CHALMERS
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Encapsulation and Controlled Release of Benzalkonium Chloride

Production of Antimicrobial Cellulose Nonwoven Fabrics for Wound Treatment

Master's thesis in Materials Chemistry

JAKOB KARLSSON

DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING
CHALMERS UNIVERSITY OF TECHNOLOGY
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Cover: Image of cellulose nonwoven fibre incorporated with microspheres produced during the project.

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Abstract

Encapsulation and controlled release of an active substance is sought-after due to its many applications, were one of them is chronic wound treatment. By having a continuous release of the antimicrobial agent the risk of infection is reduced and the lifetime of the wound dressing will be increased. The aim of this project is to encapsulate Benzyldimethylhexadecylammonium chloride (BAC) into polymeric microspheres to obtain a controlled release rate and then incorporate the microspheres into cellulose nonwoven fabrics. The microspheres are formulated using the internal phase separation via solvent evaporation methodology. Microscopic techniques are used to evaluate size and morphology of the microspheres. The encapsulation efficiency and release rate from the microspheres are evaluated both from microspheres suspensions and microspheres incorporated into fibres using UV-Vis spectroscopy and LC-MS.

Based on microscopic imagery does poly(D,L-lactic-co-glycolic acid) (PLGA) formulate microspheres of desired size, morphology and encapsulation efficiency. By adding NaCl to the continuous phase, encapsulation efficiency of BAC was increased along with an increased microsphere size, however long term stability of the microspheres was reduced. Due to BAC's poor solubility in water containing NaCl, does crystallisation of BAC occur in the continuous phase. Other factors effecting the encapsulation efficiency of BAC was type of solvent used, evaporation method and the amount of BAC added during formulation. Furthermore, incorporation of microspheres into cellulose nonwoven fabrics were successful, and the particles was well dispersed in the fibre.

The release of BAC from polymeric microspheres was slow, and it takes approximately 100 hours until a significant release can be seen. This was observed for release studies from both microsphere suspensions and microspheres incorporated into cellulose fabrics. The conclusion is then that a controlled and reduced release of BAC via microencapsulation can be obtained due to the polymeric material acting as a diffusion barrier.

Keywords: microsphere, internal phase separation method, controlled release, poly(d,l-lactic-co-glycolic acid), cellulose nonwovens by solution blowing.

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Jakob Karlsson, Gothenburg, June 2021

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1
Introduction

Due to an ageing population, the need to cure non-healing chronic wounds and specifically diabetes-related wounds has increased. A common complication from diabetes is the development of foot ulcers, and approximately 15% of all diabetes patients sustain ulcers in feet or legs [1]. These wounds have a risk of being infected and thus leading to severe consequences, such as amputation or death. One of the main treatments of chronic wounds is fabric or wound dressing impregnated with antimicrobial agents. By using impregnated wound dressings, the active substance is released quickly and can thereby kill all the bacteria present. However, long-term protection from infection can not be maintained resulting in continual replacement of the wound dressing to obtain adequate treatment [2].

Perpetual treatment can be achieved by encapsulation of the active substances into microspheres or microcapsules. Encapsulation of the antimicrobial agents results in a controlled and reduced release rate. The minimum inhibitory concentration (MIC) can then be maintained for a longer time, thus prolonging the active lifetime of the wound dressing. The slow release rate is due to the microspheres functioning as a barrier, through which the active substance needs to diffuse.

A group of active substances that has been used over the last century for their antimicrobial properties are quaternary ammonium compounds (QAC:s) [3]. The wide variety of usages, such as surfactants, dyes and most notably disinfectants, make QAC:s interesting for microencapsulation. Another aspect of using alternative antimicrobial agents is the looming threat of increasing antibiotic resistance in bacteria. By applying different ways to eliminate bacteria other than antibiotics the risk of multi-resistance is decreased. Therefore, to instead proceed with QAC:s can be a more sustainable path for antimicrobial activity. However, due to the stability and longevity the molecule, QAC resistance has also been building up at a higher pace [4]. To overcome this, less excessive concentrations has to be used while at the same time staying above the MIC. One way to achieve this is microencapsulation of the active substance.

Lastly, the biocompatibility of the microspheres must also be considered, as the shell polymer and the active substance is in contact with the skin. Formulation of microspheres comprised of biodegradable materials, the degradation will not produce harmful compounds for humans. By then integrating the microspheres into nonwoven fabric, the fabric can act as one of the layers in wound dressings, thus reducing the risk of infection.

1.1 Aims

The aim of the project is to encapsulate a antimicrobial agent into a polymer microsphere to obtain a controlled and reduced release rate, and then incorporate the microspheres into a nonwoven fibre. To formulate the microspheres, the internal phase separation by solvent evaporation methodology will be used. It is important that the microspheres have high stability over time, while at the same time being able to biodegrade. The morphology and size of the microspheres will be evaluated for multiple polymer materials, until satisfactory formation is obtained. Meanwhile, the release rate of the active substance is of utter importance and will be examined for the different microspheres. It is vital that the release rate causes the concentration to be above the inhibitory minimum concentration, and simultaneously be as low as possible.

Lastly, the microspheres will be integrated into solution blown nonwoven fibres. Morphology of the spheres will be examined along with the dispersion of the particles in the fibres to ensure even release of active substance. The release rate will also be measured from the nonwoven fibres.

1.2 Limitations

Focus of the project will mainly be to formulate polymer microspheres and incorporate them into nonwoven fibre, then measure the release rate to obtain a satisfactory concentration over time. However, only a limited amount of polymer materials and active substances will be examined due to the project's scope. The materials must be biocompatible and biodegradable, and therefore only known materials with those properties will be used.

Benzyldimethylhexadecylammonium chloride (BAC) is the active substance that will be used in this project. The anti-microbial effect will be solely assessed on the release rate of the active substance. Therefore, any microbiological test to examine the anti-microbial effect of the nonwoven fibres with microspheres will not be performed, as it is not in the time frame and thus scope of the project.

The used nonwoven fibres will be solution blown as it exhibit properties where the microspheres are dispersed and intact. The material used to spin the fibres will be cellulose, due to its renewability and biodegradability. Other techniques such as wet spinning will not be evaluated in the thesis project.

2

Theory

2.1 Internal Phase Separation by Solvent Evaporation

Formulation of microspheres and also microcapsules can be done on the basis of several methodologies, such as spray-drying, self-assembly by building layer by layer or phase separation based techniques[5, 6]. Phase separation techniques can work variously, where the separation can occur due to physical (temperature, pH, salinity) or chemical (polymerisation, emulsion or suspension) differences. One specific phase separation technique is the internal phase separation by solvent evaporation developed by Vincent and Loxley [7].This technique gained traction due to its simple methodology, only using physical phase separation with no chemical reactions. The method is based on using an oil-in-water emulsion, illustrated in Figure 2.1, where the oil phase consists of a polymer, a good solvent for the polymer, a poor solvent for the polymer and normally an active substance. The good solvent should have only partial water solubility and be volatile e.g. chloroform or dichloromethane (DCM), thus being easily evaporated.

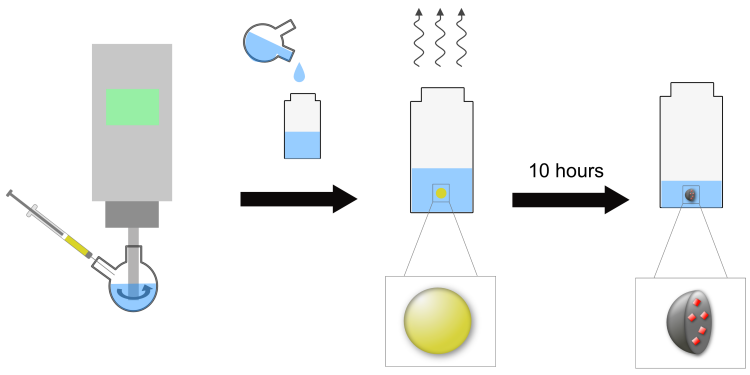


Figure 2.1: Overview of the formulation of microspheres. An oil-in-water emulsion is formed under high-speed stirring. The volatile solvent is then evaporated, until microspheres are formed.

However, the poor solvent should not be volatile or dissolve the polymer, but be soluble in the good solvent [7]. Example of such molecules are longer alkanes or triglycerides. The active substance should preferably be well soluble in the poor solvent. When all components are dissolved into an oil phase, it is added to an aqueous solution with surface-active stabilisers under high-speed stirring. After an emulsion is formed, the volatile solvent is allowed to evaporate under gentle stirring from the emulsion droplets, either under reduced or at ambient pressure, through the continuous water phase. This will cause a phase separation of the polymer and bad solvent when enough volatile solvent has evaporated, thus creating a micro-particle that can consist of a polymer shell and a liquid core containing the poor solvent.

Creation of microspheres without liquid core using internal phase separation by solvent evaporation can be done in analogous fashion [6]. The main difference is the exclusion of a poor solvent, resulting in a microsphere consisting of only polymer. In this case, the active substance has to show high affinity towards the polymer and low solubility to the surrounding water-phase to obtain successful encapsulation [8, 9]. The focus in this project will be formulation of microspheres, containing an active substance.

2.1.1 Polymeric materials

A desirable polymer has to be biocompatible and biodegradable to be applicable in this project. Other properties, for example stability and slow or controlled degradability over time is also of interest. To determine the most suitable one for the used active substance, four different polymers was utilised. The properties of the polymers are described in the upcoming section.

2.1.1.1 Poly(lactic acid)

A polymer which is well studied for microsphere formulation is poly(lactic acid) (PLA). PLA can form both amorphous and semicrystalline structures, depending on its chemical configuration [10]. A semicrystalline version of PLA is called poly(L-lactic acid) (PLLA) due to its stereospecific properties. To view the chemical structure of PLLA, see Figure 2.2. Due to the semicrystalline structure of the polymer, the release rate from PLLA microspheres has been shown to be slow. However, microspheres formulated from PLLA has still been studied in applications for drug release and cancer treatment [11, 12].

