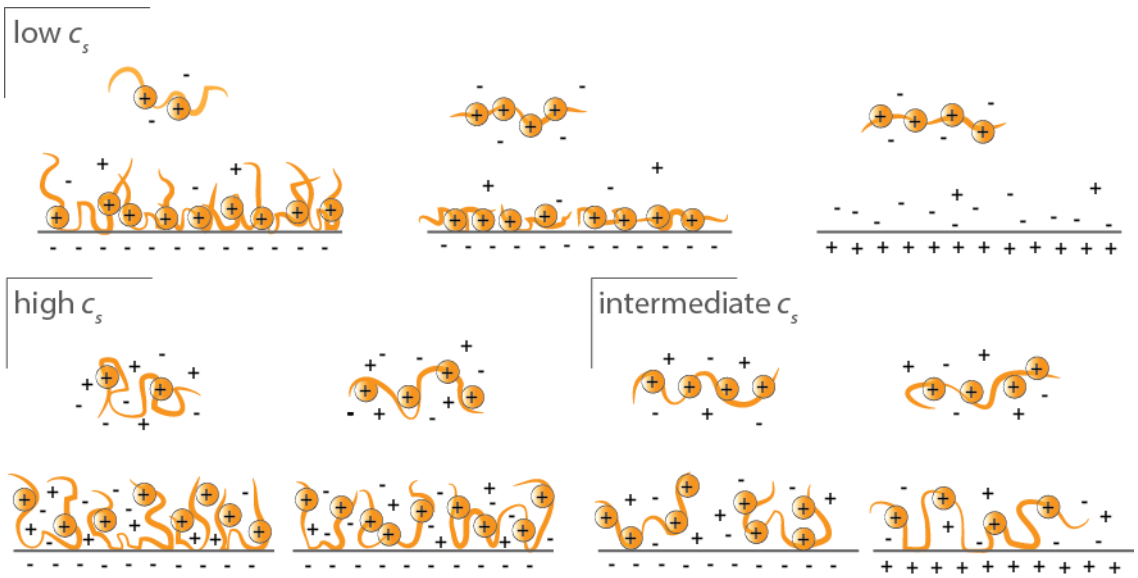


Figure 3. A schematic view of the adsorption of polyelectrolytes at a surface under various conditions for salt concentrations, c_s, polymer charge density and surface charge density.

The assembly of polyelectrolyte multilayers, so called PEMs, using the *Layer-by-Layer* (LBL) technique is achieved due to the charge inversion. This deposition technique was described by Iler [38] and developed by Decher on planar macroscopic substrates [39], and is an efficient way for controlled surface modification [40]. Möhwald and co-workers expanded the PEM assembly to microscopic colloidal surfaces [41]. To be able to build multilayers using LBL, the salt concentration is of outmost importance as described above. Also, the first charged layer needs to be anchored to the surface, e.g. by using a block-co-polymer as dispersant in the emulsification step. The reason to use polyelectrolyte multilayers in this work is to build a hydrophilic barrier at the surface of the microparticles to reduce the release of hydrophobic biocides from the microparticle to a surrounding medium.

2.4 Porosity

The release of biocide from microparticles to a surrounding medium is highly affected, not only by chemistry as described above, but also by the structure of the involved materials. In this thesis two types of porosity are considered, and here denoted *microscopic* and *macroscopic* porosity, respectively. Microscopic porosity is referred to the spaces between the polymeric chains in the microparticle, i.e. the free volume of the polymer chains, see Figure 4. This space is highly affected by the temperature and the thermal history of the polymer. Macroscopic porosity is referred as the pores between latex and pigment particles in the coating which are much larger than the

dimensions of the biocide in contrast to the microscopic pores, see Figure 4. Regarding the macroscopic porosity, the drying time of the coating is crucial, which is reported in **Paper II**.

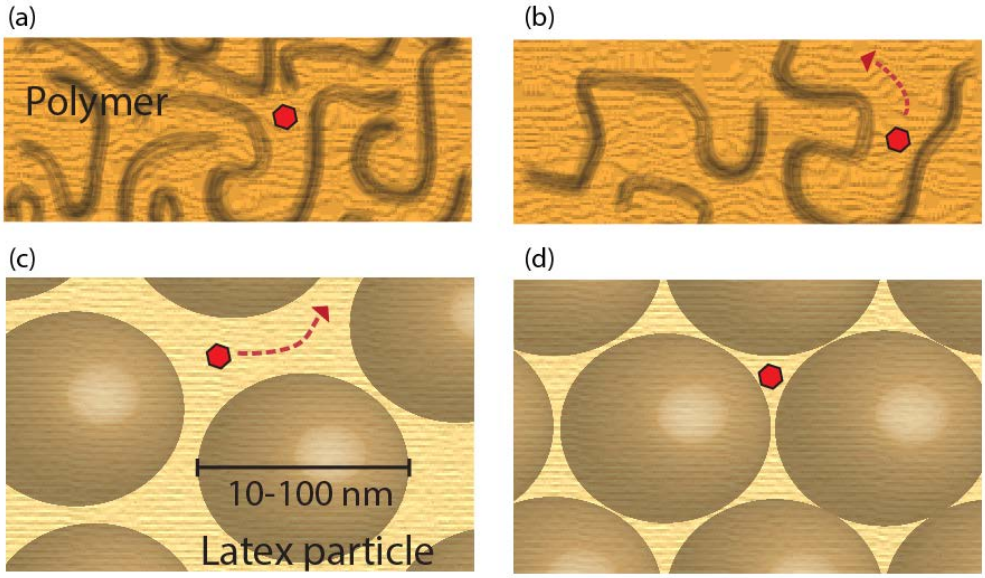


Figure 4. Illustration of microscopic pores (a), (b) and macroscopic pores (c), (d). The microscopic pores in the microspheres depend on the thermal history of the sample where (a) is a thermally relaxed state while (b) is a thermally expanded state. The macroscopic porosity in a latex coating and the particle-particle merging depends on the drying time of the coating where (c) is after short drying time while (d) is after long drying time of the paint.

The release of an active substance from a microparticle can be referred to as Fickian diffusion, which is explained in detail in Section 4.1. As stated above, the release is highly influenced by both microscopic and macroscopic pores in the system. The presence, magnitude, and distribution of microscopic porosity depends on the route of solvent evaporation, which is further explained in Section 2.4.1 below while macroscopic porosity is affected by the drying time of the paint, see Section 2.4.2.

2.4.1 Solvent Evaporation

One of the steps in the internal phase separation method is the evaporation of the volatile solvent that follows the emulsification. In this work, the evaporation time and method have been evaluated by analyzing one quick and one slow evaporation technique, respectively. If following our standard recipe (Section 3.5), the evaporation of volatile solvent is performed without instruments since the solvent is evaporated only in a fume hood under slow stirring. It takes approximately 24 hours before complete evaporation following this route. In order to decrease the time of the evaporation step in some of the experiments a rotary evaporator has been used. The

solvent is evaporated under lowered pressure which results in a much faster evaporation, a few hours in our experiments.

2.4.2 Drying Time of Paint

There are several commercial categories of paint with regard to solvent of the product. In this work the focus has been on water-borne acrylic paints intended for façade application. Paint is a complex formulation of ingredients including binder, pigment, solvent and additives. Acrylic latex is widely used as binder in paint mainly because of its stability under excess of UV, alkalinity and heat, its good exterior durability, and film clarity [42]. During paint drying, the latex particles (10 – 100 nm in size) merge together during the evaporation of the water as a reduced amount of water means a denser latex particle structure.

3

MATERIALS

In this chapter, materials used in this work – for the microparticle formulation and for the release measurements – will be stated. This includes the core and shell substances, dispersants in the water phase and information about the used biocide, 2-n-octyl-4-isothiazolin-3-one (OIT). All molecules can be found in Chart 1 including molecular weight (M_n), critical micelle concentration (CMC), and UV adsorption maximum (λ_{max}) where relevant.

3.1 THE MATERIALS FOR THE CORE AND SHELL

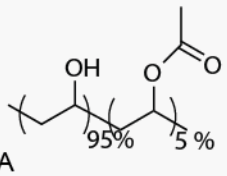
For the production of microcapsules, hexadecane has been used as oil cores. Regarding the shell material, poly(methyl methacrylate) (PMMA) has been the main choice. PMMA is a widely used polymer and goes under common brand names as Plexiglas® or Acrylite®. The main advantages are its low price, optical properties, the low sensibility to UV light, and its overall weather resistance [43], all important parameters for ingredients in façade coating. The compatibility between microparticles and the coating is very important since a homogenous mixture is crucial as final product.

PMMA is a moderately hydrophobic polymer and this can be disadvantageous with respect to the release of a given biocide. Whether this is the case or not depends in turn

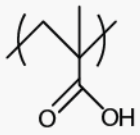
on the physicochemical properties of the biocide. Earlier studies have showed that biocides, often small semi-hydrophobic molecules, are more soluble in the PMMA shell than in the very hydrophobic alkane core, making the benefits of using core-shell structure useless [44]. This is one of the reasons why microspheres, spherical particles only consisting of PMMA and biocide, were used in several studies in this work.

Chart 1. Molecular structure and abbreviations of the chemicals used for the encapsulation and controlled release of OIT including chemical name, molecular weight (M_n), critical micelle concentration (CMC), and UV adsorption maximum (λ_{max}) where relevant.

