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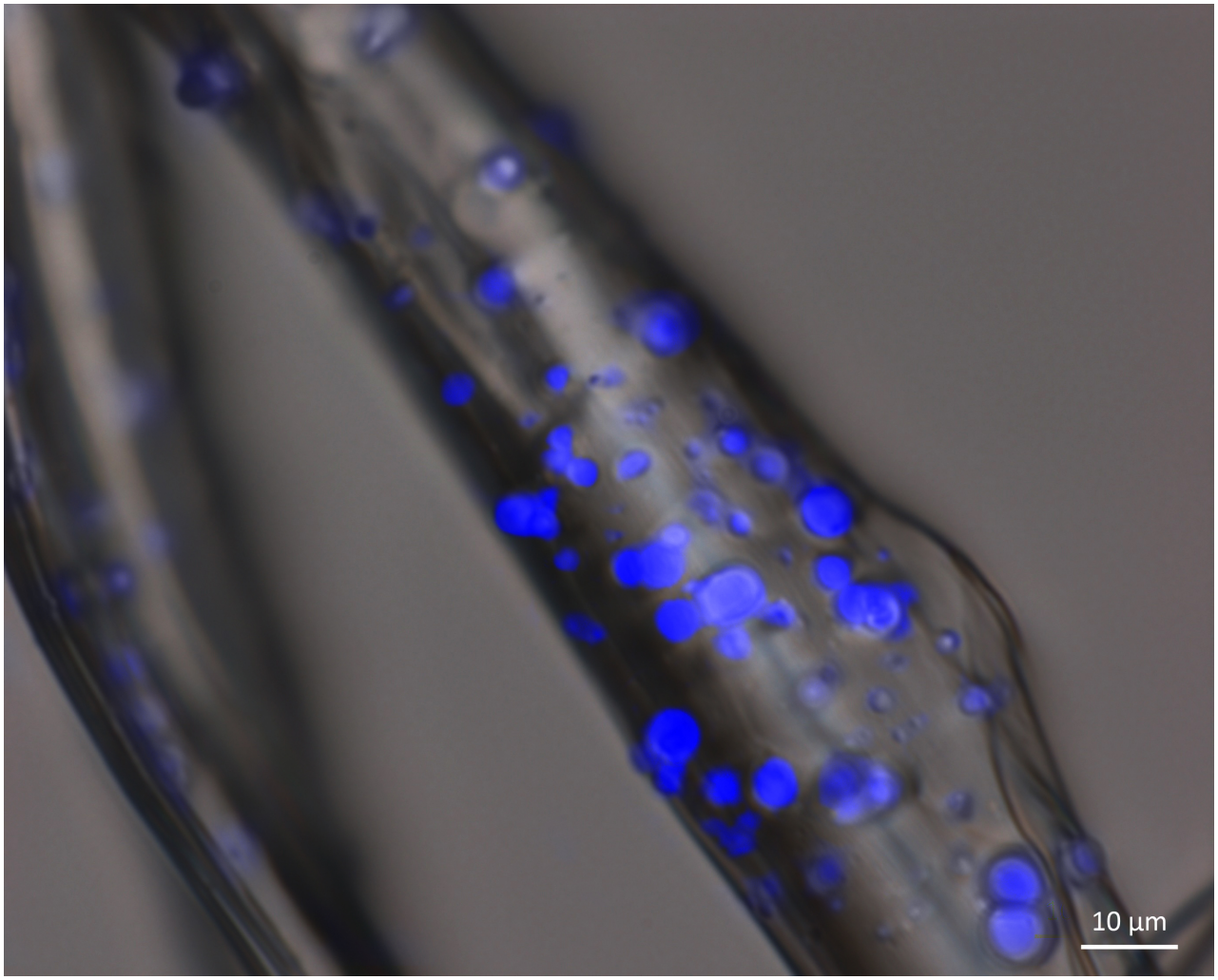

Antiseptic microspheres embedded in nonwoven fiber materials

Master's Thesis in Materials Chemistry

Petrus Jakobsen

Department of Chemistry and Chemical Engineering
CHALMERS UNIVERSITY OF TECHNOLOGY
Gothenburg, Sweden 2019

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Cover: Microscopy fluorescence picture of cellulose fibers with embedded spheres. Spheres contain the fluorescent molecule pyrene.

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Abstract

A challenge facing the medical sector is the treatment of non-healing chronic wounds and the threat of multidrug resistant bacteria strains. A potential solution to these challenges would be to create wound care bandages with better durability and reduced risk of cultivating multidrug resistant bacteria strains. In this thesis, a solution is proposed by incorporating biocompatible polymer microspheres containing the antiseptic agents octenidine dihydrochloride (OCT) and benzalkonium chloride (BAC) in a nonwoven cellulose material. The spheres were formulated using the solvent evaporation method, where the influence of emulsifier and homogenization on the sphere stability and size were investigated. It was found that a higher concentration of emulsifier counteracted aggregation of the spheres and that stirring for a short time gave the largest spheres. The mainly used polymers were poly(lactic-co-glycolic acid) (PLGA), poly(L-lactic acid) (PLLA) and bis–(2–carboxyphenyl) adipate polyanhydride (SASanhydride). The influence of the active substance on the interfacial tension was investigated with optical tensiometry by measuring the interfacial tension of dichloromethane(DCM) droplets with different concentrations of active substance to a water phase. Both OCT and BAC are surface active agents and had a considerable effect on the interfacial tension between the DCM droplets and the surrounding aqueous medium. The reduced interfacial tension due to the surface activity of BAC and in particular OCT resulted in smaller spheres. The formulated microspheres with OCT were subsequently incorporated into a nonwoven fiber material using solution-blow spinning. In this process, a solution was created containing an ionic liquid and microcrystalline cellulose where the spheres containing the active substance were dispersed. The ionic liquid, in this case, 1-ethyl-3-methylimidazolium acetate(EmimAc) was used to dissolve the cellulose. To determine the amount of active substance required in the nonwoven fiber to achieve an antimicrobial effect, the minimal inhibitory concentration (MIC) was used. At MIC the bacteria population in the wound is contained. With the proposed fabrication process, the incorporated amount of BAC in the fiber is assumed to lead to a concentration below the MIC while the concentration may be high enough for fibers with OCT containing spheres. To increase the BAC content in the fiber, a possible solution could be impregnation with BAC combined with microspheres containing BAC. A distribution coefficient, $K_{OCT} = 6.23$ and $K_{BAC} = 2.86$ for the active substances was determined with respect to the distribution between the polymer and water phase. The result indicated that there was a risk of leakage of active substance from the polymer spheres to the water phases in the emulsion and particularly in the coagulation and wash bath used in the solution-blow spinning. Solubility tests for OCT were done where it was found to have a higher solubility then values found in the literature.

Keywords: PLGA, PLLA, SASanhydride, microspheres, solution-blow spinning, solvent evaporation, emulsion, interfacial tension, distribution coefficient, octenidine dihydrochloride, benzalkonium chloride.

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Petrus Jakobsen, Gothenburg, May 2019

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1

Introduction

A challenge facing the medical sector is the treatment of non-healing chronic wounds and the threat of multidrug resistant bacteria strains [1][2]. Some of the population groups with increased risk to be affected by non-healing chronic wounds are elderly, obese and diabetes patients[3][4][5]. These groups are increasing in number and thereby the number of cases of non-healing chronic wounds. One of the reasons that diabetes patients have an increased risk for chronic non-healing wounds is flaws in blood supply which is an important part of the immune system[6]. The increase of infections could, in the end, lead to amputation of limps[6]. To treat and prevent infections in the wound, antiseptics are used. While it is important to give proper treatment, the overuse and misuse of antibiotics worldwide is the reason for the increase of multidrug resistant bacteria strains[2]. The current method for antiseptic used in bandage is to impregnate a nonwoven material with antiseptics. When the bandage is placed on the wound it gives a short-lived overdose of antiseptics but the antiseptic effect decreases rapidly. When the concentration of antiseptics is decreasing under a certain level called minimum inhibitory concentration (MIC) the risk for evolution of multidrug resistant bacteria strains is increased. The reason for this is that when the concentration drops the environment in the wound will evolutionary benefit resistant mutants[1]. The concentration is not high enough to kill the bacteria but the bacteria that are immune to the antiseptics will have favorable growing conditions, consequently multiplying and spreading the mutant gene. Figure 1.1 illustrates the concentration profile of antiseptics in the wound.

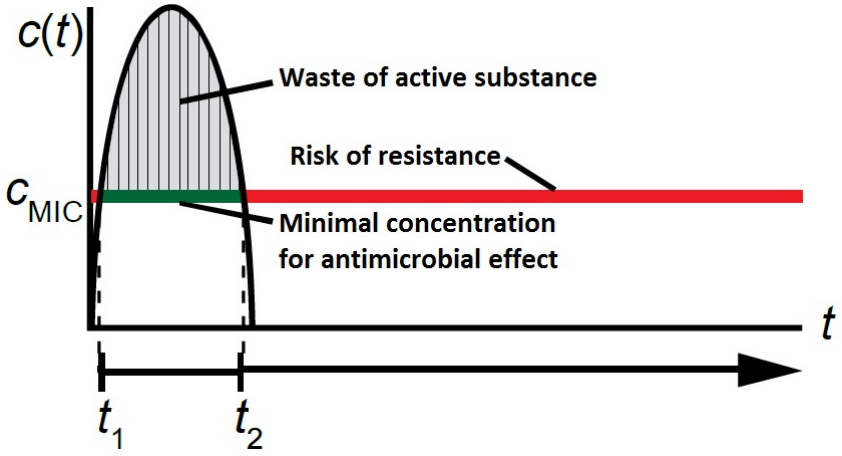

Figure 1.1: The concentration profile of antiseptic in the wound with currently used bandages. Between t_1 and t_2 there is a short-lived overdose of antiseptics. After this the concentration drops below MIC, increasing the risk of evolution of multidrug resistant bacteria strains.

A controlled release of the antiseptics that maintain the concentration in the wound just above MIC may, therefore, reduce the chemical waste as well as the risk for resistance development. Patients usually have to change the bandage once or a couple of times a week which is a cost driver, and discomfort for the patients. The frequency of bandage change is a tradeoff between costs, patient comfort, and the evolution of multidrug resistant bacteria strains.

1.1 Aim

The problem with the dosage can be improved with a modified drug delivery system. Some of the desired properties of a drug delivery system are to deliver the right amount to the right place at the right time[7]. Polymer microspheres loaded with an active substance is a commonly used drug delivery system[8]. By embedding an active substance in a sphere matrix, the release rate is reduced. Consequently by embedding microspheres into bandages a slower and controlled release of an active substance in the wound could be achieved as seen in Figure 1.2. The long-term goal of this project is to produce a nonwoven material with good antiseptic properties that could be implemented in bandages used for the treatment of non-healing chronic wounds. The specific aim of this Master thesis is to develop methods for encapsulation of BAC and OCT in different types of microspheres and to incorporate them into cellulose nonwoven materials. This would give a slower release of the active agent over a longer period, which would prolong the lifetime of the bandage, see Figure 1.2. Some main improvements with this would be the reduced frequency of bandage replacements, reducing costs and discomfort for patients. Another main benefit is the decreased risk for the development of multidrug resistant bacteria strains.

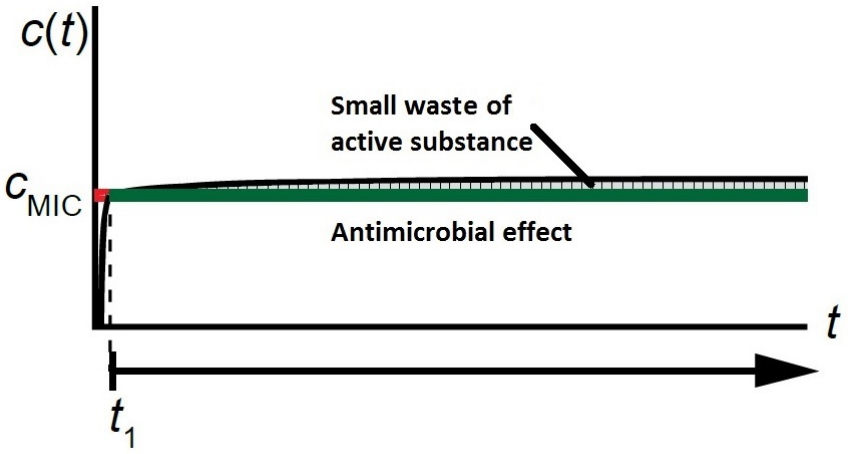

Figure 1.2: The desired concentration profile of antiseptic into the wound with a bandage with embedded microspheres containing antiseptics. Reduced waste of active substance and reduced risk of evolution of multidrug resistant bacteria strains compared to currently used bandages.

1.2 Limitations

The release process of active agent with time and its anti-microbial effect will not be studied. Other active substances then the chosen OCT and BAC will not be investigated. The overall structure and function of bandages will not be taken into consideration. If the fiber with embedded microspheres can be applied in bandages will not be considered.

1.3 Specification of the research questions

To reach the goals of this project, the following questions have to be answered.

- Is it possible to create microspheres with a sufficient amount of active agent benzalkonium chloride and octenidine dihydrochloride?
- What is the optimal formulation route?
- What polymer shows the most promising properties to constitute the microspheres?
- Are the spheres stable and with a uniform and spherical shape?
- Is it possible to incorporate the spheres in a nonwoven material with solution-blow spinning?

2
Theory

A nonwoven fiber material with spheres containing antiseptics is a system with different parts and components. The antiseptics used are benzalkonium chloride (BAC) and octenidine dihydrochloride (OCT). The antiseptics are encapsulated in polymer spheres using the solvent evaporation method. The drug loaded sphere is then incorporated into the nonwoven cellulose fiber material, using solution-blow spinning.

2.1 Antiseptic compounds

By the choice of the two active agents BAC and OCT, a broader range of antiseptic properties can be achieved compared to only one of them.

2.1.1 Benzalkonium chloride

Benzalkonium chloride (BAC) is an antibacterial quaternary ammonium compound. It is cationic and interacts with the negatively charged bacterial membrane[9]. It has the properties of a surfactant with a hydrophobic head and a hydrophobic tail[10]. BAC has a critical micelle concentration, CMC at $8.8 * 10^{-3} M$[11]. BAC has a good water solubility with above 10mg/ml and an octanol/water partition coefficient logP of between 0.59 to 2.97 depending on the length of the tail, n=12 to n=16[12][13]. Figure 2.1 shows the chemical structure of BAC.