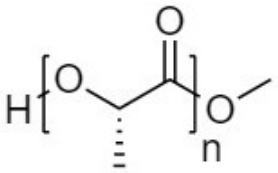


Figure 2.2: Chemical structure of PLLA.

2.1.1.2 Poly(lactic-co-glycolic acid)

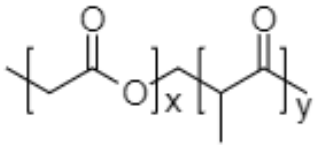


Figure 2.3: Chemical structure of poly(lactic-co-glycolic acid).

Another polymer similar to PLA is poly(lactic-co-glycolic acid) (PLGA), see Figure 2.3 [13]. PLGA is amorphous and does not form semicrystalline structures. The main mechanism of degradation for these polymers is hydrolysis, and it is heavily dependent on the lactic acid/glycolic acid ratio [14]. Larger fraction of lactic acid causes slower degradation due to a more hydrophobic polymer. Other factors that effects the degradation is the addition of additives (acidic, basic), morphology and porosity of the microspheres. Polymers consisting of only glycolic acid are possible, however these compounds have low solubility in common organic solvents (e.g. chloroform and dichloromethane) due to its high crystallinity [15, 16] making it less suitable for microsphere formulation.

2.1.1.3 Polycaprolactone

A polymer which has gained interest for microsphere formulation due to its biodegradeability is polycaprolactone (PCL) [17]. The polymer is a semicrystalline linear aliphatic ester [18] and the molecular structure is shown in Figure 2.4. PCL have relative slow degradation compared to PLGA, which can be of interest for drug delivery. The degradation does neither result in an acidic environment and the products from degradation is soluble in bodily fluids [18].

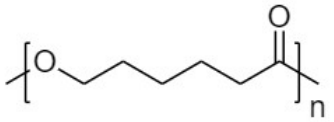


Figure 2.4: Chemical structure of PCL.

2.1.1.4 Cellulose triacetate

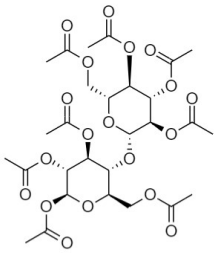


Figure 2.5: Chemical structure of CTA.

This polymer is a derivate of cellulose and one of its most important esters, see Figure 2.5[19]. The applications for cellulose triacetate (CTA) are many (microspheres, films, membranes) depending on how it has been processed. What makes CTA applicable for microsphere formulation is the low cost of the material and its non-toxicity [20]. The microspheres also gain high surface area when formulated from CTA due to high porosity. Lastly, CTA microsphere for drug release to treat diabetes has also been studied [21].

2.1.2 Benzyldimethylhexadecylammonium chloride

The active substance used in this project is benzyldimethylhexadecylammonium chloride (BAC), which is an quaternary ammonium compound (QAC), consisting of a benzyl group and an alkyl chain attached to the ammonium ion [4]. There are multiple variations of BAC, where the difference is the size of the alkyl chain, with n=5-15, as illustrated in Figure 2.6. Depending on the alkyl chain length, it will change the solubility of the compound in water and its desire to form micelles. The molecule does generally form micelles due to its amphipilic nature [4]. Another aspect that effects the solubility is the counterion, which in the case of BAC is a chloride ion. Most QAC:s use chloride or bromide as counterion, as larger ions such as iodine demonstrate lower solubility .

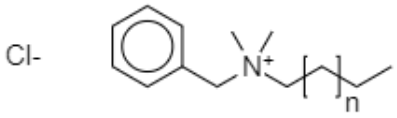


Figure 2.6: Chemical structure of BAC.

The antimicrobial effect of QAC:s was first discovered back in 1916, when Jacobs published conclusions pointing at the antiseptic properties of the quaternary ammonium ion [22, 23, 24]. Since then the applications in the commercial, industrial and healthcare sector have increased as well as focus from the academic world [25]. One of the main questions regarding the antimicrobial effects of QAC:s is the mechanism of action. As earlier concluded, the positive ammonium ion can interact with the negatively charged cellular membrane of bacteria. This will lead to breakage in the membrane causing cytoplasm leakage and ultimately lysis of the cell. The possibility of the ion to target the cellular membrane of bacteria gives QAC:s the ability to neutralise both gram-positive and gram-negative bacteria. However, QAC:s has shown to have higher efficiency versus gram-positive bacteria due to the inherent property of only having one cellular membrane, compared to gram-negative which has two [26].

2.2 Solution blowing for cellulose nonwovens

Solution blowing is a technique to produce nonwoven fibres from a polymer solution. Traditionally, a similar technique called melt-blowing has been used where the raw material is petroleum based, resulting in a product that is non-renewable [27]. By instead using renewable polymers, such as cellulose, the obtained product will contribute to a more sustainable society. Cellulose is one of the most used raw materials polymers that is considered biodegradeable and renewable [28]. One downside with using cellulose is its intermolecular bonding pattern resulting in a stable supramolecular structure that is hard to dissolve. To dissolve it, volatile and toxic solvents has traditionally been used, but have over time been exchanged for more sustainable products, such as N-methylmorpholine N-oxide monohydrate (NMMO). However, even NMMO has its downside, such as its potential for side reactions and high viscosity, and therefore has ionic liquids (IL) gained interest

as a green solvent for cellulose. These solvents have low vapour pressure, high thermal stability and most importantly great dissolution capabilities making them attractive for fibre spinning. Some of the most commonly studied IL:s are 1-allyl-3-methylimidazolium chloride ([AMIM]Cl) [29] and 1-ethyl-3-methylimidazolium acetate ([EMIM]Ac) [27, 28, 30]

The technique can be described as a combination of melt blowing and electrospinning, thus being used as an alternative to those methods to produce nonwoven fibres [31]. By using a pump to move the dope solution to a inner nozzle, the liquid can be pushed out and then exposed to a high velocity gas from an outer nozzle [32]. The gas is pumped at high pressure, thus gaining high speed at the nozzle due to a quick drop in surrounding pressure. This causes a pressure drop near the inner nozzle, also increasing the velocity of the polymer solution. Another purpose of the gas is to induce shearing at the interface between gas and dope solution resulting in conical formation of the solution leaving the inner nozzle. Thin streams of the liquid with dissolved polymer will then form, that can be collected onto a rotating cylinder in a water bath, as seen in Figure 2.7 [27, 31].

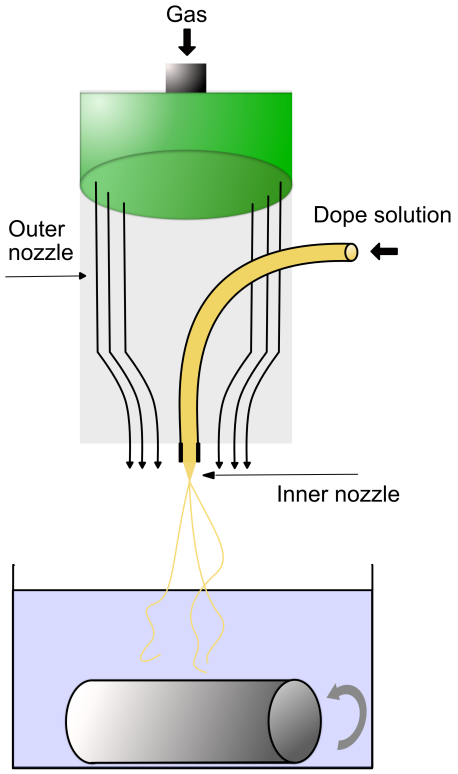


Figure 2.7: Illustration of the solution blowing technique. Dope solution is pumped through a inner nozzle, surrounded by outer nozzles with high-velocity gas. The fibres are then collected in a water bath.

The produced nonwoven fibres will have a diameter ranging in the micro- and nanoscale depending on the used polymer [31]. However, it is not only the polymer that effects the outcome of the fibre; gas pressure, solution flow, temperature and

distance to collector does also play a part [27]. With increased gas pressure and temperature, the produced fibres will be thinner while a higher solution flow leads to thicker fibres.

2.3 Analytical techniques

To be able to quantify the amount of active substance released from the microspheres and nonwoven fibres a detection method must be used. There are multiple detection methods depending on the analysed substance, however in this study two techniques are employed; UV-Vis spectroscopy and liquid chromatography-mass spectroscopy (LC-MS).

2.3.1 UV-Vis spectroscopy

This detection method is an optical spectroscopy technique based on the Lambert-Beer law, see Equation 2.1. Lambert-Beer law expresses a linear relationship between the absorbance of light and concentration of the analyte [33]. This works due to different molecular structures absorbs light at separate wavelengths, thus being possible to tell compounds apart.

$$A = \varepsilon cl \tag{2.1}$$

A is the absorbance calculated from A = -$\log_{10} I/I_0$, where I_0 is the initial light intensity and I is the light intensity after passing through the sample [33]. ε is the molar absorptivity coefficient, which is distinct for each compound at a specific wavelength. c is the concentration of the analyte and l is the length that light passes through the sample. The resulting spectrum obtained from the UV-Vis spectroscopy shows the absorbance plotted against a range of wavelengths. Example of an UV-Vis spectrum can be seen in Figure 2.8.