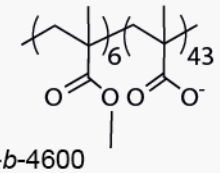
Dispersants



PVA
poly(vinyl alcohol)
M_w=95,000 g/mol

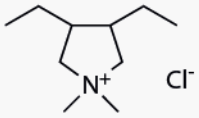


PMAA
poly(methacrylic acid)
M_w=100,000 g/mol

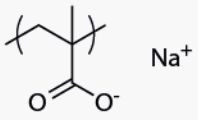


600-*b*-4600
poly(methyl methacrylate (600)-
block-*sodium methacrylate (4600))*
M_w=600+4600 g/mol

Polyelectrolytes

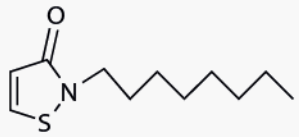


PDADMAC
poly(diallyldimethylammonium chloride)
M_w=350,000 g/mol

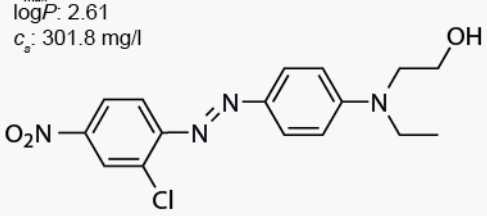


PMANa
poly(sodium methacrylate)
M_w=15,000 g/mol

Active substances

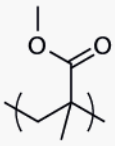


OIT
2-n-octyl-4-isothiazolin-3-one
M_w: 213.34 g/mol
λ_{max}: 276 nm
log*P*: 2.61
c_s: 301.8 mg/l



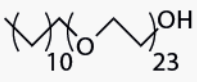
Disperse red 13
2-(4-(2-chloro-4-nitrophenylazo)-n-ethylphenylamino)ethanol
M_w=348.78 g/mol
λ_{max}=503 nm
log*P*: 4.85
c_s: 0.012 mg/l

Matrix material



PMMA
poly(methyl methacrylate)
M_w=350,000 g/mol

Release medium



Brij® L23
polyoxyethylene(23)monododecyl ether
M_w=1,199.54 g/mol
CMC =91 µM

3.2 THE BIOCIDE

Biocides have been used as protection in coatings and materials since the 1950´s [2]. As stated in the Introduction, several substances have been banned during the years due to environmental issues. One interesting group of biocides is the isothiazolinones which are heterocyclic aromatic compounds. We have focused on one of these compounds, 2-n-octyl-4-isothiazolin-3-one (OIT), which is a semi-hydrophobic biocide and often used commercially in the paint industry but also in leather and cardboard products, as well in polymeric materials as PVC [6].

OIT protects by destroying growth at the surface of the paint layer, meaning that it has to be able to move easily inside the polymeric paint matrix in order to migrate to the outermost surface [2, 6]. In current commercial products, OIT is found as a freely dispersed additive to the wet paints and a longer protection could most simply be reached by increasing the surface flux over time by increasing the biocide concentration in the paint [2]. One problem is that biocides in general, and the isothiazolinones as OIT in particular, can act as softeners which may change the thermo-mechanical properties of the paint. This, the fact that most antimicrobials are expensive ingredients, and that there actually is a maximum allowed concentration of biocide in paint products due to environmental regulations, makes encapsulation of OIT to an interesting field.

3.3 THE EMULSIFIERS

An emulsion is defined as a thermodynamic unstable heterogeneous system of two immiscible liquids, where one is dispersed in the other [45]. To stabilize an emulsion, surface-active molecules, called surfactants or more specific in this case emulsifiers, are used to support the emulsification by lowering the interfacial tension between the liquids and by rapidly diffuse to the newly created interface [37, 45]. Surfactants are amphiphilic which means that they consist of at least two parts where one is soluble in water, the hydrophilic part while the other part, which is hydrophobic, prefers non-polar environment. Many polymers are also surface active and a few have been in focus in this work.

If following the internal phase separation method (see Section 2.1.1), the water phase consists of an emulsifier, and the choice is important for the outcome of the experiment. Poly(vinyl alcohol) (PVA) has been the most used emulsifier in the encapsulation process and was also one of the studied emulsifiers in the seminal work by Loxley and Vincent [40]. However, when using PVA the microparticles will be uncharged which rules out the formation of polyelectrolyte multilayers since PEMs need a sufficiently high surface charge density [5]. Poly(methacrylic acid) (PMAA) can also be used as emulsifier. It is a weak polyacid that may be charged by alkaline

treatment [46]. Both PVA and PMAA are considered to be enthalpic steric stabilizers, which is common for aqueous dispersions.

One way to reach sufficiently high surface charge at the produced microparticles to be able to build PEMs is to use a suitable ionic amphiphilic block copolymer. Here, the hydrophobic block would be compatible with the microparticle wall while the hydrophilic block would enable a high enough negative charge at the microparticle surface. In this work, microparticles have been produced with high surface charge using the block copolymer poly(methyl methacrylate (600)-block-sodium methacrylate (4600)) (600-*b*-4600) as dispersant. The procedure has recently been reported by others in our research group [47, 48].

3.4 The Polyelectrolytes

Two different polyelectrolytes have been used to build the polyelectrolyte multilayers. The positively charged polyelectrolyte poly(diallyldimethylammonium chloride) (PDADMAC) is adsorbed to the microsphere while the next monolayer is the negatively charged polyelectrolyte poly(sodium methacrylate) PMANa. Several layers can repeatedly be adsorbed and in this work two bilayers have been standard.

When adsorbing PEMs on top of the block-copolymer the swollen polyelectrolyte block collapses. The conditions for assembly used in this work result in very dense PEMs. PEMs can be a rate-determining barrier already at very thin thicknesses [49]. The two bilayers in this work will form an approximately 5-6 nm thin barrier which is enough to be rate-determining [50]. This can be compared to if using the common dispersant PVA where a layer is approximately 20 nm thick [35].

3.5 The Standard Recipe

As mentioned, several different recipes have been tried during the work by changing e.g. emulsifier or amount of biocide. However, one standard recipe has been designed and several of the results stated in Chapter 6 are following this recipe. The standard recipe gives PMMA based microspheres consisting of 10 wt% OIT.

First the water phase, consisting of 1 wt% PVA (95 % hydrolyzed) and Milli-Q water was prepared. The oil phase consisted of dichloromethane (DCM) as volatile solvent, PMMA (350,000 Mw) as polymer matrix material and the biocide OIT. These were properly mixed before the aiding solvent acetone is added, and the oil phase is slowly added (during 120 s) under stirring at 5,000 rpm, using a homogenizer of type Silent Crusher M tool 22F (Heidolph Instruments, Germany), to 80 ml water phase. The emulsification was made in a round bottom flask, immerged in a room-temperature water bath, for an additional 60 minutes under stirring at 10,000 rpm. The emulsion was then poured into 120 ml water phase and stirred for several minutes. The emulsion

was kept in an open beaker in fume hood overnight and the DCM was allowed to evaporate under magnetic stirring at 200 rpm. To compensate for water evaporation, fresh water was added to set the final suspension to 100 ml thus giving 2.75 wt% of microspheres.

4

RELEASE METHODOLOGY

The focus of Chapter 4 is the release measurements and the theory behind the chosen setup. The release experiments include both biocide release from microparticles to the continuous phase in a suspension as well as biocide release from a dry coating immersed in water. The applied diffusion models will also be presented.

4.1 THEORY

Controlled release can be found in many different areas but was developed in the pharmaceutical industry [51]. Controlled release is a wide expression and can be e.g. triggered, fast, or sustained release [19, 51]. We are aiming for sustained release since the biocide should be released from the microparticle over a long time span. In a perfect world, the release would follow zero order, i.e. a constant release rate. This is possible, in theory, if the actives are dispersed as crystals in the core [52].

There are several different mechanisms for encapsulated active substances to be released, e.g. dissolution of the wall, mechanical rupture of the capsule wall or diffusion through the wall [11, 53]. Sustained release for an active substance is for our microparticle systems controlled by the permeation through the shell and the coating

matrix. The release rate is determined by both thermodynamic and kinetic parameters, which are described in detail below.

The solubility of a biocide in the microcapsule shell, the core material, and the surrounding medium (as a paint or an aqueous solution) will determine its distribution between the phases. This is stated by the partition coefficient, $K^i_{A/B}$, of active *i* between phases *A* and *B*:

$$K^i_{A/B} = \frac{c^i_A}{c^i_B} \tag{5}$$

where c^i_A and c^i_B are the equilibrium constants of *i* in phases *A* and *B*, respectively.

$K^i_{A/B}$ is a thermodynamic constant while the effective diffusion coefficient, *D*, is a kinetic parameter related to size of the active. It is important to distinguish between *D* and the self-diffusion of the active, D_0. *D* can be manipulated by steric factors, as crystallinity and polymer molecular weight, or interactions between e.g. active and polymer [14]. Here, the effects of pores, both microscopic and macroscopic, will be in focus and the situation can be referred as Fickian diffusion when consider diffusion from a microsphere. In porous media, the effective diffusion coefficient is given by

$$D = D_0 e^{-\frac{\gamma V_c}{V_f}} \frac{\varepsilon}{\tau}. \tag{6}$$

The effective diffusion is related to the self-diffusion coefficient D_0, the free volume of the polymer V_f, the critical volume for diffusion V_c. the porosity ε, the tortuosity τ, and the overlap factor γ, the latter being 1 for most polymers. The active is diffusing in the free volume of the polymer chains inside the microsphere if V_f is larger than V_c. *V* is the impermeable volume occupied by the polymer and V_w is the van der Waals volume [53].

$$V_f = V - V_0 \tag{7}$$

$$V_0 = 1.3V_w \tag{8}$$

The diffusion in the coating is described by the porosity ε and the tortuosity τ, and is described more in detailed in **Paper II**.

4.2 RELEASE FROM MICROPARTICLES

Release of actives from microparticles can be studied using several different methods. Independent of chosen method, at least three main steps can be crystallized: setup, analysis and evaluation. The setup includes the laboratory equipment and materials needed to perform release studies of an active from microparticles to a surrounding release medium. The analysis part includes the experimental equipment for data sampling and analytical technique to quantify the time-dependent concentration of the active. The evaluation step puts the results into perspective by the application of release models.

