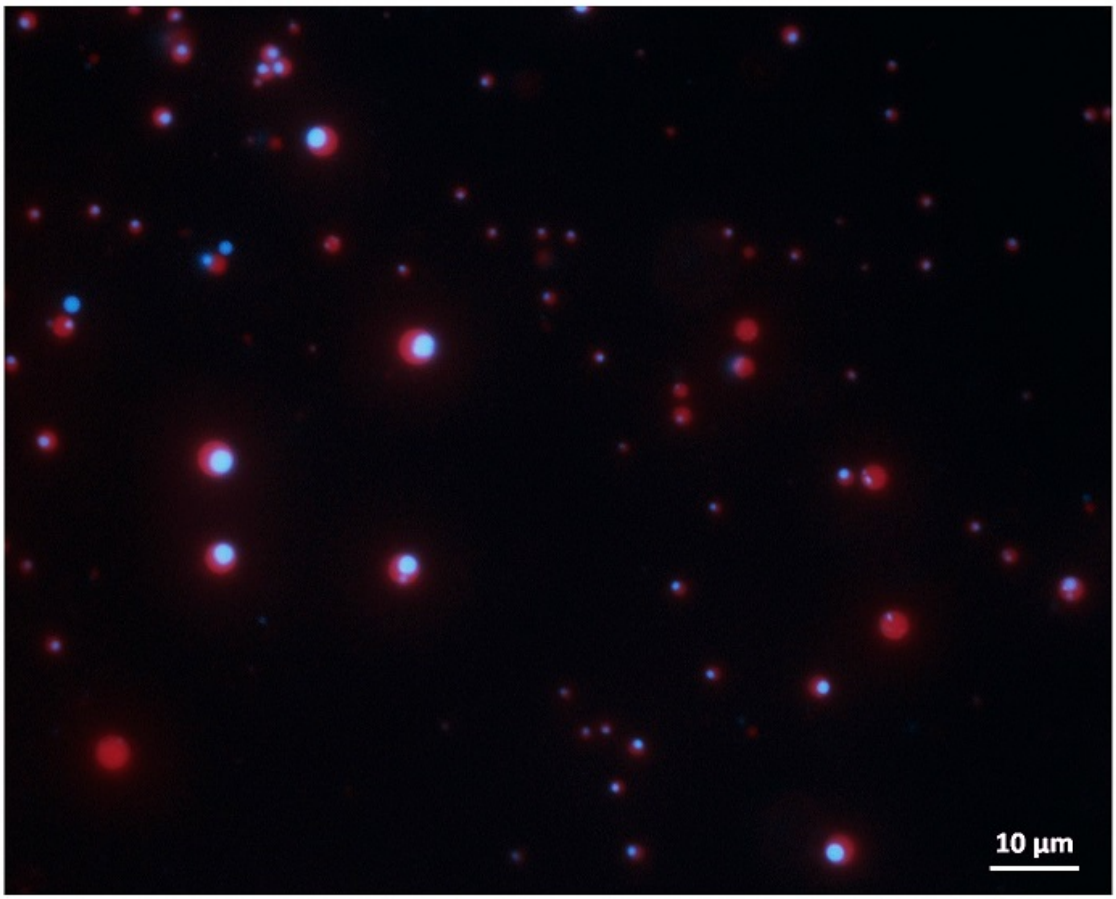
CHALMERS
UNIVERSITY OF TECHNOLOGY



Microencapsulation of actives for the healthcare of tomorrow

Formulation of core-shell microcapsules using biocompatible materials for extended and triggered release of actives

Master's thesis in Materials Chemistry

GUSTAV ERIKSSON

Department of Chemistry and Chemical Engineering
CHALMERS UNIVERSITY OF TECHNOLOGY
Gothenburg, Sweden 2018

Master's thesis 2018

Microencapsulation of actives for the healthcare of tomorrow

Formulation of core-shell microcapsules using biocompatible materials for extended and triggered release of actives

GUSTAV ERIKSSON

CHALMERS
UNIVERSITY OF TECHNOLOGY

Department of Chemistry and Chemical Engineering
Division of Applied Chemistry
Chalmers University of Technology
Gothenburg, Sweden 2018

Microencapsulation of actives for the healthcare of tomorrow
Formulation of core-shell microcapsules using biocompatible materials for extended and triggered release of actives
GUSTAV ERIKSSON

Supervisor: Lars Nordstierna, Department of Chemistry and Chemical Engineering
Co-Supervisor: Markus Andersson Trojer, Swerea IVF
Examiner: Lars Nordstierna, Department of Chemistry and Chemical Engineering

Master's Thesis 2018
Department of Chemistry and Chemical Engineering
Division of Applied Chemistry
Chalmers University of Technology
SE-412 96 Gothenburg

Cover: Core-shell microcapsules with a polyanhydride shell and fatty acid ester core. Morphology visualized using a composite image of two fluorescence filters.

Gothenburg, Sweden 2018

Microencapsulation of actives for the healthcare of tomorrow
Formulation of core-shell microcapsules using biocompatible materials for extended and triggered release of actives
GUSTAV ERIKSSON
Department of Chemistry and Chemical Engineering
Chalmers University of Technology

Abstract

The increased standard of life has during the last decades caused new challenges for our healthcare systems, mainly through the rise of so called lifestyle diseases such as diabetes. One complication of diabetes is the development of chronic wounds which are susceptible to infections. To treat such infections, the conventional method is to impregnate bandages with antimicrobial substances. However, such an approach causes a rapid release and loss of the antimicrobial substances, increasing the risk for development of antimicrobial resistance in the bacteria present. In order to prevent this, it is proposed to encapsulate the antimicrobial substances in microcapsules, that are to be incorporated into a non-woven fabric of cellulose fibers. These microcapsules will give a release profile of antimicrobial substances which reduces the risk for development of resistance.

In this project, several biopolymers have been evaluated with regard to their potential as shell materials in core-shell microcapsules. Poly L-lactic acid, Poly(lactic-co-glycolic acid), cellulose triacetate and a polyanhydride have been examined to this purpose, by attempting to covering a fatty acid ester core with the selected polymers using the internal phase separation by solvent evaporation method. To evaluate the material's potential to be used in the proposed application release studies of actives from the formulated microcapsules are carried out. It has been found that Poly(lactic-co-glycolic acid) is a promising shell material for extended release of actives while polyanhydrides have potential as a candidate for triggered release.

Keywords: chronic wounds, antimicrobial resistance, microcapsules, core-shell particles, polylactic acids, cellulose derivatives, polyanhydrides, fatty acid esters, extended release, triggered release.

Acknowledgements

Thank you to Lars Nordstierna for the opportunity to work with you for the last year as well as all your guidance. It has been a very educative and rewarding experience.

Thank you to Markus Andersson Trojer for all your guidance and excellent insight regarding all things related to microcapsule formulation.

I would also like to thank the other members of the CapCell project, Marina Craig, Kristina Hamberg and Erik Nygren for your encouragement, inspiration and helping me put my work into a context.

A special thank you to Mats Nylander for all the help with the microscope which made the visualization of the microcapsules in this project possible.

Finally, I would like to thank my family and friends for all your support and love.

Gustav Eriksson, Gothenburg, May 2018

Contents

List of Figures

List of Tables

1
Introduction

As the population grows older and the standard of life increases, new challenges arise for healthcare systems around the world. One such challenge is the rise of diabetes, for which the global prevalence of has doubled since 1980 [1][2]. Diabetes is considered a lifestyle disease and the increase is related to the increase of obesity across the world [3]. As such, the occurrence of diabetes is projected to increase worldwide and consequently so will also the strain put on healthcare systems by the disease and its complications.

One common complication of diabetes is the development of non-healing wounds in mainly the lower extremities [4]. This is due to reduced blood flow which limits the body's own ability to heal the wounds. The fact that the wounds are non-healing greatly increases the risk of infections and raises the need of antimicrobial substances to be used. Unfortunately, the widespread overuse of such substances has led to an increase in the prevalence of antimicrobial resistance in bacteria [5][6].

One strategy to use in order to control the release of active substances is to encapsulate them in microcapsules [7]. Microcapsules are spherical particles in the micrometer scale commonly used to store active compounds in order to protect them or control the release of them. To obtain an extended release of actives from the microcapsules, a core-shell morphology will be used, formulated using the internal phase separation by solvent evaporation method [8]. However, if microcapsules are to be used in a healthcare application, the traditional materials such as acrylic polymers and alkane oils have be to replaced with biocompatible alternatives.

A long lasting protection can be obtained by using a combination of microcapsules. A burst release, triggered by contact with the wound serum, to initially kill all the bacteria is desired in combination with release sustained over a long period of time to prevent the return of bacteria. The microcapsules are designed to eventually be incorporated into a non-woven fabric of cellulose fibers. The functionalized fabric can hopefully replace traditional fabrics used in conventional wound care products.

1.1 Purpose and objectives

In this project microcapsules, reservoirs for controlled release of active substances, are to be formulated using biocompatible materials. Materials and formulation conditions will be studied for production of core-shell microcapsules using the internal phase separation by solvent evaporation methodology [8]. The microcapsules will be designed in order to be used for controlled release of anti-bacterial substances.

In the first stage of the project, several biocompatible polymers will be evaluated with regard to their potential to be used as materials in core-shell microcapsules containing an antibacterial active substance. Examples of such polymers are polylactic acids, cellulose derivatives and polyanhydrides.

Later on, the release of actives from the formulated microcapsules will be studied. It is desired to find formulation strategies that yield microcapsules with a burst release triggered in contact with the wound as well as capsules with a sustained release over longer periods of time.

1.2 Limitations

The project will mainly focus on the formulation of microcapsules with regard to which polymeric shell material and formulation conditions are to be used. As core oil a single fatty acid ester will be used throughout the entirety of the project. Consequently, the formulation will not be optimized with regard to which core oil is to be used. Furthermore, no other formulation method than internal phase separation by solvent evaporation or other reservoirs than core-shell microcapsules will be considered in this project.

The microcapsule formulation will be attempted to be optimized with regard to the release of the active substance. Consequently, the release profiles of the active substance from the microcapsules will be studied. Model substances, i.e. compounds with similar structures and properties, will be used rather than antimicrobial substances. This is done in order to easier determine the morphology and study the release of actives from the produced microcapsules using varying dyes or fluorophores as the chosen model substances. However, the effectiveness of the antibacterial substances or the possibility to incorporate the formulated microcapsules into the non-woven fabric will not be examined in this project.

2
Theory

In the following chapter the theoretical background required for the project is presented. First an overview is given on the problems related to chronic wounds and antimicrobial resistance as well as current treatments and their limitations. Following this, the concept of encapsulation of actives in microcapsules is explained. Lastly, a more detailed presentation is given on the proposed materials to be used in the microcapsules formulated in the project.

2.1 Chronic wounds

One possible consequence of diabetes is a substantially increased risk of non-healing foot ulcers, which can be infected and cause the need for lower extremity amputation [1][9]. The reason that diabetes patients experience an elevated risk for chronic wounds development is that the disease can cause damage to the nerves, heart and blood vessels. This kind of damage causes a reduced blood flow to the extremities, which can hinder the healing of wounds. Non-healing wounds are susceptible to infection, which eventually can require the infected limb to be amputated [9].

Chronic wounds is a complication that is increasing in prevalence as diabetes becomes more spread across the world [1][10]. The global rate of diabetes in the adult population doubled between 1980 and 2014. The increase is correlated to life-style changes such as the increased occurrence of obesity [3].

2.2 Bacterial infections and antimicrobial resistance

As previously mentioned, one consequence of chronic wounds is the increased risk of infections. Bacteria has been confirmed to be present in the vast majority of chronic wounds [11]. Since the blood flow to the wound is reduced, the body's ability to deal with the infection on its own is diminished. The patient's inability to deal with infections raises the need for antimicrobial substances to be used. However, the increased use of antimicrobial substances, and especially the misuse and overuse, in health-care systems around the world has caused an increase in antimicrobial resistance, posing a serious concern [5][6].

Regarding wound treatment, antimicrobial substances are generally administered by impregnation of the bandage. This causes a rapid release of the substances, which when it diminishes eventually will lead to a concentration in the wound fluid below the concentration needed for the desired antimicrobial effect. The lowest concentration of antimicrobial substance allowed is called the minimum inhibitory concentration, MIC. Above this concentration bacteria growth is not possible. The presence of antimicrobial substances below the MIC on the other hand, have been shown to cause an increase in the prevalence of resistant bacteria strains [12]. In order to circumvent this, a controlled release is desired in order to prolong the administration of the antimicrobial substances. However, a delayed release causes the initial concentrations of antimicrobial substances in the wound fluid to be below the required amounts. For this reason, a combination of the rapid and prolonged release profiles is desired, as described in Figure 2.1.

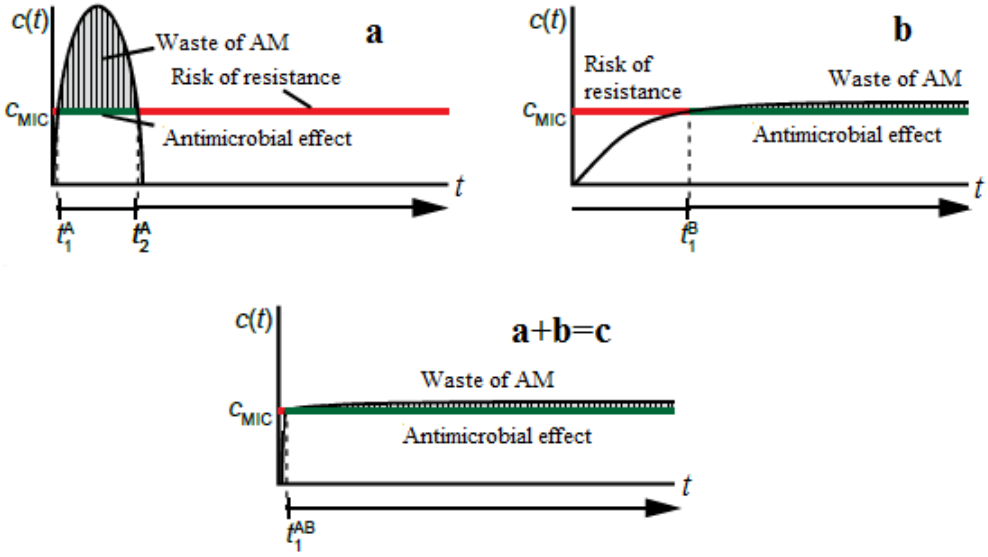


Figure 2.1: Concentration of antimicrobial substances over time depending on method of delivery. In (a), the rapid release causes the antimicrobial, AM, concentration to surpass the minimum inhibitory concentration, MIC, quickly. However, a large amount of substance is wasted and the concentration quickly decreases below the MIC. In (b), the controlled release gives a concentration of antimicrobial substances above the MIC for a sustained period of time. However, in a prolonged initial phase the concentration is not sufficient, giving the present bacteria the opportunity to adapt to the antimicrobial substance. In (c), the two previous release profiles are combined in order to give a antimicrobial concentration above MIC for the entirety of the time period.

In order to achieve the desired release profile, it is proposed to use a combination of microcapsules that degrade upon contact with the wound fluid, causing a rapid release, and microcapsules that gives a controlled and prolonged release of the active ingredient.

2.3 Microcapsules

One commonly used method to obtain controlled release of an active ingredient over a prolonged period of time is encapsulate the actives in reservoirs called microcapsules [7][13]. This method is useful in not only pharmaceutical delivery but also a number of different fields such as surface coatings, pesticide release in agriculture, fragrance release in cosmetics etc [14]. Microcapsules are defined to be solid particles, liquid droplets or gas bubbles covered with a layer of shell material or in coating the micrometer scale.

Microcapsules can be divided into three general categories based on their morphology, matrix microspheres, single core and multi core core-shell particles [15]. The different morphologies are illustrated in Figure 2.2. The microspheres are made out of a homogeneous, solid polymer matrix in which actives can be stored. Single- and multi core-shell microcapsules on the other hand, consists of a solid shell material that covers a core of a different material, either solid, liquid or gaseous.

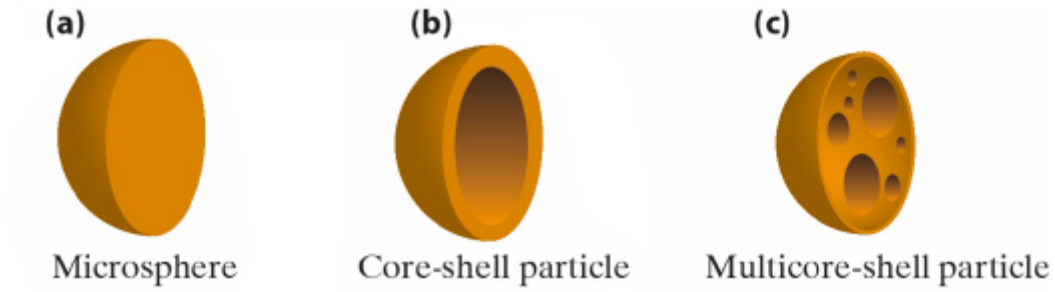


Figure 2.2: The three major categories of morphology in microcapsules. (a) the matrix microspheres, (b) demonstrates the monocored core-shell particles, and (c) displays the polycored multicore-shell particles [16].