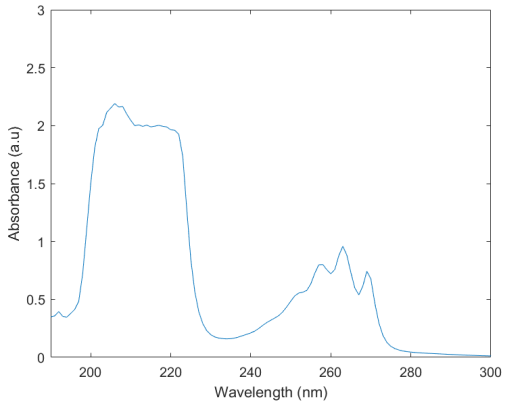
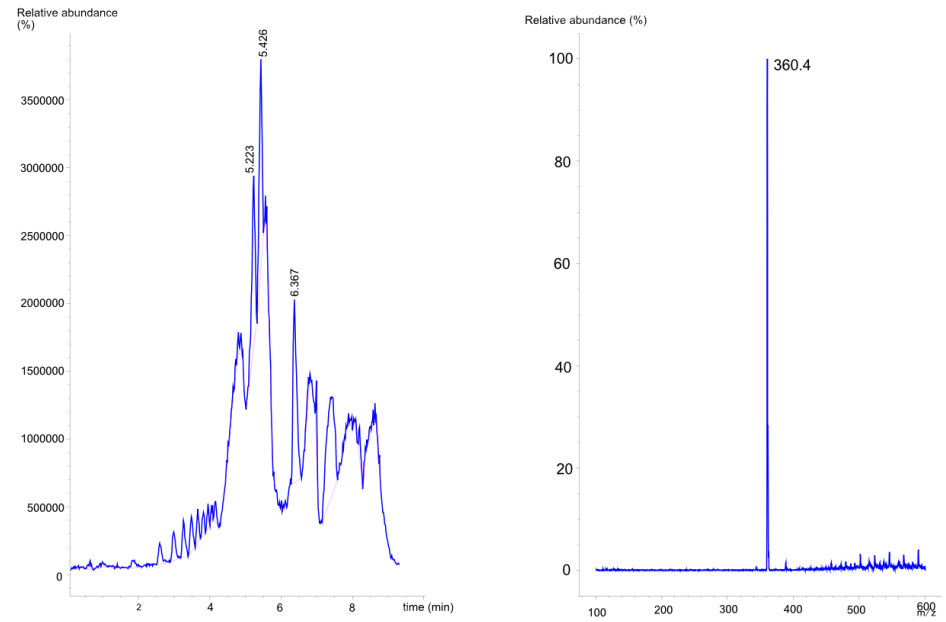


Figure 2.8: Example of a UV-Vis spectrum. The three peaks at 259 nm, 263 nm and 268 nm are typical wavelengths for BAC to absorb at.

2.3.2 Liquid Chromatography-Mass Spectroscopy

This technique combines high-performance liquid chromatography (HPLC) and mass spectroscopy (MS) to be able to quantify and qualitatively determine isolated substances [34, 35]. Each of the two parts have different roles in the liquid chromatography-mass spectroscopy (LC-MS) setup, where HPLC can separate components from an complex mixture [36]. The HPLC is a sensitive and reproducible instrument resulting in well separated solutions, where the output is an easy to understand chromatogram where elution time is plotted against relative concentration of the analytes, see Figure 2.9. Separation happens due to a liquid phase containing the analytes (mobile phase) being pushed through a colon (stationary phase) with sorbents [37]. The analytes will attach to the stationary phase differently causing a separation over time.

The next step in LC-MS is the mass spectroscopy, where the quantification and qualitative analysis of compounds occur. Firstly, the compounds has to be ionised through one of multiple methods, where one of the most common one is electronspray ionisation (ESI)[36, 38, 39].The ionised analytes then travel through an environment that will selectively let through ions of the the correct mass to charge ratio (m/z) [40]. This selection can be caused by magnetic or electrical fields, e.g. by a quadrupole. Lastly, the charged compounds will reach the mass detector that generates a mass spectrum with m/z on the x-axis and relative intensity on the y-axis, see Figure 2.9.



(a) **(b)**

Figure 2.9: (a) Example of a chromatogram obtained from LC-MS where each peak represent a separate analyte. (b) Example of mass spectrum from LC-MS. The isolated compound is detected at m/z=360.4

3

Materials and Methods

3.1 Materials

Dichloromethane (Sigma-Aldrich), chloroform ($CHCl_3$, ≥99%, Sigma-Aldrich), poly(vinyl alcohol) (95% hydrolysed, $M_w = 95\,000$, Acros Organics), Brij® L23 (Sigma-Aldrich), sodium chloride (≥99%, Sigma-Aldrich) and Benzyldimethylhexadecylammonium chloride (Sigma-Aldrich) was used in the project as received. During the project Milli-Q® water (18.2 MΩ cm, Millipore) was used. Polymeric materials used in the project was poly(D,L-lactide-co-glycolide) (70:30, $M_w \sim 10\,000$, Polysciences, Inc.), poly(D,L-lactide-co-glycolide) (75:25, $M_w \sim 97\,000$, Polysciences, Inc.), poly(L-lactic acid) ($M_w \sim 80\,000$ - 100 000, Polysciences, Inc.), polycaprolactone ($M_n \sim 45\,000$, Sigma-Aldrich) and cellulose triacetate (Sigma-Aldrich). Materials used for the fibre spinning was Avicel® PH-101 (∼50 μm particle size, Sigma-Aldrich) and 1-Ethyl-3-methylimidazolium acetate (≥98%, Proionic).

3.2 Microsphere formulation

For emulsification a *Polytron PT 3100 D* with *PT-DA 07/2EC-F101* as dispersing aggregate (Kinematica, Switzerland) was used. To get an overview of the formulation process, see Figure 2.1. The process took place in a 5 ml round bottom flask with a side neck (Ace glass, USA). There were five separate polymers analysed; PLGA ($M_w \sim 97\,000$), PLGA ($M_w \sim 10\,000$), PLLA, PCL and CTA. Firstly, 2.5 ml of 1 wt% PVA Milli-Q® water was added to the round bottom flask. Depending on the experiment, 300 mM NaCl 1 wt% PVA Milli-Q® water was used instead of 1 wt% PVA Milli-Q® water, details used for each individual batch, see Table A.1 in Appendix A. The oil phase consisting of 0.1 g polymer and BAC dissolved in DCM or chloroform was then drop wise added to the water over circa 2 minutes. Volume of the volatile solvent was 1.8 ml for all batches. Thereafter, emulsification was carried out for 60 minutes at a constant stirring speed. After an emulsion was formed, it was further diluted with 2.5 ml of 1 wt% PVA Milli-Q® water or 300 mM NaCl 1 wt% PVA Milli-Q® water. To form the microspheres, the volatile solvent was evaporated for at least 12 hours in a fume hood under magnetic stirring or with rotary evaporation at room temperature for DCM or 40 °C for chloroform at reduced pressure for 30 minutes.

3.3 Microscopy analysis

To visualize the microspheres, an Axio Imager Z2m (Zeiss, Germany) was used. Three different microscopy techniques were applied; brightfield (BF) illumination, differential interference contrast (DIC) illumination and polarised light. To confirm successful emulsification, BF illumination was used. The images obtained from BF and DIC illumination was also used to determine the size and morphology of the microspheres. Usage of polarised filter was due to detect the presence of crystalline materials in the microsphere suspension.

3.4 BAC concentration at equilibrium

To calculate the encapsulation yield of BAC, the concentration of the active substance in the continuous phase and inside the microspheres had to be determined. This was done by measuring the absorbance of BAC with UV-Vis spectroscopy in the microsphere suspension, thus being able to calculate the concentration in the continuous phase. Firstly, 1.5 ml of suspension had to be centrifuged at 17 000 g using a VWR Micro Star 17 (Avantor, USA) to remove polymeric material, and then the supernatant could be analysed. The instrument used for UV-Vis spectroscopy was an Agilent 8453 UV-Vis spectrophotometer that absorbs between the wavelengths 190 nm - 1100 nm. BAC absorbs at three separate wavelengths; 259 nm, 263 nm and 269 nm (see Figure 2.9), where the absorption at 263 nm was used to calculate the concentration. A calibration curve for BAC was performed and the linear region was between 0.01 g/L and 1 g/L, therefore resulting in the samples being diluted to be within that region. The calibration curve is shown in Appendix B Figure B.1.

3.5 Release study of microsphere suspension

For the release measurements, 2 ml of microsphere suspension was pipetted into 198 ml Milli-Q® water contaning 6 wt% Brij® L23 at room temperature. The Brij® L23 is in the release bath to be able to compare the release of BAC to the release of more hydrophobic active substances that need Brij® L23 to be soluble in water. The volumes was used to be within the linear regime of the calibration curve. After predetermined amounts of time, circa 1.5 ml of the release bath was put into a 1.5 ml Eppendorf-tube and then centrifuged for 1 minute at 17 000 g. This causes the microspheres and polymeric materials to be at the bottom of the Eppendorf-tube and the supernatant containing BAC was then pipetted to a 2 ml glass vial. This suspension consisting of BAC, Brij® L23 and Milli-Q® water was then analysed by LC-MS to quantify the amounts of BAC.

The LC-MS system used was a combination of a HPLC and mass analyser. For the HPLC, a 1290 Infinity LC System (Agilent Technologies, USA) was used in combination with a Infinitylab Poroshell 120 EC-C_{18} (Agilent Technologies, USA) 3.0 $\times$ 50 mm (2.7 μm pore size) reversed-phase column. The mass analyser used was a 6120 Single Quadrupole LC/MS (Agilent Technologies, USA) equipped with

electronspray ionisation (ESI). A combination of two mobile phases was used for the HPLC. Mobile phase A consisted of 99.96% Milli-Q® water with 0.04% formic acid and mobile phase B was composed of 99.96% LC-MS grade methanol with 0.04% formic acid. The flow rate was to 0.4 ml/min for all samples and the injection volume was set to 1 μl. For the gradient used, see Table 3.1.