When designing the release setup, two parameters need to be considered. First, the saturation concentration of the biocide in the chosen release medium has to be known. Second, the distribution of the biocide, explained by its partition coefficient, $K^{i}_{A/B}$, between the microparticle and the release medium has to be known. Directly when the microparticles are formed (Section 2.1.1) the biocide is released out in the aqueous solution until equilibrium between the concentration in the microparticles and in the aqueous solution is reached.

A common setup for release studies from particles to an aqueous solution is to use a semi-permeable dialysis tube where its pore size is smaller than the particles, see Figure 5. The dialysis tube is filled with the suspension and the concentration of released active is measured outside the membrane. However, some severe problems have been noticed when using this setup. One problem is that equilibrium inside and outside the membrane may not be established between two data samples. Also, the microparticles can agglomerate due to lack of proper mixing, affecting the release to be of hindered nature.

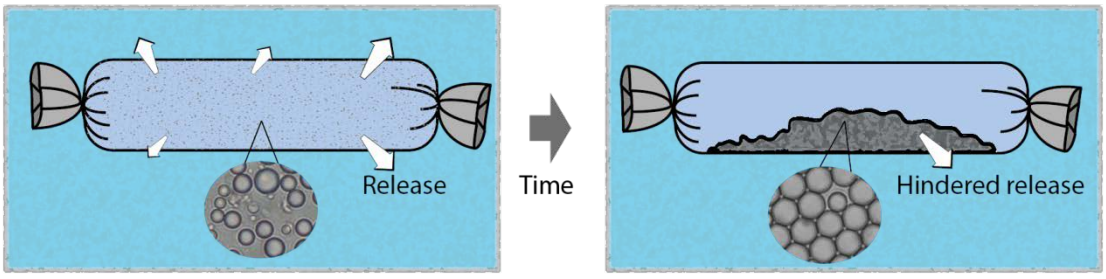


Figure 5. The use of semi-permeable dialysis membranes is a common setup for release measurements. The microcapsule suspension is put in a tube where the dialysis membrane has a pore size significantly smaller than the size of the microcapsules. Samples are taken from the surrounding medium, often an aqueous solution, and the concentration of released active substance is analysed.

To overcome these problems, focus in this work has been to design a new experimental setup which is schematically described in Figure 6. Here, a small

amount of the microparticle suspension is dispersed directly in a release medium subjected to stirring. The release of active substance out to the release medium starts subsequently to this step and small-volume samples are taken using a syringe. The sample is then pressed through a syringe filter and the filtered solution is finally analyzed. This method prevents agglomeration and permits the concentration of only released active to be analyzed.

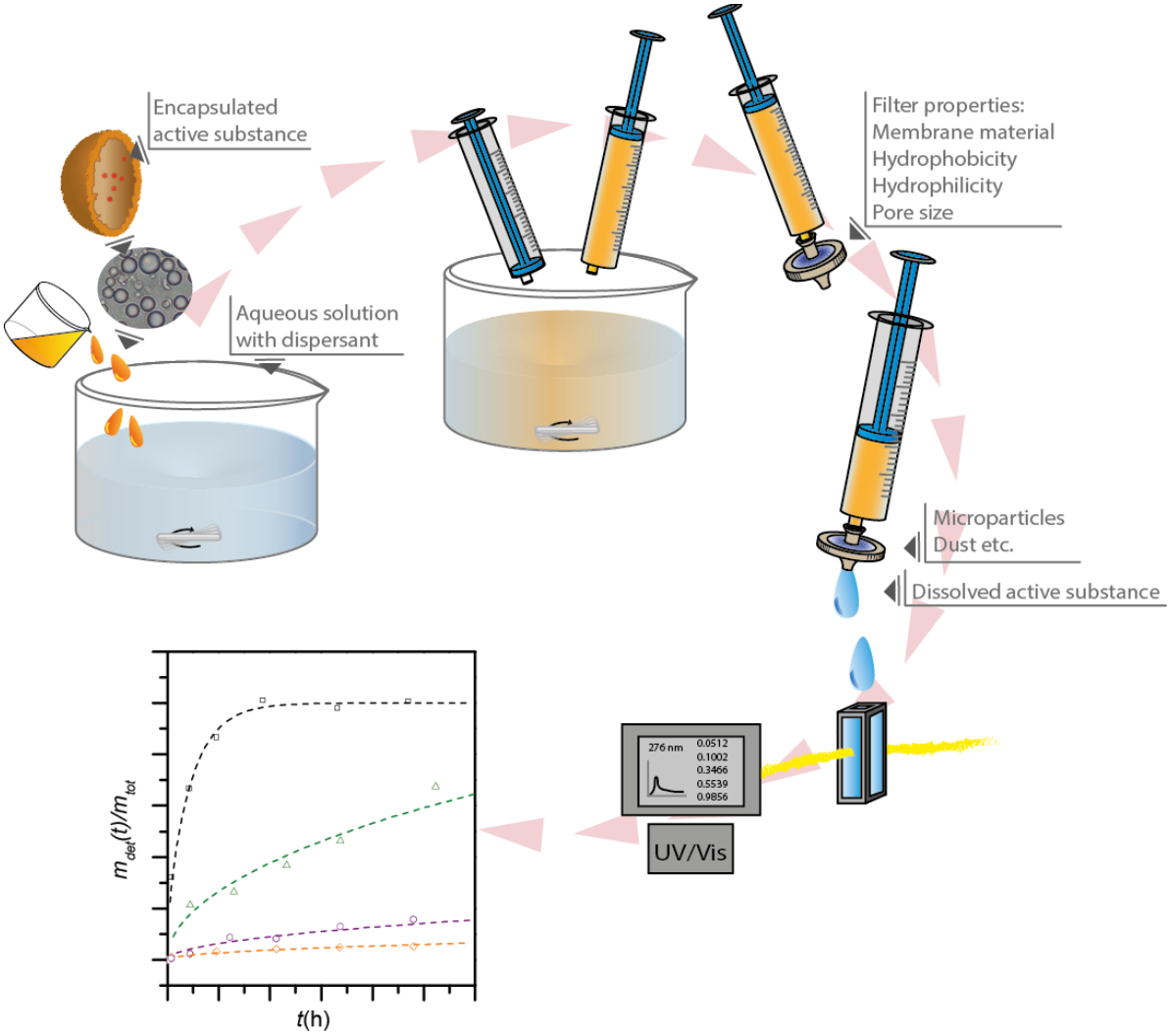


Figure 6. Experimental methodology for release measurements of active substance from microparticle suspension. The suspension is poured into an aqueous solution (consisting of dispersant if needed) at time zero and the release of biocide out from the capsules to the surrounding medium will start immediately. By using a syringe, samples are taken and pressed through a suitable filter. This removes microparticles, dust etc. giving an aqueous solution with dissolved active substance. Samples are taken after less than minutes in the beginning but the interval will increase over time. The samples are then analyzed and curves are fitted to the result using mathematical models.

As mentioned earlier, the partition coefficient of an active substance between the microparticles and the surrounding release medium is important to consider in order to set up a scientifically valid release study. The partition coefficient is experimentally determined and for a legitimate release study two criteria have to be fulfilled:

- A complete release should correspond to less than 10 % of the saturation concentration of active substance in the surrounding medium. Under this condition, the reverse flux of the active into the microparticle can be neglected.
- The final steady-state released fraction of active substance should be high, around 90 % of the theoretical maximum release concentration. Following this criterion will give well-resolved data sampling to the mathematical modelling.

When designing the experimental setup one has to consider the analytical method to quantify concentration. UV/Vis spectrophotometry is a fast and reliable method to practice though working only if the substance possesses a conjugated part. Practically, UV/Vis spectrophotometry displays a linear dependence, called the Beer-Lambert law (Equation 14), between concentration and measured quantity (absorbance) within a certain absorbance range. This means that the setup not only have to consider a minimum concentration but also a specific maximum concentration. One could of course dilute the samples but this step should be avoided to decrease the number of error sources. The range of linear dependence can differ depending on both hardware and wavelength but the absorbance range 0.1-1 is seen as standard. This means, in our setup, that the lowest concentration (determined by instrumental sensitivity) should correspond to an absorbance value of approximately 0.1 and our end value should be approximately 1 (determined by range of Beer-Lambert law linearity).

Biocides are most often small hydrophobic or semi-hydrophobic molecules. If considering a setup using only water as surrounding medium, the partition coefficient will be high, meaning a very small release of biocide out in the medium before equilibrium is established. To be able to attain well-resolved experimental data, and to implement mathematical models concerning the release of biocide, the degree of release has to be increased by altering *K*. One way to decrease *K* is to change the surrounding medium to also consist of micelle-forming surfactants. This will increase the solubility of the biocide in the release medium, highly affecting the partition coefficient and the release. In comparison to decrease *K* by the introduction of a more hydrophobic co-solvent, surfactants do not risk to alter the structure of the microparticle, e.g. by swelling.

The method described above and in Figure 6 has several positive qualities. It is reliable, straightforward and can be applied for many different systems if following the

mentioned criteria and evaluation steps. The setup can also achieve almost full release. However, there are also a few limitations. First, when collecting samples, filters are used to separate the released biocide from the microparticles. The polarity of the filter may heavily affect the result since biocide might be trapped in the filter membrane by strong adsorption. One has to find a filter that allows for complete passage for every specific substance to be analyzed. Second, the release of an active substance will not stop until the suspension has been pushed through the filter. This filter procedure for each sampling takes some time (~ seconds), which can be a limiting factor analyzing fast initial releases. Third, this setup can be sensitive for evaporation of release medium, which can give misleading concentrations, and complete coverage of all release beakers is required.