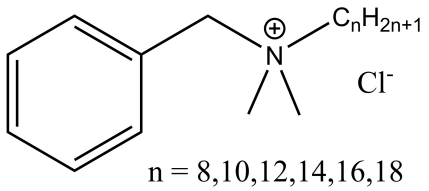

Figure 2.1: The chemical structure of benzalkonium chloride

2.1.2 Octenidine dihydrochloride

Octenidine dihydrochloride (OCT) is a bispyridinamine used for its antiseptic properties. It binds to the negatively charged sites of the bacteria membrane due to its cationic nature, with displayed antimicrobial effect against both gram-positive/negative bacteria[14][15] and fungi[16]. The reported physicochemical properties of this compound are contradictory with a water solubility of $2.6 * 10^{-8} g/ml$[17] and a CMC of

$3.79 * 10^{-3} M$[15]. A partition coefficient logP of 8.23 is reported[17]. It is stable in a pH range from 1.6 to 12.2 and is not sensitive to light[16]. The chemical structure of OCT can be seen in Figure 2.2.

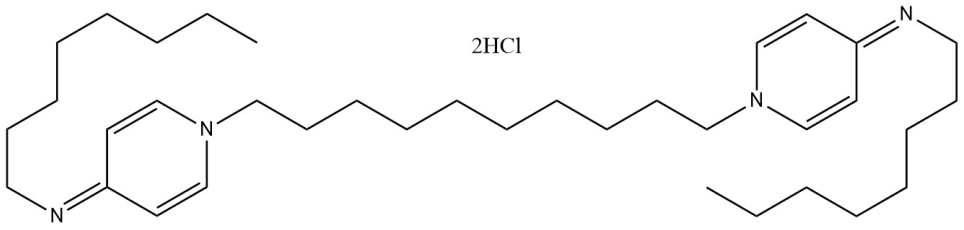

Figure 2.2: The chemical structure of octenidine dihydrochloride

2.2 Encapsulation with the emulsification/solvent evaporation method

Vincent and Loxley (1998) were the first to create liquid-core/solid-shell particles with the emulsification/solvent evaporation method, usually called the internal phase separation method[18]. This method is also a common method to produce micro/nanospheres[19]. The only difference is the absence of a liquid-core in the micro/nanospheres and the absence of internal phase separation in the oil droplets during the solvent evaporation. A modified version of the method can be described and performed in two steps to create micro/nanospheres. (i) An oil-in-water emulsion is created by stirring. The size of the emulsion droplets depends on the stirring rate. The water phase contains emulsifiers to stabilize the emulsion droplet. The oil phase contains a water-insoluble polymer and possibly a water-soluble co-solvent which decreases the droplet size and narrows the size distribution. (ii) The organic solvent in the oil phase has some solubility in the water phase and will, therefore, start do diffuse into the water phase. The organic solvent will evaporate from the emulsion solution surface. The evaporation speed can be accelerated by a lower pressure or higher temperature. Left in the oil phase is the water-insoluble polymer and possibly an active substance. The polymer will solidify and create nano/micro polymer particles with an active substance within its structure.

2.2.1 Sphere polymers

Different polymers can be used to create the microspheres, where its properties will influence the drug release system as a whole.

2.2.1.1 Polylactic acid

Polylactic acid (PLA) is a thermoplastic, biocompatible and biodegradable polymer used for different medical purposes[16][20]. In vivo, PLA degrades in the tricarboxylic acid cycle[21], into nontoxic degradation products[20][22]. The degradation time can range from days up to years[20]. Lactic acid is a chiral molecule with an asymmetric α-carbon[23]. The two enantiomers are the L and the D-lactic acid.

They polarize light differently while the 50/50 racemic mixture is optically inactive[20]. The stereochemistry will influence its properties. A pure poly(L-lactic acid) (PLLA) can have a degree of crystallinity up to 37%[20][21] whereas the racemic Poly(D, L-lactide) is amorphous. It has a glass transition temperature in the range of 60-70 °C and the semi-crystalline PLLA has a melting temperature in the range of 170-180 °C[20]. Figure 2.3 shows the chemical structure of PLA.

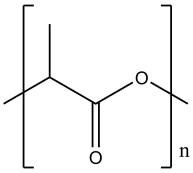

Figure 2.3: The chemical structure of polylactic acid

Poly(L-lactic acid) nanospheres with a 21wt% OCT content has been formulated by (Grit Baier, Alex Cavallaro)[16]. Where the antimicrobial effect of the spheres was tested with microbial inhibiting effect as a result.

2.2.1.2 Poly(lactic-co-glycolic acid)

Poly(lactic-co-glycolic acid) (PLGA) is a linear copolymer consisting of polyglycolic (PGA) and polylactic (PLA) acid monomeric units. It is widely used for medical applications since it is toxicological safe, biodegradable and biocompatible[24]. It is probably the best-known polymer used for drug delivery systems[19][23][25]. In the human body, PLGA will hydrolyze to lactic and glycolic acid and finally to water and carbon dioxide in the metabolic system[22][26]. PLGA is synthesized in a polycondensation reaction of lactic and glycolic acid[27]. PLGA is usually synthesized as the racemic poly(D, L-lactic-co-glycolic acid)[21][23]. Figure 2.4 shows the chemical structure of PLGA.

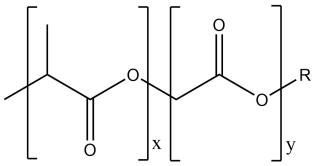

Figure 2.4: The chemical structure of poly(lactic-co-glycolic acid)

PLGA is soluble in organic solvents such as dichloromethane[28]. Depending on the ratio between PLA and PGA in the copolymer its properties changes. Where PGA is highly crystalline and more hydrophilic since its lack of methyl side group, consequently a higher ratio of PGA makes it more hydrophilic[21][23]. Since PLGA hydrolyzes in the presence of water, the degradation rate can be altered with the PLA/PGA ratio. A higher ratio of PGA leads to a larger degradation rate[23], and the maximum degradation rate has been found for a 50/50 ratio[26][29]. This is a consequence of the higher water affinity of PLGA with a larger PGA content[21]. The glass transition temperature Tg for PLGA is above 37 °C but decreases with a

decrease in lactic acid content and molecular weight. An increased molecular weight reduces the degradation rate[21].

2.2.1.3 Bis–(2–carboxyphenyl) adipate polyanhydride

Bis–(2–carboxyphenyl) adipate polyanhydride or SASanhydride is a biocompatible and biodegradable polyanhydride (PA)[30]. Figure 2.5 shows the general structure of polyanhydrides.

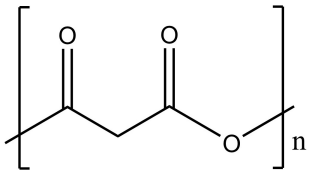

Figure 2.5: The general chemical structure of a polyanhydride.

The anhydride bond is labile and undergoes hydrolysis in the presence of water[25][30][31][32]. An anhydride bond is two acyl groups bonded to the same oxygen atom. The degradation product of PA is non-toxic small molecules that the human body easily can handle in the metabolic system[32][33]. The ability to degrade in the presence of water is useful for a drug delivery system. The degradation time can be tuned from a couple of days up to years[32][34]. The drug release properties of PA differ from those of PLGA/PLLA, which releases properties that are further discussed in chapter 2.4. PLGA and PLLA spheres release most of the active substance without losing a substantial amount of weight. Polyanhydride spheres release active substance by polymer degradation which results in matrix erosion with a linear degradation rate[25][32][34]. The drug release rate is directly proportional to the erosion of the sphere body[32]. Since polyandydrides are hydrophobic, the water penetration is mainly located close to the surface[32]. This leads to erosion and degradation mainly occurring at the surface[32][35]. These characteristics generally lead to faster release from PA spheres compared to those of PLGA and PLLA[25].

2.2.2 Emulsifier

As mentioned in section 2.2, an emulsifier is used to stabilize the emulsion. Surfactants with a hydrophilic head group and hydrophobic tail are frequently used as emulsifier[36]. Another group of emulsifiers is surface active polymers. The main difference to surfactants is the lack of distinct head/tail groups and the larger molecular weight of surface active polymers. Since the polymer has a mixture of hydrophilic and hydrophobic characters, it is attracted to the surface region. The increased concentration of polymer in the surface region will contribute to an osmotic pressure which leads to an influx of solvent. The influx of solvent causes nearby spheres or emulsion droplets to part from each other. Another effect of aggregated polymers at the surface is a steric hindrance for spheres or emulsion droplets to approach each other. These two effects will hinder aggregation and coagulation of spheres or emulsion droplets. Below is a list of the emulsifiers used, with a focus on the polyvinyl alcohol.

- Polyvinyl alcohol (PVA) is the most commonly used emulsifier to stabilize PLGA spheres[19]. It is biocompatible and therefore useful in medical application[16]. An increased concentration of PVA has been found to decrease the sphere size[19]. A higher concentration of PVA has also shown indications of a higher drug loading. This can be related to increased viscosity, a more stable emulsion, and decreased diffusion[19]. In the same way, the release rate of drugs from the spheres seems to be reduced with an increased concentration of PVA. Since a higher concentration of PVA will increase the amount of PVA associated to the surface the diffusion of the drug through the surface PVA layer will reduce the overall release rate[19].
- Poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol) (PEG-PPG-PEG) is a triblock copolymer. The central PPG block has a hydrophobic character and the PEG tails a hydrophilic character[37].
- Polyethylene glycol (PEG) is a hydrophilic homopolymer[19]. To increase the surface activity of PEG it is commonly combined with other more hydrophobic polymers or molecules[38]. When using PEG as an emulsifier in the solvent evaporation process an increased molecular weight from 6000 to 20000 DA improved its properties. This since the drug encapsulation efficiency increase, the release kinetic is slower and sphere aggregation lower[39].
- Poly(methacrylic acid) (PMAA) is a hydrophilic polymer and a weak acid[40][41]. At pH <5.5 as the carbolic groups will be uncharged, which leads to a packed structure whereas a higher pH gives a swollen structure since the carbolic groups are negatively charged and will repel each other[41].

2.2.3 Interfacial tension

The size of an average emulsion droplet, given a certain input energy, in an emulsion depends on the interfacial tension between the dispersed and the continuous phase. Surfactants and surface active polymers moves to, and by definition, reduce the interfacial tension in the interface between two interfaces. Where a decreased interfacial tension enables smaller droplets. The reason for this is the comparably lower chemical potential of the surfactants in the interfacial area compared to the corresponding bulk molecules. Molecules at an interface between two immiscible liquids have an increased chemical potential compared to the same molecules in the bulk phases. Thus leading to a surface energy. The surface energy can be described as the energy needed to create a new surface between these two liquids. High surface energy leads to a strong driving force to minimize the surface area. The surface energy can be described by the interfacial tension which can be measured and has the dimensions of force per length. Surfactants shield the two bulk phases from each other. When the surfactant concentration increases, they move to the surface area, followed by a decrease in the interfacial tension. This proceeds until the concentration reaches the critical micelle concentration, CMC. From the CMC and above the surfactants accumulates in micelles instead of increasing the concentration of surfactants in the interfacial area. By using the Gibbs adsorption isotherm the surface tension and concentration can be used to identify the surface excess concentration Γ in equation 2.1 with the unit $\frac{moles}{m^2}$.

$$\Gamma = -\frac{1}{nRT}\left(\frac{\partial\gamma}{\partial lnC_{surfactant}}\right) \tag{2.1}$$

The derivative of the surface tension γ in respect of the concentration $C_{surfactant}$ of the surfactant is measured beneath the CMC since the surface tension at that point is constant[42]. The value of the constant n depends on the surfactant. Where a nonionic surfactant has a n value of one, a fully dissociated monovalent ion the value of two, etc. If the ion is not fully dissociated, n will take a value depending on the dissociation degree. A divalent ion with a 50% dissociation degree will have a n value of 2.5. The value of the surface excess concentration indicates the surface activity of the molecule where a higher value indicates a higher tendency to decreases the surface tension.

2.2.4 Drug encapsulation efficiency and drug load

Drug load is an interesting factor to vary since it will influence the release behaviors(chapter 2.4). In the formulation of spheres, drug encapsulation efficiency is an important factor to reduce material consumption. In a literature review, Ram C Dhakar(2010) compiled a couple of factors influencing the drug encapsulation efficiency in general cases[43]. It is foremost applicable for hydrophilic drugs but the same principles are applicable for hydrophobic drugs. In general, it is a challenge to reach a high encapsulation efficiency for hydrophilic drugs.

- A higher polymer concentration during the emulsification process increases the loading efficiency. There are a couple of possible explanations for this. A higher concentration causes faster precipitation and increases viscosity which hinders leakage of the active substance. Increased polymer concentration increases particle size and consequently decreases the total surface area. Decreased total surface area increases the drug encapsulation efficiency.
- The encapsulation efficiency increases as the polymer to drug ratio increases.
- A low solubility of the polymer in the organic solvent decreases the time for the spheres to solidify, the time for it to be in a semi-solid state. Where a shorter time increases the encapsulation efficiency since less active substance migrates to the water phase. The final structure is influenced by the time the sphere is in the semi-solid state where a longer time gives a denser structure.
- A higher solubility of the organic solvent in the water phase increases encapsulation efficiency. This is an effect of a faster mass transfer of organic solvent to the water phase and consequently a faster solidification process.
- The introduction of a co-solvent increases the encapsulation efficiency. Co-solvents such as acetone, methanol or ethyl acetate decrease the solubility of the polymer in the oil phase and increases the diffusion rate of organic solvent into the water phase with the result of a faster solidification.
- A large water to oil phase increases the concentration gradient and the mass diffusion which increases the solidification rate, which consequently gives a high encapsulation efficiency and an increased particle size. The almost instantaneous solidification will lead to irregular, small, porous low density spheres. These are factors that increase the release rate of the active substance.

- A higher evaporation speed at higher temperatures is generally assumed to increase encapsulation efficiency. However, the relationship may become complicated by the fact that solubility parameters change with different temperatures. The active substance may get increased affinity to the water phase and leak out from the polymer spheres.
- Better interactions between the polymer and the active substance increase the encapsulation efficiency.
- An increased solubility of the active substance in the water phase decreases the encapsulation efficiency.
- The encapsulation efficiency increases by a decreased stirring speed. This is mainly an effect of larger spheres.
- An increased concentration of emulsifier decreased the encapsulation efficiency. This is an effect of smaller spheres formed with a higher emulsifier concentration.

Many of these factors are correlated to each other and does also affect properties like drug release and structure.

2.3 Nonwoven cellulose material

Cellulose is a natural and renewable polymer. Fiber made from cellulose has high stiffness and high strength[44] and is used as a wound dressing material in bandages. The fiber in a nonwoven has a random orientation to each other. A good wound dressing material has properties that promote tissue regeneration and inhibits infection growth. The large surface area and porous structure of nanofibrous material support adhesion and proliferation of skin cells which improves wound healing[45]. Nanofibrous material loaded with drugs has also shown antimicrobial properties towards both gram-positive and negative bacteria as a wound dressing material[45]. The large surface area enables an effective way of carrying different drugs into infected areas[45]. Electrospinning, melt blowing and solution-blow spinning are all processes that are used to create nonwoven fiber material.