Table 3.1: The gradient of mobile phases used for each sample. The total analysis of each sample was 10 minutes.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
00.00	95	5
05.00	5	95
07.00	5	95
07.01	95	5
10.00	95	5

To determine the concentration of BAC in the samples, a calibration curve was done simultaneously in the same LC-MS run. The linear region of the calibration curved was between 0.001 g/L and 0.03 g/L, and therefore the samples were diluted to be within that range. Calibration curves used for the LC-MS can be seen in Figure B.2 Appendix B. The observed elution time of BAC in the LC was circa 6.4 minutes and to determine the exact concentration the function "extract ion" was used to isolate BAC:s molecular weight of 360.4 Da plus 361.4 Da. This function makes it easier to determine the peak area of that specific molecular weight, and thus excluding signals from other compounds. To view examples of chromatograms and mass-spectra, see Appendix B

3.6 Solution blowing

To incorporate the microspheres inside the cellulose fibres, the solution blowing technique was employed. Two batches of fibres were produced, one fibre containing microspheres and one fibre without. The latter would afterwards be impregnated with BAC, and then the release between the to fibres could be compared. Initially, 12 g of cellulose was dried in an oven at 70°C for 1 hour. Thereafter, the cellulose was dissolved in 146 g of 1-Ethyl-3-methylimidazolium acetate ([EMIM]Ac) under stirring. The temperature of the solution was then increased to 70 °C in an oil bath for circa 1 hour. After the cellulose had dissolved, the solution was cooled down at room temperature and then split into two batches. The compositions of the two batches is shown in Table 3.2. Beforehand, the microsphere suspension was centrifuged at 4000 rpm for 10 minutes and the supernatant was removed. The compact mass of microspheres from the bottom of the tube used at centrifugation was then dispersed in the [EMIM]Ac/cellulose solution.

Table 3.2: The contents of the two batches used to spin cellulosic fibres.

Batch	Cellulose (g)	[EMIM]Ac (g)	Microspheres (g)	BAC (g)
1	4	48.7	0.201	0.0107
2	8	97.3	0	0

Next, centrifugation of the batches where done at 2000 rpm for 10 minutes to remove air from the samples. Lastly, the batches was added to the pump to spin the fibres using the solution blowing technique described in Section 2.2. For batch 1, 200 mM of NaCl was added to the coagulation bath of 3 litres, while for batch 2, 4 litres of distilled water was used. NaCl was added to batch 1 to decrease the solubility of BAC in the coagulation bath, and therefore minimise the loss of BAC in the bath. Afterwards was the fibres quickly washed in distilled water for 10 minutes and then dried at room temperature in a fume hood over night.

3.7 Release study of cellulose fibre

The last step was to determine the release of BAC from the microspheres incorporated into the cellulose nonwoven fibre. This was done by weighing 1,4539 g of fibre containing microspheres (Batch 1) and cut into 3 smaller pieces. The fibre pieces was then added into a release bath consiting of 100 ml Milli-Q® water containing 6 wt% Brij® L23 at room temperature. Samples of 1.5 ml was then pipetted at allotted time stamps and centrifuged for 1 minute at 17 000 g. Thereafter was the samples analysed using LC-MS with the same setup as described in Section 3.5.

A comparison was also done with the release from a fibre impregnated with BAC (Batch 2). The impregnation was done by first washing the fibre for 2 hours in distilled water to remove the [EMIM]Ac in the fibre. This was not done with the fibre incorporated with microspheres to reduce the loss of BAC from washing. Afterwards, 0.002 g of BAC was dissolved in 2.25 ml DCM and then dripped onto a fibre piece weighing 1.595 g. The DCM was then let to evaporate from the fibre for 1 hour in a fume hood. Lastly, the fibre was added into 100 ml Milli-Q® water containing 6 wt% Brij® L23 at room temperature to see the release of BAC. Samples was taken at the same time stamps as for the fibre containing microspheres, and then centrifuged at 17 000 g for 1 minute. Once again was the samples analysed using LC-MS with same setup as Section 3.5

4

Results and Discussion

This section will present the result in three separate sections. Firstly, micrographs of the formulated microspheres are presented. Afterwards, the results from the release from the microsphere suspension is discussed. From there on, microsphere incorporation into fibres and the release of BAC from the fibres are presented.

4.1 Formulation of microspheres

When evaluating the formulated microspheres there are three parameters considered; the encapsulation efficiency of BAC, the size and the morphology of the microspheres. Desired result is an even size distribution of the microspheres, with a diameter of approximately 10 µm, and a uniform morphology. Furthermore, a successful formulation of microspheres has a high encapsulation efficiency of BAC. To obtain the sought-after results, six variables were scrutinised. These variables were type of polymer used, molecular weight of polymer, salt concentration in continuous phase, type of solvent used, evaporation method and amount of BAC added during formulation.

Initially, the formulation of microspheres was evaluated based on microscopic images and the amount of BAC encapsulated. The polymers used were PCL, PLLA, CTA and PLGA with molecular weights of 10 000 Da or 97 000 Da. As seen in Figure 4.1 A, the formulation with PCL did not yield any uniform or stable microspheres. Polymeric materials can be observed in the image, however it is only consisting of small fragments that has agglomerated. The microspheres formulated using PLLA as polymer display more promising results. In Figure 4.1 B, multiple microspheres in the desired size range occur, and the morphology appear to be uniform. Nonetheless, once again can aggregation between the microspheres be observed. Micrographs of the formulated microspheres using CTA can be observed in Figure 4.1 C. Particles formed with this polymeric material was porous. Cavities and irregularities can be seen in the microspheres, but they are still circular as the general shape. The size of the particles are rather large, some above 10 µm in diameter, however no aggregation can be seen.

When using the lower molecular weight PLGA (10 000 Da), few microspheres were formed and instead mostly polymeric fragments was found, see Figure 4.2 A. Some smaller microspheres can be observed and the larger ones seem to have start to degrade. For the larger molecular weight (97 000 Da), desired shape and size of

the microspheres can be noted, see Figure 4.2 B. The size of the microspheres are between 2-10 µm in diameter and the morphology is uniform. Moreover, there is no agglomeration between the particles and they are stable by themselves. All of the aforementioned batches have had 1 wt% PVA Milli-Q® water in the continuous phase. However, formulation of PLGA (97 000 Da) microspheres with 300 mM NaCl 1 wt% PVA Milli-Q® water can be seen in Figure 4.3 A. The microspheres have a large diameter, some above 20 µm, when using NaCl in the continuous phase. Nevertheless, the particles have uniform shape and show no signs of degradation. Aggregation of the microspheres can neither be seen.

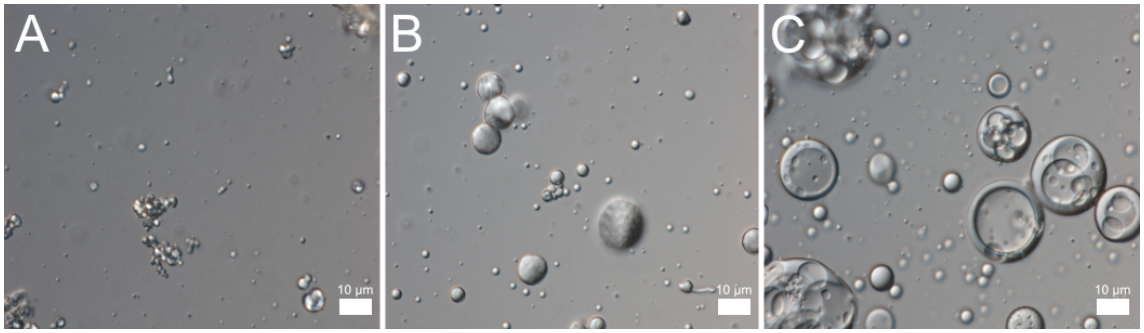


Figure 4.1: A. Image of PCL microspheres with 100x magnification. B. Micrograph of PLLA microspheres using 100x magnification. C. Image of CTA microspheres with magnification 100x.

The microspheres formulated in Figure 4.3 B had 300 mM NaCl in the continuous phase, and the volatile solvent was removed via rotary evaporation. Previous batches let the volatile solvent evaporate in a fume hood. The polymeric material used was PLGA with molecular weight of 97 000 Da. Diameter of the particles is approximately 10-15 µmand they are displaying uniform shape. No agglomeration of the microspheres is observed. PLGA (10 000 Da) microspheres when using 300 mM NaCl in continuous phase and rotary evaporation have similar size and shape to microspheres formulated with PLGA (97 000 Da), see Figure 4.3 C. The PLGA (10 000 Da) microspheres have a diameter between 15-20 µm and the morphology is uniform. Once again can no aggregation of the particles be noted.

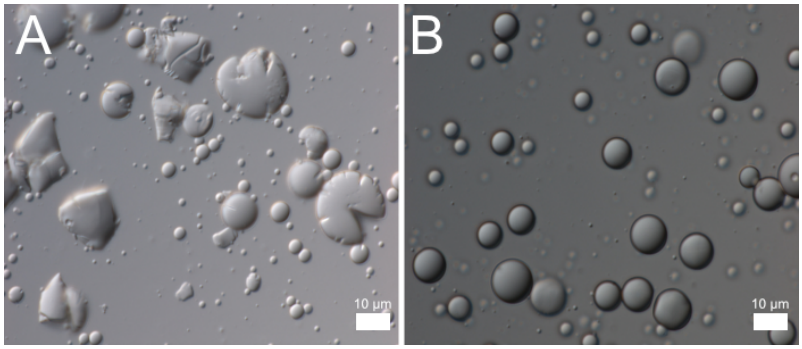


Figure 4.2: A. Image of PLGA microspheres with molecular weight of 10 000 Da. B. Micrograph of PLGA microspheres with 97 000 Da as molecular weight using 100x magnification.