4.3 Release from Coatings

To disperse biocide-loaded microparticles in a paint or varnish system is straightforward. No specific pre-treatment of the paint is needed, considering waterborne paint, and the microparticles are formulated as an additive [9].

As described in Section 3.5, the final microparticles end up as a suspension in water after formulation and the ratio between microparticle and water is typically 1:20. The water content needs to be drastically lowered before mixed into the paint, and this is achieved by centrifugation or ultrafiltration. If using PVA or PMAA as emulsifiers an excess of dispersant is important, otherwise the result will be irreversible aggregation. Highly charged emulsifiers, e.g. used when formulating microparticles with PEMs, are not dependent on excess of dispersant and are fully dispersed also from a dried state. The concentrated microparticle suspension is then mixed in the wet paint.

Similar to the release measurements described in Section 4.2, release studies from coatings can be divided in three major parts: setup, analysis, and evaluation. The steps, from encapsulating the active substance to analyzing the concentration, are described in Figure 7. The release method follows an international standard method for the release of biocides from coatings [54] but with some modifications e.g. permanent immersion instead of dipping leakage.

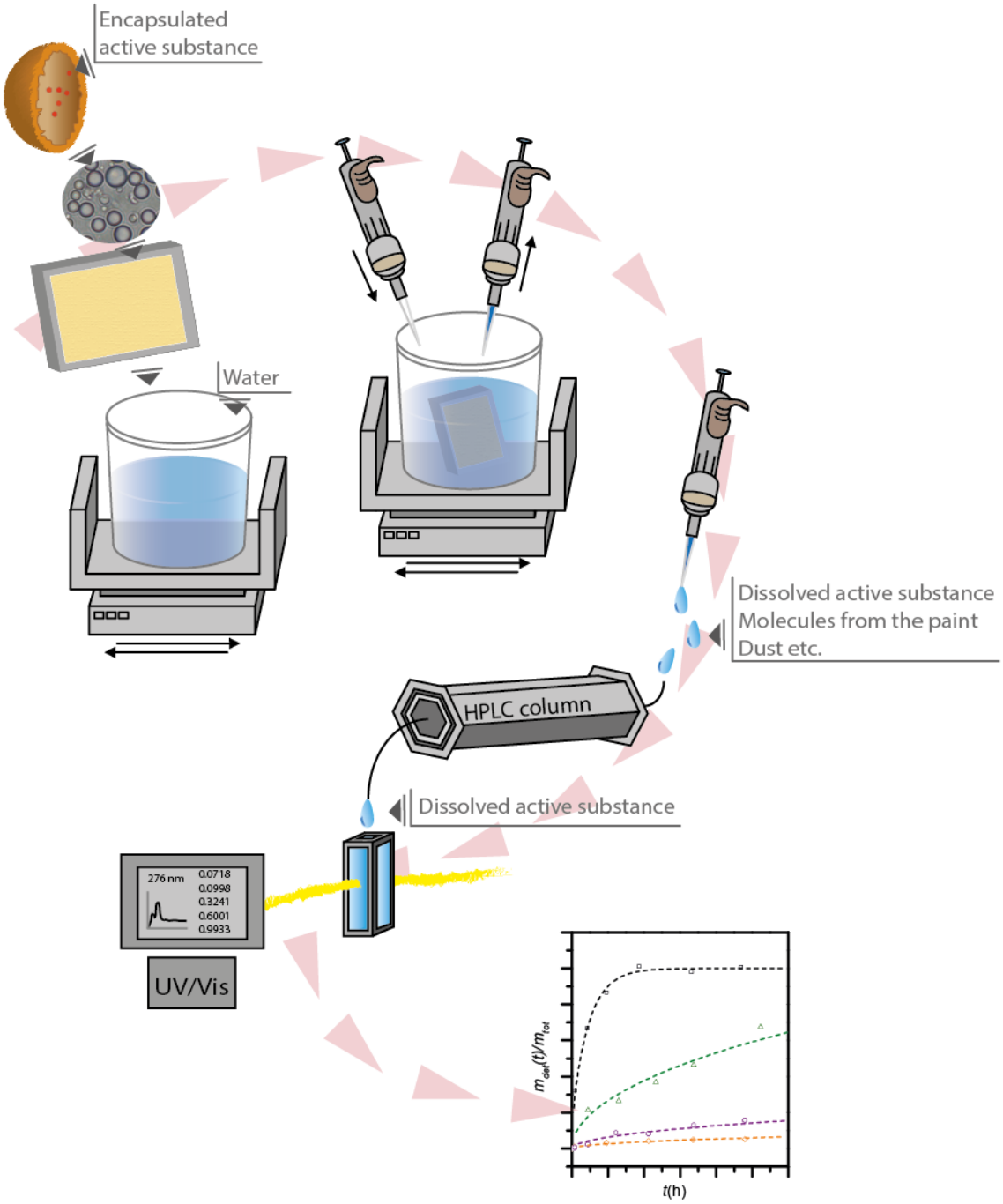


Figure 7. Experimental method to study the release of an active substance from a coating. A paint that contains microparticles is coated on a grazed polypropylene plate. After drying under controlled conditions, the coated plate is placed in a water filled beaker with lid, and stirred using a shaking table. Samples, precise volumes, are taken using an automatic pipette. Since the sample might contain also other molecules and particles from the paint, a HPLC column is used to separate the active substance before analytical quantification. Diffusion models are fitted to the experimental results.

In this work the paint, containing freely dispersed or encapsulated biocide, has been coated on polypropylene (PP) plates. It is important that the substrate is physically and chemically inert to the coating ingredients, and that it is unaffected by film drying and water contact. The PP plates are also grazed to enhance adhesion.

When applying the coating, both film thickness and coated area are parameters of utmost importance. We use an Elcometer Bird Film Applicator (Elcometer, UK) which can apply several different thicknesses, and by using tape a defined surface area can be achieved on the PP plates. To be able to control the amount of paint, and therefore also the amount of possible active substance to be released, the plates are weighed accurate both before and after applying the coating. The coated plates are then put in an incubator to dry at a constant temperature (we use 37 °C). As discussed in **Paper II**, the drying time has a great impact on the release of active. The plates are thereafter weighed again to be able to evaluate the water content in the coating.

Each dry-film coated plate was separately put in a beaker leaning downwards and tilted against the beaker wall, and tightly sealed with a lid in order to prevent evaporation of release medium. Along the progression of the release, samples were taken followed by refilling of fresh release medium. In contrast to the release studies described in Section 4.2 the concentrations of active substance are here much lower, giving conditions closely related to, and hence assumed to be, perfect sink.

Compared to the samples collected in the release studies from microparticle suspension, the samples in this study are not filtered before analyzed as the coating sticks to the substrate. A separation is however necessary since the samples not only consist of dissolved active substance and release medium but also other molecules and particles originating from the coating, e.g. fillers, and pigments. Here, only UV/Vis spectrophotometry is not a good option since other substances scatter the light signal. Instead a HPLC column is used to separate the specific active substance, as a biocide, before UV/Vis spectrophotometer can be used to analyze the absorbance at a specific wavelength.

Several external parameters can affect the release and this is important to consider when comparing different studies. Both light and temperature in the surrounding environment can change the experimental outcome. It is also important to have the same pH in all beakers since pH changes can affect the active substance, for instance by degradation.

4.4 DIFFUSION MODELS

The experimental setups described in Section 4.2 and 4.3 are of great value since the experimental outcome can be used in the fitting of mathematical diffusion models generating several important parameters, as the effective diffusion coefficient of the biocide. The applied models will be stated below and more detailed information can be found in **Paper I** and **Paper II.**

The first consideration is release from a microsphere to a surrounding medium. Here, the release, and hence analyzed, amount m over time t is expressed as fractional part of released active. The equation is derived by Crank [55]:

$$\frac{m(t)}{m_{tot}} = f(D,r,V_{sink},V_{sphere},K,t) = \frac{\alpha}{1+\alpha}\left[1-6\alpha(\alpha+1)\sum_{n=1}^{\infty}\frac{1}{9+9\alpha+(q_n\alpha)^2}e^{\frac{-Dq_n^2}{r^2}t}\right] \quad (9)$$

The equation contains the effective diffusion coefficient D and radius r of the microsphere while α is defined as

$$\alpha = \frac{V_{sink}}{V_{sphere}}\frac{1}{K} \quad (10)$$

where K is the partition coefficient, V_{sink} the volume of the release medium and V_{sphere} the total volume of microspheres. Parameter q_n is the n:th positive root of

$$\tan q_n = \frac{3q_n}{3+q_n^2\alpha}. \quad (11)$$

In all studies in this work concerning release from microspheres to a surrounding medium, a burst rate can be noticed. Burst release arises due to biocide saturation in the outermost part of the microsphere, and is an unwanted and negative effect when aiming for long time protection [49]. By also including a well-characterized size distribution $P(r)$ for microparticles and considering the burst to be a size-independent zero order release with rate constant k_{burst} and population fraction p_{burst}, the complete equation for fractional release versus time can be written as

$$\frac{m(t)}{m_{tot}} = p_{burst}k_{burst} + (1-p_{burst}k_{burst})\int f(D,r,V_{\sin k},V_{sphere},K,t)P(r)dr \quad (12)$$

When calculating the release of freely dispersed or encapsulated active from a coating, the coating is considered as plane sheets of monolithic character with homogenously embedded active. Since the final concentration of active in surrounding medium is very low, perfect sink conditions can be assumed. Released amount of active is expressed as a function of time *t*, according to Crank [55]:

$$\frac{m(t)}{m_{tot}} f(D,L,t) = 1 - \frac{8}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{(2n+1)^2} e^{-\frac{D\pi^2(2n+1)^2}{4L^2}t} \tag{13}$$

where L is the coating thickness and D the effective diffusion coefficient.

