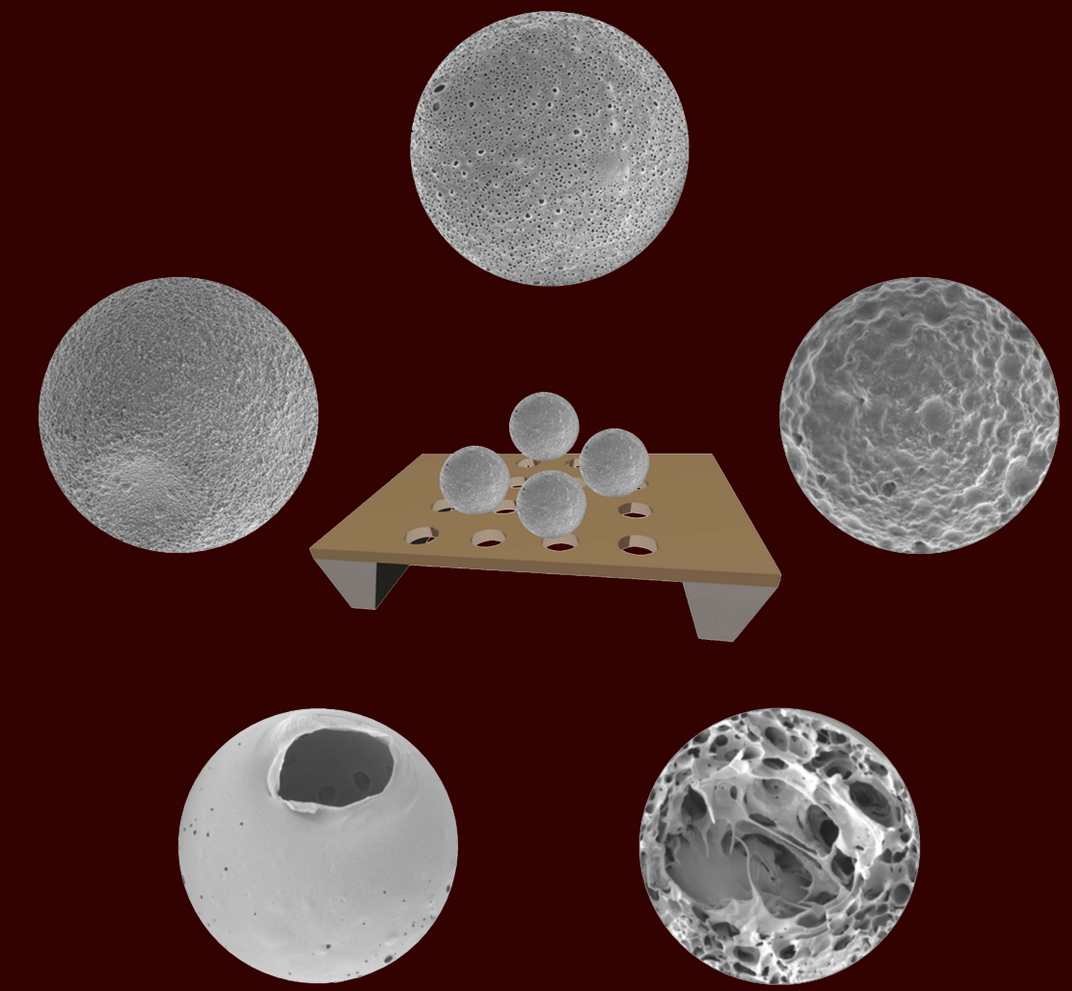
Structuring Microspheres

Nagesh A. Wagdare

2011

# Structuring Microspheres

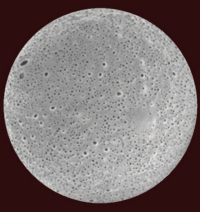


Nagesh A. Wagdare

# Invitation

You are cordially invited to attend the public defence of my PhD thesis entitled:

## Structuring Microspheres



On Friday, 23rd September 2011
at 11:00 hours in the Aula of
Wageningen University,
General Foulkesweg 1a,
Wageningen.

After the ceremony,
there will be a
reception in the Aula.
You are most welcome.

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# Propositions

1. Emulsification with high porosity microsieves is an excellent example of how micro-engineered systems can yield well-defined products at high throughputs (this thesis).

2. Phase separation in microdroplets is an elegant method to obtain microcapsules with tunable morphology of the shell and internal core structure (this thesis).

3. Microtechnology makes efficient use of energy and raw materials and therefore can contribute to a sustainable future.

4. Like the effect of gravity on physical phenomena at macroscopic scales, the influence of additives on structure formation on microscopic scale in polymeric microcapsules cannot be neglected.

5. A combined conceptual design of products and processes in academic research creates synergy and rapid innovations in industries.

6. Practicing science without a good vision is like looking into bright light with blind eyes.

7. For universal solutions of problems related to global warming people from different cultural backgrounds having different views should work together.