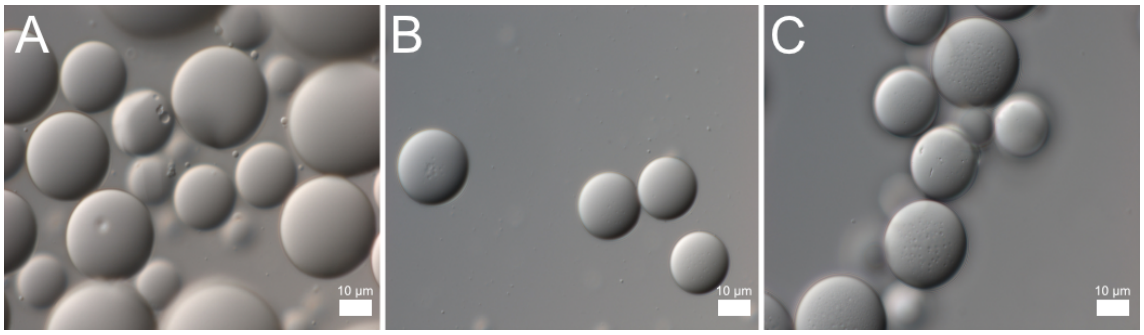


Figure 4.3: A. Image of PLGA (97 000 Da) microspheres with 300 mM NaCl in the continuous phase. B. Micrograph of PLGA (97 000) microspheres with 300 mM NaCl in the continuous phase and rotary evaporation. C. Image of PLGA (10 000 Da) microspheres with 300 mM NaCl in the continuous phase and rotary evaporation. The scale bar corresponds to 10 µm.

The same microspheres shown in Figure 4.3 B. and C. are seen in Figure 4.4, but with a time discrepancy of two weeks between the images. The microspheres formulated from PLGA (97 000 Da) can no longer be observed in the image, however crystalline material is present in the batch. The reason for this is discussed in section 4.1.2. Moreover, crystals formed have a rectangular shape and have an approximate size of 20-30 µm, see Figure 4.4 A. and B. For the microspheres formulated with PLGA (10 000 Da), crystals are noted two weeks after the batch was produced, see Figure 4.4 C. and D. The crystals have a less uniform shape, but have a somewhat rectangular morphology. Nonetheless, crystals of sizes larger than 40 µm can be seen in the micrograph.

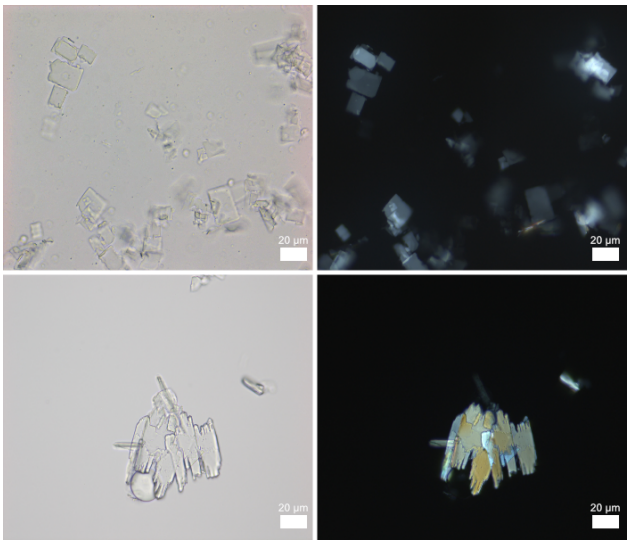


Figure 4.4: Micrographs of microspheres with BF illumination and polarised light two weeks after formulation. A. Brightfield image of PLGA (97 000 Da) microspheres. B. Micrograph of PLGA (97 000 Da) with polarised light. C. Brightfield micrograph of PLGA (10 000 Da) microspheres. D. Image of PLGA (10 000 Da) with polarised light.

PLGA (10 000 Da) microspheres formulated with 10, 5 and 2.5 wt% BAC can be seen in Figure 4.5. Another difference from earlier presented microspheres is that these three batches was formulated with chloroform as volatile solvent, compared to previous batches using DCM. Reasoning to use a different solvent is discussed in Section 4.1.2. The microspheres containing 10 wt% BAC have a uniform shape and the size ranges from 10-15 µm in diameter, see Figure 4.5 A. However, smaller particles with diameter below 10 µm can be observed as well. For the microspheres formulated with 5 wt% the diameter of the particles is circa 15 µm, with smaller microspheres occurring simultaneously, see Figre 4.5 B. The morphology of the microspheres are uniform and circular. Lastly, when using 2.5 wt% BAC to formulate microspheres the size between the particles differ significantly, see Figure 4.5 C. Lager microspheres with diameter above 30 µm can be observed along with particles having a diameter of approximately 10 µm. Nonetheless, the shape of the particles are independent of size and are circular for all cases.

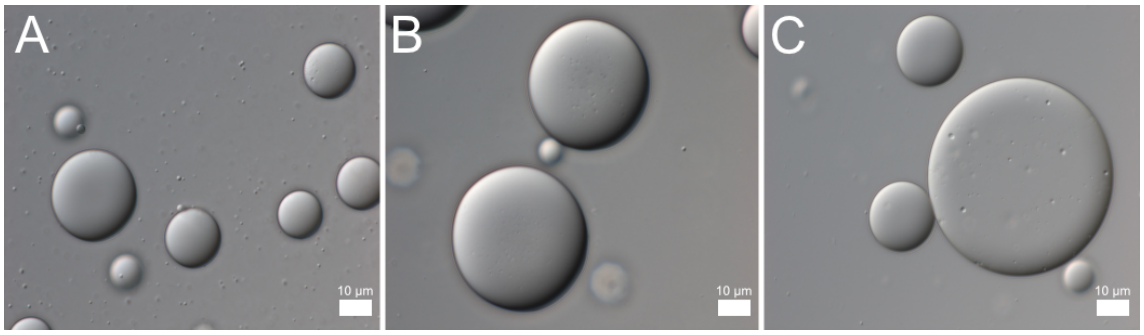


Figure 4.5: Micrographs of microspheres with 10, 5 and 2.5 wt% BAC. A. DIC image of PLGA (10 000 Da) microspheres with 10 wt% BAC. B. Micrograph of PLGA (10 000 Da) with 5 wt% BAC. C. Image of PLGA (10 000 Da) microspheres with 2.5 wt% BAC.

4.1.1 Polymeric material

As presented in Figure 4.1 and Figure 4.2, the type of polymer used to formulate the microspheres heavily effect the outcome. Using PCL as polymeric material resulted in irregular fragments, however no microspheres were formed. This was not to be expected as successful formulation has been done previously [17, 18]. The microspheres formulated with PLLA display more uniform shape compared to the particles formed by PCL. However, the size of these microspheres present a large variation, ranging from sub 1 µm in diameter to above 10 µm. The reason for this can be that the emulsion formed has varying size of droplets because of relatively high viscosity of the oil phase due to higher molecular weight (45 000 Da). The formulated microspheres from CTA are large compared to the particles formed by PCL and PLLA. Similar to PLLA, some of the microspheres are above 20 µm in diameter while others are below 1 µm. Interestingly enough does the oil phase with dissolved CTA have a high viscosity, higher than PLLA, and thus might be the reason for a large variance in size. Moreover, the particles are porous, with large cavities that is visible in the micrograph. This has been observed before by Fan et.al. and thus

yielding microspheres with high surface area [20].

Microspheres formulated with PLGA (10 000 Da) are varying in size, nevertheless larger particles have been formed but started to degrade. However, the lifetime of PLGA before it starts to break down is longer than a few minutes, and should therefore not degrade the microsphere quickly after formulation. In Figure 4.3 C, spherical and stable microsphere formulated from the same polymeric material is shown, thus questioning the results obtained in Figure 4.2 A. The instability of the larger microspheres might then be due to a less stable emulsion caused by low stirring speed. The particles produced by PLGA (97 000 Da) are more uniform in shape compared to its low molecular counterpart. The size variance between the particles is also smaller compared to PLGA (10 000 Da), ranging from 2-10 µm in diameter. If compared to results obtained with PCL and PLLA, a large difference in size can reasonably be expected as the molecular weight of PLGA is higher than the two.However, PLGA is an amorphous material and is thereby more flexible forming less rigid structures.

Adding NaCl to the continuous phase have a clear effect on the size of the microspheres, but no apparent change in morphology can be observed. The size of the particles increases with higher salt concentrations in the water, see Figure 4.3. This can be because salt effects the activity of PVA (salting-out effect), thus reducing its effect to stabilise oil droplets and microspheres [41]. Less stabilisation due to reduced PVA activity thereby increases the size of the particles. Nonetheless, due to BAC acting as an surfactant the emulsion can be stabilised, even if not as effective as PVA. There is little to no difference between the microspheres when evaporating the solvent in a fume hood or using rotary evaporation, see Figure 4.3 A. and B. If any divergence between the two evaporation methods can be noted, it is that for rotary evaporation the size of the particles are slightly smaller. This might be due to the emulsion droplets do not have time to coalesce or become larger as a result of Ostwald ripening, thereby producing smaller microspheres, as previously observed by Vincent and Loxley [7].

Observing the microspheres produced via rotary evaporation two weeks after formulation reveals that few stable microspheres are left in the suspension. Elsewise, crystalline fragments are instead present in the continuous phase, in size ranges larger than the microspheres. The crystals formed are BAC molecules crystallising in the presence of NaCl, as it is not soluble in salt water, see Table C.1 Appendix C. Nonetheless, the microspheres has aggregated due to low PVA activity and precipitated from the continuous phase. Therefore, most of particles are agglomerated on the bottom or on the sides of the vial, and not present in the micrographs. Microspheres formulated with varying amount of BAC show large difference in size. The higher the amount of BAC, the smaller particles will be formed, see Figure 4.5. This is in accordance with the surface activity of BAC, as larger concentration would create smaller oil droplets in the emulsion and thereby smaller particles.

4.1.2 Continuous phase

Observing the micrographs with micropsheres formulated without salt in the continuous phase, crystallisation in the water is not occurring. This indicates that BAC is encapsulated within the particles, soluble in the continuous phase or present in both the microspheres and the water. Moreover, when measuring the absorbance with UV-Vis spectroscopy to calculate the BAC concentration in the continuous phase, all of the BAC put into the sample could be detected. These results demonstrate that none of the BAC was encapsulated, and instead all is present in the water. This led to the conclusion that PLGA, especially molecular weight of 97 000, formulates the most desired microspheres based on size and morphology.