5 ANALYTICAL TOOLS

Several different analytical techniques have been used during this work. The size- and shape determinations of microparticles have been carried out mostly using light microscopy but to some extent also scanning electron microscopy (SEM). Differential scanning calorimetry (DSC) was used to decide and tune the setup for heat treatment experiments to study microporosity. UV/Vis spectrophotometry has been the preferred quantitative technique to analyze the concentration of OIT in the release studies.

5.1 LIGHT MICROSCOPY

A light microscope, also called optical microscope, uses visible light and a system of lenses in order to magnify images of the chosen sample. In this work, an Olympus BH-2 (Japan) light microscope equipped with an Olympus DP12 (Japan) digital camera system has been used to characterize different encapsulation batches, e.g. size and shape of the microparticles. As discussed in Section 4.4, the size distribution is an important parameter when considering the mathematical models used to describe the release. Histograms of the size distribution for different suspensions have been calculated using ImageJ (National Institutes of Health, USA), a Java-based image processing program. By measuring several hundreds of particles, a histogram was

constructed and the log-normal probability distribution was fitted to the experimental histogram data to calculate size distribution parameters.

5.2 Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) is a thermo-analytical technique. It measures the difference in amount of heat required to keep the sample and a reference in heat equilibrium as a function of temperature. Several phase transitions can be quantitatively and qualitatively analyzed, as glass transition and melting point.

DSC (Pyris1, Perkin Elmer, USA) has been used to determine the glass transition temperature (T_g) for microspheres made of PMMA. Also, the effect of OIT loading in microspheres was analyzed to be able to decide the optimal setup for the heat treatment experiments considered in **Paper II**.

5.3 UV/Vis Spectrophotometry

UV/Vis spectrophotometry (UV/Vis) is a well-established analytical tool for quantitative and qualitative determinations of samples. It uses light to radiate the sample, which absorbs the energy to excite electrons to higher anti-bonding molecular orbitals. One can use UV/Vis for direct concentration determination since the Beer-Lambert law (Equation 14) states that the absorbance is directly proportional to the concentration, specifically in a certain absorbance magnitude range with lower limit given by instrumental noise and upper limit influenced by deviation from Beer-Lambert law linearity (0.1-1 absorbance in our case). By constructing a standard curve, the absolute relationship between absorbance and concentration of the specific active was established. In Beer-Lambert law A is the absorbance, I_0 and I the intensities of the monochromatic light before and after passing through the sample, ε the extinction coefficient, c the concentration of the compound, and l the path length:

$$A = \log\left(\frac{I_0}{I}\right) = \varepsilon c l \tag{14}$$

In this work, UV/Vis (Agilent 8453, USA) has been used to analyze the concentration of OIT in the release studies described in **Paper II** and **Paper III**. The absorbance of OIT in the collected samples has been analyzed at wavelength 276 nm and each sample has been analyzed three times and the mean value for the absorbance provided the concentration value using the constructed standard curve. The release studies where freely and encapsulated OIT was released from painted surfaces were also analyzed using UV/Vis but with a connected HPLC column, see Section 4.3.

6 RESULTS AND DISCUSSION

In this work polymeric microparticles have been formulated and loaded with the biocide OIT. The microparticles have also been modified in terms of internal porosity or by assembling polyelectrolyte multilayers on the surface. The outcome of these two modifications has been analyzed by release measurements and will be presented in this chapter.

6.1 MICROENCAPSULATION

Both microcapsules and microspheres have been formulated using the internal phase separation method. As described in Section 3.3, several different emulsifiers have been studied during the encapsulation process, especially PVA and PMAA, see micrographs of microcapsules in Figure 8. Charged/Uncharged PMAA and the hydrolysis degree of PVA have been shown to affect the shape and uniformity of the microcapsules.

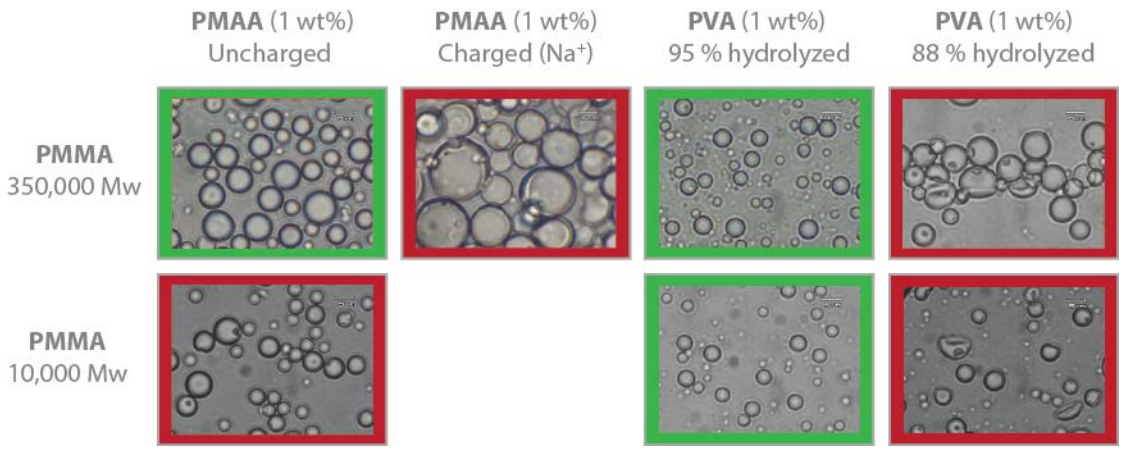


Figure 8. Micrographs of microcapsules using different emulsifiers and different molecular weight of the shell polymer PMMA. The green frames are successful batches while red frames are unsuccessful batches.

As can be seen in Figure 8, uncharged PMAA and PVA of high hydrolysis degree provided core-shell capsules of high uniformity. Charged PMAA and PVA of low hydrolysis degree resulted in non-spherical capsules or shape similar to a blueberry. The blueberry capsules have a single inward bend still not understood. One idea is that it arises during the evaporation step when the polymer migrates to the interface. An effort has been put to follow this step microscopically but more work is needed. However, non-uniform shaped particles can in some cases be advantageous. So called *Janus particles* are popular due to their non-uniform morphology and can be found in applications in e.g. the pharmaceutical and agriculture area. Janus particles can, in a controlled way, be formulated using the internal phase separation method depending on the emulsifier [56].

6.1.1 Encapsulating OIT

OIT has been the biocide of choice in this work, since it is common in the paint industry. An early scientific aim in this work was to encapsulate OIT in microcapsules. However, it was shown that OIT possesses surface-active properties which made it impossible to form microcapsules. The interfacial surface tension between oil and water was lowered, and the spreading conditions could not be fulfilled. More detailed surface-active properties of OIT have still not been evaluated.

To be able to encapsulate OIT, monolithic microspheres were formulated instead of microcapsules, see Section 3.5. The idea was to load the microspheres with a quite high amount of OIT and up to 20 wt% was possible. The distribution of OIT inside the homogenous polymer matrix has not been studied in detail but our assumption, from a chemistry point of view and the application of various diffusion models, is that OIT is homogenously distributed in the entire PMMA microsphere matrix.

6.2 POROSITY

The porosity was particularly investigated in **Paper II**. OIT loaded microspheres following our standard recipe were formulated and used in release studies to evaluate the role of both microscopic and macroscopic porosity, see Figure 9.

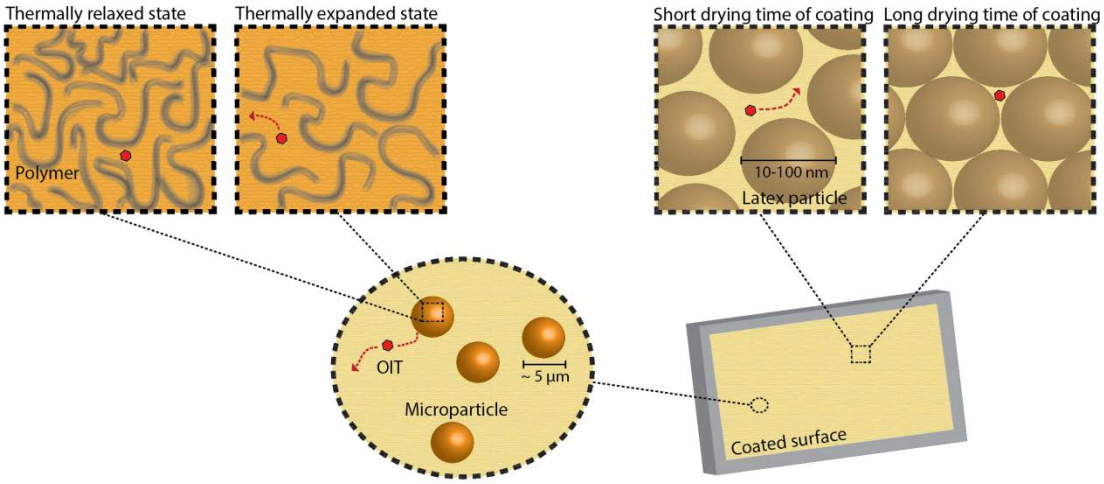


Figure 9. The microscopic porosity in the microspheres depends on the thermal history of the microparticle and the release rate of OIT is lower in a thermally relaxed state. The macroscopic porosity in a latex coating depends on the drying time of the coating. When latex binder has merged together, after long drying time of the paint, a decrease in release rate of OIT occurs.