8. Foods are not healthy or unhealthy, it is the diet that a consumer chooses which is healthy or unhealthy.

Propositions belonging to PhD thesis

**"Structuring microspheres"**

Wageningen, 23rd September 2011

Nagesh A. Wagdare

# Structuring Microspheres

Nagesh A. Wagdare

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# Structuring Microspheres

Nagesh A. Wagdare

## Thesis

Submitted in fulfilment of the requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus
Prof. dr. M.J. Kropff,
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Friday 23 September 2011
at 11.00 a.m. in the Aula.

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**To my beloved parents**

# Contents


| | | |
|---|---|---|
| **Chapter 1** | General introduction | 01 |
| **Chapter 2** | High throughput microsieve emulsification | 17 |
| **Chapter 3** | Porous microcapsule formation with microsieve emulsification | 35 |
| **Chapter 4** | Hollow capsules: influence of the encapsulated oil on capsule morphology | 47 |
| **Chapter 5** | Structured microspheres from polymer blends | 61 |
| **Chapter 6** | Discussion and outlook | 77 |
| | **Summary** | 93 |
| | **Summary in Dutch (Samenvatting)** | 97 |
| | **Appendix** | 101 |
| | **Acknowledgments** | 103 |
| | **Curriculum vitae** | 107 |
| | **Publication list** | 109 |
| | **Overview of completed training** | 111 |

# Chapter 1

## *General Introduction*


## Abstract

In this Introduction Chapter some basic principles are described for obtaining and structuring microspheres and hollow microcapsules. These microparticles are becoming increasingly important as vehicles for encapsulation of sensitive products in food, pharmaceutical, drug and cosmetics-related applications. New routes for production and encapsulation with different micro-technological tools are elaborated. Especially, a combination of microsieve emulsification and phase separation is explored, since it may yield microcapsules and microspheres with controlled size, shape and morphology. This is also the subject of this thesis and the thesis outline is therefore presented at the end of this chapter.


"*Small is beautiful"*
E. F. Schumacher

## Introduction

Improving the health and quality of life of an ever increasing and more prosperous world population demands continuing improvement of the production and quality of many consumer products. The quality of many products related to food, pharmaceuticals, cosmetics, detergents and many other sensitive products benefits from encapsulation. This can lead to a more efficient use, requiring less of an active component in the product, delivery at a required site, yielding better effect with a given dose, or a longer shelf life, as sensitive components are shielded from their environment. Therefore, improving the design of new materials for microspheres is attractive for future applications *[1]*.

This thesis aims at obtaining more insight in conditions and processes for preparing and structuring microcapsules and microspheres by using phase separation in polymeric solutions combined with a mild microsieve emulsification process.

## Microencapsulation and controlled release

### *Encapsulation*

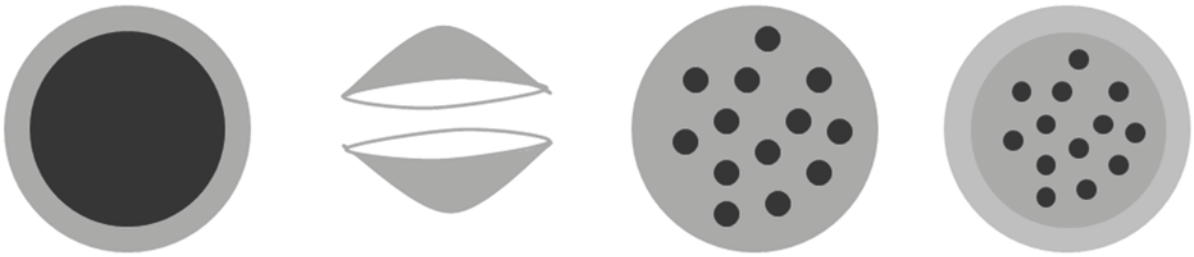
Encapsulation is a process by which one material or mixture of materials is entrapped within another material for protection of these materials. Encapsulation is originally not invented by man. Life would not be possible without encapsulation. Examples of large scale encapsulated objects in nature include eggs, seeds and fruits. On a smaller scale, cells, viruses, and cell nuclei can also be considered as encapsulated systems.

By mimicking nature, we can make better use of sensitive or active materials. These materials can be liquid, solid or even gaseous. A microencapsulate would have a core that is rich in this encapsulated material, surrounding by a thin shell made of a material that gives protection and mechanical stability. A wide range of active materials have been encapsulated for all kinds of applications, including adhesives, agrochemicals, living cells, enzymes, flavours, fragrances, pharmaceuticals, etc. Capsule shell materials can be synthetic polymers, natural polymers, fats, waxes and even inorganic materials like ceramics.