2.3.1 Solution-blow spinning

Solution-blow spinning(SBS) has two fundamental components, a polymer solution called a dope and a pressurized gas stream[46]. The pressurized gas and the polymer solution are mixed inside the outer nozzle. The polymer solution is then extruded out of the inner nozzle in the direction of the gas. The fiber material is then usually collected on a moving belt[47], creating a nonwoven microfiber. Cellulose is under normal conditions insoluble in water and many organic solvents. There are a couple of reasons for this. Cellulose has a high degree of polymerization and there is a small gain in entropy of dissolution compared to molecules with a small degree of polymerization[48]. The hydrogen and carbon atoms, which are hydrophobic gives the molecule an amphiphilic character[48]. The high amount of hydroxyl groups and oxygen enables strong inter-molecular bonds[48]. Different methods are used to solubilize cellulose polymer solution[49]. One of them is to use low-melting-point organic salts, called ionic liquids[48][49]. They have advantages

such as recycle-ability, a liquid state in room temperature, high thermal stability, low vapor pressure, and tuneable solubility properties[47][49]. Many other methods to dissolve cellulose will damage its structure while dissolution with ionic liquids will cause no or little degradation[50]. Bulky ions combined with delocalized and/or sterically shielded charges make a liquid state favored for ionic liquids[50]. Their melting point is below 100 °C[50]. One of the most commonly used salts is 1-ethyl-3-methylimidazolium acetate (EmimAc) where both the acetate anion and the imidazolium cations interact with the hydrogen bonds of the hydroxyl groups of the cellulose structures thus leading to dissolution[49].

2.4 Drug release

The release behavior from polymer microspheres is complicated and depends on various factors. The release of the encapsulated active agent can vary from a couple of days to months[22]. A triphasic release profile has been suggested for PLGA microspheres[51][52][53][54]. During the first burst release phase, a substantial amount of drug is released. This is mainly active substance sited at or close to the surface of the spheres, a burst release up to 40% of the loaded active substance is possible[52]. The initial burst phase can be described by a first-order kinetics[55]. It describes the dissociation of active substance from the sphere surface and the mass diffusion process from the surface to the bulk phase, see equation 2.2.

$$\frac{\mathrm{d}m}{m\mathrm{d}t} = kS(c_o - c) \tag{2.2}$$

c is the concentration of the active substance in the bulk phase, which can be assumed to be close to 0. c_o is the saturation concentration of the active substance in water. k describes the diffusion kinetics of the surface and diffusion in the solution. S describes the specific surface properties, where smaller particles give a larger specific surface area and therefore faster release properties. Both k and S changes with time when properties like drug/polymer composition and porosity changes with the dissolution of active substance[55]. After the initial burst release, the behavior goes into a diffusion dependent release behavior[51][56][57]. The active substance diffuse though the pores and water diffuses into the porous structure of the spheres. The diffusion length of the active substance is increasing while water penetrates deeper into the sphere structure[51]. All diffusion processes are influenced by the solubility of the drug in the water phase[43]. The last step of drug release is characterized by a breakdown of the spheres[51][54]. The breakdown is a consequence of degradation and erosion[53]. As mentioned in chapter 2.2.1.2 PLGA hydrolysis in the presence of water. The PLGA chain decomposes to its monomeric building block. The reaction is autocatalytic since the monomers are of acidic character and the reaction rate is increased by a decreased pH level[29][52][53][54]. The pH level will be lower inside the sphere structure compared to the bulk phase. Lower molecular weight molecules will result in a higher amount of carboxylic chain ends which in turn may result in a lower pH and faster degradation[29]. The erosion process is the result of mass transport inside the sphere, where monomers, small oligomers, and active substance is transported out of the sphere and water transported into the

sphere structure[53]. The overall release profile is heavily influenced by the porosity, where a high porosity increases the release rate[58]. An increased drug loading means that the active substance is connected to each other and therefore creates a porous network inside the sphere structure[58]. A consequence of a porous structure is a diffusion controlled release in contrast to erosion and degeneration controlled release[59]. The porosity also depends on the size of the active substance where a large active substance gives larger pores[59]. When formulating the spheres with the solvent evaporation method, the water to oil phase ratio, have an impact on the porosity. Where a higher water/oil phase ratio increases the porosity[43]. In Figure 2.6 the fraction of initial active substance in the spheres released is displayed as a function of time with a triphasic release profile.

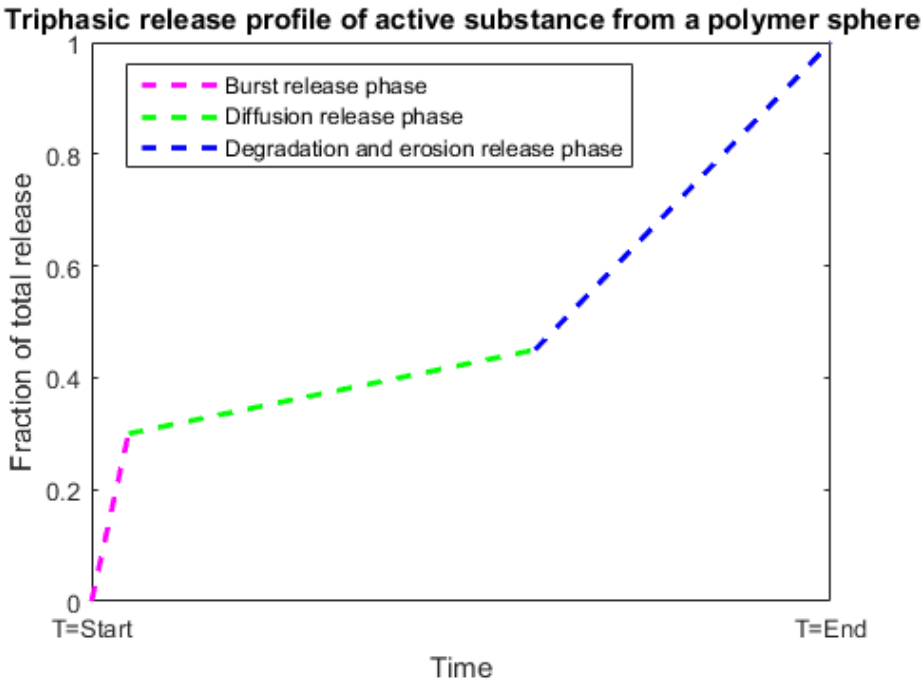

Figure 2.6: Triphasic release profile of active substance from a polymer sphere. Three different release phase. Burst release, diffusion and degradation/erosion release phase. Fraction of active substance released as a function of time

3
Methods

3.1 Chemicals and materials

- Deuterium Oxide(D_2O) (99.9 atom% D, Aldrich)
- Acetone ($\geq$ 99.8%, VWR Chemicals)
- Chloroform-d ($\geq$ 99.5%, Sigma-Aldrich)
- Dichloromethane(DCM) (Sigma-Aldrich)
- Poly(vinyl alcohol)(PVA) (95% hydrolyzed, M_w 95 000 g/mol, Acros Organics)
- Poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol)(PEG-PPG-PEG) (M_n:14 500, Aldrich)
- Poly(ethylene glycol)(PEG) (M_r:35 000,Sigma)
- Poly(methyl methacrylate)(PMMA) (M_w:350 000 g/mol)
- Poly(L-Lactic Acid)(PLLA) (M_w 40 000-70 000 g/mol, Polysciences)
- Poly(L-Lactic Acid)(PLLA) (M_w 700,000 g/mol, Polysciences)
- Poly(adipic anhydride)(Polymer source)
- Bis-(2-carboxylphenyl) Adipate Polyanhydride(SASanhydride) (M_n:5800 Polymer source, P20098A-SASanhydride)
- Poly(D,L-lactide-co-glycolide)(PLGA) (70:30, M_w 10 000 g/mol, Polysciences, Inc.)
- Poly(D,L-lactide-co-glycolide)(PLGA) (75:25, M_w 97 000 g/mol, Polysciences, Inc.)
- Poly(sebacic acid), diacetoxy terminated (Sigma-Aldrich)
- Benzalkonium Chloride(BAC) ((M_w:360 g/mol, Sigma)
- Octadinine dihydrochloride(OCT) ($\geq$ 97%, M_w:623.83 g/mol AmBeed)
- Microcrystalline cellulose, Avicel PH-101(MCC) ($\sim$50µm, Sigma-Aldrich)
- 1-ethyl-3-methylimidazolium acetate(EmimAc) ($\geq$96.5%, Sigma-Aldrich)
- Pyrene ($\geq$ 99.0%, Sigma-Aldrich)

3.2 Microsphere formulation

Polymer (PLGA, PLLA, SASanhydride, poly(adipic anhydride) or poly(sebacic acid)), active substance (octenidine dihydrochloride or benzalkonium chloride), the co-solvent acetone and the organic solvent dichloromethane were mixed in a beaker under rigorously stirring. The water phase with an emulsifier was loaded into the reaction flask. The emulsifiers and the concentrations of the emulsifier are described in chapter 3.2.3. The recipe for the sphere formulation can be seen in Table 3.1. All formulations were based on this recipe, where ratios between the different components could be shifted. The polymer was the basis for the recipe, where a small

batch contained 0.1g and a large batch 1.5g. The amount of active substance was based on the weight of the polymer. The weight of DCM is based on the total weight of the sphere phase (polymer+active substance). The volume of the PVA solution is based on the total weight of the dispersed phase. To create the emulsions three methods were used, homogenizing, vortex and only magnetic stirring. They are described in chapter 3.2.2.

Component	Amount	Ratio
Polymer	0.1g	Small batch
Active substance	0.01g	10wt% of polymer
DCM	2.912g	26.475 x weight of sphere phase
PVA solution	5.41ml	0.8949 x weight of dispersed phase
Acetone	0.2ml	Co-solvent

Table 3.1: Basic recipe for the emulsion

3.2.1 Octenidine dihydrochloride concentration

The spheres consist of polymer and active substance, where the ratio between them can be varied with different loads of active substance. Different loads of OCT were tried on PLGA and PLLA spheres to investigate its influence on the properties of the system. PLGA was tested with 0, 5, 7.5, 10 and 15wt% OCT while PLLA was tested with a load of 5 and 10wt%. All of the batches were created with the same procedure to only have the drug load differentiating them.

3.2.2 Homogenization

Three different methods to create emulsions were used; homogenizer, vortex, and magnetic stirring.

- **Homogenizer**
  The basic architecture of the homogenizer is a rotating shaft within a cylinder. Stirring and holes at the upper part of the cylinder create a flow of liquid in the vessel. The homogenizer was turned on before the organic phase was carefully dripped into the water phase in the vessel. The small $5ml$ vessel could only fit half of the water phase according to the recipe, and the rest was added after the homogenization. The homogenizer was set on a fixed stirring speed for 80 minutes. Different homogenizers and settings were used as seen in Table 3.2. When the (*Heidolph, Germany*) was used a small volume of about $5ml$ was used while the two (*Kinematica, Switzerland*) took a larger volume of about $50 - 150ml$. Every 10-20 minutes the solution was macroscopically mixed with a syringe to ensure that every part of the emulsion got stirred. After 80 minutes the emulsion was poured into a new beaker without lid and with a magnetic stirrer. The DCM evaporated from the emulsion over night. When the DCM had evaporated the lid was sealed and the suspension was magnetically stirred.

Company and model name	Stirrer diameter [cm]	Stirring speed [RPM]
Heidolph, Silent crucher M	0.4	5000
Kinematica, Polytron PT 3100 D	2.2	500
Kinematica, Polytron PT 3000	2.2	50

Table 3.2: The different homogenizer used to create stirring for the emulsion.

- **Vortex**
  All of the water phase was mixed with the dispersed phase in a beaker. The volume of the batch could range from $5ml$ to $150ml$. It was shaken vigorously with a *Tecno Kartell TK3S* for 10 or 20 seconds at a frequency of $30Hz$. The DCM/acetone was then evaporated with the rotary evaporator for 2.5 hours. The weight before and after evaporation was noted to control that the weight loss corresponded to the weight of the DCM and acetone. The sample could then be transferred to a new beaker with a lid and with a magnetic stirrer for storage.
- **Magnetic stirring**
  All of the water phase was mixed with the dispersed phase into a beaker. The beaker was shaken carefully, not enough to create a homogeneous emulsion. The beaker was then put on stirring with a magnetic stirrer on low speed, evaporating the DCM over night.

3.2.3 Emulsifier

Different emulsifiers at different concentrations were investigated as constituents of the water phase. The suspensions with different emulsifiers and concentrations were made under the same conditions to make them comparable. The different emulsifiers and concentrations tested is displayed in Table 3.3

Emulsifier	Concentration [wt%]
PVA	1
PVA	2
PVA	5
PMAA	1
PEG-PPG-PEG	1
PVA and PEG	1 and 0.75
PVA and PEG, PEG added a day after	2 and 0.5

Table 3.3: The different emulsifier used and their concentration in the water phase.

3.3 Solution-blow spinning

Fiber spinning consists of two steps, the preparation of the dope solution and spinning. Fibers containing OCT and BAC spheres were spun in different ways and are

described separately. OCT fibers were done in the manner that was first conceived, while BAC fibers were done by impregnation without any spheres.

3.3.1 Dope solution preparation for OCT fibers

To spin fibers, a dope solution was first prepared. The main ingredients of the dope were microcrystalline cellulose (MCC) that was dissolved in 1-ethyl-3-methylimidazolium acetate (EmimAc). To improve the dissolution of MCC in EmimAc it was done at increased temperature, around 60 °C. When the dope was yet again at room temperature the spheres could be dispersed in the dope. The recipe for a fiber with a 7wt% sphere content is displayed in Table 3.4. Fiber with sphere content of 5, 7 and 10wt% was created.

Material	Mass[g]	At dry fiber[mass %]
Microcrystalline cellulose (MCC)	15.55	93
EmimAc	155.5	-
Sphere dry mass	1.167	7

Table 3.4: Recipe for a fiber with a dry 7wt% sphere content

Since the spheres were created and stored in a PVA water solution, a work-up of the spheres by centrifugation had to be done. There is a risk of MCC solidifying when the water concentration in the dope increases or cellulose coming into contact with water droplets. It was, therefore, essential to reach a low water content in the sphere slurry that was poured into the dope. The work-up of the spheres is done by multiple centrifugation steps. The centrifugation time was minimized and done with cooling to reduce the risk of aggregation or decomposition of the spheres, making it impossible to redisperse them. After centrifugation, the spheres would occur as sediment on the bottom and the water phase could be poured off, or centrifuged yet again. To get a good sedimentation large spheres were beneficial, where a shorter centrifugation time was needed and a higher yield was achieved. The sediment was dispersed by stirring into a liquid slurry. The slurry was cooled in an ice bath and some cool EmimAc was added to the slurry. The sphere slurry with EmimAc was added to the dope and was stirred to disperse the spheres evenly. Afterward, the air bubbles in the dope were removed by centrifugation.