The difference in microscopic porosity in the microspheres arises due to the choice of evaporation method. During the formulation the volatile solvent, DCM in this case, evaporates and the evaporation rate has been found crucial in terms of microscopic porosity. A thermally relaxed state of the microparticle polymer was achieved if evaporating DCM in an open beaker in fume hood over 24 hours (denoted *F*). This gave a more dense structure, or more specifically a decrease in free volume of PMMA. A thermally expanded state is achieved if accelerating the evaporation using a rotary evaporator (denoted *R*). It was clearly stated that microspheres with polymers in a thermally relaxed state lowered the OIT release, see Figure 10. The model-fitted diffusion coefficient of OIT is significantly larger for the rotary evaporated microspheres *R* compared to the standard microspheres *F*.

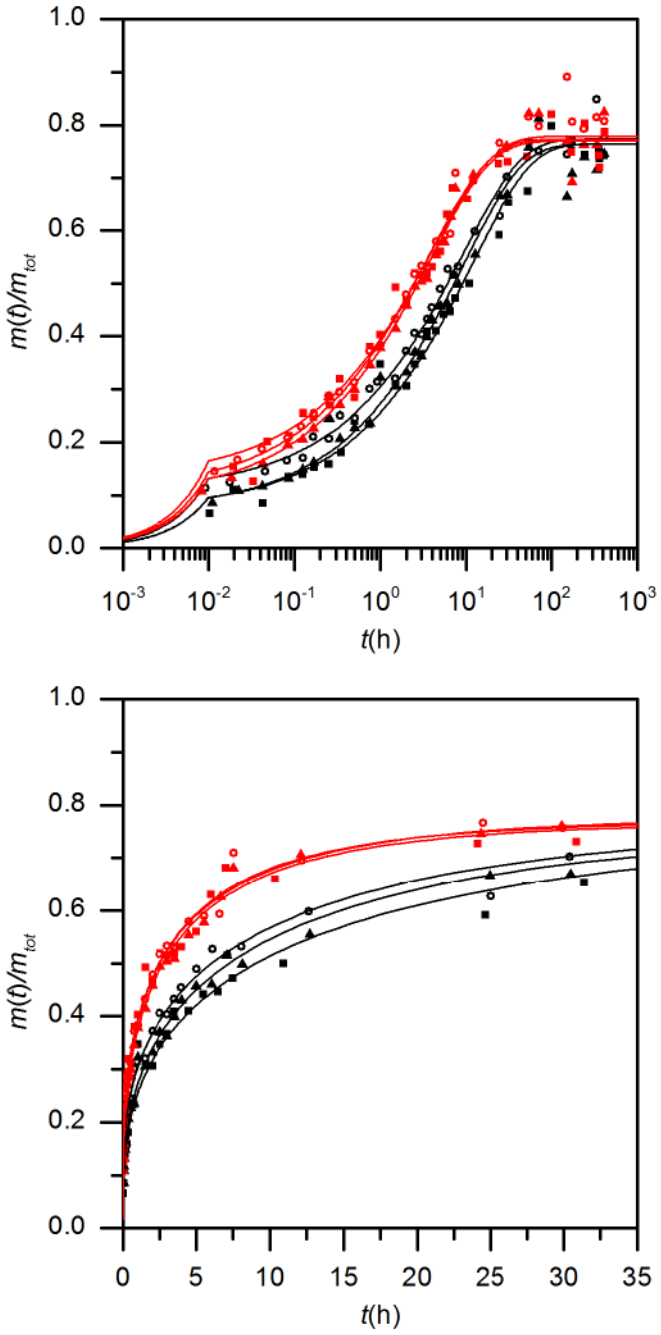


Figure 10. The suspension release of OIT for three replicas with slow evaporation of DCM (■▲○) and three replicas with fast evaporation of DCM (■▲○). The top figure shows the entire release using a logarithmic time scale while the first 35 hours of the experiment are presented on a linear time scale in the bottom figure.

In order to strengthen these results, heat treatment experiments were carried out using an autoclave. This was performed on suspensions *R* and *F*, respectively and the heat treated suspensions were denoted *RA* and *FA*. Using an autoclave (for more detailed information, see **Paper II**), the microspheres could be treated in temperatures well

above their glass transition temperature, T_g. Above T_g the polymer chains can reshape and conform in an energetically more stable microstructure. The heat treatment of *F* microspheres did not alter the release pattern, see Figure 11. The *R* microspheres, on the other hand, were significantly modified in microscopic porosity by the heat treatment, as seen in Figure 12. Heat treatment at elevated temperature well above the glass-transition temperature thus proved to generate a relaxation pathway of the polymer chains to a more favorable and hence less porous state. These findings strongly support the findings above on the relationship between solvent method and microscopic porosity.

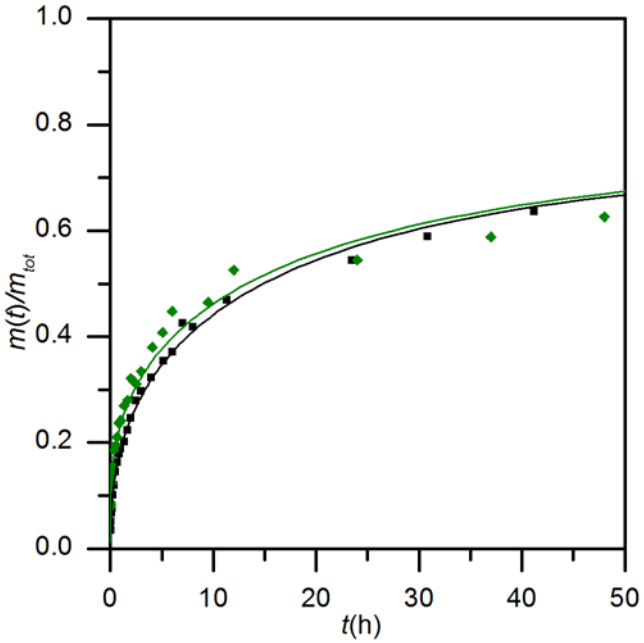


Figure 11. The suspension release of OIT with slow evaporation of DCM (■) and the same suspension but heat-treated (◆).

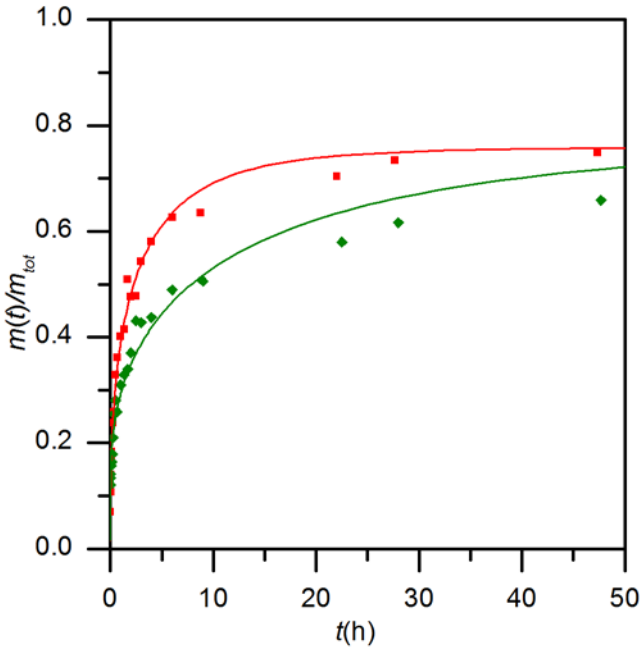


Figure 12. The suspension release of OIT with fast evaporation of DCM (■) and the same suspension but heat-treated (◆).

The macroscopic porosity of the coating was investigated in **Paper II** by release studies of both freely and encapsulated OIT from waterborne varnish coatings. The results can be found in Figure 13 where the left figure is the first 25 hours of the release study and the right figure is the entire study.

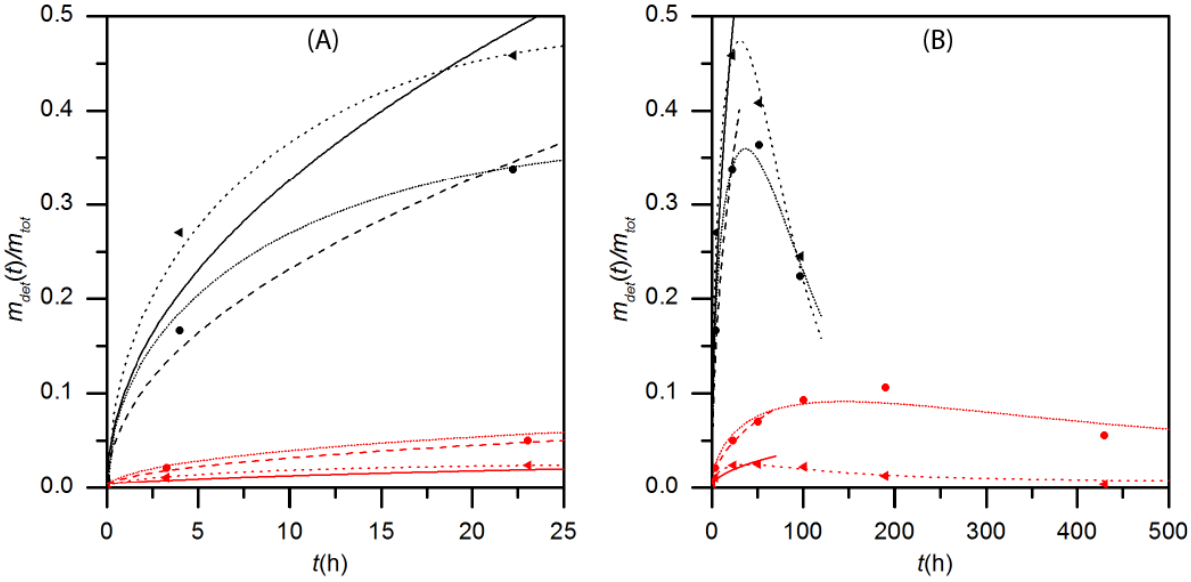


Figure 13. The release of OIT from varnish coatings. (A) The first 25 hours. (B) The entire experimental time. (◀) Free OIT after 7 days drying with decay fitting (•••) and no-decay fitting (—). (●) Encapsulated OIT after 7 days drying with decay fitting (···) and no-decay fitting (- - -). (◀) Free

OIT after 28 days drying with decay fitting (•••) and no-decay fitting (—). (●) Encapsulated OIT after 28 days drying with decay-fitting (···) and no-decay fitting (- - -).