### *General types of encapsulated systems*

Encapsulates are usually in the micrometer range (1 – 100 μm). The general types of encapsulated systems are shown in figure 1. If the core, which can be an active ingredient or contain one, is uniformly surrounded by a thin shell, it is usually referred to as a microcapsule

(figure 1a) *[2]*. When the core is empty, a hollow capsule (figure 1b) is formed. These kind of hollow capsules are interesting for loading with functional materials at a later stage or they can be used as such for ultrasound-mediated diagnostics or therapy *[3]*. In microspheres (figure 1c), an active ingredient can be encapsulated as dispersed material in the solid sphere. For obtaining more tailored (and more complex) release profiles microspheres made from polymer blends are used (figure 1d). For example, an active ingredient such as a solid drug can be encapsulated within the core of one polymer, which is then additionally coated with a second polymer as a shell *[4-6]* for extra protection or to allow for release only under specific conditions.



a) b) c) d)

*Figure 1. General types of encapsulation systems a) Microcapsule in which a liquid core is encapsulated by a solid (polymeric) shell b) Hollow capsule obtained after taking out the template c) Microsphere in which a solid ingredient is encapsulated d) Microsphere from polymer blends in which a solid ingredient is encapsulated in the core and additionally protected with an extra layer to fine tune the release. Note that the encapsulation systems shown here are quite general and even much complex structures will be discussed in this thesis. (For color picture see Appendix on page number 101).*

***Classical encapsulation methods***

Encapsulates can be produced by many different mechanisms. One can use the phase behaviour of solutions of polymeric shell-forming materials, through complex coacervation, phase separation, interfacial polymerization, in-situ polymerization, and thermal and ionic gelation in liquid media. On the other hand, one can make use of the mechanical and rheological properties of concentrated matrices, using spray drying, spray chilling, fluidized bed, electrostatic deposition, centrifugal extrusion or pressure extrusion. However, many encapsulation processes used so far in industries are energy consuming and usually result in

polydisperse capsules showing a range of release properties. Thus, there is a need to improve or even replace them by new methods.

### *Applications of encapsulation*

The first application of encapsulation was in the field of printing. Nowadays, there is a diversity of encapsulated products available on the market, ranging from foods, pharmaceuticals, cosmetics, perfumes or detergents to many other consumer products *[7, 8]*. A vast amount of literature is available on encapsulation for pharmaceutical applications. For each application different materials and suitable processes are required. Therefore, there is scope for further studies and attention should be paid to designing new encapsulation materials and developing new sustainable production processes.

### *Controlled release mechanisms*

Controlled release is the predetermined release of the active material from an encapsulate under specific conditions. One mechanism involves diffusion-controlled release; with this mechanism the release of active material is controlled by the diffusion of the active substance from its location inside the capsule to the outside of the capsule. The bulk of the capsule itself may act to control the release (matrix-type controlled release). Important applications are the controlled release of artificial fertiliser or of pesticides in agriculture. In addition to relying on matrix-controlled release, a membrane may be added around the capsule for controlling release (membrane-type controlled release). Examples of this are controlled release of strawberry aroma across a polysaccharide membrane *[9]*, and encapsulation and release of fragrances, deodorants, pheromones, mainly in cosmetic applications.

Triggered release is the release of a component upon a specific stimulus given to the encapsulate. Common methods used for triggering release are a change in temperature, pH and water activity (relative humidity).

Pressure- or force-activated release systems are found in e.g. carbonless copy paper, in which an ink is encapsulated in a dense but brittle shell material, which is crushed during writing. This releases the ink *[10, 11]*. A similar mechanism is used in swipe-sensitive samples that are sometimes included in magazines, holding samples of a perfume. Rubbing with a finger over the sample breaks the encapsulates and releases the perfume. Plucking or peeling-activated release has been used in delivering fragrances and aromas *[7, 8]*. This creates a sensation of freshness to the consumer.

In solvent-activated release, a capsule shell is being dissolved liberating its content, or the capsule simply swells to start or enhance the release of its active substances. It is most commonly used as release mechanism in the food industry. Here the encapsulating matrices are most often water-soluble and dissolve in the presence of water. In the case of osmotically controlled release, the core of the particles will take up solvent (e.g. water) over time and swell until the capsule bursts or swells so much that it allows release of the encapsulated component. This release method can be used for any active ingredient that is first encapsulated in a hydrophilic carrier and then secondly coated with a hydrophobic polymer matrix.

There are many poorly water-soluble active ingredients like vitamins and flavours that need to be protected (e.g., from oxidation) but should be released on demand (e.g., during ingestion or preparation). Although an approach to obtain microsphere-based delivery systems by emulsification processes is relatively simple, new structured materials are required for better performance and longer shelf life of these products. Even after tremendous progress in encapsulation research, it still remains a major challenge to encapsulate and improve the shelf life and controlled release of sensitive ingredients *[12, 13]*.

***Encapsulation materials***

In the remainder of this Chapter we will focus on polymers as the materials for obtaining microspheres and microcapsules. Ultimately, the properties of the polymer material determine the type of protection and release of encapsulated ingredients. Therefore, a careful selection of the polymer with desired properties is essential for each application (Table 1).