3.3.2 OCT sphere fiber spinning

The dope solution was poured into a cylinder where it was pushed by a piston under pressure to the nozzle. The pressure on the piston was up to $400kg$. The nozzle consisted of nine small pipes. The dope was forced through the pipes by the pressure of the piston and high-pressure air. The pressure of the air ranged from 0.2 to 0.7 bar. The dope was sprayed on a rotating cylinder standing in the coagulation bath. The size of the coagulation bath was $0.04L$ per gram of dope solution. As the dope collected on the cylinder reaches the water surface, the EmimAc dissolved into the coagulation bath, leaving the fiber on the cylinder. The fiber was then removed from the cylinder and washed in a $2L$ water bath for 30 minutes and then dried.

3.3.3 Dope solution preparation and fiber spinning of BAC impregnated fiber

The preparation of BAC impregnated fiber followed the same logic in the procedure but there were some differences. Before adding any cellulose to the EmimAc, $4.5g$ BAC was added. The temperature was increased to 60 °C and stirred for one hour. When the BAC was dissolved the dope was cooled to room temperature. $18g$ of MCC was added, the temperature increased to 60 °C and stirred for one hour. Observe that no spheres were added in this experiment. When the fiber was spun $15.5g$ BAC was added to the coagulation bath of $8L$ and $3.88g$ BAC was added to the $2L$ wash bath.

3.3.4 Control of drug content in fiber

To control if there were any active substances in the fiber after the fabrication a leaching test was performed. A piece of BAC impregnated fiber was weighed and then dried and weighed again to analyze the amount of water in the wet fiber. $0.01g$ of the fiber was then leached in $1ml$ of deuterated water for a couple of days. A piece of fiber containing OCT spheres was leached in deuterated water and one sample in deuterated chloroform. Reference samples for both BAC and OCT in deuterated water and OCT in deuterated chloroform with known concentrations were prepared. The concentration in the liquid phase of all the samples was investigated with NMR spectroscopy.

3.3.5 Coagulation bath leakage test

To test if the active substance was leaking out of the coagulation and wash bath a sample was taken before and after the spinning. The water sample was then investigated with liquid chromatography–mass spectrometry, LC-MS.

3.3.6 Fluorescent spheres

To be able to observe the spheres in the fibers in a good way a sample with the fluorescent molecule pyrene was created. The batch consisted of PLLA spheres with a 5wt% OCT load. The fiber was created according to the procedure described in chapter 3.3. The fiber was then observed with the optical light microscopy with a fluorescent radiating light source.

3.4 Distribution coefficient

The idea in this thesis was to encapsulate active substance in polymer spheres. Since a water phase is used both in the emulsion and in solution-blow spinning it is of interest to know how both BAC and OCT will distribute between the polymer and water phase. Since BAC has a large water solubility it was interesting to see how it was distributed between the polymer phase and the surrounding PVA water

phase. The water solubility of OCT is lower but still interesting to investigate. The distribution is defined as K in equation 3.1.

$$K = \frac{C_{PolymerPhase}}{C_{WaterPhase}} = \frac{\frac{mActiveSubstance_{in}Spheres}{mSpheres}}{\frac{mActiveSubstance_{in}WaterPhase}{mWaterPhase}} \tag{3.1}$$

To determine the concentration in both of the phases, a sphere suspension was prepared in the usual manner described in chapter 3.2, with the only difference that the PVA water phase was made out of deuterated water. The suspension was then centrifuged at 4000 RPM for 15 minutes to sediment most of the spheres at the bottom. The supernatant was then filtered with a *PALL Fluorodyne 0,2µm acrodisc syringe filter*. This was done to remove the spheres leaving the PVA water phase. As a reference sample $0.01g$ BAC was dissolved in $5.41ml$ deuterated water and $0.0079g$ OCT in $2ml$ deuterated water. To investigate the influence of the filter two samples of the BAC reference were prepared, one where the sample was filtered and one where it was not. $1ml$ of each sample was then analyzed with NMR.

3.4.1 BAC spheres with equilibrated emulsion

When the distribution coefficient was calculated it was possible to prepare an emulsion where the polymer phase and the water phase would be in equilibrium. With the standard receipt seen in Table 3.1 scaled for 1.5 grams of PLLA, 2.56 grams of BAC was added instead of the original 0.15 grams that usually make a 10wt% BAC load. The amount of BAC needed to equilibrate the water phase with the polymer phase was calculated with equation 3.2 which is derived from equation 3.1. In the end, the amount of BAC for the spheres and the water phase was added up.

$$mBAC_{in}WaterPhase = \frac{mBAC_{in}Spheres * mWaterPhase}{K * mSpheres} \tag{3.2}$$

3.4.2 OCT solubility

As mentioned in chapter 2.1.2 there is contradictory information about the water solubility of OCT in literature. If the solubility is high there is an increased risk of OCT leakage into the water phase. The solubility of OCT was tested for in deuterated water and in 5wt% PVA solution made from deuterated water. Three samples were prepared. A sample with an oversaturated amount of OCT in deuterated water i.e. all of the OCT was not dissolved, and a similar sample but in 5wt% PVA was made. The reason an oversaturated amount was used was to ensure the limit of solubility was reached. As a reference a sample with $0.0079g$ OCT in $2ml$ deuterated water was prepared. In the reference sample, all of the OCT got fully dissolved. All of the samples were put on magnetic stirring over night to minimize the time dependence of the solubility. The amounts can be seen in Table 3.5
All of the samples were then filtered with a *PALL Fluorodyne 0,2µm acrodisc syringe filter* to remove OCT that had not been dissolved. The samples were then analyzed with NMR.

Sample	Amount[g]
Reference sample, OCT in deuterated water, dissolved	0.0079
Oversaturated amount of OCT in 5wt% PVA deuterated water solution	0.0079
Oversaturated amount of OCT in deuterated water solution	0.0237

Table 3.5: Receipt for solubility testing of OCT. Amount of OCT in $2ml$ of deuterated water or 5wt% PVA deuterated water solution.

3.5 Characterization

Optical light microscopy, optical particle size measurement, and optical tensiometry were the three most recurring analyze methods in this thesis and a short review of them are found here.

3.5.1 Optical light microscopy

All microspheres and nonwoven materials were analyzed with optical light microscopy. The microscope was a *Zeiss Axio Image 2* from *Carl Zeiss* with the *Zeiss ZEN Pro software*. By analyzing images of the sphere suspension in the microscopy, size distribution and potential aggregates could be examined. To do a statistical analysis of the sphere size distribution, spheres in microscopy pictures had to be counted in the *ImageJ* software.

3.5.2 Optical particle size measurement

To determine the size distribution of the spheres in the suspension a particle size method was used. To do this the *Mastersizer Microplus* by *Malvern Panalytical* was used. It is based on a light scattering technique. It uses different theories to relate the scattered light to the sizes of the particles.

3.5.3 Optical tensiometry

To determine the impact of the active substance on the surface properties, the interfacial tension was measured. Since no measurement could be done on the emulsion droplet or sphere directly, the surface interface of the emulsion droplet was modeled, this by measuring the tension at the dichloromethane and water interface. The experiment type to determine the interfacial tension was pendant drop. A drop of the heavy phase was created on a needle inserted in the light phase, where the angle between the two phases was continuously measured over a short time. A series of solutions containing DCM, PLGA and OCT were prepared, in this case, the heavy phase. The amount of OCT was varied to equivalent the amount of OCT used to create spheres with a drug load from 0wt% to 15wt%. A series for BAC was also prepared but without PLGA. It contained DCM and BAC in the same amounts corresponding spheres with a drug load from 0wt% to 15wt%. The light phase was a 5wt% PVA solution. Some separate samples were also measured: Pure DCM in the heavy phase and MilliQ water in the light phase to compare with reference values.

A sample with PLGA and no active substance. A 10wt% OCT sample without PLGA to determine the effect of PLGA. The equipment used was a *Theta* from *Biolin Scientific* with a *OneAttension* software.

4

Results and discussion

4.1 Microsphere formulation

The formulation process of the microspheres was analyzed with respect to the homogenization and emulsifier type. While the properties of the spheres were analyzed regarding the different concentrations of OCT, polymer type and surface activity.

4.1.1 Verification of mastersizer and repeatability of vortex method

Spheres formulated with the solvent evaporation method were analyzed with respect to the sphere diameter with both mastersizer and optical microscopy. To verify the mastersizer, its size distribution was compared with a size distribution produced from optical microscopy images. The images were processed in *ImageJ* (see chapter 3.5.1) and then recounted to volume fraction. Since the two samples were done the same way the repeatability of the vortex method. Both batches were made of PLGA, 10wt% OCT in 5wt% PVA solution and with a vortex time of 20 seconds. In Figure 4.1 the two samples 16/04 and 17/04 measured with the mastersizer is plotted with the result from the pictures taken with the optical microscopy and then analyzed with *ImageJ*. The line representing the microscopy is a merge of the two batches. This was performed to increase the sample size, in this case, 650 spheres. Nonetheless, the standard deviation is visually high. The peak at $\sim 57\mu m$ represents only one sphere but makes a large impact on the total volume. This implies the need for large sample size analyzing pictures from microscopy to achieve a reliable size distribution. Besides the large standard deviation, it can be used to confirm the reliability of the mastersizer. When excluding the larger diameters which would need a larger sample size it is clear that the peak of the microscopy/*ImageJ* is related to the two peaks of the mastersizer measurements. The two batches 16/04 and 17/04 were made in the same way and the two mastersizer size distributions indicate a good overlap between the two batches.

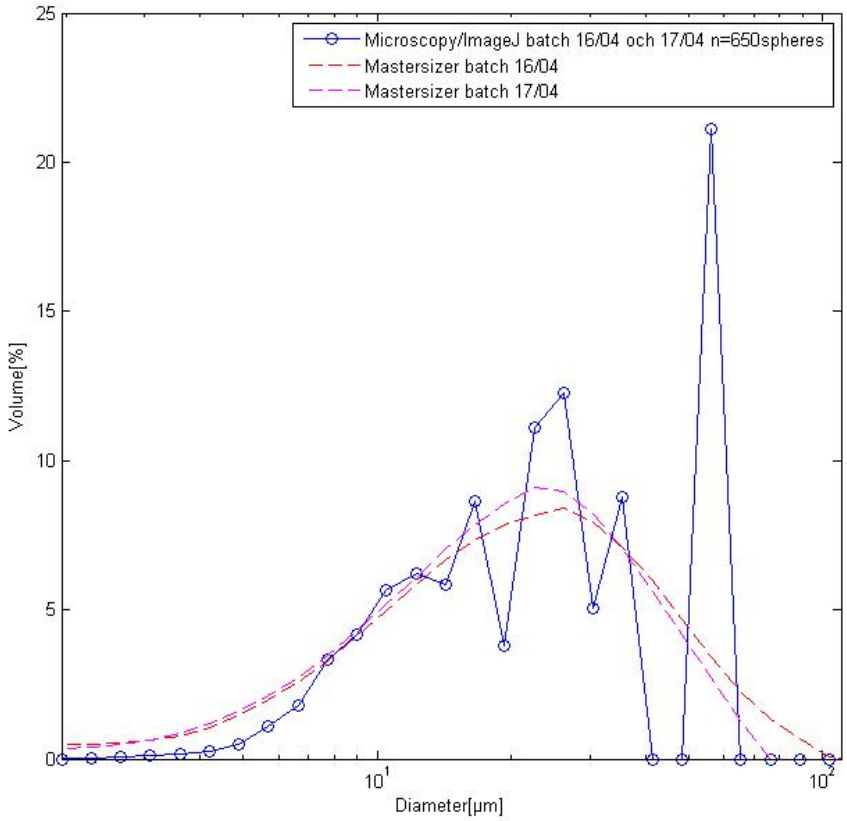

Figure 4.1: Volume weighted size distribution of spheres in suspension counted with mastersizer or optical microscopy, processed in *ImageJ*

4.1.2 Effect of homogenization

Throughout the experiments, different stirring methods and stirring conditions were tested. As shown in Figure 4.2 the spheres created with the vortex method consistently showed a larger diameter, while the spheres created with homogenizers were comparably small. For the vortex method there was a clear difference in the sphere diameter depending on the time the emulsion was shaken in the vortex, indicated by the shift of the peaks between the two 20 second and the 10 second vortex batches in Figure 4.2. When comparing two batches formulated with the homogenizer, with the same components but a difference in reaction volume, 125 and 85ml and stirring rotation speed, 50 and 500RPM there was no clear difference between the two batches. The expected result would be decreased sphere diameter with increased rotation speed. The similarity between batches could be explained by differences in reaction volume but since a lower volume is expected to reduce the sphere size this is not a likely explanation. In conclusion, the rotation speed in itself does not influence the sphere diameter in contrast to previous results. The homogenizing time is probably long enough to reach a steady state in sphere diameter. In this steady state, the sphere diameter probably depends on other factors such as the very high PVA concentration and the surface activity of the active ingredient.

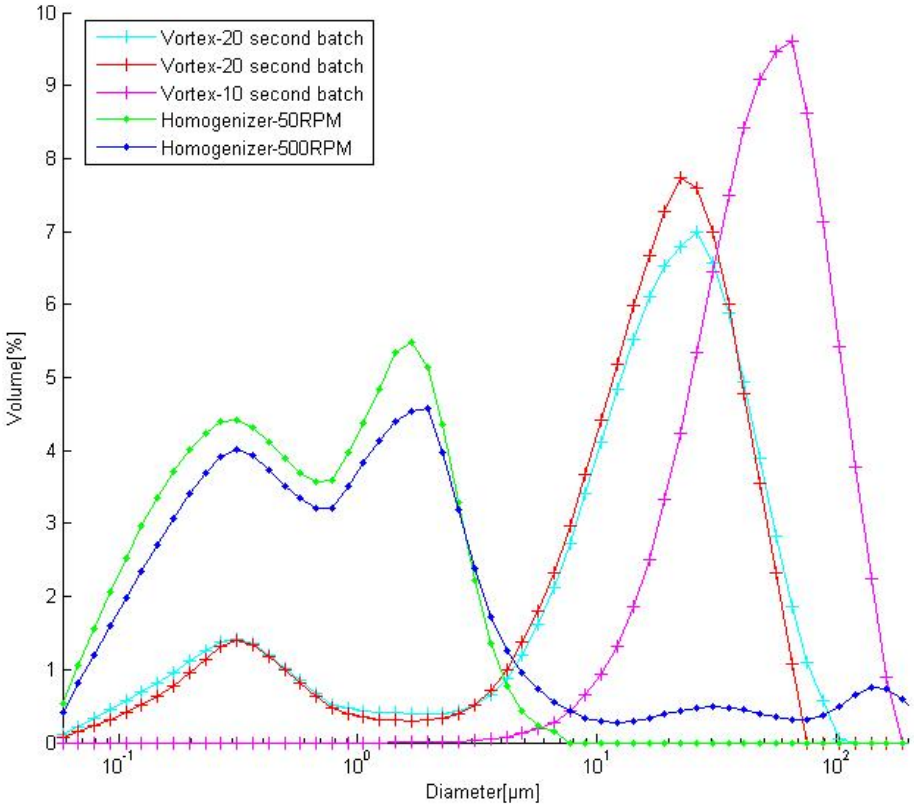

Figure 4.2: Plot showing the volume weighted diameter of the spheres created with the homogenizer or vortex method.