2.3.1 Release of actives from microcapsules

One of the main applications of microcapsules is to control the release of an active substance. To use microcapsules as reservoirs for actives it is a necessity to understand the release of said actives from the capsules. The release is controlled by the diffusion of the active substance from the core oil, through the polymer shell, out in the surrounding medium [16]. This process is regulated by the solubility of the active substance in the core oil, microcapsule shell and surrounding medium as well as the kinetic diffusion coefficients.

Depending on the morphology of the microcapsules, different release kinetics are expected [13]. The variation of the released amount and release rate over time for different microcapsule morphologies can be seen in Figure 2.3. Consequently it is, by controlling the morphology of microcapsules, possible to affect the release of an active substance.

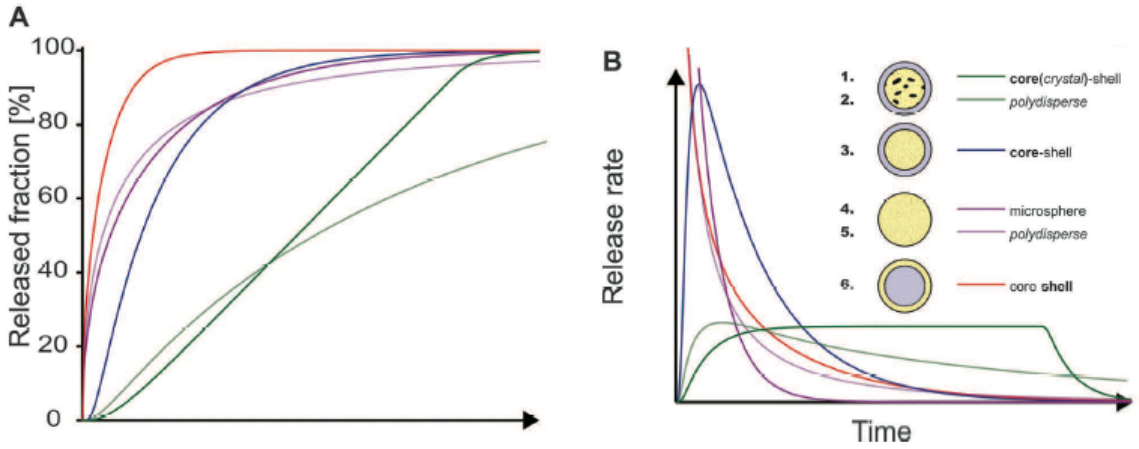


Figure 2.3: Graphs depicting (a) the released fraction of an active substance over time for different microcapsules morphologies and (b) the corresponding release rate [13].

In Figure 2.3, it is worth noting that the most prolonged release is obtained when the active is suspended as crystals inside the core oil. This will yield a zero-order release profile of the active substance. This can be explained by the fact that the in order for the active substance to precipitate as crystals inside the core oil it needs to saturate the oil. When the core oil is saturated with the active substance the concentration will be constant, causing the diffusion rate to also be constant. However, there are several challenges related to formulation of microcapsules with actives suspended as crystals in the core. For this reason prolonged release is generally obtained by dissolving the active in the core oil of core-shell microcapsules.

2.4 Formulation of microcapsules

Formulation of microcapsules can be carried out using one of several different methods. The available methods can be divided in two general categories, physical or chemical [15]. For a microcapsules formulation strategy to be considered chemical some chemical reaction is required to take place during the process. Common examples of such reactions are polymerization of the shell material or some other formation of the shell using a precursor. The physical formation process, sometimes called mechanical, is consequently the formulation strategies where no chemical reaction takes place. The most commonly used method of microcapsule formation is the physical process of phase separation.

2.4.1 Internal phase separation by solvent evaporation

One method for formulation of core-shell microcapsules that has gained in popularity in the recent years is the physical strategy of internal phase separation by solvent evaporation, which was developed by the Vincent group in 1998 [7][8]. In this method, an emulsion is formed with a continuous phase of water containing a

surface active agent or polymer, and a dispersed phase of a volatile solvent containing a polymeric shell material, non-volatile core oil and possibly an active substance. As volatile solvents, chlorinated hydrocarbons such as dichloromethane or chloroform is commonly used.

The capsules are formed by drop wise addition of the dispersed phase to the continuous phase under high shearing, which will lead to formation of an emulsion of the two immiscible solutions. The size of the eventual microcapsules is constrained by the emulsion droplet size. Consequently, it is possible to control the microcapsule size by controlling the size of the emulsion droplets, which can be done by altering the shearing rate and amount of surface active agent in the continuous phase. After the emulsion is formed the volatile solvent is allowed to evaporate, during which the polymer and non-volatile solvent will phase separate, leading to solidification of core shell microcapsules. The process of core-shell capsule formation during the evaporation of the volatile solvent is shown in Figure 2.4.

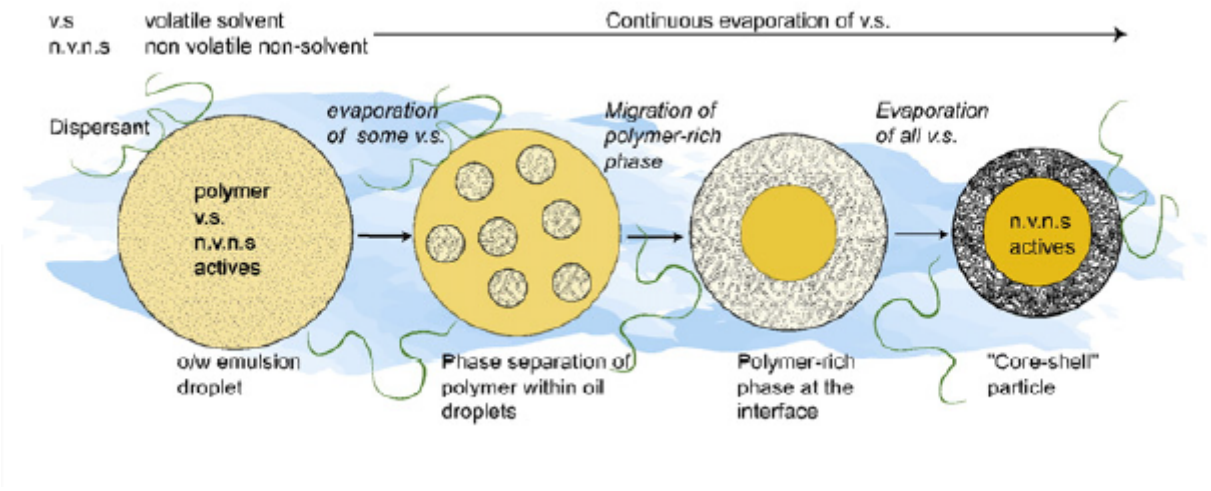


Figure 2.4: Process of core-shell capsule formation during volatile solvent evaporation [7].

For core-shell microcapsules to successfully form by the use of this method, some basic conditions needs to be fulfilled. The volatile solvent needs to solve the non-volatile solvent, the polymer and the active substance. The active substance needs to be solvable in the non-volatile solvent but not in the polymer, in order to be encapsulated in the core of the microcapsules. The non-volatile solvent and polymer has to be immiscible. Neither the polymer, non-volatile solvent or the active substance is allowed to be solvable in water. However, some solubility of the volatile solvent in water is required to facilitate the evaporation.

To successfully form core-shell microcapsules using the internal phase separation method there are also some conditions regarding the spreading coefficients that are to be fulfilled as formulated by the Vincent group. The spreading coefficient in a

three phase system, S_i is defined by Equation 2.1, where γ_{ij} is the interfacial tension between the phases i and j.

$$S_i = \gamma_{jk} - (\gamma_{ij} + \gamma_{ik}) \tag{2.1}$$

Using this definition, the constrains of the spreading coefficients can be stated. In order for core-shell microcapsules to be formed the core oil, o, and water, w, is not allowed to wet the other components of the system while it is required that the polymer, p, wet the oil and water. It causes the spreading coefficients to be as stated in Equation 2.2. If the spreading coefficient of the polymer also is negative, so called acorn particles will be formed.

$$S_p > 0; S_w < 0; S_o < 0; \tag{2.2}$$

2.5 Choice of core oil

In the first core-shell microcapsules formulated by the Vincent group using the interal phase sepearation by solvent evaporation method the liquid alkanes hexadecane and decane were used as the core material [8]. The hydrophobic alkanes satisfies the conditions regarding solubility in the volatile solvent and polymer as well as the spreading conditions. However, alkanes are generally not suitable for health care applications, thus attention has been turned to biocompatible oils such as fatty acid esters. Microcapsules have previously been successfully formulated by internal phase separation using the plant oils rapeseed oil and linseed oil [17].

As an alternative to vegetable oils, polyunsaturated fatty acid esters, FAEs, are proposed as a core oil for microcapsules. Fatty acid esters are the esters of fatty acids, and are shown to have beneficial health effects [18]. These effects, such as anti-inflammatory properties, are especially prominent in polyunsaturated fatty acids [19]. One example of a FAE is ethyl linoleate, the molecular structure of which can be observed in Figure 2.5. Ethyl linoleate is the result of the esterification of linoleic acid when reacted with ethanol [20].

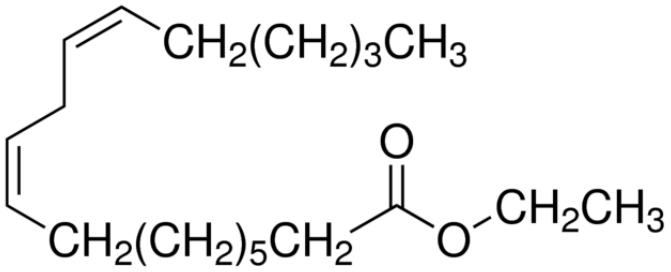


Figure 2.5: Molecular structure of ethyl linoleate.

2.6 Choice of polymer

The previously discussed choice of core material affects the choice of which material is to be used as the microcapsule shell. The Vincent group used PMMA when they developed the internal phase separation by solvent evaporation methodology for microcapsule formulation [8]. However, when a core oil more hydrophilic than alkanes is being used to solve an active substance, a more hydrophilic polymer is required to fullfil the solubility and spreading coefficient requirements in order to form core shell microcapsules [7][13].

In order to use the microcapsules in healthcare applications some further demands are put on the properties of the polymers used, in addition to being able to formulate microcapsules by interal phase separation. The materials used are required to be compatible with the body, characterized by their ability to degrade into benign compounds when in a biological environment [21][22].

2.6.1 Polylactic acid

One commonly used biopolymer is polylactic acid, PLA, which is a polymer consisting of repeating lactic acid units [23]. They are biodegradeable esters, making them friendly to both patients and the environment, and thus interesting to replace conventional plastics with [7]. Polylactic acids has previously been used for core shell microcapsules prepared by solvent evaporation [14][24]. The molecular structure of the repeating units in PLA can be seen in Figure 2.6.

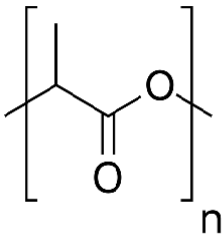


Figure 2.6: Molecular structure of Polylactic acid.

In the structure of the lactic acid monomer it can be noted that the compound is chiral [25]. Consequently, two enantiomers of the acid exists, the L-lactic acid and D-lactic acid. Depending on whether a racemic mixture of the monomer is used or not, the properties of the polymer, such as degree of crystallinity, will vary.

Polylactic acids are soluble in chlorinated solvents, making them candidates for shell materials in core shell microcapsules [24][26].

2.6.1.1 Poly(lactic-co-glycolic acid)

An alternative to PLA is the copolymer Poly(lactic-co-glycolic acid), PLGA, which is a polymer consisting of repeating lactic and glycolic acid uints [26]. PLGA is biodegradable in the same way as PLA is, and has also previously been used to formulate microspheres, making it a candidate for microcapsules used in healthcare application [27]. The molecular structure of PLGA can be observed in Figure 2.7.

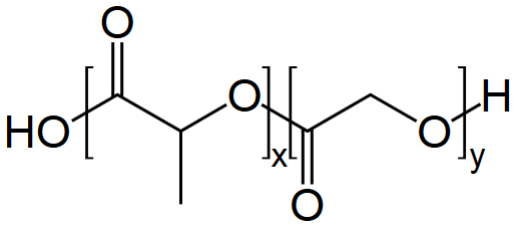


Figure 2.7: Molecular structure of the copolymer Poly(lactic-co-glycolic) acid. In the figure, the lactic acid monomer is found to the left and the glycolic acid monomer to the right.

Since glycolic acid is somewhat more hydrophilic than lactic acid, PLGA is a more hydrophilic polymer than PLA [7]. Being a copolymer, the properties of PLGA is depending on the ratio between lactic and glycolic acid in the molecule, as well as if they are structured in an alternating, block or random fashion [28].

2.6.2 Cellulose

Cellulose is the most abundant polymer in nature and the material that gives strength to plant cell walls. The polymer is of great interest as a material since it is renewable and lately a lot of research has been carried out regarding new applications of cellulose. The cellulose polymer consists of repeating D-glucose molecules that are $\beta 1 \rightarrow 4$ linked [29]. The molecular structure of the repeating unit can be seen in Figure 2.8. The way the monomers are connected, in combination with being able to form hydrogen bonds, gives cellulose some of its characteristic properties, such as being able to form microfibrils with high tensile strength. These microfibrils can form a network, which is the basis of plant cell walls.

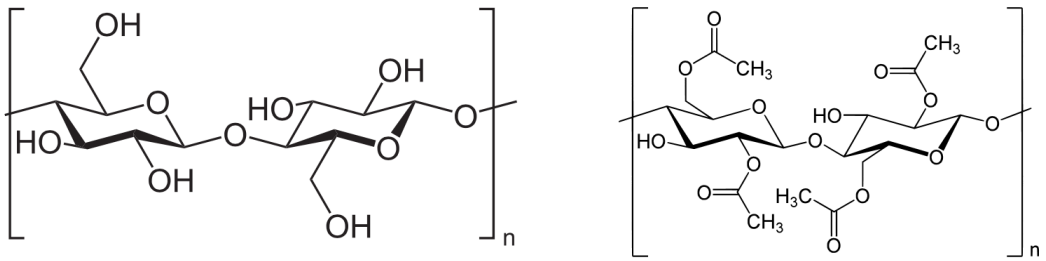


Figure 2.8: Molecular structure of the repeating unit in the cellulose polymer and cellulose diacetate respectively.

2.6.2.1 Cellulose acetate

One commonly used cellulose derivative is cellulose acetate, in which the hydroxyl groups are substituted with acetate groups [30][31]. The degree of substitution affects the properties of the polymer in terms of for example solubility and hydrophilicity. If the degree of substitution is around 2.5, meaning that roughly 80% of the hydroxyl groups are replaced with acetate groups, the polymer is called cellulose diacetate and is solvable in acetone [30]. The molecular structure of the repeating unit in cellulose diacetate can be seen in Figure 2.8. When all three hydroxyl groups on the repeating cellulose units are substituted with acetate the polymer is called cellulose triacetate. This polymer is solvable in chloroform [32]. The higher degree of substitution, the more hydrophobic the polymer is [7].

2.6.3 Polyanhydrides

To achieve the rapid release of the active antimicrobial substance required to prevent development of resistance in the early stages a shell material that will degrade upon contact with the wound is proposed. One candidate for such a class of polymer is poly anhydrides, which are polymers containing anhydride linkages that are able to undergo hydrolysis [33][34]. The molecular structure of such a polymer can be observed in Figure 2.9.

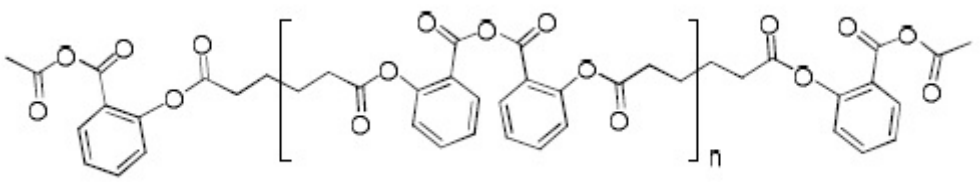


Figure 2.9: Molecular structure of Bis–(2–carboxyphenyl) Adipate Polyanhydride, called SASanhydride [35].

In the molecular structure above, it is worth noting the anhydride bond in the middle of the repeating unit, which is the location of the degradation of the polymer [36].