Stimuli-responsive polymers have gained specific interest for controlled release. With careful choice of polymers, several micro- and nano-containers can be prepared, that are responsive to temperature, pH, etc. Polymers composed of a wide range of building blocks can be prepared with different properties *[14-20]*. pH-triggered release of encapsulated materials can be obtained when the polymeric capsules respond to changes in the pH of the environment.

Temperature-sensitive release is possible due to the unique ability of some polymeric materials to either collapse or expand in a solvent (such as water) at a specific temperature; the permeation properties of the encapsulation matrix will change at this temperature and increase the release of the active material. Temperature-activated release can also be obtained by melting. Melting of the capsule wall results in disintegration of the capsule and release of the active material. This type of release can be achieved with oils in the presence of gelators,

which melt at a specific minimum temperature (especially at body temperature). Hybrid systems are also known that make use of a combination of release mechanisms to provide unique release properties. This is used for the release of some flavours, which are sometimes coated with two types of materials. First, the outer coating of the capsules melts and the remaining hydrophilic shell of the capsule may be degraded by dissolution in saliva to release the active flavour ingredients *[21]*.

**Table 1.** Some examples of synthetic polymers used in controlled or triggered release

| Polymer (synthetic) | Properties/release mechanism | Applications |
|---|---|---|
| Polyesters e.g. polylactide, polylactide-*co*-glycolic acid, | Biodegradable, release occurs by diffusion | Drug delivery |
| Polyacrylate copolymer e.g. Eudragit | Responsive to pH changes | Oral drug delivery |
| Poly N-isopropylacrylamide | Responsive to temperature changes | Cosmetic applications |
| Polyurethane, polyurea, urea-formaldehyde polymer | Diffusion-controlled release or by crushing of a polymer shell | Perfumes, detergents, ink-free paper, pesticides and other agrochemicals |

Oral delivery systems are often complex since the active ingredients need to be released at a specific location in a desired manner, while withstanding processing and transportation conditions. Therefore, a wide range of pH-sensitive materials have been explored for targeting a specific location in the gastro-intestinal tract. For example, Eudragit is a commercial pH-sensitive polymer available in different forms. It is a (meth)acrylate based copolymer with either some acrylic acid groups or cationic acrylate groups. Eudragit copolymers with acrylic acid are insoluble at acidic pH and soluble at neutral to alkaline pH *[22, 23]*. On the other hand Eudragit copolymers with dimethylaminoethyl ester groups are soluble at acidic pH and insoluble at alkaline pH. By careful choice of the pH-sensitive functional groups and the percentage of these groups, materials with different solubility properties at different pH values can be obtained. Therefore, these are potentially useful materials for oral delivery systems *[24-26]*. For cosmetic applications, the active components need to be released after application. In this case one may for example use a temperature-responsive poly(N-isopropylacrylamide) *[27-29]*.

When sustained drug delivery is required, one may use a polymer that is degradable in the body and releases its active material by slow out-diffusion. A well-known degradable polymer

is polylactide. For other application like perfumes, inks, detergents or agrochemicals, polymers such as polyurethane, polyurea or urea-formaldehyde are often used *[30-32]*. While for pharmaceutical and cosmetic applications, synthetic polymers may be used, for foods this is not an option. Instead one may choose biopolymers having similar properties.