The batch created by stirring by magnetic stirrer over night (see chapter 3.2.2) shows the same sphere diameters as batches created with homogenizers. This does also indicate that the stirring speed at itself does not play any major role in the sphere diameter when the stirring time is long enough to reach a steady state. After a batch has been homogenized by the homogenizer it is put on magnetic stirring over night. There is a possibility that there is a difference between the different rotation speeds but that the magnetic stirring over night evens out the differences. This reasoning is not valid for the vortex method where the stirring heavily influences the sphere diameter. It is reasonable to assume that if the vortex time was long enough the sphere diameter would not decrease any further then for the homogenizer and reach a steady state. The vortex method was preferred since it created larger spheres. Larger spheres were desired for two main reasons. Larger spheres give a smaller total surface area compared to many small spheres, which in turn gives a slower total release of the active substance. In the fiber spinning process, one of the steps involves centrifugation of the suspension to separate the spheres from the water phase. This was a much faster and efficient process with larger spheres.

4.1.3 Effect of the octenidine dihydrochloride concentration

The concentration of OCT or drug load was tested to investigate its impact on the suspension and spheres. In Figure 4.3 four batches with a drug load of 0, 5, 7.5 and 10wt% are shown in a series. The concentration for OCT in the spheres had a great influence on the sphere size distribution.

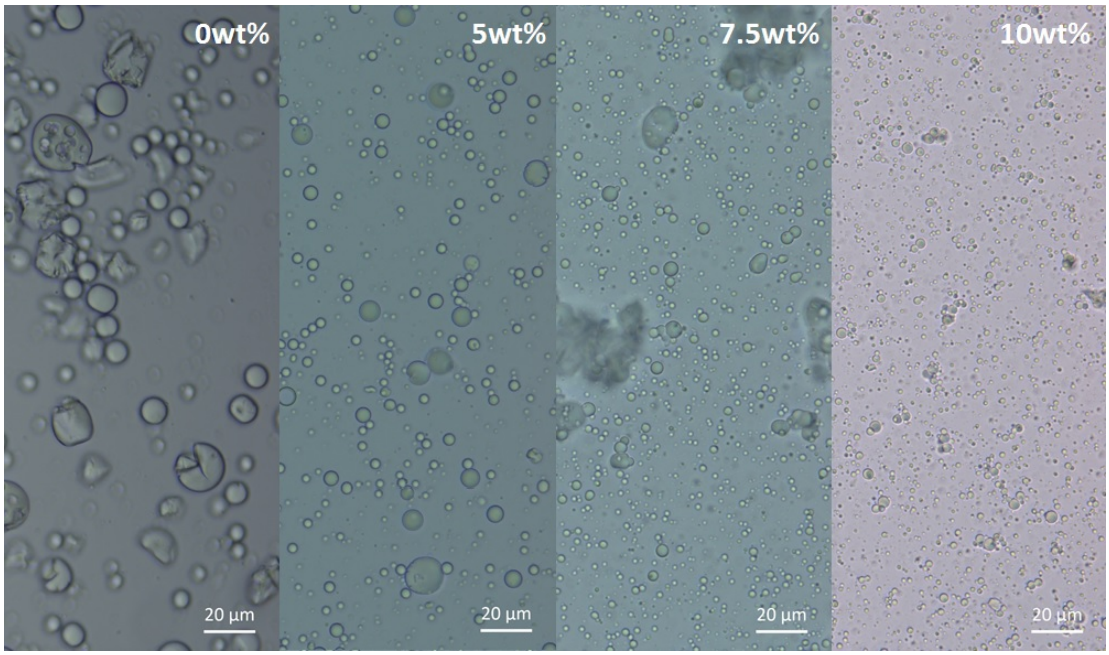

Figure 4.3: Optical microscopy picture of a series of suspensions with PLGA spheres containing 0, 5, 7.5 and 10wt% OCT.

The reason for this is probably the fact that OCT is a surface active molecule. The surface active molecules reduce the surface energy and thus allows for a greater total surface area. The surface activity of the active substances is further discussed in chapter 4.1.6. A sufficient concentration or load of OCT in the spheres were desired to achieve MIC in the wound. A low concentration in the spheres would require an increased amount of spheres incorporated in the fiber material. From a material efficiency perspective, a high drug load is desirable. Since the sphere radius and release properties are affected by the drug load there is likely a maximum desired drug load. The release rate are affected by the sphere radius since the release is proportional to the specific area of the spheres, $Release \propto A/V_{sphere}$. The volume of the spheres does increase faster than the area with an increased sphere radius, $V_{sphere} \propto r^3_{sphere}$ and $A_{sphere} \propto r^2_{sphere}$. An increased diameter gives the sphere a larger volume to store the active substance compared with a relatively small surface area for the active substances to diffuse through. The higher drug content in the spheres should also increase the release rate as discussed in chapter 2.4, since a higher drug load would increase the porosity of the spheres. If the porosity was increased by the drug load could not be determined from these tests. With these two factors in mind, it is not obvious that a higher drug load would be beneficial.

4.1.4 Effect of polymer type

Different polymers were used to create spheres. PLGA, PLLA, and SASanhydride were all valid polymer to create spheres. Poly(adipic anhydride) was at first not soluble in DCM but could dissolve when the acetone amount increased from 200µl to 800µl in a small batch. The sphere suspension that was created was full of fragments with few complete spheres. Poly(sebacic acid) was not able to dissolve in DCM even though the acetone concentration was increased. Poly(adipic anhydride) and poly(sebacic acid) were not further investigated.

4.1.4.1 PLGA

PLGA was the polymer in the majority of the experiment since it increases the comparability between different experiments, and it has been studied and used in previous works. Figure 4.4 shows a suspension with PLGA spheres, loaded with 10wt% OCT in a 5wt% PVA solution. The homogenization method was, in this case, the vortex.

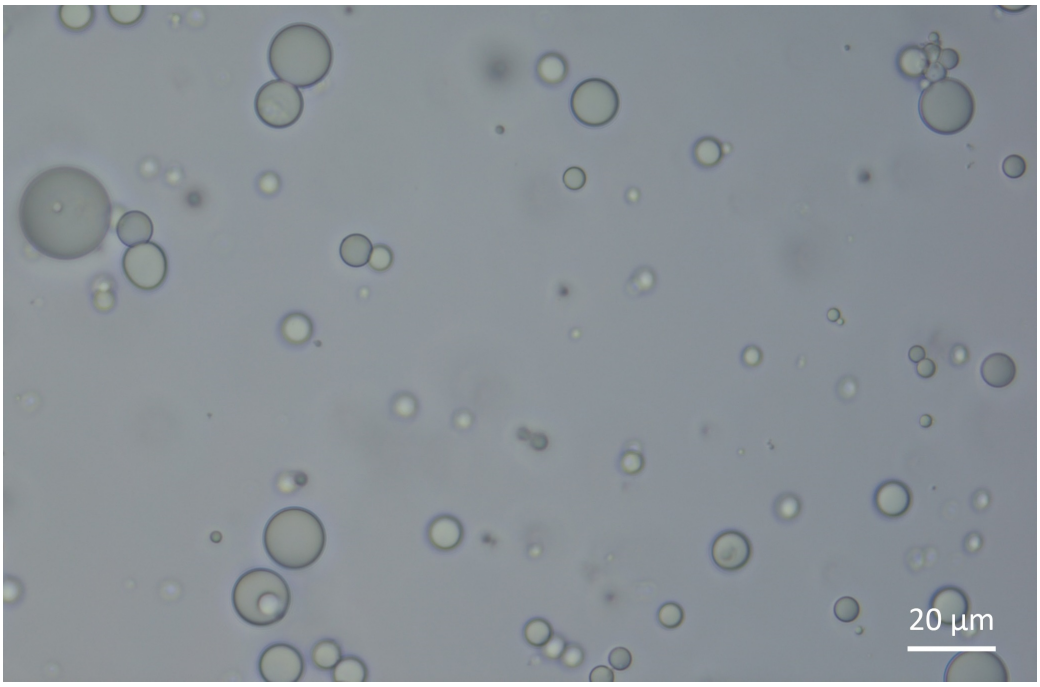

Figure 4.4: Microscopy picture of a PLGA 10wt% OCT load sphere suspension in a 5wt% PVA solution.

An issue with the PLGA spheres containing OCT was that the spheres accumulate in aggregates and after a day substantial amount would be aggregated at the bottom and side of the beaker. There would be no aggregation when it was not loaded with any OCT, suggesting a clear relationship between aggregation and the presence of OCT. There may also be a correlation between more aggregation and higher OCT concentration but this could not be ensured since the level of aggregation is difficult to determine. The issue and solution to aggregation are further discussed in chapter 4.1.5. The spheres in suspensions with PLGA, stored for a couple of months did

completely disappear. As described in chapter 2.2.1.2 the polymer should have been hydrolyzed into smaller units as lactic and glycolic acid and finally to water and carbon dioxide.

4.1.4.2 PLLA

Suspensions with PLLA spheres were made in different compositions. Compared to the PLGA suspension the PLLA microspheres behaved differently. The PLLA spheres did not aggregate but formed a sediment on the bottom of the vial after a couple of days. The sediment could be redispersed by shaking the vial. It is possible that PLLA spheres did aggregate while the physical or chemical bond was weak compared to the ones between PLGA spheres since PLGA is likely to have more polar intermolecular bonds. There may also be an electrostatic effect dependent on the concentration of OCT at the surface. An increased concentration of OCT at the surface leads to an increased concentration of counterions and consequently a higher electrostatic stabilization. PLLA spheres stored up to three months did not show the same trait of hydrolysis as PLGA and seemed to stay intact. It was expected that the degradation of PLLA would be slower than the one seen for PLGA. This related to the crystallinity and hydrophobicity of PLLA. PLLA is partly crystalline which hinders the water to penetrate the tight crystal structure and thus reducing the hydrolyzing rate. The methyl group in the PLLA structure increases the hydrophobicity compared to PLGA, which also reduces the water penetrability and the hydrolysis rate of the PLLA spheres. There is a possibility that the active substance would interrupt the structure and thus decrease the crystallinity. This could neither be confirmed nor denied by the observations. If there were any differences in diameter between PLGA and PLLA spheres could not be determined from observations.

4.1.4.3 SASanhydride

It was possible to make suspensions with SASanhydride spheres. The spheres did after a day aggregate but in a different manner then the PLGA/PLLA. While PLGA/PLLA aggregated at the bottom and surface of the beaker SASanhydride formed single larger aggregates. Indicating lower adhesion to the surface. SASanhydride was investigated in a less rigorous way than PLGA/PLLA but seemed to be a good candidate for the purpose.

4.1.5 Effect of emulsifier

At first, a 1wt% PVA solution was used as the continuous phase. It was noted that the spheres after a day started to accumulate in aggregates. This aggregates would build upon the bottom and the sides of the storage vessel. Figure 4.5 shows aggregates of PLGA spheres in a 1wt% PVA suspension.

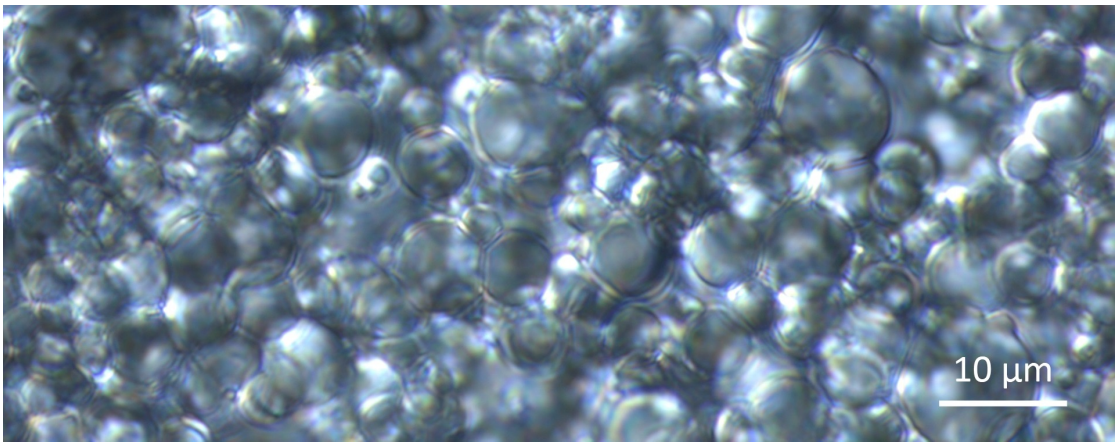

Figure 4.5: Microscopy picture of aggregate of PLGA spheres in a 1wt% PVA suspension.

Since spheres were accumulated in aggregates, the concentration of freely floating spheres in the suspension gradually decreased. To reach a more stable suspension different emulsifiers were investigated.

- With Poly(methacrylic acid), PMAA as the emulsifier a suspension with spheres was not reached. The sample contained fragments.
- The 1wt% PVA and 0.75wt% PEG sample created a viscous suspension with a surface layer.
- With PEG-PPG-PEG as an emulsifier, a stratification was created without any emulsion.
- When 0.5wt%PEG was added to an emulsion 2wt% PVA as emulsifier an emulsion was created but did not improve the stability.
- Increasing of the amount of PVA to 2wt% did not improve the stability while a 5wt% PVA solution seemed to increase the stability to the degree that the suspension would be stable weeks after the emulsion was created. The reason for this could be that an increased concentration of PVA in the suspension will increase the concentration of PVA close to and on the surface of the spheres. This will sterically hinder spheres approaching each other, reducing aggregation. There may also be an osmotic effect, since the increased concentration of PVA close to the surface will increase the influx of water from the surroundings, pushing two nearby spheres from each other.

A 5wt% PVA solution was after this confirmation used as the standard continuous water phase.

4.1.6 Surface activity of active substance

The surface activity was investigated by the measuring of the interfacial tension for a series of samples containing different concentrations of BAC and OCT.