2.7 Choice of dispersant

In order to facilitate the emulsion formation and increase the stability of the formulated microcapsules some dispersant is used. The Vincent group used an emulsifier such as poly vinyl alcohol, PVA, in the aqueous phase of the emulsion to achieve this effect [8]. However, there are some difficulties related to the use of an emulsifier such as PVA dissolved in the aqueous phase, such as particle aggregation [37][38]. To circumvent this the formulation strategy can be modified by adding a block-co-polymer in the disperse phase. The block-co-polymer is suggested to contain the same monomer used for the shell material and a surface active block, generally

polyethylene glycol, PEG [39]. If for example, PLA is used as the shell material it is suggested to use a PEG-PLA block-co-polymer, as illustrated in Figure 2.10.

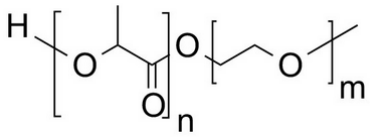


Figure 2.10: Molecular structure of a PLA-PEG co-block-polymer.

In previous studies the surface of microcapsules have successfully been covered with block-co-polymers but it is now suggested to replace the surface active polymer in the aqueous phase with a block-co-polymer in the disperse phase. This change is proposed to give a microcapsule system that is stable irregardless of surrounding medium, as opposed to require the presence of an excess of surface active polymer in the surronding medium to be stable.

2.8 Choice of active compounds

In the context of microencapsulation, active substances are molecules that are stored inside the capsules to be protected and possibly released in a controlled manner. The term active substance is commonly used in the pharmaceutical field to refer to the active ingredient in a pharmaceutical drug.

When studying the encapsulation and release of active substances it is advantageous to use dyes as model substances rather than biocides [7]. As a consequence of the fact that coloring agents often are UV active, they are suitable as model substances for studies of the release of actives from microcapsules. The concentration of actives in the medium can in these cases easily be followed by taking samples and measuring the absorbance using UV-spectrophotometry.

Coloring agents can also be used to efficiently study and visualize the morphology of the formulated microcapsules [40]. By using two coloring agents with different hydrophilicity the morphology of core-shell capsules can be visualized since the more hydrophilic compound will be found in the shell while the more hydrophobic compound will be partitioned in the core oil.

2.8.1 Pyrene

Pyrene is one of the most used fluorescence probes [41]. The compound is used in several applications, such as determination of critical micelle concentration and detection of specific molecules in biological systems [42]. Pyrene is UV-active with several excitations between 200 and 400 nm, two strong signals are found at 240 and 272 nm. Pyrene consists of four combined benzene rings, forming a flat polycyclic aromatic hydrocarbon compound, as can be seen in the molecular structure illustrated in Figure 2.11. As such, the compound is high hydrophobic.

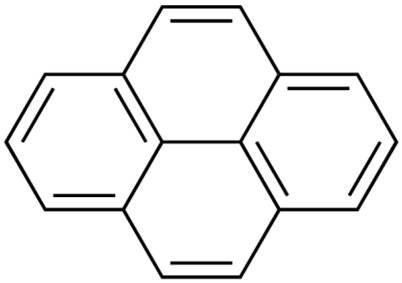


Figure 2.11: Molecular structure of pyrene.

2.8.2 Methylene Blue

One often used dye is methylthioninium chloride, commonly called methylene blue [43]. It is used both as a dye and has beneficial medical applications. As a dye the compound gives a blue color and can be used as a marker in biological systems. Since methylene blue is a salt, it is significantly more hydrophilic than pyrene. This will cause the compound to accumulate in the semi-hydrophilic shell of core-shell microcapsules rather than the hydrophobic core.

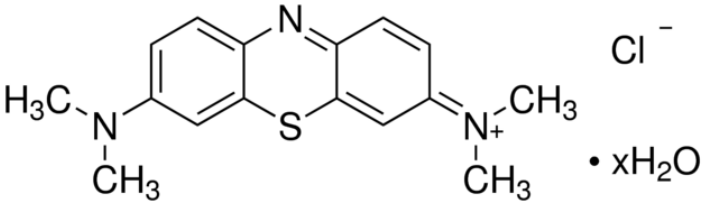


Figure 2.12: Molecular structure of methylene blue.

2.8.3 Sudan I

Sudan I is a common azo dye, i.e. an organic compound with a double bond between two nitrogen atoms that can be used as a colouring agent [44]. Sudan I gives a red color and is often used as a lipophilic marker, making it suitable for marking of the hydrophobic core of core-shell microcapsules. The compound is also UV-active, with an excitation at 480 nm. The molcular structure of Sudan I can be seen in Figure 2.13.

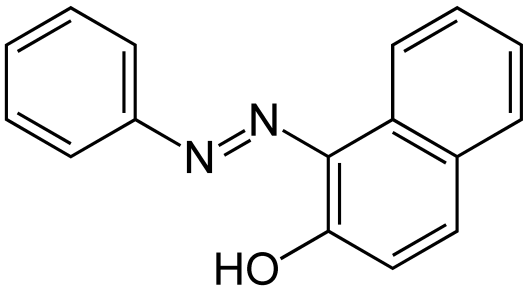


Figure 2.13: Molecular structure of Sudan I.

3
Methods

The experimental procedure of the project consisted mainly of formulation of microcapsules.

3.1 Chemicals and Materials

The following chemicals were used to carry out the experimental work of the project.

- β-Carotene, $\geq$ 93 %, Sigma-Aldrich (C9750)
- Acetone, $\geq$ 97 %, Sigma-Aldrich (W332615)
- Bis–(2–carboxyphenyl) Adipate Polyanhydride, $M_n \approx 1\ 000$, Polymer Source (P20098A-SASanhydride).
- Brij L23, Sigma-Aldrich (P12554)
- Cellulose triacetate, Sigma-Aldrich (22199)
- Dichloromethane, Sigma-Aldrich (L090000)
- Ethyl linoleate, $\geq$ 99, Sigma-Aldrich (L1751)
- Hexadecane, $\geq$ 99 %, Sigma-Aldrich (H6703)
- Methylene Blue, hydrate redox indicator, Riedel-de Haën (32723)
- Methanol, $\geq$ 99.5 %, Fluka (24228)
- Milli-Q water
- PEG(5000)-b-PLA(1000), Diblock polymer, Polysciences, Inc (24386).
- Poly(D,L-lactide-co-glycolide), $M_w \approx 10\ 000$, Polysciences, Inc (19247).
- Poly(L-lactic acid), $M_w \approx 40\ 000$ - 70 000, Polysciences, Inc (06529).
- Poly(vinyl alcohol), 95 % hydrolyzed, $M_w \approx 95\ 000$, Acros Organics (183290010)
- Pyrene, $\geq$ 99 %, Sigma-Aldrich (82648)
- Sudan I, $\geq$ 95 %, Sigma-Aldrich (103624)

Some of the chemicals used require special care, especially the volatile and toxic chlorinated solvent dichloromethane, DCM. This is important to consider during the evaporation process of the microcapsule formulation, which is to be carried out inside a fume hood. Further more, care is required when handling acetone and methanol which are flammable and hexadecane as well as the coloring agents which are toxic. The polymers used are generally considered benign as they are biopolymers. This also applies for the fatty acid ester used, ethyl linoleate. It is worth noting that Milli-Q water was used for the entirety of the project.

The following material and equipment was used during the project.

- Heidolph Silent Crusher homogenizer model M tool 6F
- Hamilton syringe 1 mL model 81320
- 5 ml 14/10 round bottom flask with side neck, Ace Glass (9592-35)
- UV Spectrophotometer HP8453
- Zeiss Axio Imager 2

3.2 Formulation of microcapsules

Microcapsules were formulated using the internal phase separation by solvent evaporation technique described in the previous chapter. It is worth noting that the formulation carried out in this project was scaled down from the original formulation postulated by the Vincent group.

In parallel with the microcapsule formulation, a solubility study was carried out on select dyes, fluorophores, and active compounds in the fatty acid ester ethyl linoleate, further described in Appendix I.

3.2.1 Polylactic Acid

The first batch, A, of microcapsules were formulated using the L-enantiomer polylactic acid, Poly(L-lactic acid), PLLA, as the polymeric shell material. The disperse phase was prepared according to Table 3.1. 2.5 ml of this dispersed phase was added drop wise to 2.5 ml of a continuous phase consisting of 1 weight-% polyvinyl alcohol, PVA, in water in a two headed pear beaker under shearing by a *Heidolph Silent Crusher homogenizer model M tool 6F* at 5000 rpm to create an emulsion. Upon addition of the dispersed phase the rotational speed of the homogenizer was increased to 10 000 rpm. After one hour of emulsification was stopped and the emulsion was allowed to stand in a fume hood under stirring over night to evaporate the volatile solvent.

Table 3.1: List of the batches with microcapsules produced using Poly(L-lactic acid) as shell material and the composition of the dispersed phases used during formulation.

Batch label	DCM	PLLA	Core oil	Acetone	Coloring agent
A	3.2 g	0.21 g	0.16 g hexadecane	None	None
B	2.4 g	0.093 g	0.062 g ethyl linoleate	190 μl	None
C	3.23 g	0.1 g	0.022 g ethyl linoleate	200 μl	1.28 μg Pyrene

For the second batch of microcapsules, B, the hexadecane was replaced with the fatty acid ester ethyl linoleate. The amount of microcapsule material used was reduced, but the ratio between polymer and core oil was identical. Acetone was added to the disperse phase in order to decrease the size distribution of the microcapsules [8]. The disperse phase was transferred using a syringe of model *Hamilton 81320* to 3 ml of an identical continuous phase as previously used under shearing at 9000 rpm.

Upon addition of the dispersed phase, the rotational speed was decreased to 5000 rpm in order to increase the size of the microcapsules for easier evaluation. After one hour the emulsion was transferred to an additional 3.4 ml continuous phase under stirring in a fume hood to allow for complete evaporation of the volatile solvent over night.

The third microcapsule batch, C, was prepared in order to be used for release studies. The ratio between the polymer and core oil was set to 5:1. 6 weight-% pyrene was dissolved in the ethyl linoleate prior to the addition to the DCM. The disperse phase was transferred to 3 ml of the same continuous phase as previously used under shearing at 7500 rpm. Upon addition the rotational speed was decreased to 5000 rpm and emulsification carried out for one hour. The emulsion was then transferred to an additional 3 ml continuous phase for evaporation of the volatile solvent as previously.

3.2.2 Poly(lactic-co-glycolic acid)

In order to evaluate Poly(lactic-co-glycolic acid), PLGA, as a potential shell material several batches of microcapsules were formulated. The same equipment and procedure was used as when producing the PLLA microcapsules. In order to determine the morphology of the capsules and determine their potential to be used in the final product the disperse phase was tuned between the different batches. A select amount of the disperse phases is prestented in Table 3.2.

Table 3.2: List of the batches with microcapsules produced using PLGA as shell material and the composition of the dispersed phases used during formulation.

Batch label	DCM	PLGA	Ethyl linoleate	Acetone	Coloring agent
D	3.98 g	0.075 g	0.05 g	200 μl	None
E	3.98 g	0.09 g	0.06 g	200 μl	4.5 μg Methylene Blue
F	3.23 g	0.1 g	0.022 g	200 μl	10 μg Methylene Blue
G	3.23 g	0.1 g	0.022 g	200 μl	1.4 μg Pyrene, 10 μg Methylene Blue
H	3.23 g	0.1 g	0.022 g	200 μl	1.4 μg Pyrene

Batch D was formulated using the same 3:2-ratio between the polymer and core oil as previously used. The microcapsules were formulated using the same procedure as used for batch B.

In order to determine the morphology of the microcapsules an amount of methylene blue was added to the disperse phase equivalent to 5 weight-% of the polymer in batch E. No other changes were made to the procedure.

Batch F was prepared using a 5:1-ratio between the polymer and core oil in order to increase the shell thickness. By doing so it was hypothesized that the morphology

would be easier to determine. Since the microcapsules are 3D particles, a larger volume of polymer is required to increase the radius of the particle shell which is in one dimension. The amount of methylene blue added was doubled to 10 weight% of the PLGA, to ensure that enough of the substance would be present in the capsules to be visible. The shearing was decreased to 7000 rpm during the addition of the dispersed phase, in an effort to increase the microcapsule size. In all other regards the formulation strategy was identical to previous batches.

In batch G another coloring agent, pyrene, was added. Pyrene was selected due to its solubility in ethyl linoleate, see Appendix I. 6 weight-% pyrene was solved in the ethyl linoleate. The reason that two different coloring agents were used was in order to easily visualize the morphology of the microcapsules due to the different solubilities of the compounds.

The final PLGA microcapsules batch, H, was formulated to carry out release studies. The Methylene Blue was removed to avoid disturbance when measured the released pyrene amount.

3.2.3 Cellulose triacetate

A number of microcapsule batches with cellulose triacetate as the shell material was formulated. The dispersed phase was added to the same continuous phase as previously used and the formulation was carried out in an identical fashion. During the preparation of the dispersed phase in the first two batches no acetone was used, since it is a poor solvent for cellulose triacetate [32].

Table 3.3: List of the batches with microcapsules produced using cellulose triacetate as shell material and the composition of the dispersed phases used during formulation.

Batch label	DCM	CTA	Ethyl linoleate	Acetone	Coloring agent
J	2.425	0.075 g	0.05 g	None	2.48 μg Sudan I, 3.76 μg Methylene Blue
K	2.425 g	0.075 g	0.05 g	None	3.76 μg Metyhlene Blue
L	3.32 g	0.1 g	0.022 g	200 μl	5 μg Methylene Blue
M	3.23 g	0.1 g	0.022 g	200 μl	1.4 μg Pyrene

Batch J was formulated with 5 weight-% Sudan I in relation to the ethyl linoleate and 5-weight-% methylene blue in relation to the cellulose triacetate. The formulation strategy was identical to the one used in batch E.

In batch K the Sudan I was removed, in all other regards the formulation was carried out in an identical fashion.

When formulating batch L, the amount of oil was reduced to obtain a 5:1-ratio in relation to the amount of polymer. This was done in order to increase the shell

thickness, so the possible core-shell morphology more clearly could be visualized. The shearing was decreased to 7000 rpm during the addition of the dispersed phase, similar to the formulation of batch F. It was also attempted to add acetone to the disperse phase.

In batch M pyrene was used as fluorophore in order to study the release. 6 weight-% was dissolved in ethyl linoleate prior to addition to the disperse phase. No other changes were made to the formulation strategy from the previous batches intended for release studies.

3.2.4 Polyanhydride microcapsules

To obtain microcapsules that will give a burst release of active ingredient, anhydride polymers are proposed as a possible shell material [45]. As polymeric shell material Bis–(2–carboxyphenyl) Adipate Polyanhydride, called SASanhydride, was selected, due to its solubility in chlorinated solvents [35]. The formulated batches are presented in Table 3.4.

Table 3.4: List of the batches with microcapsules produced using SASanhydride as shell material and the composition of the dispersed phases used during formulation.

Batch label	DCM	SAS	Ethyl linoleate	Acetone	Coloring agent
N	2.87 g	0.0887 g	0.059 g	160 μl	None
O	2.425 g	0.0741 g	0.01482 g	160 μl	0.95 μg Pyrene, 7.4 μg Methylene Blue
P	3.23 g	0.1 g	0.022 g	200 μl	1.4 μg Pyrene

The first batch, N, was prepared with a 3:2-ratio between the polymer and oil and without any coloring agents. The dispersed phase was added to the continuous phase under shearing at 7500 rpm, which was decreased to 5000 rpm upon the addition.

In batch O, the ratio between the polymer and oil was changed to 5:1 and methylene blue as well as pyrene was added in the same fashion as in the corresponding PLGA-batch, G.

The final anhydride batch, P, was formulated in the same fashion as the previous batches intended for release studies.

3.3 Microcapsule formulation using block-co-polymer dispersants

To study the possibility to use a surface active block-co-polymer in disperse phase rather than a surface active polymer in the continuous phase in order to stabilize the microcapsules two batches were prepared. The disperse phases prepared are listed in Table 3.5. The first disperse phase, Q, was formulated in order to obtain

1.65 % block-co-polymer in relation to the shell material and a 3:2-ratio between the polymer and core oil. In the second batch, R, the proportion of block-co-polymer in relation to the shell material was increased to 16.4 %.

Table 3.5: List of PLLA microcapsules batches prepared using the surface active block-co-polymer PEG-PLA in the disperse phase, and the composition of the dispersed phases used during the formulation.

Batch label	DCM	PLLA	Ethyl linoleate	Acetone	PEG-PLA
Q	2.53 g	0.0789 g	0.0526 g	160 μl	1.3 μg
R	1.90 g	0.0575 g	0.0385 g	120 μl	9.445 μg

Batch Q was prepared by the addition of the disperse phase to 3 ml pure water under shearing at 7500 rpm. Following the addition, the rotational speed was increased to 8000 rpm and the emulsification was carried out for one hour.

During the formulation of the second batch, R, the shearing was carried out at 10 000 rpm for the entirety of the emulsification.

3.4 Analysis of microcapsules

To determine the morphology and size the produced microcapsules optical light microscopy was used. A microscope of the model *Zeiss Axio Imager 2* from Carl Zeiss in Germany was used to analyze the microcapsule suspensions. Digital images were acquired using the *Zeiss ZEN Pro* software. The same software was used to enhance the images, in combination with *Adobe Photoshop CS6* from Adobe Systems in the US.