First of all, significant degradation of OIT occurs in the release medium of the coating studies after approximately 50 hours. No degradation was detected at long experimental time during release of OIT in suspension. Three possible reasons have been discussed. First, and perhaps most importantly, the analytical technique used to quantify OIT from coating release was HPLC while release from microsphere suspension was UV/Vis spectrophotometry. Since HPLC separates ingoing components depending on its adsorption to chosen supporting material, it is highly sensitive to e.g. molecular structure or polarity. A decrease in detected concentration of OIT when analyzing the release from coatings indicates that degradation has occurred during the experiment. The degradation pathway of OIT is not clearly stated in the literature, however, the conjugated part of the isothiazole ring might still be intact since no degradation could be seen using UV/Vis spectrophotometer as analytical technique. Second, the time-scale of the two different studies was not the same and the release from coatings was evaluated at longer times. Third, the aqueous environment differed between the two studies with respect to pH, light influence, and presence of other dissolved species (primarily from the coating). The mathematical models considering degradation used to fit the data samples are stated in **Paper II**.

The results show that encapsulated OIT releases slower than freely dispersed OIT from a varnish coating. Also, the drying time of the varnish influences the macroscopic structure of the coating. Here, a longer drying time decrease the release rate and the effective OIT diffusion coefficient significantly. This results in slower release when the paint is formulated with microparticles, compared to freely dispersed biocide, as the microparticle become the rate-limiting structure with regard to diffusion. However, an opposite effect was considered after very long (28 days) drying time. Here the release rate was lower for the freely dispersed OIT compared to that of encapsulated OIT. A plausible explanation is the disruption of the merging close-packing of latex particles when microspheres were embedded in the coated film. The size range of the latex particles is 10–100 nm while the microspheres are a couple of micrometers in diameter. This difference in length scales may affect the close-packing of the 28 days dried paint, see Figure 14, resulting in a structure not allowing for perfect homogenous film formation.

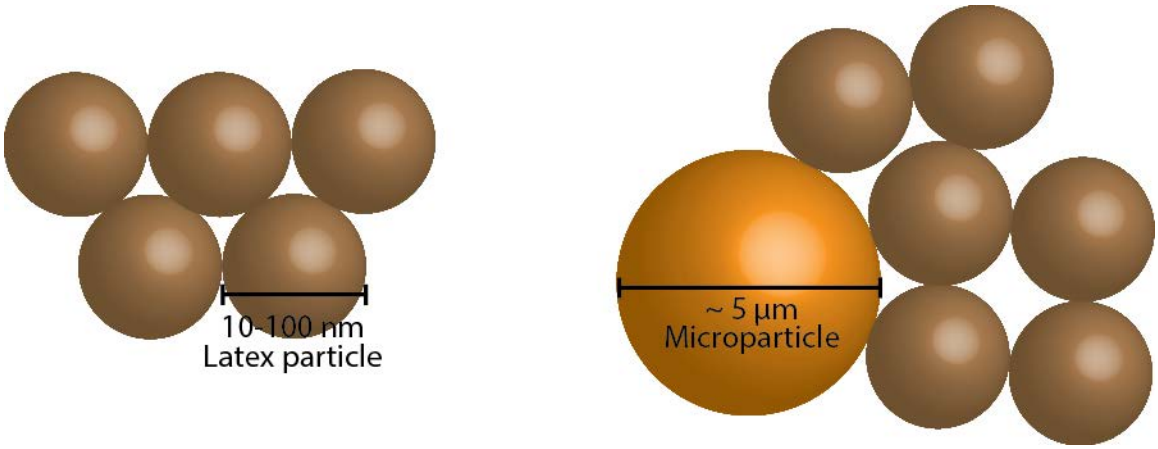


Figure 14. The size of the microspheres may affect the close-packing of the smaller latex particles.

6.3 Polyelectrolyte Multilayers

In both **Paper I** and **Paper III**, polyelectrolyte multilayers (PEMs) were built at the surface of microspheres. The microspheres are produced by following our standard recipe, but with some modifications. To be able to build PEMs, a block-copolymer, poly(methyl methacrylate (600)-block-sodium methacrylate (4600)) (600-*b*-4600) was used as emulsifier in the water phase. The latter makes the surface of the microsphere highly negatively charged, see Figure 15, and the first and oppositely charged polymer can then be adsorbed as a layer. This surface modification has been shown to result in a substantial decrease in release rate out from the microparticles.

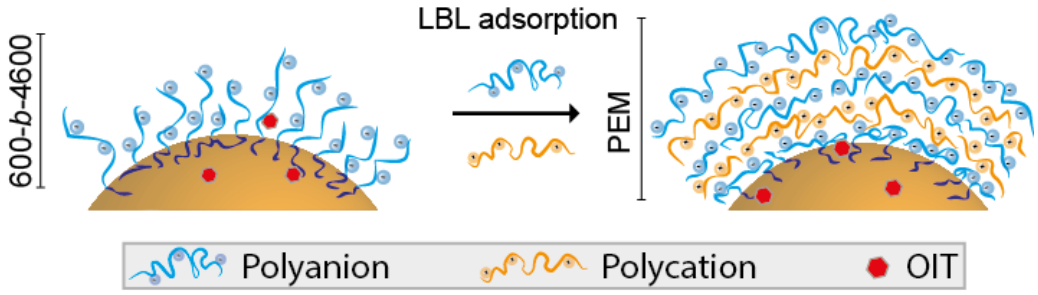


Figure 15. The layer-by-layer adsorption. The 600-*b*-4600 block-copolymer makes the microsphere surface highly negatively charged. When adsorbing polyelectrolyte multilayers on top of the block-copolymer the brush-like structure collapses and gives a dense structure. The hydrophobic biocide OIT will only solubilize in negligible low amounts in the multilayers.

In **Paper I** and **Paper III**, different active substances were used. In **Paper I** Disperse Red 13, a hydrophobic dye, was used in order to evaluate the release while OIT was the chosen active substance in **Paper III**. OIT is almost 1000 times more hydrophilic than Disperse Red 13 and a positive result for one of them might not lead to success in

the other case. The hydrophilic barrier of the PEM-modified microspheres will for instance affect the release of Disperse Red 13 to a larger extend. Also, the burst release is lowered for Disperse Red 13 since the burst release is due to accumulation and saturation in the outermost part of the microparticle and the solubility of Disperse Red 13 is here very low.

In **Paper III**, the release of OIT from microspheres with PEMs to an aqueous suspension was compared to the release out from microspheres produced using the standard recipe and the results are stated in Figure 16. Clearly PEM-modified microspheres decrease the release rate of OIT. The diffusion coefficient is lowered 40 times, but the burst rate is similar in the two cases.

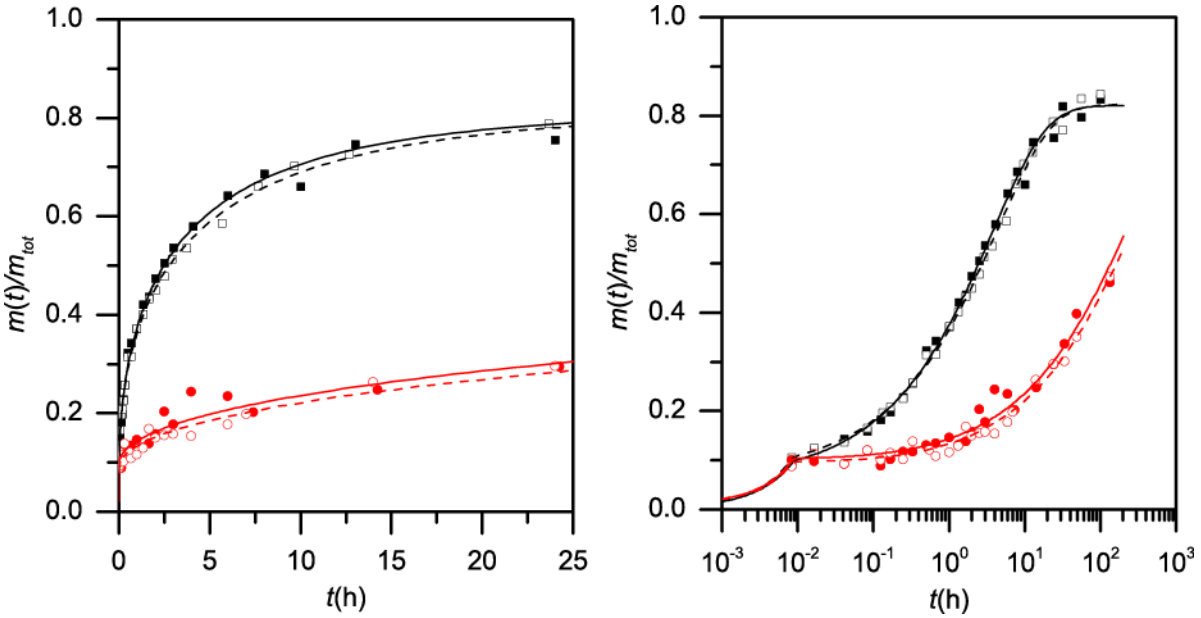


Figure 16. The release of OIT from PVA-based (■□) and PEM-modified (●○) microspheres. Filled and unfilled symbols are duplicates. The left figure shows the first 25 hours on a linear scale while the entire release using a logarithmic time scale are showed in the right figure. Full and dashed lines are fitted diffusion models.