4.1.6.1 Surface activity of OCT

The interfacial tension was measured for a series of solutions containing DCM, PLGA, and OCT. The series contained OCT of the concentrations 0.025, 0.25, 2.5,

3.3, 4.1, 5, 7.5, 10, 13, and 15wt% made in the proportions described in Table 3.1. The weight percentage that describes the amount of active substance relative to the polymer was converted to concentration of the DCM volume. The concentration was then logarithmized and plotted towards the interfacial tension, displayed in Figure 4.6.

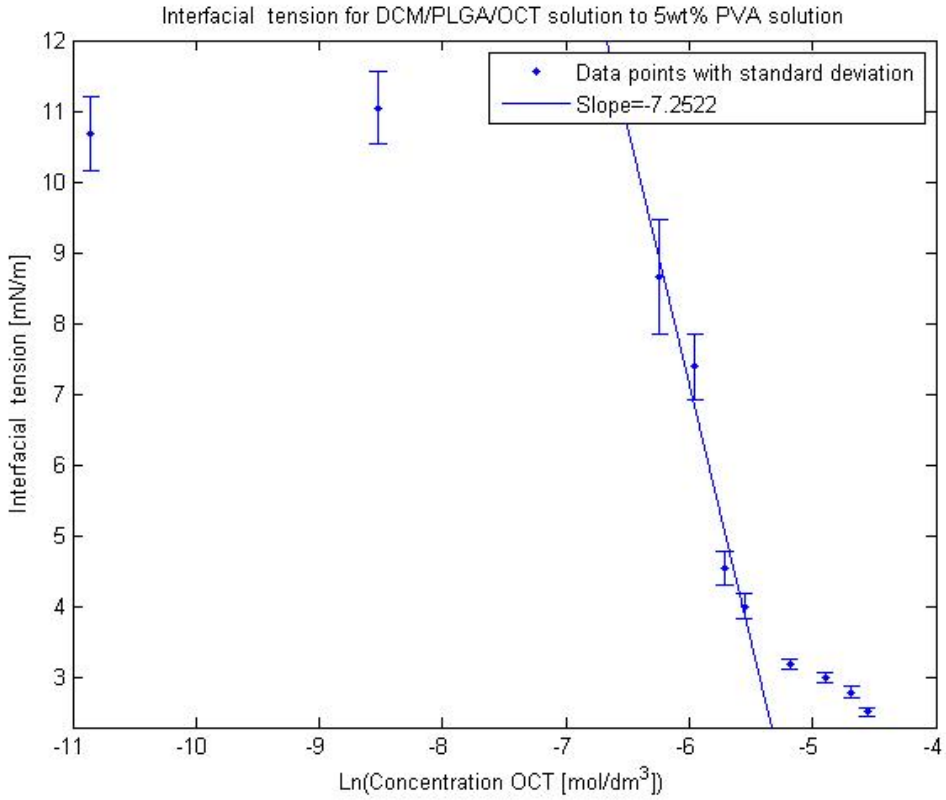

Figure 4.6: Plot of the interfacial tension with respect to the natural logarithm of the OCT concentration in the heavy DCM phase. Plotted with a slope before the plateau. Standard deviation displayed for every data point.

The Figure indicates a sharp decline in the interfacial tension between the heavy and light phase with an increasing OCT concentration. The light phase was, in this case, a 5wt% PVA solution. The interfacial tension is expected to plateau when the CMC is reached. If this is the case when lnC is around -5 in the graph is difficult to determine but the decline in interfacial tension does seem to ease off. The interfacial tension was linearized in the region where the decline in interfacial tension is rapid. This gave a slope of -7.2522. By using the slope in equation 2.1 at a temperature of $T = 293K$, a surface excess of $\Gamma = 9.924 * 10^{-7}\ \frac{moles}{m^2}$ is calculated. n is assumed to be 3 as OCT is a divalent ion with two monovalent counter ions. OCT has a poor water solubility and should in the DCM phase be neutrally charged. But when it reaches the interfacial area it is assumed to fully dissociate and have a charge of two. To be certain if this is the case, other experiments have to be done. The surface excess can be recalculated to an area per molecule of $167.3\frac{\text{Å}^2}{molecule}$. This can be compared with one of the most used surfactants sodium dodecyl sulfate (SDS),

whose area per molecule of around $33\frac{Å^2}{molecule}$ at a water-air interface[60]. This is a consequence of the bulky molecular structure of OCT with two side chains.

4.1.6.2 Surface activity of BAC

The interfacial tension was measured for a series of solutions containing DCM and BAC. The series contained BAC in the concentrations 0.025, 0.27, 1.5, 2.5, 2.87, 3.3, 4.17, 5, 7.5, 10, 12.5, and 15wt% made in the proportions described in Table 3.1. The weight percentage that describes the amount of active substance relative to the polymer was converted to concentration of the DCM volume. The concentration was then logarithmized and plotted towards the interfacial tension in Figure 4.7.

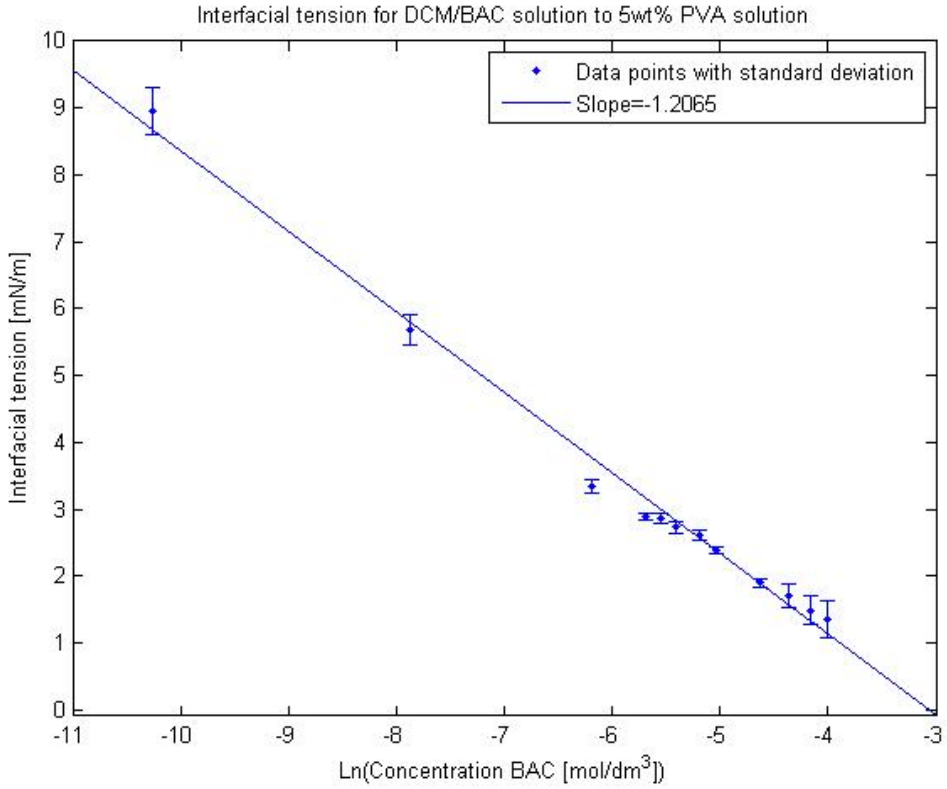

Figure 4.7: Plot of the interfacial tension in respect to the natural logarithm of the BAC concentration in the heavy DCM phase. Plotted with a slope. Standard deviation displayed for every data point.

As for OCT, it is clear that BAC decreases the interfacial tension. No plateau is visible in the examined concentration range of BAC. A linearization is done between the points in the plot and gives a value of, slope=-1.2065. By using the slope in equation 2.1 at a temperature of $T = 293K$ a surface excess of $\Gamma = 2.477 * 10^{-7}\ \frac{moles}{m^2}$ is calculated. n is assumed to be 2 as BAC is a monovalent ion. At the interface, BAC is assumed to fully dissociated and have a charge of one. The surface excess can be recalculated to an area per molecule of 670.5 $\frac{Å^2}{molecule}$. Comparing the surface excess and area per molecule of OCT and BAC it is clear that OCT is the more surface active molecule and should influence the sphere formation in a higher degree.

4.1.6.3 Influence of emulsifier and polymer on the interfacial tension

Some further samples where the influence of the emulsifier and PLGA were of interest can be seen in Table 4.1. The pure DCM without PLGA and active substance and with MilliQ water as light phase had an interfacial tension of $25.94\frac{mN}{m}$ which can be compared with 28.31 $\frac{mN}{m}$ found in literature[61]. A sample with pure DCM with no PLGA or active substance was tested with MilliQ and another with 5wt% PVA as the light phase. There was a clear difference between the samples where the one with MilliQ had a value of $25.94\frac{mN}{m}$ and the one with 5wt% PVA a value of $12.16\frac{mN}{m}$. This was expected since the PVA concentration is high and is a surface active polymer. The sample without active substance had a value of $9.68\frac{mN}{m}$. This is the value both of the plots should have started at, but since there is no natural logarithm of zero it highlighted here. All of the measured values in the BAC series are below this value which is expected. The interfacial tension should decrease when BAC is added. Some of the measured data points in the OCT series are above the zero concentration value. The reason for this is unclear but the measurement uncertainty at low concentrations may be a reason. This value can also be used to understand the impact of PLGA on the interfacial tension. Where the interfacial tension is reduced from 12.16 to $9.68\frac{mN}{m}$ by adding PLGA. The two 10wt% samples with or without PLGA can also be compared for this purpose. The response of this system is, however, the opposite where the addition of PLGA increases the interfacial tension. Emil Lukasiewicz (2019) did a similar measuring series of OCT at different concentrations. The interfacial tensions that were measured showed a consistently lower interracial tension than the once measured in this thesis, indicating that the interfacial tension should increase when PLGA is added to the system[62].

Heavy phase	Light phase	Interfacial tension [mN/m]
Pure DCM without PLGA and active substance	MilliQ water	25.94
Pure DCM without PLGA and active substance	5wt% PVA	12.16
PLGA without (0wt%) active substance	5wt% PVA	9.68
10wt% OCT with PLGA	5wt% PVA	3.00
10wt% OCT without PLGA	5wt% PVA	2.27

Table 4.1: Different compilations in the heavy and light phase with their measured interfacial tension

4.1.7 BAC sphere suspension at equilibrated conditions

A sphere suspension was prepared with the vortex method, where the water and polymer phase was in equilibrium. The formulation procedure is described in chapter 3.4.1. At equilibration, there should be no diffusion of BAC from or to the polymer phase from the surrounding water phase in the suspension. A microscopy picture can be seen in Figure 4.8.

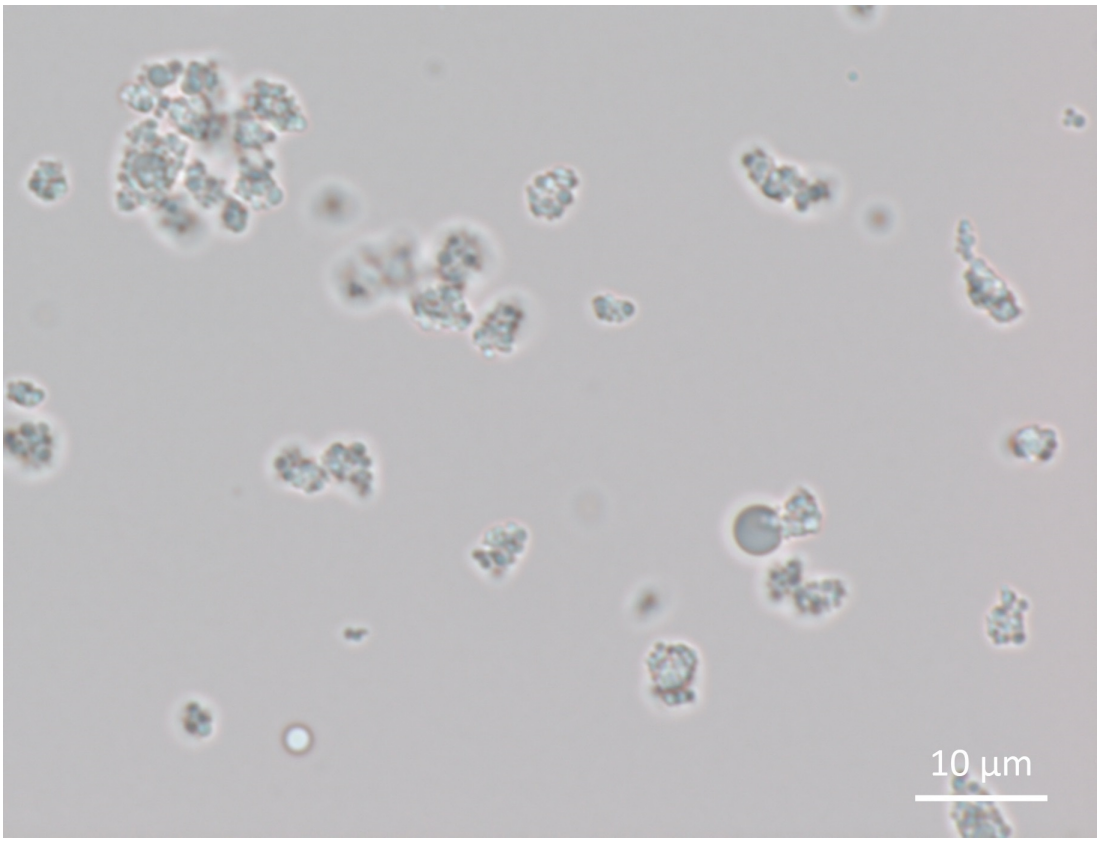

Figure 4.8: PLLA spheres with BAC, created in emulsion at equilibrated conditions

There were few spheres with an intact structure with moderate size. What can be seen in larger quantities are particles with an uneven structure. These particles can either be spheres which are very porous or large quantities of small spheres aggregates in larger structures. Both explanations are reasonable consequences of a high BAC concentration. The high BAC concentration would reduce the interfacial tension, which would enable spheres of smaller radius. Comparing the concentration in this experiment with the one in Figure 4.7, the natural logarithm of the concentration lnC is -1.5. This value is far from within the range of the plot and at this concentration, the CMC value would have been reached. A high concentration of active substance would make the polymer structure porous[58]. The small radius and therefore a large total area or a porous structure would enable a fast release.

4.2 Solution-blow spinning

The solution blown spinning procedure can be divided into two steps, a dope preparation before the spinning. The theoretical possibilities of a sufficient drug load were then investigated and compared to analyses of the content of the active substance in the fiber.