## Microcapsule preparation with micro-technological devices

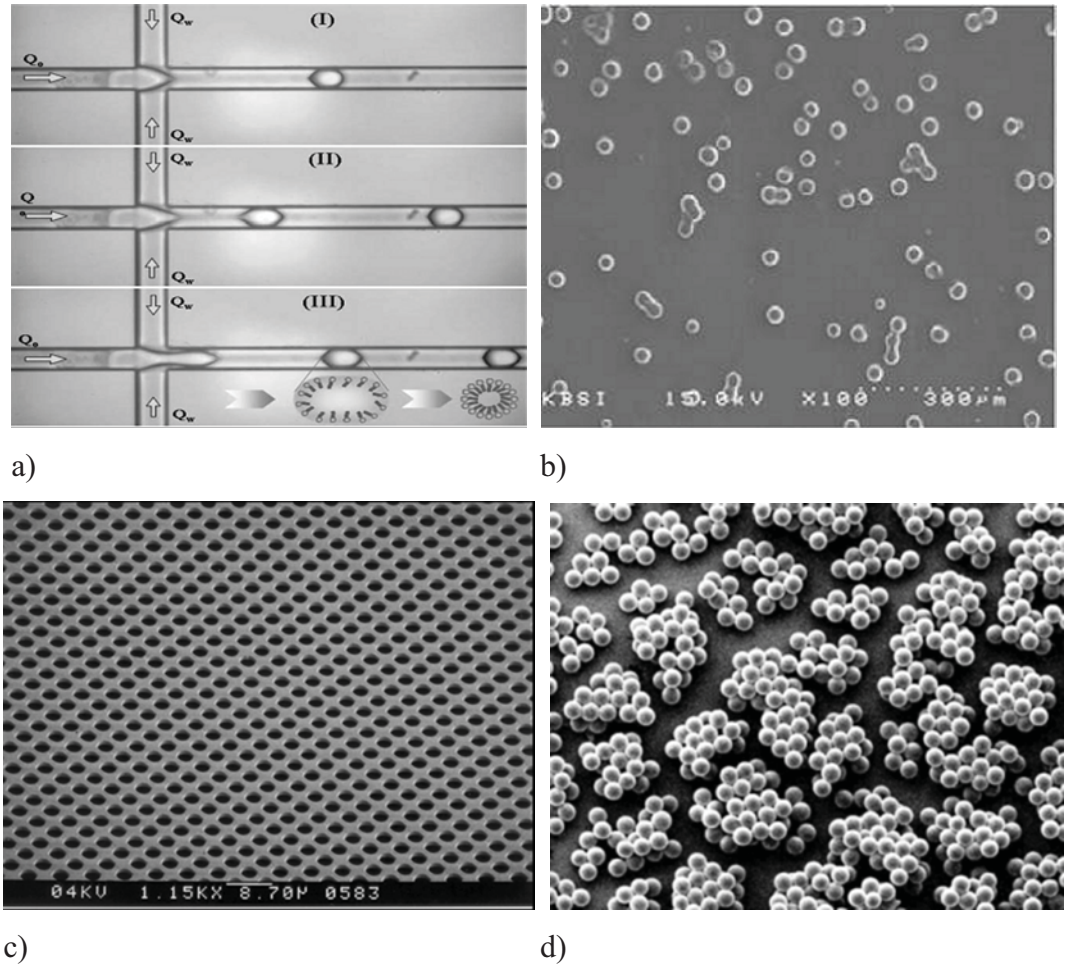
Many conventional encapsulation methods do not allow for the production of encapsulates with well-defined properties; for example the primary droplet size in a spray dryer features a wide size distribution, while droplet coalescence may make the final size distribution even wider (Table 2).

For microcapsule formation with phase separation, the to-be-encapsulated component (e.g. oil) is typically soluble in an organic solvent, but is not well-miscible with the shell-forming polymer. This solution is then emulsified into a liquid that is a strong non-solvent for all components (e.g., water). The organic solvent has certain solubility in water, which leads to an increasing concentration of the oil and polymer in the droplets. Now phase separation between polymer and oil occurs, followed by solidification of the polymer leading to formation of core-shell capsules. Since the phase separation occurs after the formation of the organic phase droplets (i.e. the precursors for the capsule formation), control over the uniformity and size of the precursor droplets also give control over the uniformity and size of the resulting microcapsules.

**Table 2.** Comparison of different emulsification techniques

| Emulsification technique | Energy consumption | Size distribution | Scalability | Cost |
|---|---|---|---|---|
| Conventional emulsification (e.g., homogenization ) | High | Poor | Yes | Economic |
| Microfluidic devices | Low | Good | No | High |
| SPG membrane emulsification | Low | Sufficient | Feasible | Acceptable |
| Microsieve emulsification with | | | | |
| a) Auto break up | Low | Good | Feasible | Acceptable |
| b) Cross-flow | Low | Sufficient | Feasible | Acceptable |

Recent years have seen the emergence of emulsification methods using micro-engineered systems that are able to produce rather monodisperse emulsions, with a minimum of energy input. This makes these techniques potentially interesting for encapsulation of shear-sensitive components such as enzymes, living cells and probiotics (Table 2). Microsieve emulsification is potentially a good and mild method for the preparation of the initial template. By first emulsifying a polymeric solution into water, phase separation is induced to create a polymeric shell around the active component. This would yield monodisperse encapsulates, provided that the primary emulsions droplets are monodisperse and stable against coalescence.




a) b)

c) d)

*Figure 2. Comparison of microparticle preparation by a flow-focussing microfluidic device (S. Abraham et al.,) reproduced with permission [35] and by microsieve emulsification, a) Droplet generation in a flow-focussing microfluidic device b) Microspheres obtained from flow-focussing microfluidic device c) SEM image of a microsieve (courtesy Aquamarijn BV) d) Microspheres prepared with microsieve emulsification (courtesy Nanomi BV).*

Microfluidic routes offer good possibilities for obtaining a high control over the size, shape and uniformity of the primary emulsion droplets. However, they cannot be easily scaled up to commercial production rates and making products in practical quantities is quite difficult. Membrane emulsification, on the other hand, has more potential for scalability of the process as shown by R. A. Williams and others *[33, 34]*. However, with use of membrane emulsification the produced microparticles are not as monodisperse as those obtained with microfluidic techniques.

Figure 2a) shows a flow-focusing microfluidic device *[35]* for the production of well-controlled monodisperse microspheres (figure 2b). Although the microspheres have a very uniform size the productivity is low. Upscaling of this process by using many nozzles in parallel is sensitive to differences in flow rates and thus may influence the droplet size.