3.5 Release studies

In order to assess the potential of the formulated microcapsules to be used as reservoirs for controlled release of the active ingredients release studies were carried out on the microcapsules loaded with actives. To facilitate the release, an aqueous medium containing a surface active compound, such as Brij, was prepared with a concentration well above the critical micelle concentration.

Initially, a calibration curve was prepared by the procedure described in Appendix II in order to be able to determine the pyrene concentration in a 6% Brij L23 aqueous solution based on the UV absorbance at specific wavelengths using the Beer-Lambert law, Equation 3.1.

$$A = \varepsilon cl \tag{3.1}$$

Release studies were carried out on the microcapsules produced in batch C, H, M and P. Prior to the release studies the amount of microcapsules in the suspension was set to 2 weight-% by weighing the suspension and adding additional water. The studies were initiated by the addition of 2 ml microcapsule suspension to 198 ml 6% Brij L23 aqueous solution. The ratio of suspension and aqueous Brij solution was calculated using the calibration curve to yield an absorbance from which the concentration could be calculated using the Beer-Lambert law upon complete release of the encapsulated pyrene. The release was followed by extracting samples from the release bath, immediately filtrating them in order to remove the microcapsules, and measuring the pyrene concentration by measuring the absorbance at 242 nm.

The release profile was determined by plotting the released fraction against the elapsed time. In order to calculate the released fraction, the absorbance value when all pyrene in the sample had been released was required. This was obtained by extracting a 2 ml sample of the release medium containing microcapsules and resuspending it in 6 ml methanol, in order to extract all the pyrene out of the microcapsules. Following this, the sample was filtrated and the absorbance measured to yield the maximum released pyrene concentration possible in the release bath.

4

Results and Discussion

In the following chapter the results from the microcapsule formulation are presented using microscopy images. The results from the release studies are also presented and discussed.

4.1 Formulation of microcapsules

The formulated microcapsules were mainly analyzed using optical light microscopy. By this method both the quantity and quality of the microcapsules can be evaluated. Using the fluorescence microscopy, the morphology could be observed thanks to the coloring agents and their varying distribution in the shell and core respectively.

4.1.1 Polylactic Acid

The microcapsules with a PLLA shell and hexadecane core produced in the first batch can be observed in Figure 4.1.

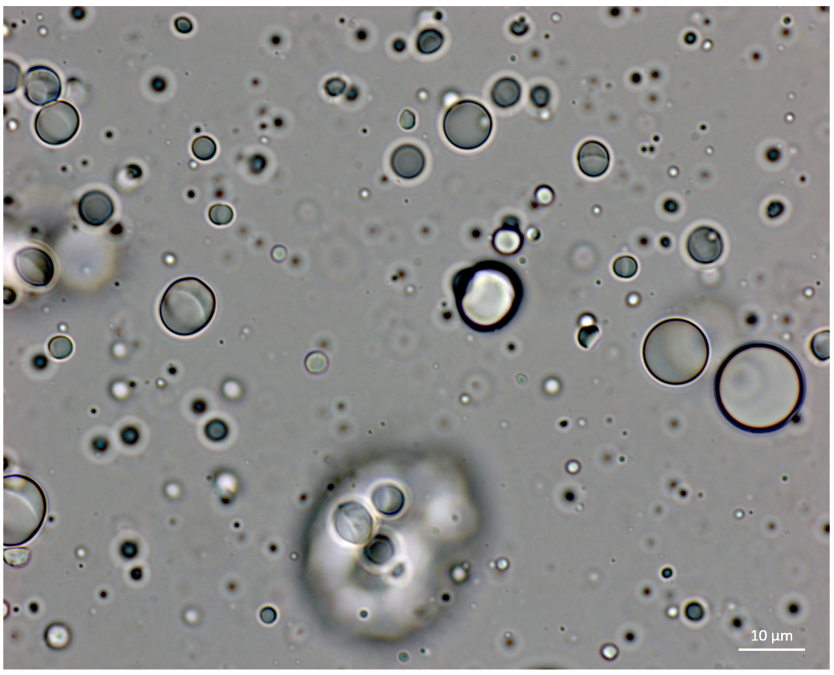


Figure 4.1: Microcapsules from batch A observed using optical light microscopy.

The capsules containing hexadecane are mostly spherical and appear to have a smooth surface. A number of them show an acorn shape, possibly indicating that the spreading coefficient for the polymer is negative [7]. Following this successful formulation, a similar batch was prepared were the hexadecane was replaced with the fatty acid ester ethyl linoleate. The capsules in this batch can be seen in Figure 4.2.

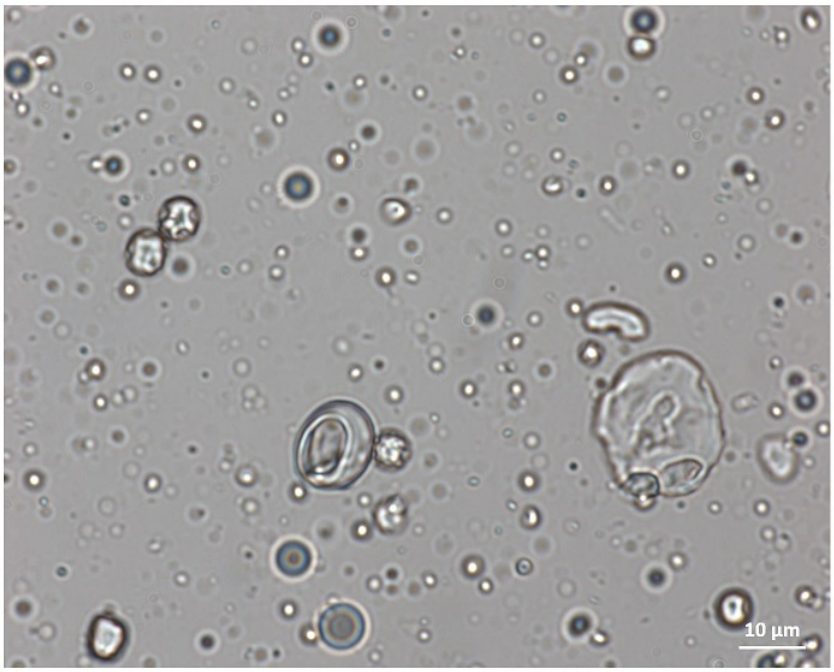


Figure 4.2: Microcapsules from batch B observed using optical light microscopy. The capsules are clearly deformed and a number of oil droplets are visible.

In this batch the capsules are deformed as opposed to the capsules containing hexadecane. This can be attributed to the chemical similarities between the polymer and oil. A possible consequence of the chemical similarity is that the constraints on the spreading coefficients required for core-shell particles are not fulfilled. More specifically, it is possible that the interfacial tension between the polymer and water is too high to compensate for the lower tension between the oil and water, causing the spreading coefficient of the polymer, S_p, to be negative.

Another possible contributing factor to the deformed microcapsules seen in Figure 4.2 can be found in the characteristics of the shell polymer. Since the L-enantiomer of the lactic acid is used exclusively, the polymer will be more crystalline than it would be if a mixture of enantiomers were used. The higher degree of crystalline can possibly hinder the formation of a complete shell around the core oil during the phase separation process. It is possible that a complete shell has not had time to form before complete evaporation of the volatile solvent has occurred, due to the higher viscosity.

The microcapsules in batch C can be seen in Figure 4.3. Once again somewhat

deformed microcapsules can be observed. Using a fluorescence filter a core-shell morphology can be determined in some of the microcapsules by noting the higher intensity in core compared to the shell, due to the affinity of the probe pyrene to the more hydrophobic core oil rather than the polymer shell.

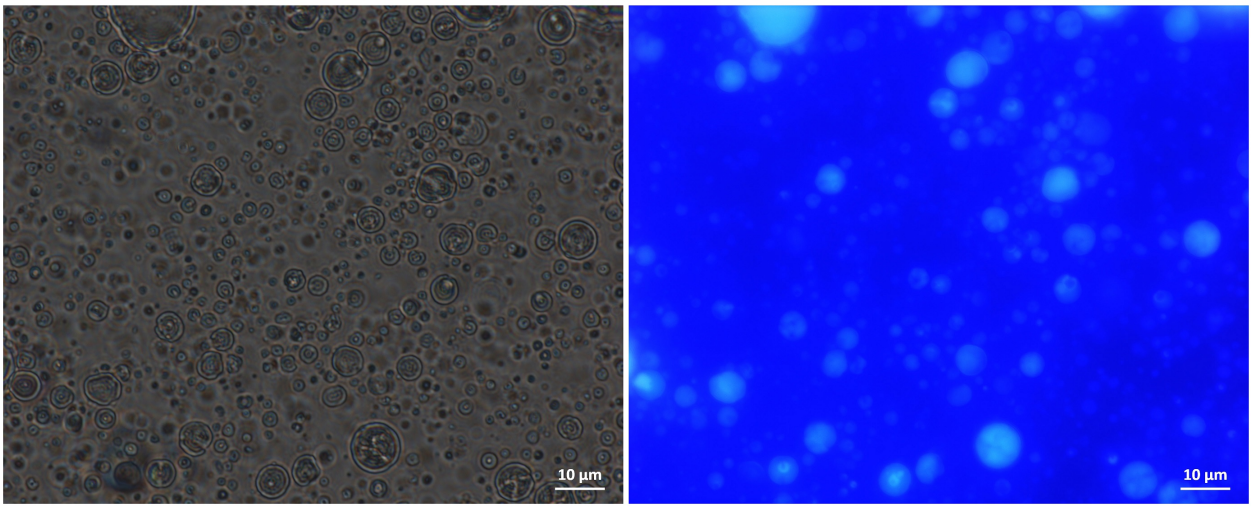


Figure 4.3: Microcapsules from batch C observed using optical light microscopy to the left and with a fluorescence filter to the right.

After approximately one week the microcapsules in batch C were observed once again to study the stability of the capsules. The result can be observed in Figure 4.4.

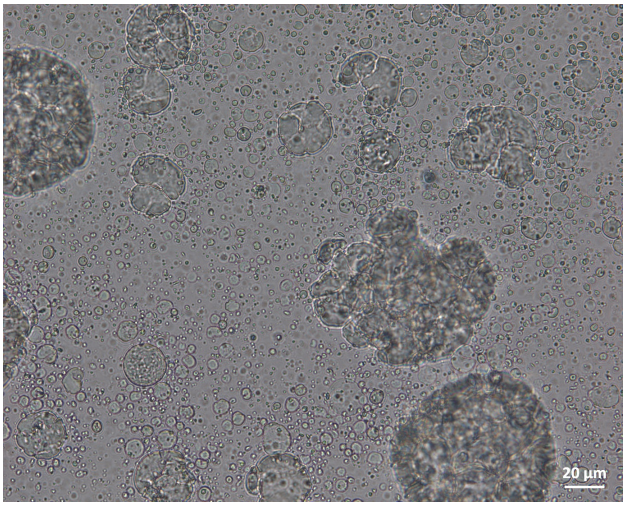


Figure 4.4: Microcapsules from batch C observed one week after formulation using optical light microscopy.

One week after the formulation of batch C, phase separated structures can clearly be seen. This further strengthens the theory that the polymer shell is incomplete, which would put the core oil in direct contact with the surrounding water. As a consequence, the oil can come in contact with oil from other microcapsules, causing larger, phase separated structures to form.

4.1.2 Poly(lactic-co-glycolic acid)

The batches of microcapsules with a PLGA shell formulated can be found in table 3.2. The first batch was formulated using the same procedure as the PLLA capsules with ethyl linoleate. The produced microcapsules can be observed in Figure 4.5. The capsules are clearly spherical and appear to be somewhat monodisperse in size.

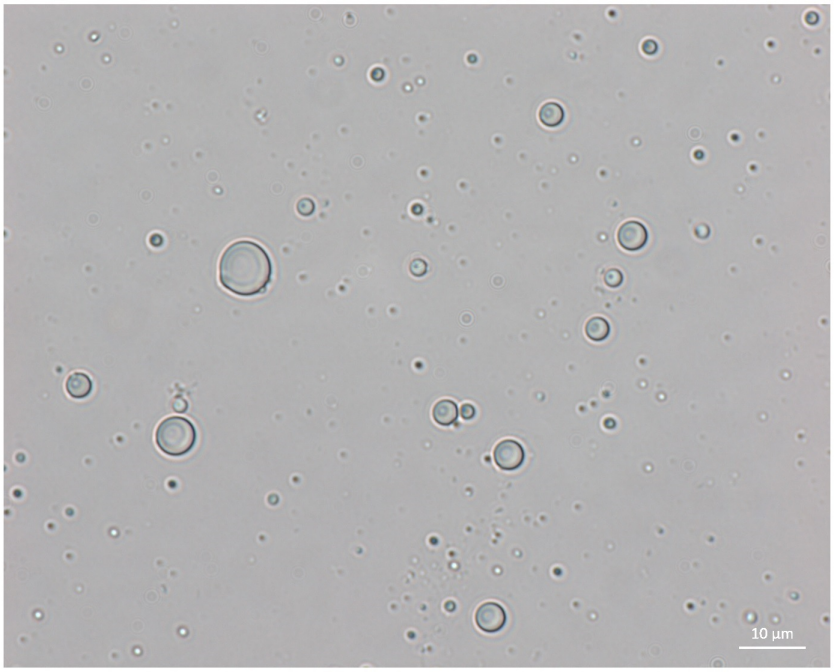


Figure 4.5: Microcapsules from batch D observed using optical light microscopy.

A likely explanation for the improved quality of microcapsules when PLLA is replaced with PLGA is found in the hydrophilicity of the polymers. PLGA is a more hydrophilic polymer than PLLA, which further facilitates the phase separation between the polymer and core oil. When considering the definition and constraints of the spreading coefficient, the increased hydrophilicity reduces the interfacial tension between the polymer and the water, compensating for the low oil and water tension. This allows the spreading coefficient of the polymer to remain positive, causing core-shell capsules to be formed. It is also worth noting that the amorphous nature of the polymer can ease the formation process compared to the more crystalline PLLA.

The following batch, D, was formulated using 5 weight-% Methylene Blue. In order to determine the morphology, the microcapsules were observed using fluorescence microscopy. The acquired images can be seen in Figure 4.6.

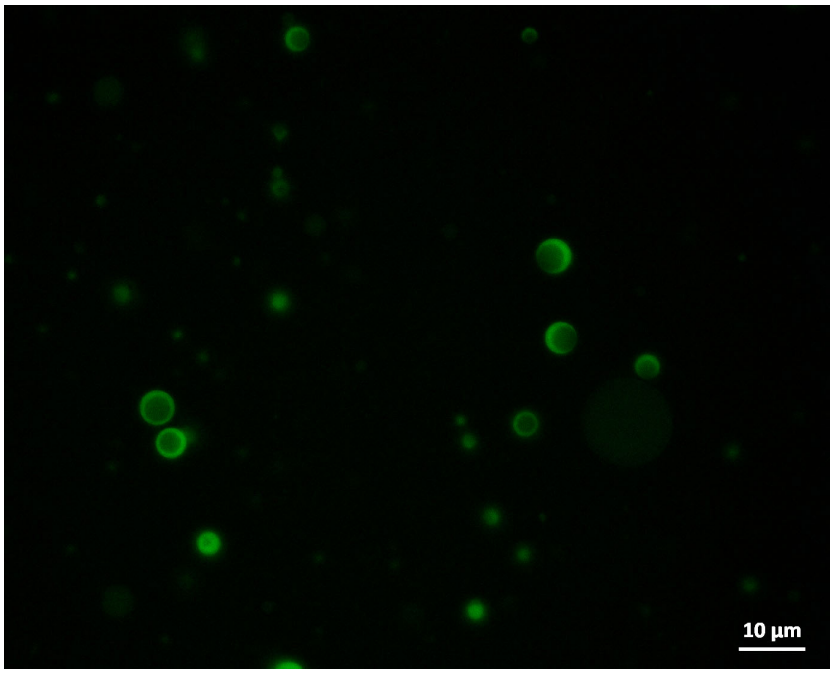


Figure 4.6: Microcapsules from batch E observed using fluorescence microscopy.

It can clearly be observed that the microcapsules have a core-shell morphology by studying the figure. The increased intensity in the polymer shell is due to the distribution of methylene blue between the shell and core regions being shifted to the shell.

Batch F was formulated using a 5:1-ratio between PLGA and ethyl linoleate as well as 10 weight-% methylene blue in proportion to the amount of polymer used. The microcapsules observed using fluorescence microscopy can be seen in Figure 4.7.