The images displaying microspheres directly after formulation, but with NaCl in the continuous phase show no signs of crystallisation. Furthermore, when evaluating the concentration in the water for those samples using UV-Vis spectroscopy, the BAC concentration was zero or extremely low. That implies that all or most of the active substance is encapsulated within the microspheres. Nonetheless, observing the samples two weeks after formulation reveals formation of crystals in the continuous phase sparking the question when does crystallisation occur. As BAC is not soluble in 300 mM NaCl, it should not be present in the continuous phase without crystallising. However, when saturating the salt solution with DCM, the BAC will become soluble in the continuous phase and thus not visible as crystals in the micrographs, see Appendix C. This results in a crystallisation process when all DCM is removed, and does continue with the controlled release of BAC from the microspheres. Therefore, when a small volume of the suspension is used for centrifugation, the DCM evaporates and the BAC crystallises in the continuous phase. The crystals will then be centrifuged together with the microspheres as a sediment, and the BAC concentration in the supernatant (continuous phase) will be low.

Microspheres formulated with chloroform as solvent displayed similar characteristics to the ones produced with DCM. Crystallisation after two weeks could be observed for the samples, and without a clear difference in the micrographs. However, as chloroform is less soluble in water compared to DCM (see Appendix C), the amount of BAC dissolved in the continuous phase saturated with chloroform is expected to be less than for DCM. Nevertheless, by observing the images, no such conclusion could be done.

4.2 Release study of microsphere suspension

A release study of the microsphere suspension was performed on six batches, and then analysed with LC-MS to quantify the amount of BAC in the continuous phase, see Figure 4.6. Two of the batches was formulated using PLGA with molecular weight of 97 000 Da and was loaded with 5 wt% BAC. The difference between the samples were the solvents, were one used chloroform and the other DCM. Furthermore, the other four batches used PLGA (10 000 Da) as polymeric material, but was loaded with varying amounts of BAC (2.5 wt%, 5 wt% and 10 wt%), see Figure

4.6. Similar to PLGA (97 000 Da), two batches were prepared with 5 wt% BAC, but with different solvents. The samples loaded with 2.5 wt% respectively 10 wt% BAC were both formulated with chloroform as solvent. Moreover, all of the batches had 300 mM NaCl in the continuous phase during emulsification and were prepared via rotary evaporation.

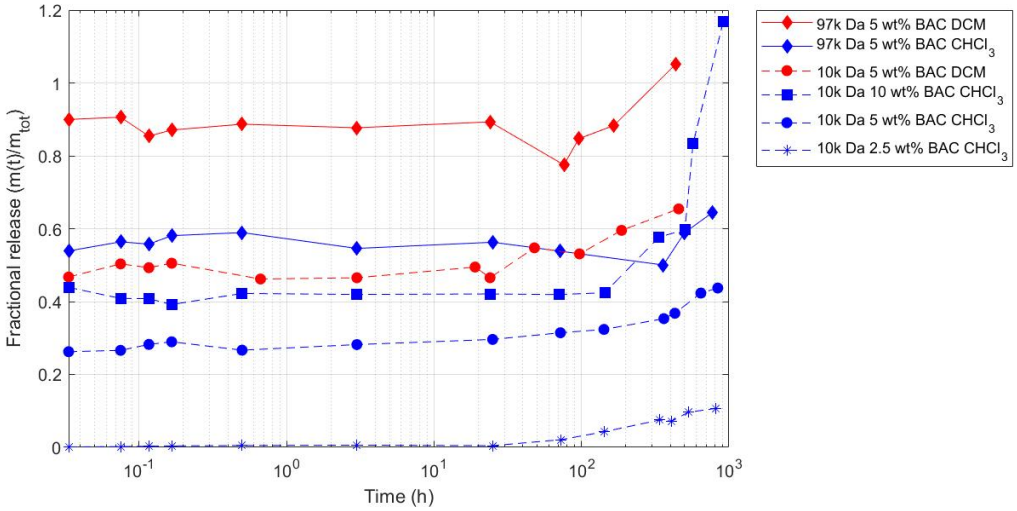


Figure 4.6: The release of BAC from microspheres. The x-axis is time (hours) in logarithmic scale and the y-axis is the fractional release of BAC in relation the theoretical maximum concentration.

The lowest fractional release can be observed for PLGA (10 000 Da) encapsulated with 2.5 wt% BAC, see Figure 4.6. The burst (measured concentration of BAC at the first data point) is zero, which is a desired property for encapsulation. However, the release rate is slow and it takes circa 100 hours until BAC starts to release from the microspheres. From there on, the BAC is continuously released and after 800 hours, 11% of the theoretical maximum of BAC was released from the particles. The batch formulated with PLGA (10 000 Da), 5 wt% BAC and chloroform as solvent had an approximate burst of 26 %, but after that a slow release of the active substance. Similar to the microspheres loaded with 2.5 wt% BAC, an increase in release rate is noted after 100 hours and 44 % was released after 850 hours. The release curve for PLGA (10 000 Da), 5 wt% BAC and DCM as solvent display the same characteristics as when using chloroform, but the burst was higher (circa 45 %). This leads to that 65 % of the theoretical maximum was released after 450 hours. When loading PLGA (10 000 Da) with 10 wt% BAC, the burst was 44 % and once again does the release start after 100 hours. However, in this case, the release rate was higher resulting in a high BAC concentration measured to 117 % of theoretical maximum after 900 hours. Obtaining a concentration 17 percentage points above theoretical maximum can seem unreasonable, however errors when weighing, evaporation of the samples and the accuracy of the analytical method might effect the result. Noteworthy is that the fractional release is based on the theoretical maximum of BAC, due to the actual total concentration of BAC in the samples is unknown. Therefore can the absolute values measured not be fully trusted. Nonetheless, the

relationship between the samples is not affected by this as the experimental method are the same for all samples. Moreover, the microspheres formulated with PLGA (97 000 Da) displayed a higher burst compared to their lower molecular counterpart. Microspheres that was formulated with chloroform as solvent had a burst of 54 %, while when DCM was used as solvent the burst was at 90 % of theoretical maximum. For the particles formulated with DCM, it takes 100 hours until an increase in BAC concentration can be observed. When using chloroform, the release rate is slower and no increase in BAC concentration can be seen until 300 hours.

Firstly, a clear difference between PLGA (97 000 Da) and PLGA (10 000 Da) can be observed. Using PLGA (97 000 Da) as polymeric material lowers the encapsulation rate of BAC, as more active substance is present in the continuous phase at the first data point. The difference in burst between the two polymers with the same formulation process is between 30-40 %, resulting in PLGA (10 000 Da) yielding higher encapsulation efficiency. The reason for this might be that BAC has the ability to interact with the end-groups of the polymer forming intermolecular bonds, such as electrostatic interactions or hydrogen bonding. When using PLGA (10 000 Da) there are more end-groups present, increasing the possibility for BAC to interact and thus improve the encapsulation. The formation of intermolecular bonds can also be the explanation for the low release rate in the first 100 hours. Because there is a strong interaction between the active substance and the polymeric material, there will be no diffusion and release through the microspheres. However, after approximately 100 hours the microspheres start to degrade, releasing the BAC into the continuous phase.

Another observation is that the encapsulation efficiency is heavily dependant on the weight percentage (wt%) BAC used during formulation. Using a lower wt% increases the encapsulation rate, based on the PLGA (10 000 Da) batches using 2.5, 5 and 10 wt% BAC, see Figure 4.7. This indicates that there is a maximum concentration of BAC that is able to be encapsulated in the microspheres. Normally is the encapsulation efficiency expected to be independent of the amount of active substance used, due to an equilibrium between the continuous phase and the microspheres. The reason for a higher encapsulation efficiency with lower wt% of BAC can be the size of microspheres. As mentioned in Section 4.1.1, lower amount of BAC used during formulation will increase the size of the microspheres. Larger microspheres is formulated due to larger oil droplets in the emulsion, where the BAC is dissolved. Then when the solvent is evaporated, BAC needs to diffuse a longer distance to reach the interface between the microsphere and the continuous phase, thus increasing encapsulation efficiency. Moreover, because higher amounts of BAC yields smaller microspheres, there is a strong indication that the surface active BAC is at the interface in the emulsion, and therefore closer to the continuous phase. This will in turn lead to a lower encapsulation efficiency. Conclusively, a higher wt% of BAC used will result in a lower encapsulation efficiency which is an undesired property for microencapsulation.

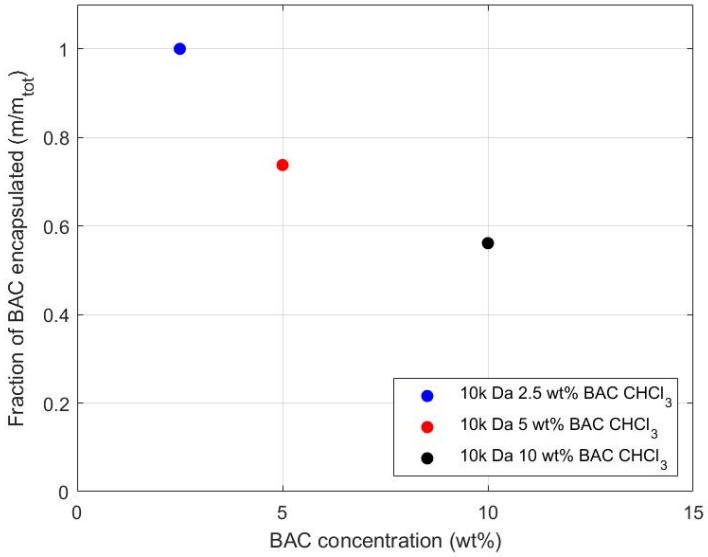


Figure 4.7: The release of BAC from microspheres. The x-axis is time (hours) in logarithmic scale and the y-axis is the fractional release of BAC in relation the theoretical maximum concentration.