A recent trend for producing emulsions of microspheres is by using microsieves or micro-engineered membranes (figure 2c). Membrane emulsification with microsieve is also used for producing monodisperse emulsion in ultrasound contrast imaging *[36]*. The microsieve is made from silicon nitride, in which very uniform pores are fabricated by a photolithographic technique. These microsieves are very thin; much thinner than a conventional membrane. Therefore, high dispersed phase fluxes can be obtained at a relatively low transmembrane pressure. Membrane emulsification with microsieves can also lead to rather uniform microspheres (figure 2d), as each pore has exactly the same dimensions. Microsieve emulsification is usually carried out in either dead-end or cross-flow mode. In cross-flow emulsification the to-be-dispersed phase (oil) is pressed through a microsieve, and the cross-flowing continuous phase (water) shears the droplets off and carries them away (figure 3). Cross-flow microsieve emulsification, being a regular membrane based process, can easily be scaled up.

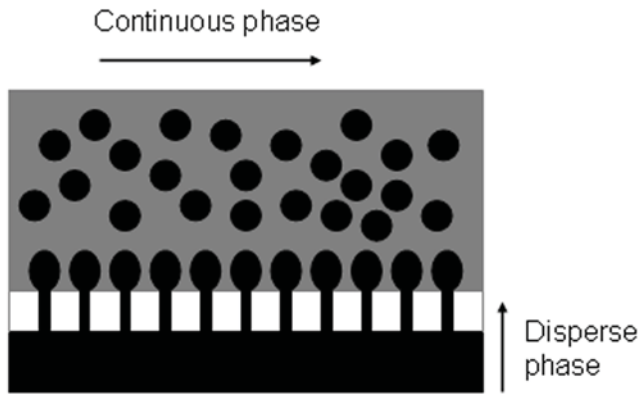



*Figure 3. Cross-flow microsieve emulsification.*

Emulsification with various types of membranes, such as porous glass (SPG) have also been explored to prepare emulsions and microspheres with narrow size distribution *[37-40]*. The micro-engineered (microsieve) membranes have well-defined uniform pores, controlled geometries and very small thickness which makes the microsieve emulsification a unique method for obtaining uniform droplets and microspheres at high-throughputs.

## Impact of the encapsulation process on the performance

The uniformity of the microspheres (which is achievable with microsieve emulsification) is important for the release behaviour. Recently, it was shown that the release properties of microspheres prepared with a microfluidic device were much better than of those prepared with conventional techniques such as homogenization. Poly(lactic-*co*-glycolic acid) (PLGA) microspheres, prepared with a conventional device showed a much more gradual release in time than those prepared with a microfluidic device. The release rate depends on the size of the microspheres; smaller microspheres give a faster release than bigger microspheres *[41]*.
Mild emulsification conditions as obtained with a microfluidic device are essential for encapsulation of shear-sensitive biological samples, such as enzymes and live cells, as they may be damaged by strong mechanical forces. Careful control of the fluid flow rates has been used to control the number of cells encapsulated in microspheres *[42, 43]*. Some flavours are volatile and can easily evaporate during encapsulation by conventional emulsification. Conventional emulsification devices are not very suitable for preparation of double emulsion microcapsules, since during the second stage of the emulsification the high shear used can easily break up the primary emulsion. Therefore, microdevice-based emulsification which employs low shear forces has great potential for these types of encapsulation. Although the production costs will be higher, encapsulation with microtechnological devices will allow for a precise control of the encapsulation process and properties of the products, which is important for applications with high added value.

## Encapsulation by means of a structuring process

After the formation of the primary droplet, it has to be structured into a capsule. This can be achieved by phase separation of the shell-forming components from the to-be-encapsulated components, followed by a solidification of the shell-formers. This is most easily achieved by using the phase behaviour of polymeric solutions. Therefore, in this thesis a new approach

will be explored for structuring microcapsules by a combination of microsieve emulsification and phase separation.

## New approach - Combination of microsieve emulsification and phase separation

Phase separation is a method that has been shown to yield core-shell capsules *[44, 45]*. In a typical process, a shell-forming component (e.g. a polymer) is dissolved in a volatile organic solvent and mixed with a to-be-encapsulated component (e.g. oil or another polymer). The components should be chosen such that the to-be-encapsulated component is soluble in the solvent, but is not well-miscible with the shell-forming polymer. This solution is then emulsified to form droplets, into a liquid that is a strong non-solvent for all components (e.g. water).

The volatile solvent (which has a low solubility in the non-solvent) will diffuse somewhat into the surrounding non-solvent phase, and evaporate at the surface of the bath. Thus, the droplets will slowly lose solvent, decrease in size and become more concentrated in shell-forming polymer and encapsulated component. As the extraction of solvent proceeds, the droplet becomes unstable at some point and the two components start to phase separate. Depending on the properties of the two components, especially their polarity, the more apolar component has a tendency to nucleate in the center, while the more polar, shell-forming component will migrate to the surface to form a shell where it is in contact with water. When a relatively polar shell-forming polymer and an apolar encapsulated component are used, this process results in the formation of microcapsules with an encapsulated droplet surrounded by a polymeric shell.