The PEM-modified and OIT loaded microspheres were mixed in a commercial water-borne and acrylic façade paint and the release of OIT out from the coating to the surrounding water reservoir were analyzed and compared to the results from freely dispersed OIT in the paint. Another parameter was in the same time considered: the drying time of the paint. The central question in **Paper II** was the rate-determining properties with regard to release of OIT: the drying time of the paint compared to the influence of PEM particles. The results are given in Figure 17.

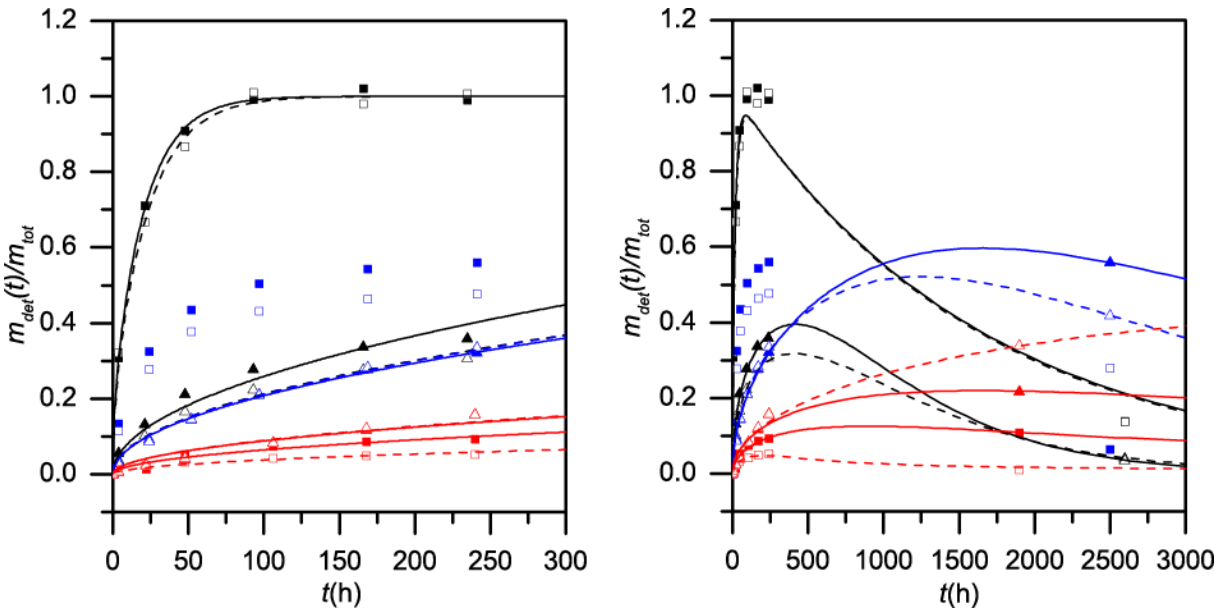


Figure 17. The fractional release $m(t)/m_{tot}$ of detected OIT from a latex paint as a function of time t. In left the first 300 hours of the experiment are displayed and the data has been fitted according to the diffusion models in Equation 12. In right figure, the long-term release is displayed which includes the degradation of the biocide. Freely dispersed OIT is marked with ■ and □, and encapsulated OIT with ▲ and△. Filled and unfilled symbols are duplicates. The different drying times are marked with, black (one day), blue (seven days) and red (33 days). The fitting for the curve corresponding to free OIT dried for one week was poor and has consequently been excluded.

It was clear that freely dispersed OIT in the coating release faster than encapsulated OIT. It was also noticed that increased drying time of the coating decreased the release rate significantly for free OIT but only to a small extent for encapsulated OIT, the reasons are discussed in Section 6.2. Polyelectrolyte multilayers provide rate-determining properties for OIT and a release system more or less independent of the drying time of the paint. As in the case in Section 6.2 degradation was considered in the coating experiments but not in the suspension experiments. The reasons are the same as stated above.

6.3.1 Antimicrobial Properties

In **Paper III**, a study in order to evaluate the antimicrobial properties of coatings, including freely or encapsulated OIT was carried out. The mold resistance was evaluated by visual inspection of *Aspergillus Niger* surface growth. *Aspergillus Niger*, also known as black mold, is a fungi and common biofouler [57]. The antimicrobial effect of a biocide-containing coating is determined by the surface concentration of the biocide. This concentration is primarily decided by the surface flux, which is the amount of biocide that leaves the coated surface per unit area and unit time. The surface concentration is also affected by the rate of the biocide removal, e.g. leaching,

degradation, and microbial adsorption. By time, the surface concentration reaches eventually an antimicrobial minimum level. From this follows that the surface flux must be kept above a specific critical surface flux in order to maintain protection against growth. The surface flux $f_{flux}(t)$ is equivalent to the derivative of the diffusion models presented above with normalization of biocide mass m and surface area A_s, respectively, according to

$$f_{flux}(t) = \frac{m}{A_s}\frac{d}{dt} f_{release}(t)\,. \tag{15}$$

Results are presented in Figure 18 and discussed more detailed in **Paper III**. The grey horizontal line in the bottom figure is the approximation of a critical surface flux in this experiment. Two systems, encapsulated OIT after 7 and 33 days of drying, were found to show a surface flux above this critical value at time corresponding to antimicrobial tests. These two samples also displayed full antimicrobial properties visualized in the top part of Figure 18. Coating formulations containing encapsulated OIT provided a more superior antifouling protection than coatings with free OIT, regardless of drying time.

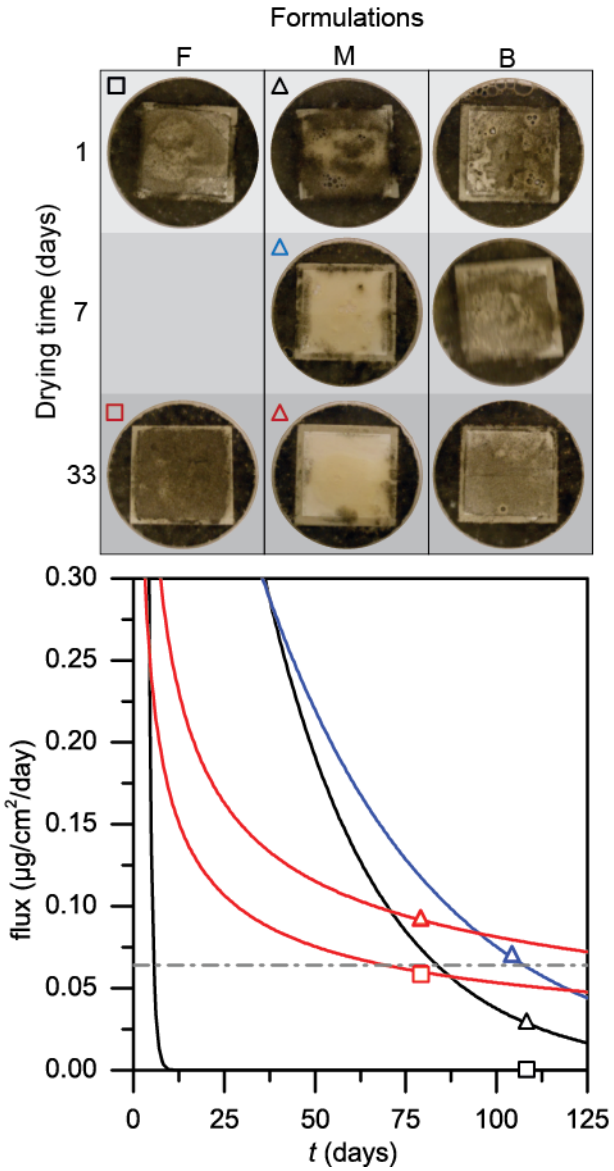


Figure 18. Top: the antifouling effect for various coatings, with free (F) or encapsulated (M) OIT, visually provided by photographs showing the growth of *Aspergillus niger*. Coatings containing no OIT were used as blank reference (B) for all drying times. Bottom: time-dependent OIT surface flux calculated from diffusion-models release shown in Figure 17. Freely dispersed OIT is marked with □, and encapsulated OIT with △. The different drying times are marked with black (1 day), blue (7 days) and red (33 days). The grey horizontal line in the graph illustrates the critical surface flux needed to stay above the critical surface concentration and prevent fungal growth. Each symbol corresponds to the specific time when the fouling of *Aspergillus niger* was analyzed for each coating.

7

CONCLUDING REMARKS

In this work, microparticles loaded with the biocide OIT have been formulated using the internal phase separation method and the release of the biocide has been evaluated from both microparticles and coatings. Core-shell type microcapsules can only be formulated by the internal phase separation method if the shell polymer, the core material, and the continuous aqueous phase, all fulfill specific spreading coefficients. Studies in this work showed that incorporation of OIT prevented formation of core-shell particles due to the surfactant-like properties of OIT. Highly loaded microspheres were used instead. In future work, new studies to understand these properties will be considered. One way to succeed to encapsulate OIT in core-shell microparticles might be to change the shell polymer or the core oil.

The effect of microscopic porosity of the microparticle polymer material needs to be considered when designing microparticles for sustained release. It was shown that a thermally relaxed state of the polymer decrease the release rate significantly. Also, the macroscopic porosity in the coating will matter when analyzing and evaluating the outcome of release studies.

Polyelectrolyte multilayers have here been proven to be an effective barrier against hydrophobic actives, as many of the commercial available biocides.

The experimental design of the release methodology that has been formulated is a robust and straightforward tool, suitable for many different systems.

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