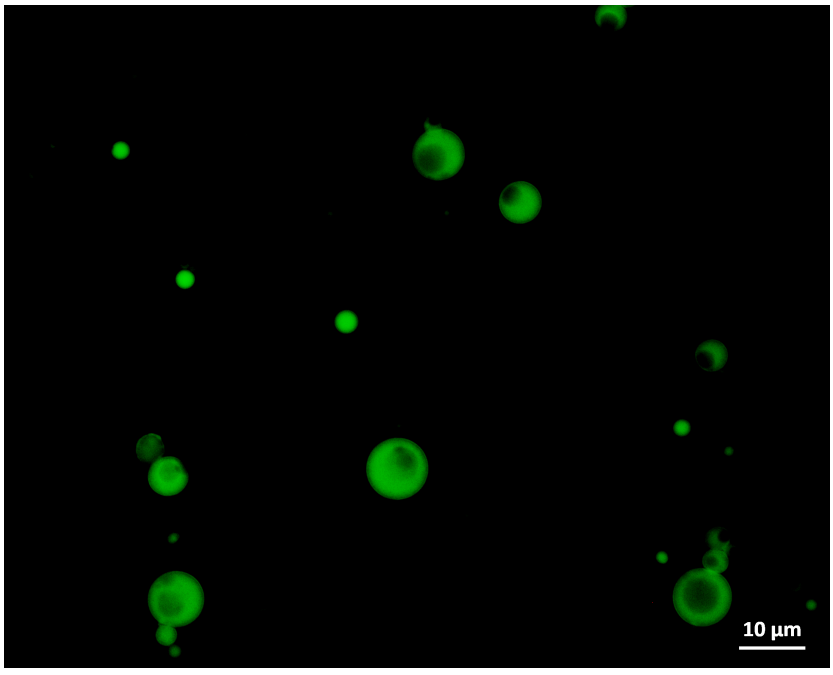


Figure 4.7: Microcapsules from batch F observed using fluorescence microscopy.

Once again core-shell morphology can clearly be observed. As expected, the microcapsules have a noticeably thicker shell when a higher ratio between the PLGA and ethyl linoleate is used. It was also observed that some of microcapsules display a multicore-shell morphology when the amount of oil used in relation to shell material is reduced.

Based on the promising results a batch was formulated using the same strategy with 6 weight-% pyrene in proportion to the amount of ethyl linoleate used. The microcapsules were observed once again using fluorescence microscopy and can be seen in Figure 4.8.

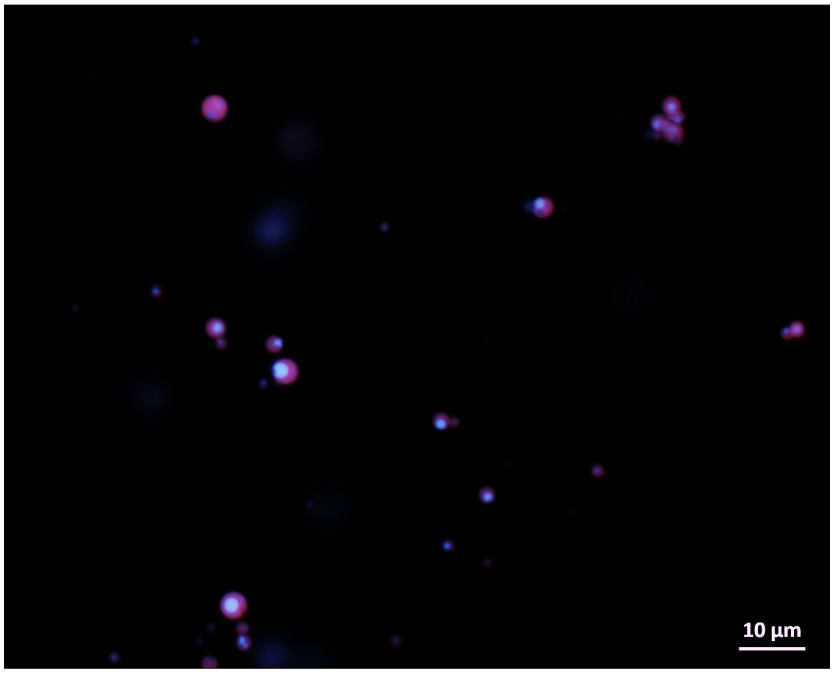


Figure 4.8: Microcapsules from batch G observed using a composite image of two fluorescence filters.

The morphology is especially clearly visualized with the shell due to the affinity of pyrene to the more hydrophobic core and of methylene blue to the hydrophilic polymer shell. The fact that the compounds are fluorescent at different wavelengths allows for the use of different fluorescence filters in order to visualize the morphology in a clear manner.

The final PLGA batch, H, was formulated according to the same procedure as previously, with the methylene blue removed. The produced capsules can be observed in Figure 4.9. It is worth noting that some of the microcapsules display a multicore-shell morphology, this is speculated to be due to the high polymer to oil ratio used.

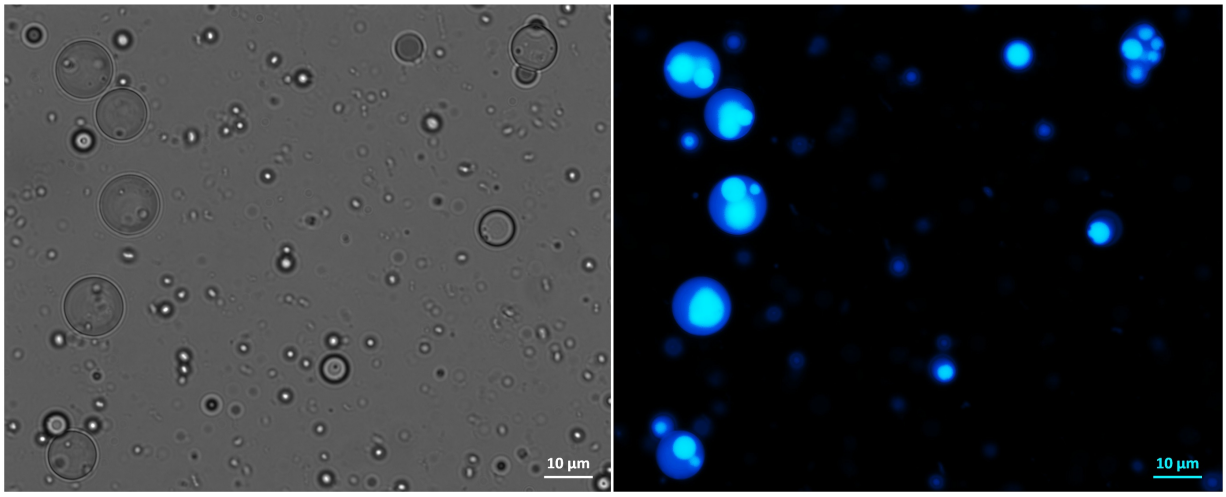


Figure 4.9: Microcapsules from batch H observed using optical light microscopy to the left and by a fluorescence filter to the right.

In batch H core-shell morphology could clearly be observed once again. It is worth noting that the size distribution in the different batches of PLGA microcapsules is roughly the same and varying between 1 - 10 μm. The microcapsules in batch H were observed once again, approximately one week after the formulation, in order to assess the stability of the system. The result can be seen in Figure 4.10.

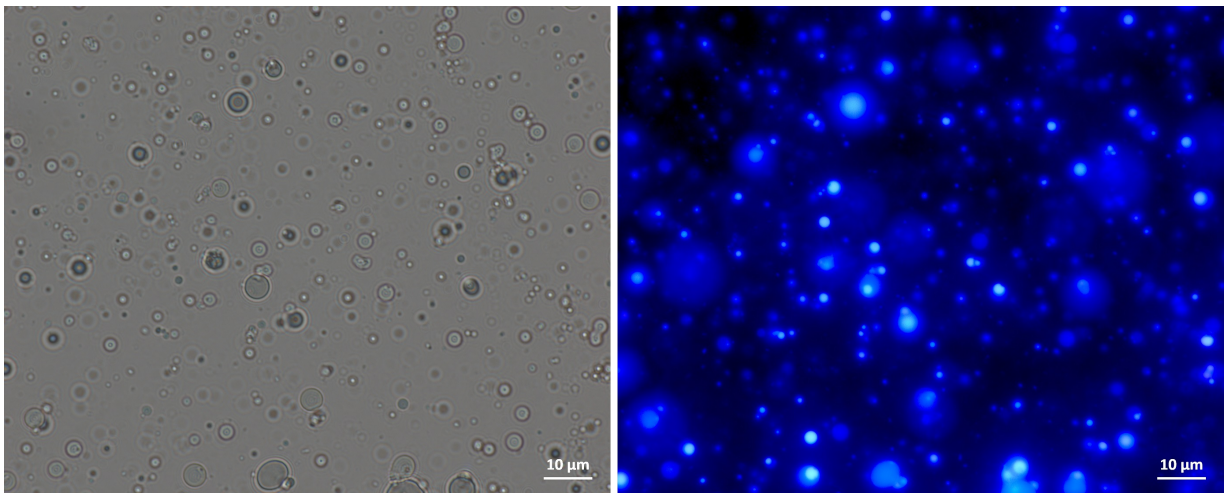


Figure 4.10: Microcapsules from batch H observed one week after Figure 4.9 using optical light microscopy to the left and by a fluorescence filter to the right.

As can be observed, the microcapsules appear to be in a similar condition as when just formulated. The core shell structure can still clearly be seen using the fluorescence filter since the pyrene can still be observed solved in the ethyl linoleate core. This indicates that the PLGA microcapsule system is stable, even when suspended in water. Since PLGA is a more hydrophilic polymer than PLLA, this is to be expected, as the reduced interfacial tension between the polymer and water will compensate for the lower tension between the water and the core oil.

4.1.3 Cellulose triacetate

The microcapsules formulated using cellulose triacetate as the selected shell material are presented in Table 3.3. In the first batch the coloring agents Sudan I and Methylene Blue were used. As can be seen in Figure 4.11, spherical particles were formed, quite uniform in size. When observed using a fluorescence filter, no core-shell structure was visible. It appeared that the coloring agent was uniformly distributed in the particles, suggesting that the particles were homogeneous or that the coloring agents were present in both the core and shell of the microcapsules. Due to the different polarity of the polymer and oil, the first alternative is more likely.

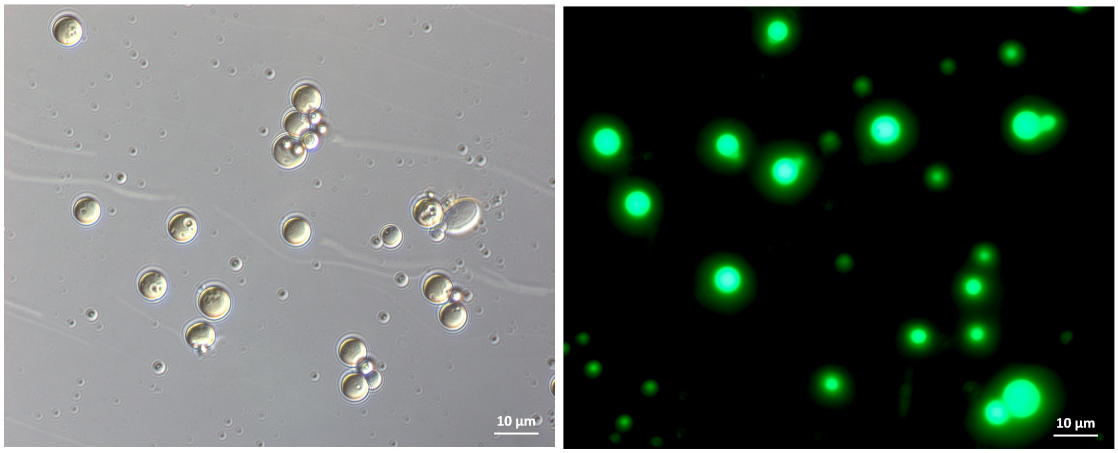


Figure 4.11: Microcapsules from batch J observed using a reflective differential interference contrast, DIC, filter and a fluorescence filter.

However, after approximately a week a change of color was noted in the microcapsule suspension. When microcapsules once again were studied it was observed that the spherical particles were suspended in oil that had leaked out, as shown in Figure 4.12.

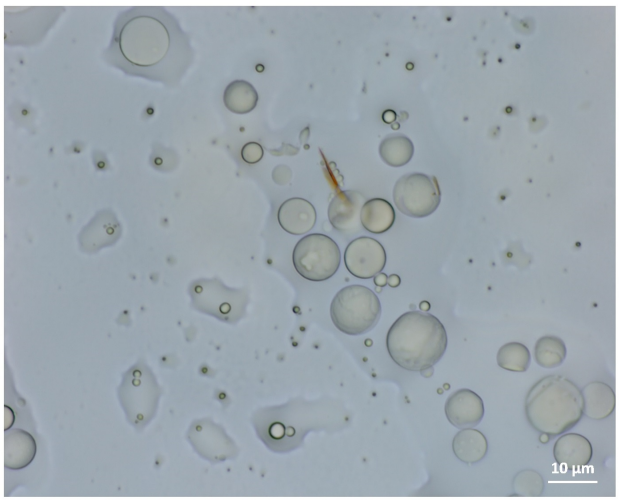


Figure 4.12: Microcapsules from batch J observed a week later using brightfield optical light microscopy. The leaked oil can clearly be seen engulfing the spherical particles.

The leakage of oil from the microcapsules could indicate that the demands on the spreading coefficients are not met when using cellulose triacetate and ethyl linoleate or that the polymer shell is incomplete, similar to when PLLA was used. This would once again cause the microcapsule system to be unstable over time.

In the following batch, K, the Sudan I was removed since it was not possible to determine the morphology of the capsules in batch J. The formulated microcapsules can be observed in Figure 4.13.

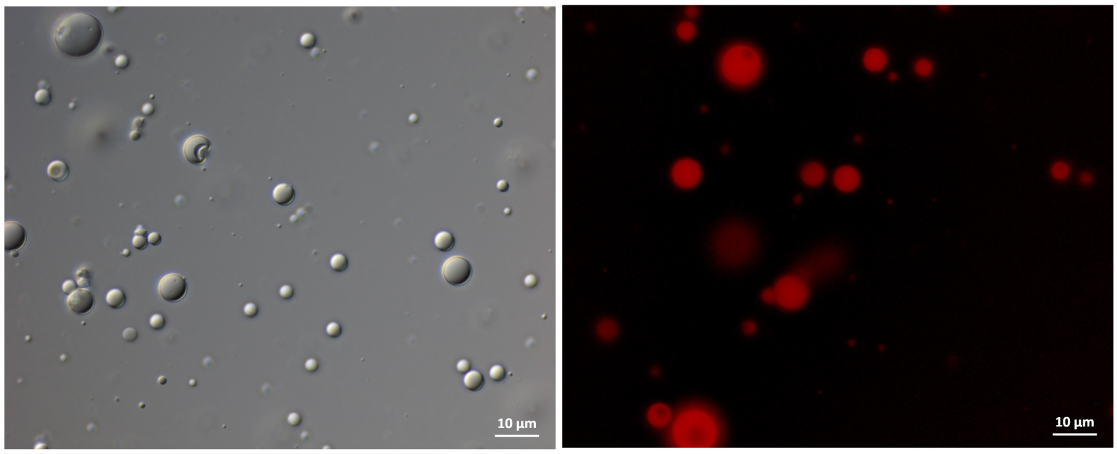


Figure 4.13: Microcapsules from batch K observed using a reflective DIC filter and a fluorescence filter.

Once again, spherical microcapsules are observed. Using the fluorescence filter, some of the microcapsules can be suggested to have a core-shell morphology due to the higher intensity of the fluorescence compound methylene blue in the outer region of the microcapsules.

In order to ease the determination of morphology, the amount of core oil used was reduced in the following batch, L, which can be studied in Figure 4.14.

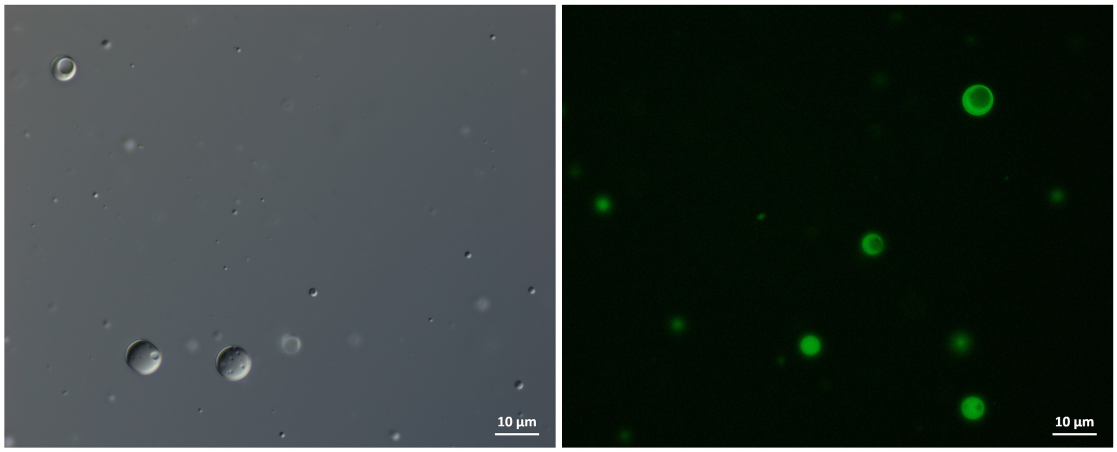


Figure 4.14: Microcapsules from batch L observed using a reflective DIC filter and a fluorescence filter.