Some combinations of components suitable for such a phase separation process include poly(methyl methacrylate) and poly(lactide) as shell-forming components, and different alkanes or oils *[45, 46]* as encapsulated components. To our knowledge, the combination of so-called enteric coating material (pH-sensitive Eudragit) as a polymer and vegetable oils, e.g. sunflower oil are not yet studied for the preparation of core-shell microcapsules via this phase separation process. This combination is interesting since such microcapsules have potential for delivering oil-soluble active components (e.g. nutrients) to the colon.

As the phase separation process depends on the mutual interaction betweens the components, the process depends on the properties of the components that are encapsulated. With proper choice of parameters, like solubility, spreading coefficient and other thermodynamic

parameters, oil-soluble active ingredients can be encapsulated in an Eudragit shell. If the Eudragit is mixed with a second polymer (immiscible with Eudragit) instead of oil, the emulsification and phase separation results in the formation of structured double-walled microspheres. This method can be used to incorporate solid active materials in the core. With proper choice of polymers the release of such active ingredients can be tuned.

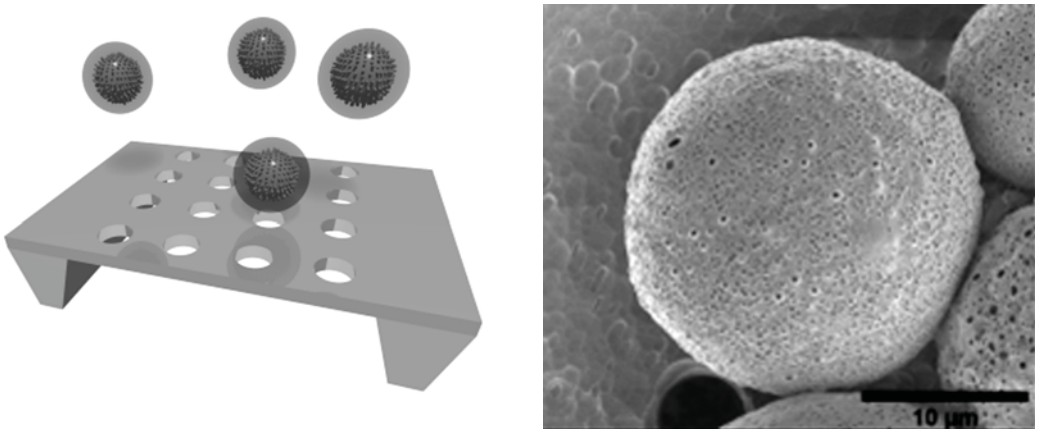



*Figure 4. a) Microsphere formation with microsieve emulsification and phase separation. b) SEM image of a double-walled microsphere with PMMA encapsulated in Eudragit; the microsphere shell is porous due to the presence of Eudragit in the outer layer of the microsphere.*

## Objective and outline of thesis

The objective of the research described in this thesis is to explore the properties of microcapsules that can be employed for oral delivery of active ingredients, for targeted delivery in the lower gastrointestinal tract. The contents of capsules therefore have to be protected in the upper gastrointestinal tract at acidic pH, and be subsequently released in the extreme lower part of the gastrointestinal tract. Besides the material properties of the shell, the uniformity of the size of the capsules, the shell thickness, the pore size and porosity of the shell are important for the release properties. The pH-dependent solubility of Eudragit makes it interesting as a shell-forming polymer for protecting the capsules from the acidic conditions in the stomach, and delivering or releasing the contents at the alkaline conditions in the lower intestine. Thus, Eudragit FS30D polymer (a commercial copolymer of poly(methyl acrylate-co-methyl methacrylate-co-methacrylic acid) 7:3:1)) was used as shell-forming polymer in this thesis. It is used here as a model component, even though it is not food grade. For food

applications one may substitute it for a food grade biopolymer with similar properties. On the other hand Eudragit is approved and extensively used for oral delivery in pharmaceutical applications.

In this thesis, use is made of knowledge of phase separation processes in membrane films for fabrication of microcapsules and microspheres with desired surface morphologies. The combination of phase separation with microsieve emulsification is expected to result in structured microcapsules and microspheres with a narrow-size dispersion and well-defined surface morphology.