A difference between the samples using DCM and chloroform can also be observed. Both for the microspheres formulated with PLGA (10 000 Da) and PLGA (97 000 Da), the particles that had chloroform as solvent display lower burst. The difference in burst is 40 percentage points for PLGA (97 000 Da) and 20 percentage points for PLGA (10 000 Da), which indicates a more successful encapsulation with chloroform. The reason for this might be, as mentioned in Section 4.1.2, that chloroform is less soluble in water, and thereby decreasing the solubility of BAC in the continuous phase. This will lead to an higher concentration of BAC inside the microspheres and lower burst.

4.3 Cellulose nonwovens by solution blowing

Two batches of cellulose fabrics were produced via solution blowing, one with microspheres incorporated and one without. The microspheres used for incorporation was formulated with PLGA (10 000 Da), 5 wt% BAC and DCM. 300 mM NaCl was used in the continuous phase and the solvent was evaporated using rotary evaporation. This microsphere system was chosen due to displaying sufficient encapsulation efficiency to be able to detect the release of BAC from the microspheres, without losing to much BAC during the formulation process. These microspheres also had the desired size for incorporation. An overview of the produced fabric without microspheres can be seen in Figure 4.8. A difference between the two fabrics produced was the thickness, as the fabric without microspheres was thicker than the one with microspheres. This is most likely due to the amount of cellulose used for the fabric without microspheres were twice as much, therefore forming a thicker fiber.

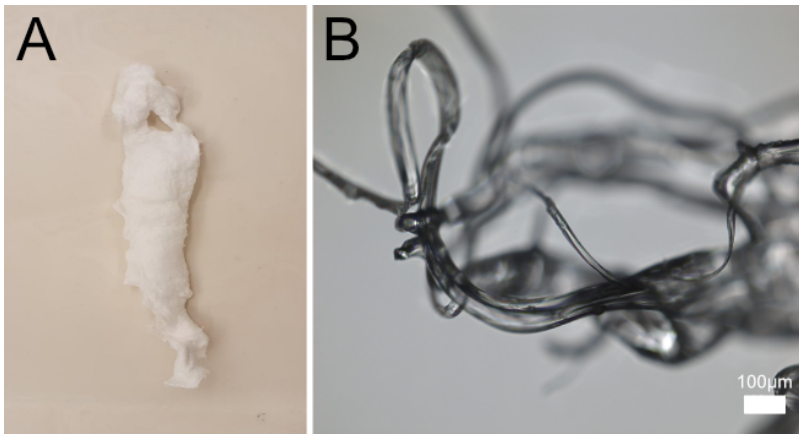


Figure 4.8: A. The produced fabric with approximately 20x4 cm in dimensions. B. Micrograph of the fabric with 10x magnification.

Single strands of fibres were put under the microscope to compare the batches, and to determine if incorporation of microspheres was done successfully. For the fabric with micropsheres, separate and clusters of particles are visible in the micropgraphs, see Figure 4.9. Arrow 1 and 3 indicates the separate microspheres that are well incorporated. The 2nd and 4th arrow points at aggregated spheres, however are still incorporated into the fibre. Nonetheless, the particles are well dispersed all over the fabric based on the micrographs resulting in successful incorporation.

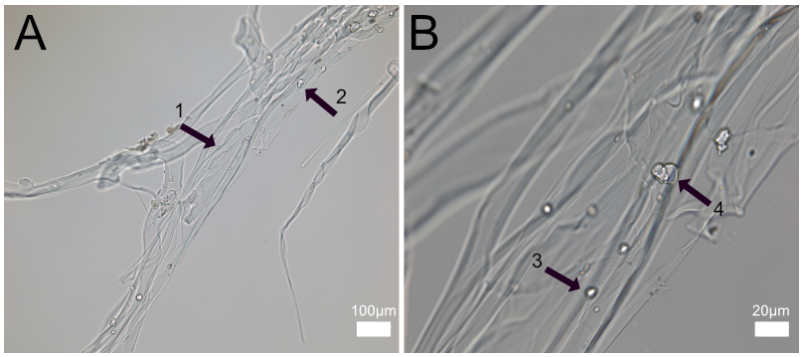


Figure 4.9: Micrographs of nonwoven fibre incorporated with PLGA microspheres. A. Image of fibre strand with BF illumination with 10x magnification. B.Image of fibre strand with BF illumination with 40x magnification.

Micrographs of the fabric produced without microspheres have similar appearance to the fabric incorporated with microspheres, see Figure 4.10. The main difference observed between the two fabrics is the absence of particles in the image. Therefore, this indicates that incorporated microspheres will be clearly visible using microscopic imagery.

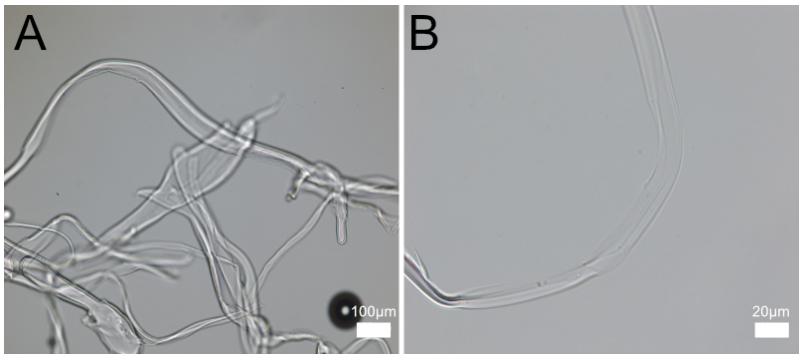


Figure 4.10: Micrographs of nonwoven fibre without microspheres. A. Image of fibre strand with BF illumination with 10x magnification. B.Image of fibre strand with BF illumination with 40x magnification.

4.3.1 Release study of cellulose fibre

The release of BAC from the impregnated fibre is quick and everything is released after approximately 5 minutes, see Figure 4.11. This can be concluded because a plateau is reached after 5 minutes, indicating that all of the BAC is released. Comparably, the release from the fibre incorporated with microspheres is slower and it takes 150 hours for 10 % of the theoretical maximum of BAC to be released. For all the data points from the fibre incorporated with microspheres, the detected amount of BAC in the release bath is low contributing to a large noise-to-signal ratio. This will lead to an uncertainty when determining the concentration and therefore make it hard to draw any conclusions based on the obtained values. However, an increase of released BAC after 100 hours would be expected as it has been observed in the previous release study on microsphere suspension. Moreover, it is clear that the microsphere acts as diffusion barriers resulting in a lower concentration of BAC released when compared to the impregnated fibre.

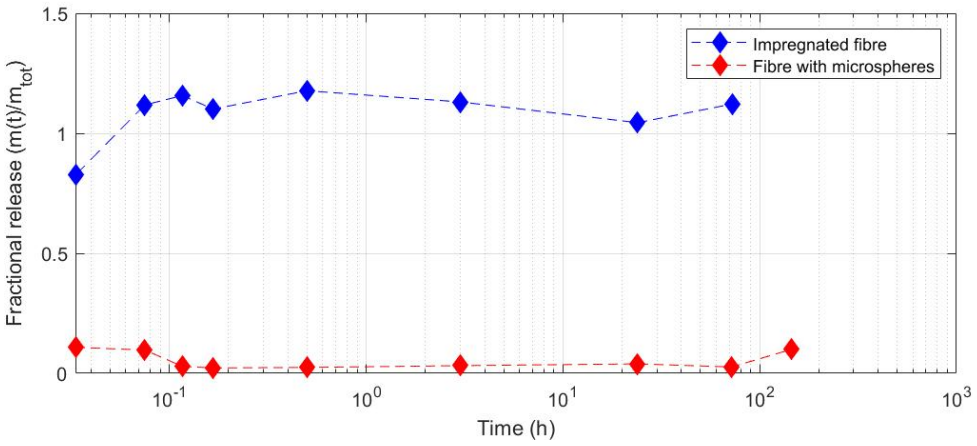


Figure 4.11: Release of BAC from fibre incorporated with PLGA microspheres and fibre impregnated with BAC.

5
Conclusion

By encapsulation the active substance can get a controlled and reduced release rate. Nevertheless, successful encapsulation of BAC with solvent evaporation via internal phase separation has shown to be difficult. The best results based on size, morphology and encapsulation efficiency were acquired when PLGA was used as polymeric material. PLGA (97 000) displayed desired size and morphology with and without NaCl in the continuous phase. Likewise does PLGA (10 000 Da) microspheres formulated with NaCl exhibit desired size and morphology, but with a higher encapsulation efficiency concluding that PLGA (10 000 Da) yields the most successful microspheres.

The addition of NaCl to the continuous phase increased the encapsulation effeciency of BAC into the microspheres. Moreover, the size of the microspheres were increased with the drawback of less stable particles. However, crystallisation of BAC in the continuous phase did occur in the presence of NaCl. Other parameters shown to effect the encapsulation efficiency is type of solvent used, evaporation method and BAC loading in the microspheres. Using chloroform as solvent display the highest encapsulation efficiency which can also be observed when rotary evaporation is used as evaporation method. Furthermore, a lower weight percentage of BAC used during formulation exhibit higher encapsulation efficiency.

Incorporation of microspheres into cellulose nonwoven fibres is possible. Microspheres are well dispersed in the fibre, concluding that the release of BAC from the microspheres should occur evenly from the fabric. However, aggregation of microspheres on the fibre can be observed but this should not effect the release of BAC.

A controlled and reduced release of BAC from the microspheres can be observed. It takes approximately 100 hours until a release of BAC is detected, which is seen when the release is measured from both microsphere suspension and cellulose fibre. Furthermore, after 100 hours does the polymer microspheres start to degrade, and therefore releases the BAC into the continuous phase. This is a strong indication that a controlled release of BAC via microencapsulation can be obtained due to the polymeric material acting as a diffusion barrier.