Yet again spherical particles are observed. In both batch K and L, the DIC images reveal holes or unevenness on the microcapsule surfaces. As in the PLLA case, this can be explained by the semi-crystalline nature of the polymer [46][47]. Using fluorescence microscopy, some of the capsules appear to have a core-shell morphology. However, it is worth noting that the shell does not appear to be thicker than in the previous batch, despite the increased ratio between the polymer and oil used.

Since there were concerns regarding the stability of the cellulose triacetate microcapsules, batch L was studied once again one week after formulation, which can be seen in Figure 4.15.

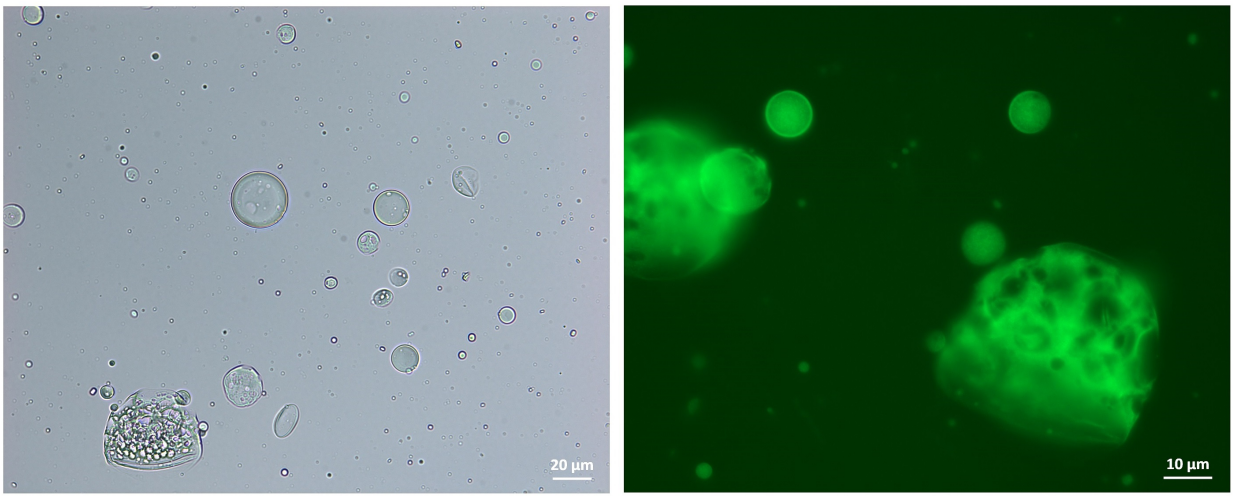


Figure 4.15: Microcapsules from batch L observed a week later using brightfield optical light microscopy.

Just as in the first cellulose triacetate batch, the microcapsules seems to be unstable. In this batch, some of the microcapsules have collapsed into gel-like structures, possibly due to the surrounding water entering the particles. If the some of microcapsules have holes in the surface this is a possible consequence since the water and oil will be in contact with each other. It is possible that this behavior can be explained by semi-crystallinity in the polymer, as is the case with PLLA.

Batch M was formulated in order to study the release of actives form the cellulose triacetate microcapsules. The encapsulation of pyrene can be observed in Figure 4.16.

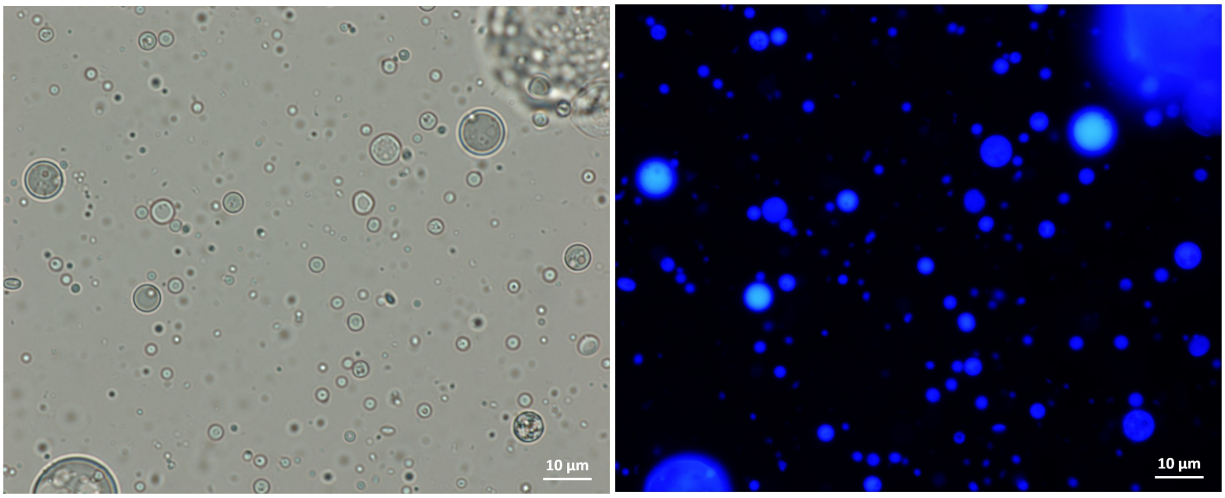


Figure 4.16: Microcapsules from batch M observed using brightfield optical light microscopy to the left and with a fluorescence filter to the right.

By observing fluorescence image in Figure 4.16, it can be noted that the pyrene appears to be evenly distributed throughout the particles.

As previously, the stability of the microcapsules were examined by observing them once again one week after formulation, which can be seen in Figure 4.17.

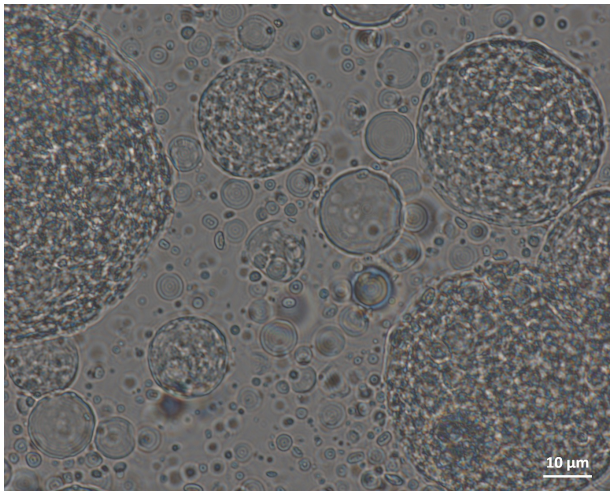


Figure 4.17: Microcapsules from batch M observed one week after Figure 4.16 using brightfield optical light microscopy.

All in all, the cellulose triacetate microcapsules appear to be a challenging system to work with. The system is unstable and the formulated microcapsules tend to aggregate into gel like particles. This is possibly due to holes in the polymer shell, causing the fatty acid ester to be in direct contact with the surrounding medium. Using methylene blue as a fluorophore, core-shell structure can be observed. However, as this result is inconsistent with the batches where pyrene or Sudan I is used, it can possibly be explained by methylene blue's affinity for the surrounding water as well.

4.1.4 Polyanhydride microcapsules

The microcapsules formulated in the first anhydride batch, N, can be observed in Figure 4.18. Spherical microcapsules can be observed that appear to have a smooth surface and roughly the same size distribution as in previous batches.

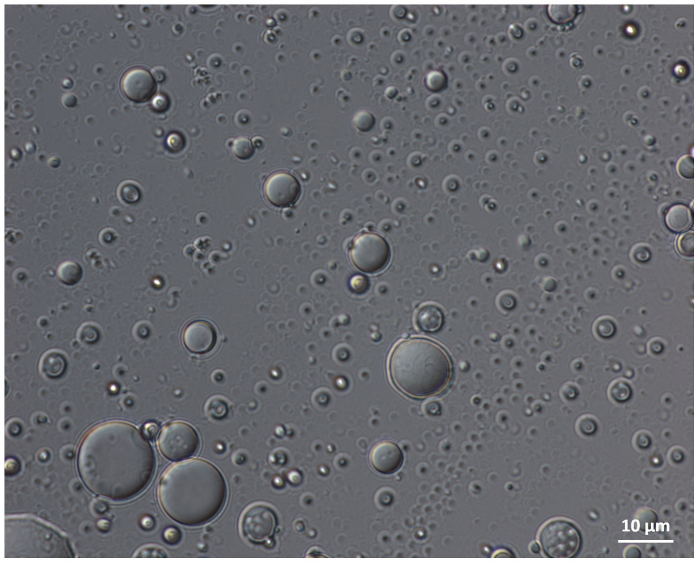


Figure 4.18: Microcapsules from anhydride batch N observed using optical light microscopy with a DIC filter.

Based on this result a second microcapsule batch, O, was formulated using the same amount of polymer and oil, with coloring agents added. The microcapsules produced in this batch can be studied in Figure 4.19.

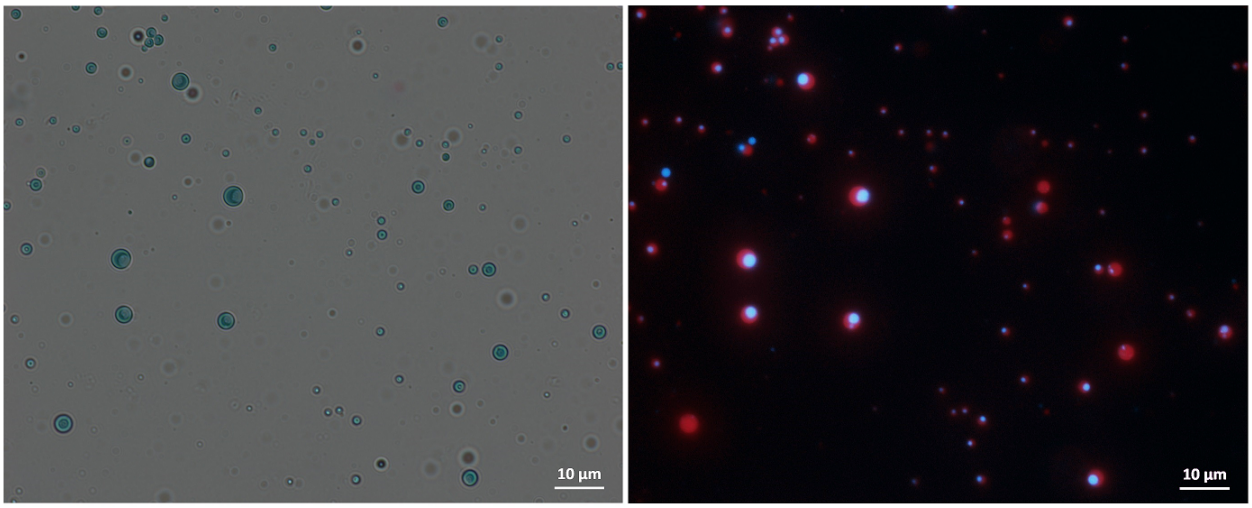


Figure 4.19: Microcapsules from anhydride batch O observed using optical light microscopy to the left and with a composite image of two fluorescence filters to the right.

The core shell morphology can clearly be seen aided by the fluorescence filter. The microcapsules are more clearly blue than in previous batches using the same ratio

between the polymer and methylene blue, indicating a different equilibrium of the distribution of the coloring agent between the polymer and surrounding medium when the polyanhydride is used rather than PLGA. This is most likely due to the more hydrophobic nature of the polyanhydride compared to PLGA.

The final anhydride batch, P, was formulated in a similar fashion as the other batches intended for release studies. The formulated microcapsules can be observed in Figure 4.20.

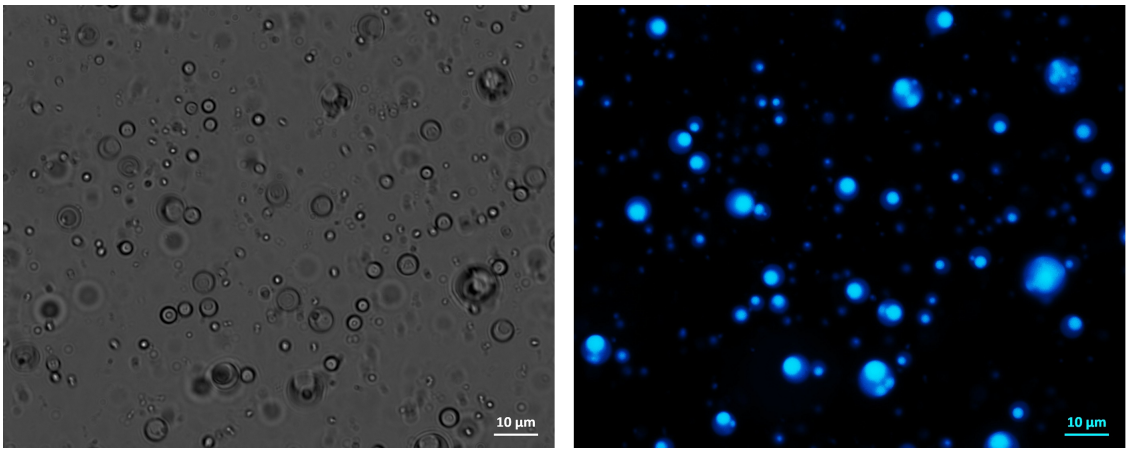


Figure 4.20: Microcapsules from anhydride batch P observed using optical light microscopy to the left and with a fluorescence filter to the right.

As expected, core-shell microcapsules were formed once again. In some of the microcapsules, a multicore morphology can be noted, consistent with previous results when a high ratio between polymer and core oil has been used.

Just as the previous batches intended for release studies, the stability of the microcapsules in the suspension was observed approximately one week later. The microcapsules in batch P one week after formulation can be seen in Figure 4.21.

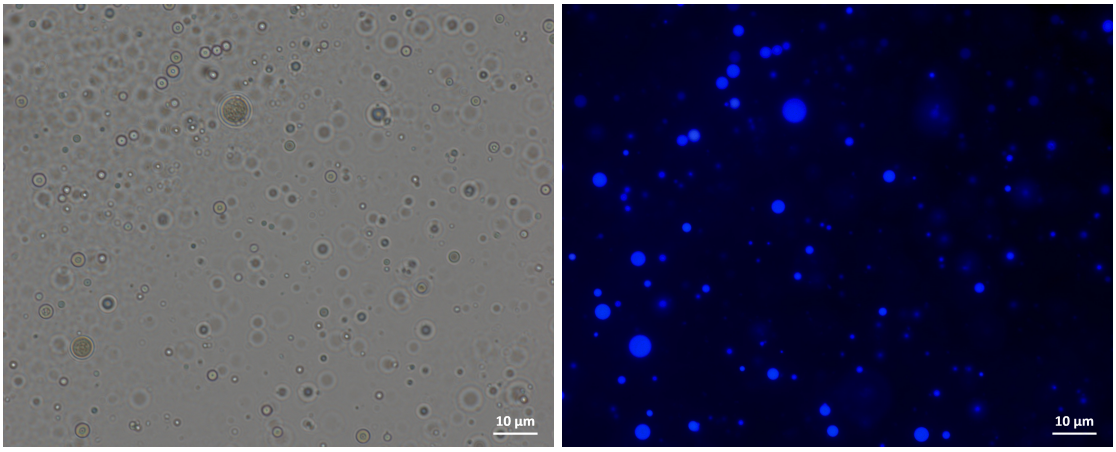


Figure 4.21: Microcapsules from anhydride batch P observed one week after Figure 4.20 using optical light microscopy to the left and with a fluorescence filter to the right.

It can be observed that the microcapsules are still spherical and in the same size distribution. However, using the fluorescence filter it can be noted that the fluorophore appears to be evenly distributed within the microcapsules. This can possibly be attributed to hydrolysis of the polymer shell at the anhydride bonds, causing cavities in the shell that the fatty acid ester containing the fluorophore have filled.

4.1.5 Block-co-polymer as dispersant

When attempting to formulate microcapsules using a surface active co-block-polymer in the disperse phase rather than a surface active polymer in the continuous phase no emulsion was formed. In Figure 4.22 it can be seen how the continuous and disperse phase remain phase separated after attempted emulsification for one hour.