4.2.1 Dope solution formulation

The dope solution was successfully prepared with well dissolved cellulose, dispersed and intact spheres. A problem could have been dissolution or damage on the spheres in the dope solution and its making. This since EmimAc is an aggressive solvent that dissolves cellulose. Despite this, the spheres were intact and well dispersed as observed in Figure 4.9. This may be because the nonpolar structure of the sphere, not interacting with the polar ionic structure of EmimAc, and is therefore not dissolved or damaged. The MCC does seem to be well dissolved. Theses spheres are SASanhydride spheres with a drug load of 10wt% OCT.

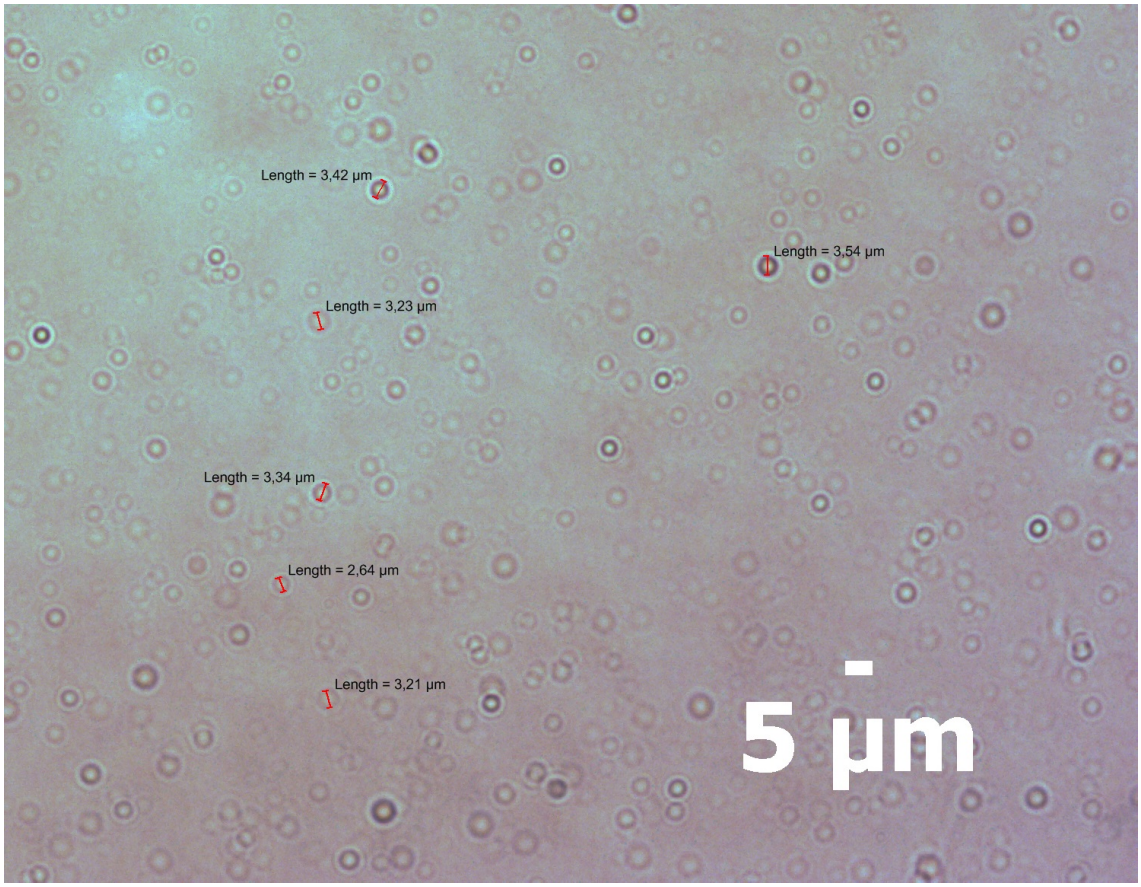

Figure 4.9: Dope solution with 10wt% OCT in dispersed SASanhydride spheres.

When dispersing the spheres in the dope it was important to follow the procedure described in chapter 3.3.1, to avoid water in the dope as coagulation was observed when this was the case. To have a large diameter, $D \geq 5\mu m$ was essential in the dope solution preparation process. This since the suspension with spheres was centrifuged to create a sediment with spheres at the bottom of the vial. When the spheres had cemented at the bottom the water phase could be poured of and the sphere slurry could be inserted into the dope. Smaller spheres were difficult to collect by centrifugation since the sedimentation rate is proportional to the diameter of the spheres according to Stokes law. Consequently, multiple centrifugation steps had to be done, increasing the water content in the dope since the sphere slurry contains

water. With larger spheres almost all of the spheres could be collected from the suspension with one centrifugation step, leading to high efficiency and reducing the water content in the dope.

4.2.2 Fiber spinning

Fibers with spheres of PLGA, PLLA, and SASanhydride were created. All of the polymers were valid since they created fibers with well dispersed spheres in the fiber structure. The sphere content of the fibers probably affected the physical properties of the fiber material, where a higher sphere content should have made the material weaker by interrupting the cellulose structure. This was not investigated further but 5, 7 and 10wt% all seemed like valid options. It was clear that suspensions with smaller spheres did not achieve a high enough yield in the centrifugation step described in chapter 3.3.1. This since the visible amount of spheres were much smaller than the amount seen in fibers created with larger spheres. Fiber with a 7wt% PLGA sphere content is displayed in Figure 4.10. The spheres contained 10wt% OCT and had a volume-averaged diameter of about 15µm.

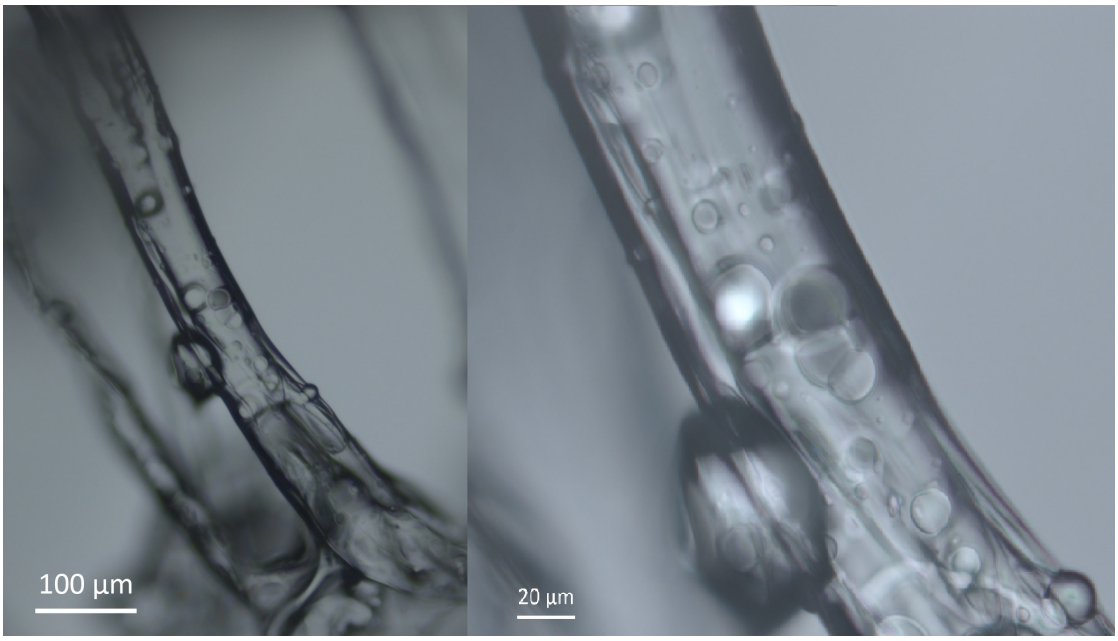

Figure 4.10: Fiber with PLGA spheres. 10 and 40 times magnification.

The spheres were intact and well dispersed. While the spheres seem to be well dispersed, it is difficult to determine if the spheres are on the surface or inside the fiber. Fibers with PLLA spheres containing pyrene was created and photographed with optical microscopy. An example can be seen in Figure 4.11. It shows the fiber structure with spheres dispersed in its structure.

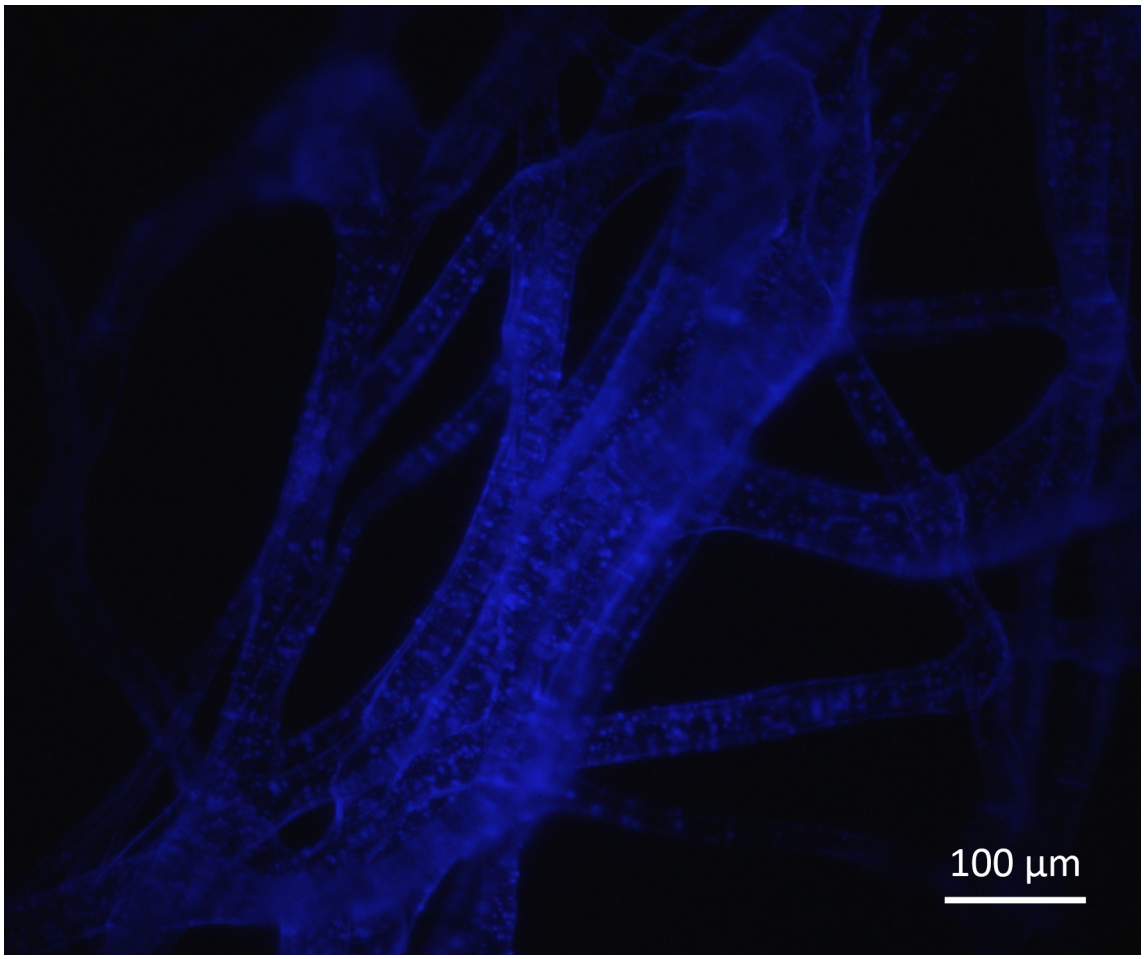

Figure 4.11: Fiber with PLLA spheres containing pyrene. Florescent microscopy picture

4.2.3 Theoretical possibility of sufficient drug load

An import aspect is the theoretical possibility to have a sufficient amount of active substance in the fiber. As a benchmark the minimal inhibitory concentration, MIC was used. At this concentration of the active substance, the growth of bacteria is inhibited. Two bacteria species and their MIC for OCT and BAC that was used and can be seen in Table 4.2[63].

Active substance	*S.aureus*	*P.aeruginosa*
BAC	$1 * 10^{-3}$M	$6.3 * 10^{-4}$M
OCT	$5 * 10^{-7}$M	$3 * 10^{-5}$M

Table 4.2: The minimal inhibitory concentration of BAC and OCT for the two bacteria species *S.aureus* and *P.aeruginosa*[63]

The fiber must have a sufficient amount of active substance to reach MIC in the exudate, this by diffusion of the active substance from the fiber to the exudate. To model a wound environment a Franz cell can be used[64]. This was not done

in this thesis but the principal is here used to calculate a theoretical amount of active substance that can be incorporated via microspheres. The Franz cell has an $11ml$ volume which adsorbs active substance from a $1.77cm^2$ fiber piece. From these numbers, a minimal amount of active substance per area of fiber $\frac{g}{cm^2}$, can be calculated. These are compiled in Table 4.3.

Active substance	*S.aureus*	*P.aeruginosa*
BAC	$0.002237288\frac{g}{cm^2}$	$0.0014095\frac{g}{cm^2}$
OCT	$1.94 * 10^{-6}\frac{g}{cm^2}$	$1.16 * 10^{-4}\frac{g}{cm^2}$

Table 4.3: Minimal amount of active substance per area unit of fiber $\frac{g}{cm^2}$

A potential amount of active substance per area unit is calculated to $0.0018\frac{g}{cm^2}$, see appendix A.1 for calculations. When comparing this with the values in Table 4.3 it is clear that BAC does not archive a high enough amount to reach the MIC, while OCT seems to be able to reach a high enough concentration. Since this assumes that all the active substance is released from the fiber and not consumed with time it is reasonable to have a higher amount than just the amount that in an optimal scenario would satisfy the MIC. A solution to this problem is to impregnate the fiber and use the spheres as reservoirs to maintain the concentration above the MIC. This calculation assumes a perfect manufacturing process of the fibers with spheres to get a concentration of $0.0018\frac{g}{cm^2}$ active substance in a fiber piece. This may not be the case since there may be a leakage of the active substance in the manufacturing process, this is further discussed in chapter 4.4 and 4.5.