Moving forward, a longer release study should be evaluated to obtain the full release curve of BAC over time. This would lead to a deeper understanding regarding the total time needed for all the BAC to release and how it correlates to the burst. Moreover, using different salts during formulation to increase encapsulation efficiency and to have less impact on destabilising the microspheres should be of interest. This also correlates to try other polymeric stabilisers instead of PVA, that show less dependence of salt concentration. Lastly, it is of interest to perform microbiology studies to determine if fibres incorporated with BAC loaded microspheres can prevent bacteria growth. There, a combination of impregnation and microsphere incorporation would be of interest to possibly obtain the best results.

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A

Appendix 1

Table A.1 display details for each experiment performed to formulate microspheres. The amount of polymer, volatile solvent and continuous phase used was constant for all batches. Variables that was changed was type of polymer, molecular weight of polymer, amount of BAC used, which solvent used, if salt was used, stirring speed during emulsification and the type of method used to evaporate the volatile solvent.

Table A.1: Details of each experiment for microsphere formulation.

Polymer	Molecular weight (Da)	BAC (wt%)[1]	Solvent[2]	NaCl (mM)	Stirring speed (rpm)	Evaporation[3]
PCL	45 000	10	DCM	0	1 000	Fume hood
PLLA	55 000	10	DCM	0	1 000	Fume hood
CTA	-	10	DCM	0	1 500	Fume hood
PLGA	10 000	10	DCM	0	1 000	Fume hood
PLGA	97 000	10	DCM	0	2 000	Fume hood
PLGA	97 000	10	DCM	300	2 000	Fume hood
PLGA	97 000	5	DCM	300	2 500	Rotary evaporation
PLGA	10 000	5	DCM	300	2 500	Rotary evaporation
PLGA	10 000	10	Chloroform	300	2 500	Rotary evaporation
PLGA	10 000	5	Chloroform	300	2 500	Rotary evaporation
PLGA	10 000	2.5	Chloroform	300	2 500	Rotary evaporation
PLGA	97 000	5	Chloroform	300	2 500	Rotary evaporation

1. The weight percentage (wt%) is calculated in proportions to the total amount of polymer used.
2. The two solvents used were DCM and chloroform.
3. Evaporation of volatile solvent was either done in a fume for at least 12 hours or by using rotary evaporation for circa 30 minutes. Rotary evaporation took place at room temperature for DCM and at 40°C when evaporating chloroform.

B

Appendix 2

A calibration curve for BAC in Milli-Q® water was performed with UV-Vis spectroscopy. The blue dots are predetermined concentrations and their corresponding absorbance, with the red dashed line as linear regression fit. The equation obtained for the linear regression model was $y(absorbance) = 0.9123 \times x(concentration)$ with R^2=0.9975.

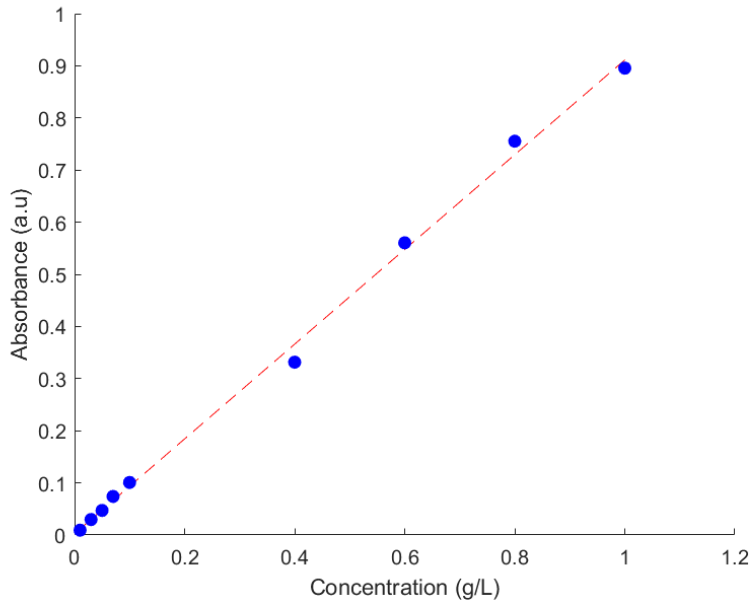

Figure B.1: Calibration curve for BAC in Milli-Q® water with UV-Vis spectroscopy. Blue dots represent the data points and the red dashed line is a linear regression fit.

There were three LC-MS runs and a corresponding calibration curve was performed each time to ensure correct measured concentration of BAC, see Figure B.2. The first LC-MS run had the equation $y = 2{\times}10^8x{+}34085$ based on the linear regression. The second run had $y = 4{\times}10^8x{+}287775$ and the third had $y = 1{\times}10^8x{+}110424$. The R^2 for the three runs were 0.9995, 0.9993 and 0.9988 respectively.

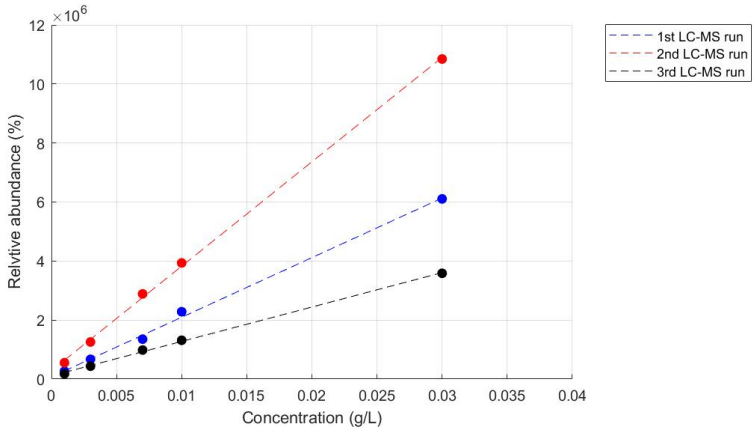

Figure B.2: Calibration curve for BAC in 6 wt% Brij® L23 Milli-Q® water with LC-MS. Dots represent the data points and the dashed line is a linear regression fit for the three different LC-MS runs.

Chromatograms obtained from LC-MS are displayed in Figure B.3. The total chromtogram is shown in Figure B.3 A. and a distinct peak for BAC is seen 6.367 minutes. In Figure B.3 B. the BAC molecular weight m/z=360.4 ha been extracted, resulting in only one visable peak in the chromatogram. This is done to ensure no other ions are considered when the BAC concentration is caluculated.

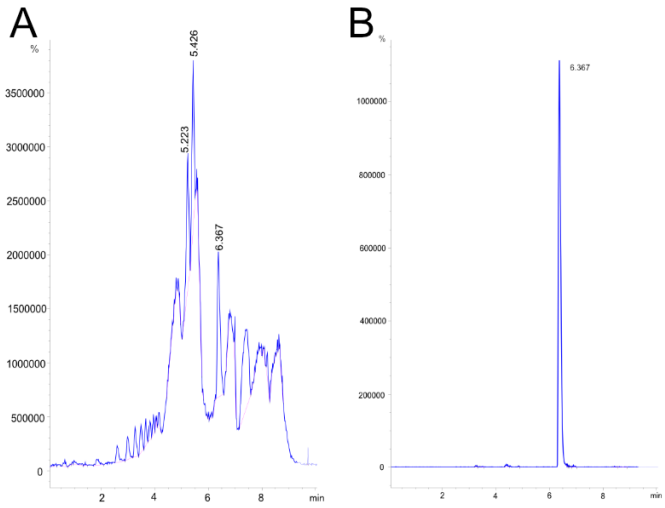

Figure B.3: Chromatograms from LC-MS to determine the concentration of BAC in a suspension release study. A. The entire chromatogram acquired from a LC-MS run. The BAC elution time is observed at 6.367 minutes. B. An extracted ion chromatogram, where only the elution time of the sought-after analyte is analysed. In this case, BAC with at elution of 6.367 minutes with m/z=360.4.

The mass spectra is used to determine which ions are detected at which elution time, see Figure B.4. The highest peak for BAC is observed at m/z=360.4, which is to be expected. Mass spectra is obtained from the extracted ion chromatogram, thus revealing no other ions had been detected at that elution time and molar mass.

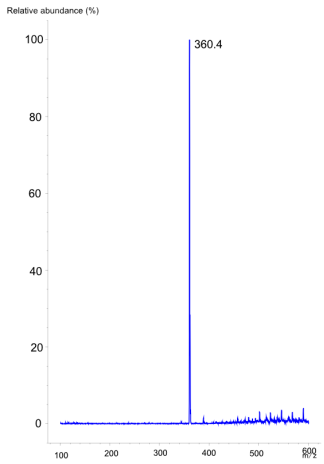

Figure B.4: Mass spectra of BAC ion with m/z=360.4. The x-axis displays the molar mass and the y-axis shows an arbitrary unit of relative abundance between the peaks.

C
Appendix 3

The solubility of BAC at multiple concentrations of NaCl is displayed in Table C.1. There is a heavy influence of BAC solubility dependent on the salt concentration in Milli-Q® water, with a decreased solubility with higher salt concentrations. The solubility was determined by dissolving BAC on salt solution and mix for several hours. The samples was then centrifuged and the BAC concentration in the supernatant was determined by UV-Vis spectroscopy.

Table C.1: Solubility of BAC at different NaCl concentrations.

NaCl concentration (mM)	BAC concentration (g/L)
0	4.17
25	0.45
50	0.024
100	0.021
200	0
300	0

To determine of there was any difference in solubility of BAC when the salt water was saturated with DCM, two samples was prepared. One sample contained BAC dissolved in 300 mM NaCl Milli-Q® water and the second contained BAC dissolved in 300 mM Milli-Q® water saturated with DCM. The samples was then stirred for several hours, and thereafter visually inspected. In the sample without DCM, precipitation could be observed, while the sample with DCM was much clearer. This indicates that BAC has a higher solubility in salt water saturated with DCM.

The same experiment was performed with chloroform instead of DCM, but in this case did not the sample saturated with chloroform become clear. However, there was still a difference between the samples with and without chloroform, but it was less significant compared to experiments with DCM. This indicates that salt water saturated with chloroform does increase the solubility of BAC, although not as much as with DCM.

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