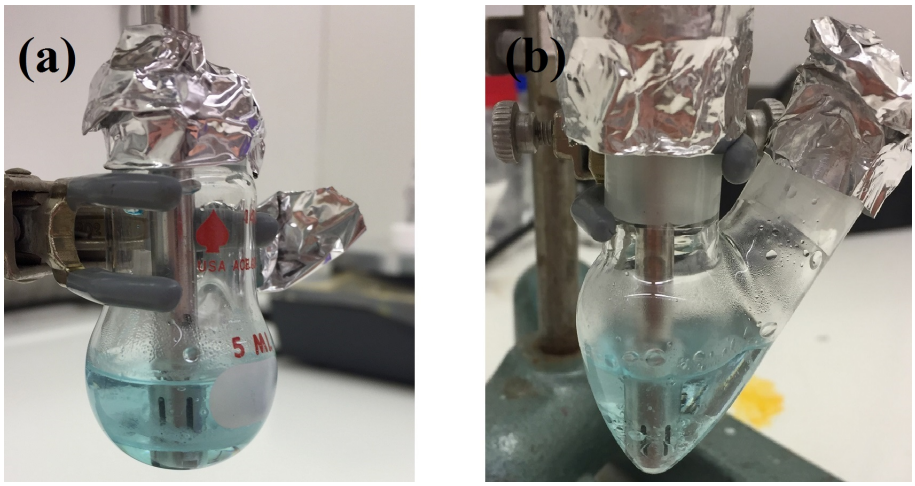


Figure 4.22: Round bottom flask containing phase separated continuous phase and disperse phase following failed emulsification in (a) batch Q and (b) batch R. In both cases it can be observed that the heavier dispersed phase is separated below the aqueous phase.

In batch R the amount of co-block-polymer used was 16.4 weight-% of the amount of shell material used, far above the amount required to cover the surface of the microcapsules. Furthermore, the attempted emulsification was carried out at significantly higher rotational speeds than in previous microcapsules batches. This indicates that replacing the surface active polymer in the continuous phase with a surface active co-block-polymer in the disperse phase might prove futile. It is worth noting that in order to reach the same amount of surface active polymer in the system as when 1 weight-% is used in the continuous phase during the emulsification an amount of PEG-PLA corresponding to roughly 50 weight-% of the PLLA used would be required.

4.2 Release studies

The release of pyrene from the microcapsules in batch C, H, M and P was studied using the method described in the previous chapter. The release profile is illustrated by following the released fraction of pyrene in the release bath over time. In order to obtain the released fraction value for a specific time point, the measured absorbance value was divided by the maximum absorbance. The maximum absorbance was obtained by extracting all of the pyrene out of the microcapsules by resuspending them in methanol.

The obtained released fraction values were plotted against the elapsed time and the obtained release profiles for the studied batches can be found in Figure 4.23.

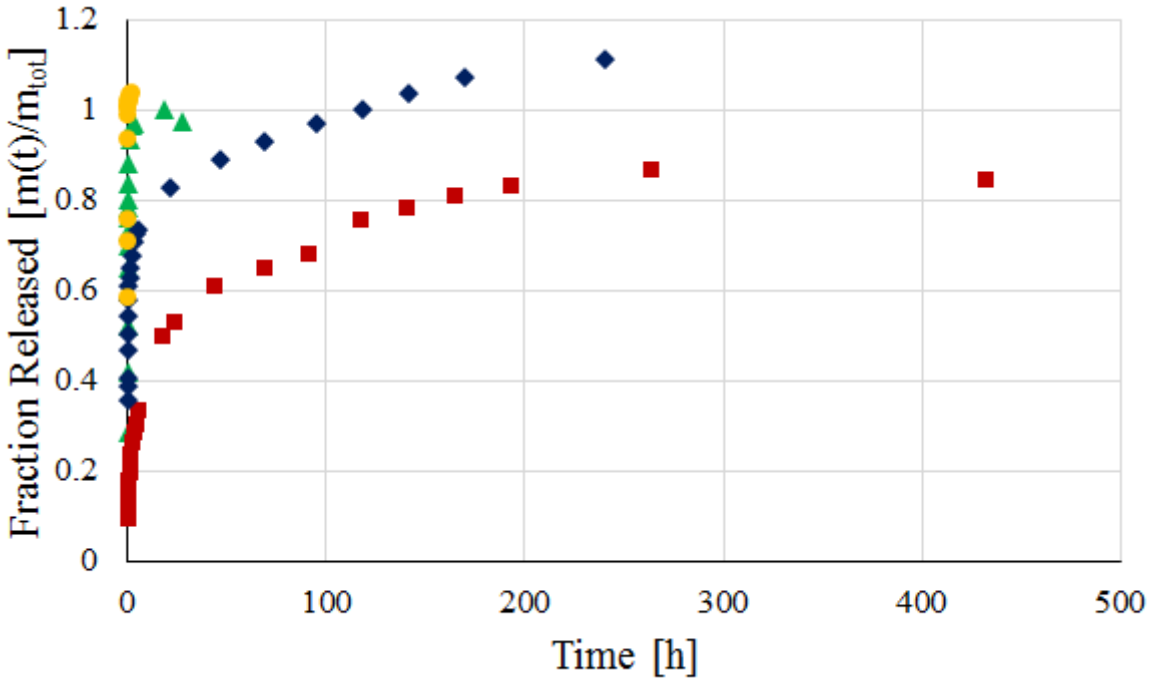


Figure 4.23: The release fraction of pyrene over time from the microcapsule batches C, H, M and P. The yellow data corresponds to the PLLA-batch, the green data to the cellulose triacetate-batch, the blue data to the anhydride-batch, and the red data to the PLGA-batch.

It can immediately be noted that the release from the PLLA and cellulose triacetate microcapsules are far more rapid than from the anhydride and especially the PLGA batches. To ease the comparison of the different profiles, the initial release and release fraction were plotted against the logarithmic value of the elapsed time, as shown in Figure 4.24.

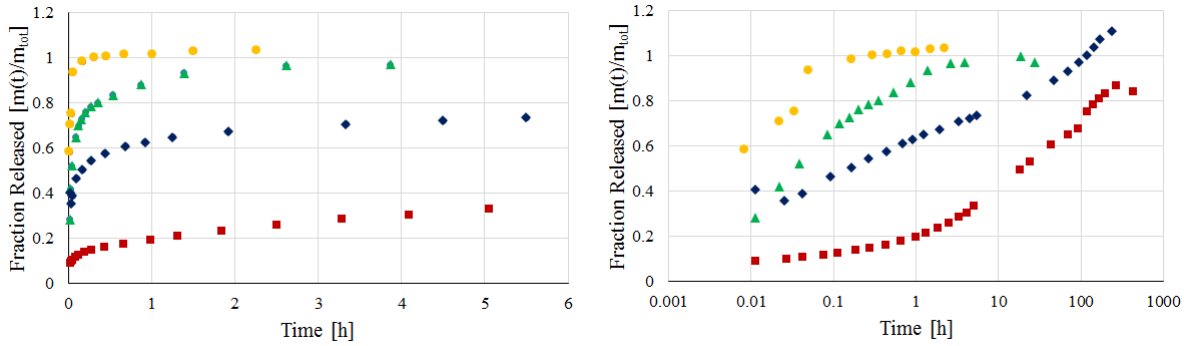


Figure 4.24: The release fraction of pyrene over time from the microcapsule batches C, H, M and P. In the left graph the initial release can be observed, and in the right graph the entire release against the logarithmic value of the elapsed time. The yellow data corresponds to the PLLA-batch, the green data to the cellulose triacetate-batch, the blue data to the anhydride-batch, and the red data to the PLGA-batch.

The release of actives from the PLLA microcapsules in batch C is far more rapid than from the other microcapsule systems. All of the encapsulated pyrene is released within 20 minutes. A possible explanation for this is found in the characteristics of the shell polymer. As discussed when analyzing the formulated microcapsules, incomplete shell formation is a possible consequence that can be said to have occurred due to crystallinity in the polymer. If the shell is incomplete, the release will occur directly from the oil to the surrounding medium, which will be a far faster process since the active compound does not have to diffuse through the polymer shell.

The results from the release study on cellulose triacetate microcapsules are consistent with previous, unpublished results from release studies carried out within the research group on cellulose triacetate microcapsules with a plant oil core. As seen when observing the microcapsules using a fluorescence filter, the capsules appear to be homogeneous particles with the active compound evenly distributed rather than core-shell particles. The obtained release profile further indicates that the system consists of isotropic cellulose triactete and ethyl linoleate gel particles rather than phase separated core-shell particles. This behavior can be explained by the semi-crystalline nature of the polymer. It is also possible that hydrogen bonds have formed between the polymer and the core oil, causing gel-like particles to form rather than core-shell microcapsules.

Regarding the release from the anhydride microcapsules, the release profile is as expected from core-shell microcapsules in which the active is solved inside the core. Worth noting is that the release was not carried out in an acid environment, in which the degradation of the polyanhydride shell would be far more rapid. In the logarithmic scale graph in Figure 4.24, the released fraction of pyrene from the microcapsules exceeds one, which is most likely due to evaporation of water from the release bath, consequently increasing the pyrene concentration.

Finally, the release of pyrene from the PLGA microcapsules in batch H shows the most extended release profile by a significant margin. This confirms that the active ingredient is only found in the core of the microcapsules and that the polymer shell acts as a rate determining barrier. The extended release profile is comparable to the profile found in microcapsules with polyelectrolyte shells, which are some of the most prolonged profiles known [13]. The release appears to have stopped at a fraction release of 0.8, which can be explained by a equilibrium partitioning of pyrene between the microcapsules and the surrounding medium.

Following the release studies of batch H and P, a sample from the release bath was observed using microscopy to determine whether the pyrene could be confirmed to have diffused out of the capsules or not. The PLGA and anhydride microcapsules can be observed in Figure 4.25 and 4.26 respectively.

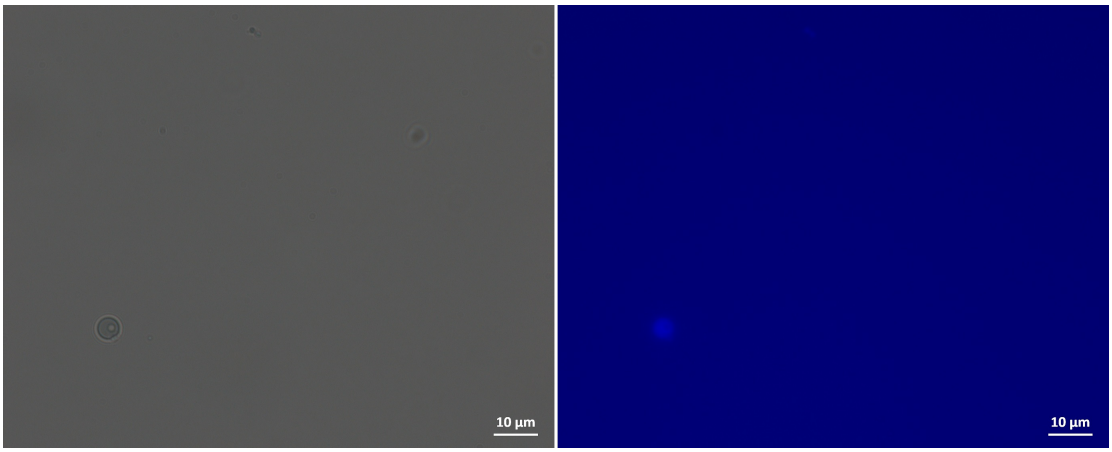


Figure 4.25: Microcapsules from batch H in the release bath after release studies had been carried out, observed using optical light microscopy to the left and by a fluorescence filter to the right.

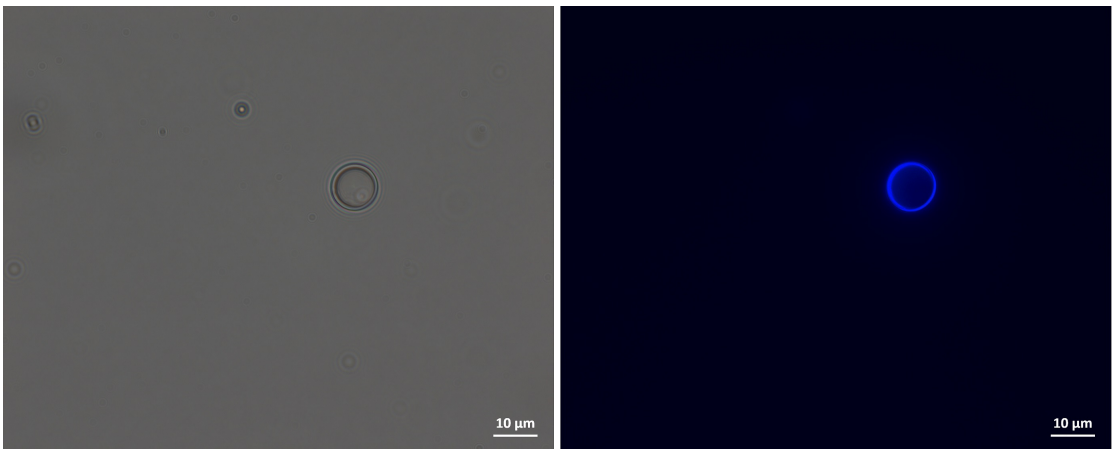


Figure 4.26: Microcapsules from batch P in the release bath after release studies had been carried out, observed using optical light microscopy to the left and by a fluorescence filter to the right.

In both Figure 4.25 and 4.26 it can be observed that the amount of pyrene in the microcapsules drastically had reduced after the release studies had been carried out. In the PLGA batch, the microcapsule is barely distinguishable using fluorescence microscopy, while in the anhydride batch, the pyrene is seen in a higher concentration at the outer shell of the microcapsules.These images further confirm that the encapsulated pyrene indeed has been released from the microcapsules. The differences between the systems can be attributed to the difference between the polymers, the polyanhydride used is more hydrophobic than PLGA and contains aromatic rings, affecting the partitioning of pyrene.

5
Conclusions

From the experiment work carried out in this project several conclusions can be drawn.

When attempting to formulate PLLA microcapsules with a fatty acid ester core challenges were encountered and no stable system was successfully formulated. The release of actives from the system was faster than any of the other system evaluated. A possible explanation for this is found in the properties of the polymer, the semi-crystalline nature of PLLA could have caused the encapsulation of the core oil to be incomplete. For this reason, it is recommended that further studies evaluate the potential of the racemic PLA, which is more amorphous, as a shell material in core-shell microcapsules containing a fatty acid ester core oil. It is also recommended to carry out general studies on how the properties of the polymer affects the encapsulation, as this area is rather unexplored.

The PLGA microcapsule system developed in this project shows great promise in field of extended release of actives. The formulated microcapsules display a clear core-shell morphology and appear to be stable over time. Furthermore, the release from these microcapsules is far more extended than what normally is observed. Future work could study how the composition of the PLGA co-polymer affects the encapsulation and release of actives.

Regarding the use of polyanhydrides as shell material for microcapsules the results obtained in this project are promising. Core-shell microcapsules were successfully formulated which displayed a release profile. Future studies could also include release studies carried out in different media with varying pH, in order to further study the effects of hydrolysis of the polymer. If the release can be triggered by altering the pH of the environment, polyanhydrid microcapsules can be a promising candidate for triggered release of actives.

The cellulose derivative cellulose triacetate was also evaluated as a component of microcapsules. With this polymer a number of challenges were encountered. Firstly, the morphology of the microcapsules could not clearly be said to be of core-shell nature. Rather, it would appear as if they were homogeneous gel particles. However, the results open up the possible for cellulose triacetate microcapsules to be used for burst released triggered by aqueous environments. Furthermore, the particles does not appear to be stable over time, but will rather collapse, possibly due to the entry of water into the particles. Finally, the release of actives from the particles is rapid and difficult to control. It is recommended that future studies evaluate the potential of other cellulose derivatives for microencapsulation, such as the more amorphous ethyl cellulose.

References

(1) Global Report on Diabetes., *World Health Organization*, ISBN 978 92 4 156525 7, 2016.

(2) Egro, F. M. Why is type 1 diabetes increasing? *Journal of Molecular Endocrinology* **2013**, *51*, R1–R13.

(3) Sheehan, J.; Ulchaker, M. M., *Obesity and type 2 diabetes mellitus*; New York Oxford University Press: 2012.

(4) Frykberg, R. G.; Banks, J Challenges in the Treatment of Chronic Wounds. *Advances in Wound Care* **2015**, *4(9)*, 560 –582.

(5) Nikaido, H. Multidrug Resistance in Bacteria. *Annual review of biochemistry* **2009**, *78*, 119 –146.

(6) Antimicrobial resistance., *World Health Organization*, `http://www.who.int/mediacentre/factsheets/fs194/en/`, [cited: 2018-03-02].

(7) Andersson Trojer, M.; Nordstierna, L.; Bergek, J.; Blanck, H.; Holmberg, K.; Nydén, M. Use of microcapsules as controlled release devices for coatings. *Advances in Colloid and Interface Science* **2015**, *222*, 18–43.

(8) Loxley, A.; Vincent, B. Preparation of Poly(methylmethacrylate) Microcapsules with Liquid Cores. *Journal of Colloid and Interface Science* **1998**, *208*, 49 –62.