4.2.4 BAC impregnated fiber

It was possible to create fibers with a BAC impregnation seen in Figure 4.12. See appendix A.2 for a detailed description of the calculations of the values in this chapter. A concentration of $0.311\frac{g}{dm^3}$ was measured with NMR in the leached deuterated water phase. This gives the fiber a BAC content of $3.11wt\%$. When the water evaporated after the wash step, BAC is assumed to be left in the dry fiber. The wash bath will work as an impregnation step. The concentration of BAC in the fiber caused by evaporated wash bath is $1.64wt\%$. The rest of the $3.11wt\%$, the $1.48wt\%$ can be attributed to the BAC dissolved in the dope solution. There is a possibility that some of the BAC in the fiber come from the coagulation bath. As discussed in chapter 4.2.3 it would probably not be enough with spheres as a source of active substance since the content of in the fiber would be too low. A solution could

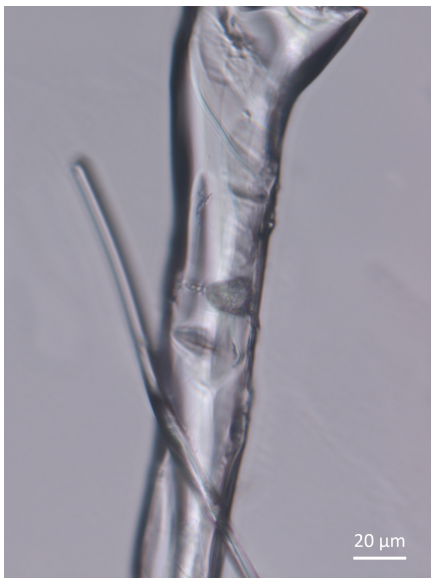

Figure 4.12: BAC impregnated fiber

be to impregnate fiber with BAC as done here. With a BAC content of $3.11wt\%$ this gives a estimated BAC content of $0.003321\frac{gBAC}{cm^2}$. Comparing this with the required concentration in Table 4.3 the concentration is above the required values of $0.002237288\frac{gBAC}{cm^2}$ and $0.0014095\frac{gBAC}{cm^2}$.

4.2.5 OCT content in fiber

Fiber samples leached in deuterated chloroform and deuterated water was analyzed using NMR and compared to references. Neither the sample in chloroform or heavy water did show any sign of OCT content. Since the concentration of OCT was expected to be low with a concentration below $0.35\frac{mg}{mlDeuteratedChloroform}$ and $0.23\frac{mg}{mlDeuteratedWater}$ it is possible that the NMR signal was too weak to be detected. It is also possible that the solubility of OCT in deuterated water and deuterated chloroform wasn't high enough to leach OCT. If there was any OCT in the fiber and the spheres could not be concluded with this experiment.

4.3 Solubility of OCT in water and PVA solution

A high solubility is expected to lead to higher leakage to the water phases form the polymer spheres, and are therefore an important factor to analyze. The solubility of OCT was investigated by the preparation of three samples. One reference and two solutions with oversaturated amounts of OCT, see chapter 3.4.2. By comparing the NMR signal area of the reference and the two oversaturated samples concentrations could be calculated and are compiled in Table 4.4.

Sample	Concentration $\frac{g}{ml}$
Reference sample, OCT in heavy water, dissolved	0.00395
Oversaturated amount of OCT in 5wt% PVA heavy water solution	0.00332
Oversaturated amount of OCT in heavy water solution	0.0125

Table 4.4: OCT concentrations in a heavy water reference, a 5wt% PVA heavy water and a heavy water sample.

The water solubility calculated of $0.01245\frac{g}{ml}$ is much higher than the one found in literature of $2.6 * 10^{-7}\frac{g}{ml}$[17]. A high water solubility may be a problem since the encapsulation efficiency should decrease with increased water solubility of the active substance[43]. An increased water solubility does also increase the release rate of active substance to the water phase as discussed in chapter 2.4. The reason OCT shows a lower solubility in the PVA solution may be an effect of oversaturation since the total concentration of solute is high.

4.4 Distribution coefficient of BAC and OCT between polymer and water phase

The idea behind this thesis was to encapsulate the active substances in polymer spheres and then in nonwoven fiber. This is done by the solvent evaporation method

and solution-blow spinning. In these steps, it is assumed that all or most of the active substance will stay in the polymer phase. Since BAC is very water-soluble it was interesting to see how it would distribute between the polymer and water phase. As discussed in chapter 4.3, OCT had a higher water solubility then suggested by the literature, and it is, therefore, interesting to investigate considering a distribution coefficient between the polymer and water phase.

4.4.1 Distribution coefficient for BAC

A detailed description of the calculations of the values in this chapter can be seen in appendix A.3. The filter did not absorb any active substance and did therefore not influence the results. A distribution coefficient of $K = 2.86$ could be calculated with equation 4.1.

$$K = \frac{mBAC_{in}Spheres * mWaterPhase}{mBAC_{in}WaterPhase * mSpheres} = 2.86 \tag{4.1}$$

A distribution coefficient of 2.86 indicates a slight affinity of the BAC to the polymer phase. If this was not the case, it would be impossible to create spheres with a drug load since it leads to a low drug encapsulation efficiency and the release from the spheres would be instantaneous in contact with water. Considering the large amount of water used in the sphere formulation and fiber spinning compared to the polymer phase the calculated distribution coefficient is probably to low too yield a fiber with spheres containing active substance. While it seems like BAC has an affinity to the spheres, it may be explained by the surface activity of BAC. It is possible that BAC resides on the surface of the spheres instead of inside it. The concentration of PVA should be greater at the surface of the spheres and it is possible that BAC binds to the PVA molecules. In the fiber spinning process, a coagulation bath and a wash bath is used. To counteract the diffusion of BAC from the spheres to the water phase, the coagulation and wash bath can be equilibrated with the polymer phase according to the distribution coefficient in equation 4.2.

$$mBAC_{in}WaterPhase = \frac{mBAC_{in}Spheres * mWaterPhase}{K * mSpheres} \tag{4.2}$$

For a standard sample of 18g fiber with 1.8g spheres and 0.18g BAC, a total amount of around 360g BAC would have to be used to equlibirate the water phases. When comparing the amount of BAC inside the spheres and the amount in the water phase this procedure seem to be very inefficient.

4.4.2 Distribution coefficient for OCT

The process to determine the distribution coefficient for OCT between the polymer and water phase is similar to chapter 4.4.1 and the calculations can be seen in appendix A.4. A distribution coefficient of $K = 6.23$ is calculated with equation 4.3.

$$K = \frac{mOCT_{in}Spheres * mWaterPhase}{mOCT_{in}Water * mSpheres} = 6.23 \tag{4.3}$$

A distribution of $K = 6.23$ indicates an affinity to the polymer phase and compared to BAC, OCT has a larger affinity to the polymer phase. This is also expected since the water solubility of OCT is lower. The surface activity of OCT is larger than the one of BAC and may explain the larger distribution coefficient compared to BAC. OCT may in a larger degree be attracted to and pack well at the surfaces of the spheres. In this case, the surface may not only be the external surface but the internal surface created by the porous structure. For a standard sample of $18g$ fiber with $1.8g$ spheres containing $0.18g$ OCT a total amount of around $170g$ OCT would have to be used calculated with equation 4.2. This if OCT in the water phase in the emulsion, coagulation and wash bath would be in equilibrium with OCT in the polymer phase according to the distribution coefficient. As for BAC, the distribution coefficient seems to be too low to achieve an efficient encapsulation, fiber spinning and to yield a product containing spheres with adequate amounts of active substance.

4.5 Leakage of active substance

The coagulation bath of a batch with OCT sphere fibers, and the coagulation and wash bath for the batch of BAC impregnated fibers was investigated with LC-MS. The result can be seen in Table 4.5

Sample	Concentration $[\frac{g}{dm^3}]$
OCT:Coagulation bath	Below limit of detection
BAC:Coagulation bath before spinning	0.10091
BAC:Coagulation bath after spinning	0.02514
BAC:Wash bath before wash	0.03085
BAC:Wash bath after wash	0.02782

Table 4.5: Concentrations of OCT or BAC in the coagulation and wash bath. Measured with LC-MS.

The OCT sample was below the limit of detection of $4.4 * 10^{-4} \frac{g}{dm^3}$, with the conclusion that the concentration is below this value. In this case, this would mean a total amount of $7.92 * 10^{-4} g$ OCT since a $1.8L$ coagulation bath was used. This can be compared with the theoretical amount of OCT that should have been inside the fiber of $0.025g$ OCT. As concluded in chapter 4.4.2 the amount of OCT in the fiber is probably much lower than the theoretical. It is therefore hard to determine the reason for the low concentration of OCT in the coagulation bath. Low leakage or no leakage since there is no OCT from the start are both valid explanations. The BAC concentration decreases in both the coagulation and wash bath after spinning and washing. This indicates that the fiber absorbs BAC and therefore decreases the amount in the water phases. There are reasons to take the exact values from the LC-MS with care. This since the concentration in the coagulation and wash bath should have been $1.94 \frac{g}{dm^3}$ before the spinning and washing.

5
Conclusion

The purpose of this thesis was to investigate the possibilities to incorporate spheres containing antiseptic agents in nonwoven fiber materials. This would enable bandages with increased durability and decrease the risk of multidrug resistant bacteria strains. To reach the main goal some sub-goals and questions had to be answered.

- It was possible to formulate polymer microspheres with BAC and OCT content. The content in itself would probably not be enough to reach the desired minimal inhibitory concentration (MIC) in a wound. The reason is leakage of active substance to the water phase, making the encapsulation and spinning process inefficient. A solution to this could be to impregnate fiber with active substance, which was done for BAC where a sufficient drug content was reached.
- It was concluded that a high PVA concentration of 5wt% was beneficial for the stability of the suspension. Larger spheres are beneficial in the fiber spinning step since it is easier to centrifuge larger spheres. Larger spheres should also have slower release properties. The best way to formulate large spheres was with the vortex method, i.e. a very short stirring time. The concentration of the active substances did influence the interfacial tension since they are surface active agents. This leads to decreased sphere size at increased active substance concentration.
- PLGA, PLLA, and SASanhydride did all seem to be promising polymer to create spheres containing active agents. Whereas one of them is preferred over the others was difficult to determine but, PLLA was less eager to aggregate and did not hydrolysis.
- It was possible to incorporate spheres in nonwoven material with solution-blow spinning. The spheres were well distributed in the fiber material and were visible with microscopy.

5.1 Future works

Whereas some questions had been answered there is still work to be done. The combination of spheres with BAC content and BAC impregnated fiber was not tested but should be. It would be beneficial to find a way to formulate spheres and incorporate them in fibers with reduced water amount contact since it would reduce leakage of active substance to the water phase. A way to achieve this would be to incorporate the spheres in the fiber after the spinning and washing step since this probably is the step in the production where the largest leakage is occurring, due to the large water phases. Another way to reduce leakage would be to find

other active substances with a higher distribution coefficient, shifted to the polymer phase. Since none of the polymers was found to have any large advantage over the others more research should be put into finding differences in their properties. Where the release properties of spheres created of different polymers is a major factor. The morphology should have a great impact on the release, drug load and efficiency of the spheres. It would be interesting to investigate which factors affect the morphology of the spheres and how the morphology of the sphere, in turn, affect the drug release system.

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A
Appendix

A.1 Calculation of active substance per fiber area

The amount of active substance that is incorporated in the fiber material can be calculated and compared with the values in Table 4.3. If the fiber material weighs $18g$ and has a sphere content of 10wt% of that, there is $1.8g$ spheres. These spheres have an active substance of 10wt% of that as standard, meaning $0.18g$ active substance. The fiber material would have a total area of around $100cm^2$. This gives an amount of $0.0018\frac{g}{cm^2}$.

A.2 Calculations for BAC impregnated fiber

A piece of fiber had been leached in $1ml$ of deuterated water and then tested in NMR. As a reference, the reference sample without filtration seen in Table A.1 was used. The peak area for the leached sample came in as 17% of the reference sample, i.e. 722 compared to 123. This gives a concentration of $0.311\frac{g}{dm^3}$ or $4.99 * 10^{-4}\frac{moles}{dm^3}$. Assuming all BAC in the fiber had been leached, the BAC in the water phase can be attributed to the fiber piece of $0.01g$. This gives the fiber a BAC content of $3.11wt\%$. Since the fiber piece was weighted before and after it was dried a water content could be estimated. The water content was around 90wt% of the fiber after it came out of the wash bath. The dry fiber weighed $10.681g$ indicating a water content of around $90g$ after the wash bath. The wash bath had a concentration of $0.00194\frac{gBAC}{gWater}$. The water in the washed fiber should in total contain $0.1746g$ BAC. When the water evaporated BAC is assumed to be left in the dry fiber. The concentration of BAC in the fiber caused by evaporated wash bath is $\frac{0.1746gBAC}{10.681gFiber} = 1.64wt\%$. The rest of the $3.11wt\%$, the $1.48wt\%$ can be attributed to the BAC dissolved in the dope solution. This fiber weighted $10.681g$, assuming a total area of $100cm^2$, there is $0.10681\frac{gFiber}{cm^2}$. With a BAC content of $3.11wt\%$ this gives a BAC content of $0.003321\frac{gBAC}{cm^2}$.

Sample name	Peak area
Reference, no filter	722
Reference, filter	729
Suspension supernatant, filter	683

Table A.1: Peak area of the aromatic signal peak from the NMR spectra

A.3 Calculations of the distribution coefficient for BAC

The suspension with spheres had been filtered and it was assumed that all the spheres were caught in the filter. This would leave the water phase with dissolved BAC in the supernatant. The BAC residing in the spheres and the water phase would, therefore, be separated and a distribution between the polymer and water phase could be calculated. The NMR signal peak area of the aromatic groups was compared to each other in Table A.1. The two reference samples show a similar result, implying the filter does not absorb BAC and will therefore not decrease the concentration of BAC in the liquid after filtration. The averaged signal peak area for the two references was 725.5. The area of the signal is directly proportional to the concentration in the sample. The signal from the supernatant was 94.14% of the reference which means the concentration was 94.14% of the one in the reference. The concentration in the reference was 0.00183 g/ml and consequently, the concentration in the supernatant was 0.00172 g/ml. A water phase of $5.41ml$ gives a total weight of $0.00932g$ BAC in the water phase. This leaves $0.000480g$ BAC in the PLLA polymer since a total of $0.00980g$ BAC was used in the formulation. With a PLLA weight of $0.0975g$ a distribution coefficient could be calculated with equation A.1.

$$K = \frac{mBAC_{in}Spheres * mWaterPhase}{mBAC_{in}WaterPhase * mSpheres} = \frac{0.000480 * 5.41}{0.00932 * 0.0975} = 2.86 \qquad \text{(A.1)}$$

A.4 Calculations of the distribution coefficient for OCT

The process to determine the distribution coefficient for OCT between the polymer and water phase is similar to chapter 4.4.1. The concentration in the reference sample was $0.00395\frac{gOCT}{ml}$ and the NMR signal of the filtered sphere suspension 42% of this, equal to a concentration of $0.001659\frac{gOCT}{ml}$. With a volume of $5.41ml$ in the suspension the water phase contains $0.00897519g$ OCT, leaving $0.00102481g$ OCT in the polymer phase of $0.0992g$ PLLA. With these numbers, a distribution coefficient of $K = 6.23$ is calculated with equation A.2.

$$K = \frac{mOCT_{in}Spheres * mWaterphase}{mOCT_{in}Water * mSpheres} = \frac{0.00102481 * 5.41}{0.00897519 * 0.0992} = 6.23 \qquad \text{(A.2)}$$