(9) Moxey, P. W.; Gogalniceanu, P.; Hinchliffe, R. J.; Loftus, I. M.; Jones, K. J.; Thompson, M. M.; Holt, P. J. Lower extremity amputations — a review of global variability in incidence. *Diabetic Medicine* **2011**, *28*, 1144–1153.

(10) GBD Compare Data Visualization., *University of Washington*, `https://vizhub.healthdata.org/gbd-compare/`, [cited: 2018-03-12].

(11) Management of Biofilm., *World Union of Wound Healing Societies (WUWHS)*, `http://www.wuwhs2016.com/files/WUWHS_Biofilms_web.pdf`, [cited: 2018-05-02].

(12) Gullberg, E.; Cao, S.; Berg, O. G.; Ilbäck, C.; Sandegren, L.; Hughes, D.; Andersson, D. I. Selection of Resistant Bacteria at Very Low Antibiotic Concentrations. *PLOS Pathogens* **2011**, *7*, 1–9.

(13) Andersson Trojer, M.; Nordstierna, L.; Nordin, M.; Nyden, M.; Holmberg, K. Encapsulation of actives for sustained release. *Phys. Chem. Chem. Phys.* **2013**, *15*, 17727–17741.

(14) Liu, B.; Wang, Y.; Yang, F.; Wang, X.; Shen, H.; Cui, H.; Wu, D. Construction of a controlled-release delivery system for pesticides using biodegradable PLA-based microcapsules. *Colloids and Surfaces B: Biointerfaces* **2016**, *144*, 38 – 45.

(15) Dubey, R.; Shami, T.; Bhasker Rao, K. Microencapsulation Technology and Applications. **2009**, *59*, 82–95.
(16) Bergek, J., *Evaluation of Biocide Release from Modified Microcapsules*; Doktorsavhandlingar vid Chalmers tekniska högskola. Ny serie, no: 4205; Institutionen för kemi och kemiteknik, Teknisk ytkemi, Chalmers tekniska högskola, 2017.
(17) Mirabedini, S.; Dutil, I.; Farnood, R. Preparation and characterization of ethyl cellulose-based core-shell microcapsules containing plant oils. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **2012**, *394*, 74 –84.
(18) Bhatnagar, D.; Hussain, F. Omega-3 fatty acid ethyl esters (Omacor®) for the treatment of hypertriglyceridemia. *Future Lipidology* **2007**, *2*, Copyright - © 2007 © Future Medicine Ltd; Last updated - 2012-06-29, 263–270.
(19) Calle, D.; Negri, V.; Ballesteros, P.; Cerdán, S. In *Theranostics*, 2015.
(20) Cabarcos, P.; Tabernero, M. J.; Otero, J. L.; Míguez, M.; Bermejo, A. M.; Martello, S.; Giovanni, N. D.; Chiarotti, M. Quantification of fatty acid ethyl esters (FAEE) and ethyl glucuronide (EtG) in meconium for detection of alcohol abuse during pregnancy: Correlation study between both biomarkers. *Journal of Pharmaceutical and Biomedical Analysis* **2014**, *100*, 74 –78.
(21) Niaounakis, M. In *Biopolymers: Applications and Trends*, Niaounakis, M., Ed.; William Andrew Publishing: Oxford, 2015, pp 1 –90.
(22) Niaounakis, M. In *Biopolymers: Applications and Trends*, Niaounakis, M., Ed.; William Andrew Publishing: Oxford, 2015, pp 291 –405.
(23) Garlotta, D. A Literature Review of Poly(Lactic Acid). *Journal of Polymers and the Environment* **2001**, *9*, 63–84.
(24) Xiao, C.-D.; Shen, X.-C.; Tao, L. Modified emulsion solvent evaporation method for fabricating core-shell microspheres. *International Journal of Pharmaceutics* **2013**, *452*, 227 –232.
(25) Garlotta, D. A Literature Review of Poly(Lactic Acid). *Journal of Polymers and the Environment* **2001**, *9*, 63–84.
(26) Makadia, H. K.; Siegel, S. J. Poly Lactic-co-Glycolic Acid (PLGA) as Biodegradable Controlled Drug Delivery Carrier. *Polymers* **2011**, *3*, 1377–1397.
(27) Mao, S.; Shi, Y.; Li, L.; Xu, J.; Schaper, A.; Kissel, T. Effects of process and formulation parameters on characteristics and internal morphology of poly(d,l-lactide-co-glycolide) microspheres formed by the solvent evaporation method. *European Journal of Pharmaceutics and Biopharmaceutics* **2008**, *68*, 214 –223.
(28) In *Compendium of Polymer Terminology and Nomenclature: IUPAC Recommendations 2008*, Jones, R. G., Kahovec, J., Stepto, R., Wilks, E. S., Hess, M., Kitayama, T., Metanomski, W. V., Eds.; The Royal Society of Chemistry: 2009, pp 3–21.
(29) Klemm, D.; Heublein, B.; Fink, H.-P.; Bohn, A. Cellulose: Fascinating Biopolymer and Sustainable Raw Material. *Angewandte Chemie International Edition* **2005**, *44*, 3358–3393.
(30) Zugenmaier, P., *Crystalline Cellulose and Derivatives: Characterization and Structures*; Springer Series in Wood Science; Springer Berlin Heidelberg: 2008.

(31) Fundueanu, G.; Constantin, M.; Esposito, E.; Cortesi, R.; Nastruzzi, C.; Menegatti, E. Cellulose acetate butyrate microcapsules containing dextran ion-exchange resins as self-propelled drug release system. *Biomaterials* **2005**, *26*, 4337 – 4347.

(32) Howard, P.; Parikh, R. S. Solution properties of cellulose triacetate. II. Solubility and viscosity studies. *Journal of Polymer Science Part A-1: Polymer Chemistry* **1968**, *6*, 537–546.

(33) Albertsson, A.-C.; Eklund, M. Influence of molecular structure on the degradation mechanism of degradable polymers: In vitro degradation of poly(trimethylene carbonate), poly(trimethylene carbonate-co-caprolactone), and poly(adipic anhydride). *Journal of Applied Polymer Science* **1995**, *57*, 87–103.

(34) Tamada, J.; Langer, R. The development of polyanhydrides for drug delivery applications. *Journal of Biomaterials Science, Polymer Edition* **1992**, *3*, PMID: 1350734, 315–353.

(35) Bis–(2–carboxyphenyl) Adipate Polyanhydride., *Polymer Source Inc*, `https://polymersource.com/dataSheet/P20098A-SASanhydride.pdf`, [cited: 2018-05-03].

(36) Berkland, C.; Kipper, M. J.; Narasimhan, B.; Kim, K. K.; Pack, D. W. Microsphere size, precipitation kinetics and drug distribution control drug release from biodegradable polyanhydride microspheres. *Journal of Controlled Release* **2004**, *94*, 129 –141.

(37) Andersson Trojer, M.; Li, Y.; Abrahamsson, C.; Mohamed, A.; Eastoe, J.; Holmberg, K.; Nyden, M. Charged microcapsules for controlled release of hydrophobic actives. Part I: encapsulation methodology and interfacial properties. *Soft Matter* **2013**, *9*, 1468–1477.

(38) Trojer, M. A.; Mohamed, A.; Eastoe, J. A highly hydrophobic anionic surfactant at oil–water, water–polymer and oil–polymer interfaces: Implications for spreading coefficients, polymer interactions and microencapsulation via internal phase separation. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **2013**, *436*, 1048 –1059.

(39) Pisani, E.; Ringard, C.; Nicolas, V.; Raphael, E.; Rosilio, V.; Moine, L.; Fattal, E.; Tsapis, N. Tuning microcapsules surface morphology using blends of homo- and copolymers of PLGA and PLGA-PEG. *Soft Matter* **2009**, *5*, 3054–3060.

(40) Wan, X.; Xu, J.; Xie, X.-M.; Guo, B.-H. Revealing formation process of microcapsules during in situ polymerization via confocal laser scanning fluorescence microscopy. *Colloid and Polymer Science* **2011**, *289*, 1719–1728.

(41) Piñeiro, L.; Novo, M.; Al-Soufi, W. Fluorescence emission of pyrene in surfactant solutions. *Advances in Colloid and Interface Science* **2015**, *215*, 1 – 12.

(42) Fu, J.; Cai, Z.; Gong, Y.; O'Reilly, S.; Hao, X.; Zhao, D. A new technique for determining critical micelle concentrations of surfactants and oil dispersants via UV absorbance of pyrene. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **2015**, *484*, 1 –8.

(43) Rafatullah, M.; Sulaiman, O.; Hashim, R.; Ahmad, A. Adsorption of methylene blue on low-cost adsorbents: A review. *Journal of Hazardous Materials* **2010**, *177*, 70 –80.

(44) Abraham, M. H.; Amin, M.; Zissimos, A. M. The lipophilicity of Sudan I and its tautomeric forms. *Phys. Chem. Chem. Phys.* **2002**, *4*, 5748–5752.
(45) Albertsson, A.-C.; Carlfors, J.; Sturesson, C. Preparation and characterisation of poly(adipic anhydride) microspheres for ocular drug delivery. *Journal of Applied Polymer Science* **1996**, *62*, 695–705.
(46) Roche, E.; Chanzy, H.; Boudeulle, M.; Marchessault, R. H.; Sundararajan, P. Three-Dimensional Crystalline Structure of Cellulose Triacetate II. *Macromolecules* **1978**, *11*, 86–94.
(47) Wolf, R. M.; Francotte, E.; Glasser, L.; Simon, I.; Scheraga, H. A. Computation of low-energy crystalline arrangements of cellulose triacetate. *Macromolecules* **1992**, *25*, 709–720.
(48) Koburger, T.; Hübner, N.-O.; Braun, M.; Siebert, J.; Kramer, A. Standardized comparison of antiseptic efficacy of triclosan, PVP–iodine, octenidine dihydrochloride, polyhexanide and chlorhexidine digluconate. *Journal of Antimicrobial Chemotherapy* **2010**, *65*, 1712–1719.
(49) Tezvergil-Mutluay, A.; Mutluay, M. M.; Gu, L.-s.; Zhang, K.; Agee, K. A.; Carvalho, R. M.; Manso, A.; Carrilho, M.; Tay, F. R.; Breschi, L.; Suh, B.-I.; Pashley, D. H. The anti-MMP activity of benzalkonium chloride. *Journal of dentistry* **2011**, *39*, 57–64.
(50) Sreenivasan, P.; Haraszthy, V.; Zambon, J. Antimicrobial efficacy of $0 \cdot 05$ % cetylpyridinium chloride mouthrinses. *Letters in Applied Microbiology* **2013**, *56*, 14–20.

A

Appendix I - Solubility of coloring agents and active ingredients in ethyl linoleate

In order to use a coloring agent to visualize the core in microcapsules or to store an active ingredient it is required to know the solubility of the compound in the core oil. In the project several coloring agents and active ingredients are of interest. The candidates for coloring agents to be used are Methylene Blue, Sudan I, β-carotene, Pyrene, Lycopene and Butylated hydroxytoluene, BHT.

The solubility was determined by attempting to solve a certain weight-% of the coloring agent in ethyl linoleate, beginning with 1 weight-%. Whether the compound would dissolve or not was noted and if it did, a higher concentration was attempted. If 1 weight-% would not dissolve, no lower concentration was attempted as the compound was deemed not useful in the project. The results are presented in Table A.1.

Table A.1: Determined intervals for the solubility point of coloring agents in ethyl linoleate.

Compound	Soluble	Not soluble
Methylene Blue	<1 wt%	1 wt%
Sudan I	2.4 wt%	4 wt%
β-carotene	<1 wt%	1 wt%
Pyrene	8 wt%	10 wt%
Lycopene	<1 wt%	1 wt%
BHT	15.5 wt%	>15.5 wt%

The solubility of Pyrene and BHT is by far higher than for the other compounds. This can be speculated to be due to the higher degree of aromaticity in BHT and pyrene compared to the other studied compounds.

The active ingredients studied are the quaternary ammonium compounds, QACs, octenidine dihydrochloride, benzalkonium chloride and hexadecylpyridinium, which are known to have antimicrobial properties [48][49][50]. Initially, however, the solubility of the surface active agent hexadecyl-trimethyl-ammonium bromide, CTAB, was studied due to its chemical similarity to the antimicrobial compounds of interest.

To determine the solubility of the active ingredients the same procedure as when determining the solubiltiy of coloring agents was carried out. The results are presented in Table A.2.

Table A.2: Determined intervals for the solubility point of active ingredients in Ethyl linoleate.

Compound	Soluble	Not soluble
CTAB	<1 wt%	1 wt%
Octenidine	<1 wt%	1 wt %
Benzalkonium	<1 wt%	1 wt %
Hexadecylpyridinium	<1 wt%	1 wt%

CTAB is virtually not soluble in ethyl linoleate, incidiating that the fatty acid ester is a poor solvent for salts. Similar results were also observed for the studied QACs, further indiciating that the fatty acid ester is indeed a poor solvent for salts. Consequently, if ethyl linoleate, or similar compounds, are to be used as core oils in core-shell microcapsules the compounds to be encapsulated are limited to highly hydrophobic or aromatic compounds such as pyrene or BHT. QACs, or other salts, would then have to be converted into their corresponding acids in order to possibly be used.

B

Appendix II - Pyrene calibration curve

To be able to carry out release studies of pyrene a calibration curve was to be prepared first. The calibration curve was prepared by formulating solutions with varying pyrene concentration in 6% Brij L23. The absorbance of the UV-active pyrene was measured using a *UV-Spectrophotometer HP8453* and within the region of absorbance values between approximately 0.05 and 1.5 the relationship between the absorbance and the concentration is linear [16]. This allows for determination of the concentration of a UV active compound by measuring the corresponding absorbance and using the Beer-Lambert law, Equation 3.1. The absorbance of pyrene was measured at 240 or 272 nm [42].

The prepared samples with varying pyrene concentration are presented in Table B.1.

Table B.1: List of the pyrene solutions prepared and their corresponding absorbance at 242 and 274 nm respectively.

Pyrene concentration [mg/l]	Absorbance at 242 nm	Absorbance at 274 nm
0.05	0.00456	-0.008
0.2	0.15089	0.10186
0.5	0.32968	0.27568
1	0.40432	0.27091
2.5	1.11766	0.87073
5	1.5151	0.92647

By plotting the absorbance against the concentration and using linear regression, the k-value can be obtained as the slope of the curve. The data selected for the calibration curve was the obtained values between 0.2 and 2.5 mg/l, since the values below and above seems to be outside the linear region. The calibration curves obtained from the selected data can be observed in Figure B.1.

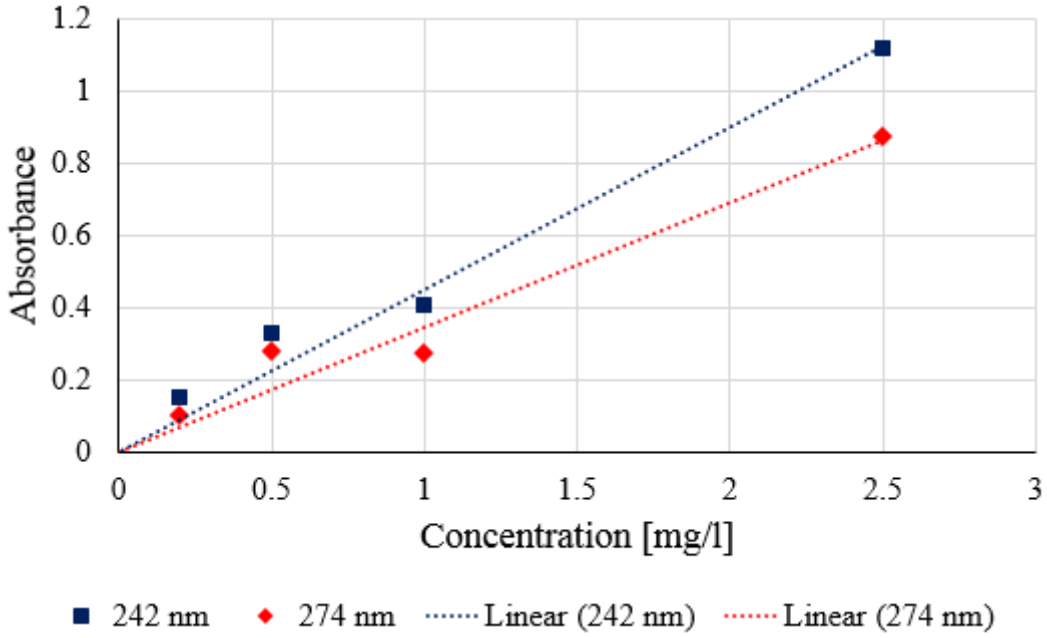


Figure B.1: Obtained absorbance values at 242 and 274 nm, of varying pyrene concentration between 0.2 and 2.5 mg/l in 6% Brij L23 solution.

Using linear regression on the data, a k-value of 0.4501 l/mg is obtained for the signal at 242 nm and 0.3456 l/mg for the signal at 274 nm. The R^2-values for the linear models are 0.969 and 0.9493 respectively.