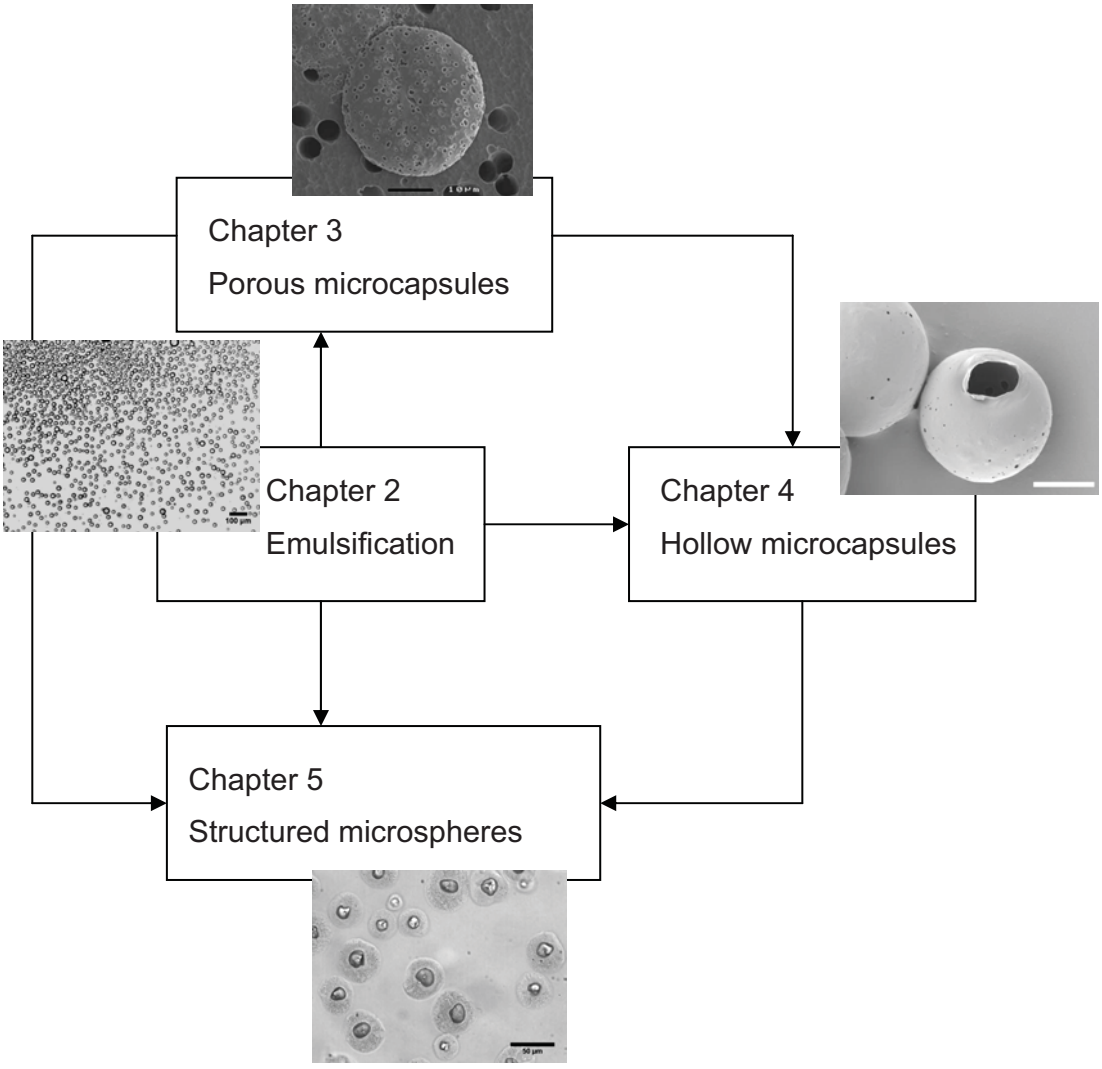



*Figure 5. Schematic representation of the coherence between the chapters of the thesis. Emulsification is the basic processing tool (chapter 2) and with use of phase separation in a four component system different encapsulation structures are formed as discussed in the remaining chapters (chapter 3-5).*

**In Chapter 2** emulsification of sunflower oil in water is reported using a high porosity microengineered membrane. A method was developed for production of narrow size-dispersed sunflower oil-water emulsions with the microsieve cross-flow emulsification technique. The effect of various surfactants on the droplet formation was studied. Conditions for obtaining high throughput and narrow size-dispersed oil-water emulsions were explored and discussed.

**In Chapter 3** the emulsification method developed in Chapter 2 was used to prepare Eudragit microcapsules. Hexadecane was encapsulated in an Eudragit-rich shell as a result of phase separation induced by liquid-liquid demixing. A mechanistic formulation of Eudragit microcapsule formation is presented. Various microscopic techniques such as optical, electron, confocal laser scanning and atomic force microscopy were used for the characterization of these microcapsules. The pH-dependent dissolution behavior of the Eudragit shells of these microcapsules was investigated.

**In Chapter 4** the encapsulation technique developed in Chapter 3 was further explored by using various edible oils. The resulting microcapsules were characterized with various microscopic techniques. A relation between the properties of the oil and Eudragit microcapsule formation is investigated. Furthermore, hollow porous capsules can be prepared after removing the oil template with an organic solvent.

**In Chapter 5** microspheres are described that were prepared from binary mixtures of poly methyl methacrylate (PMMA) and Eudragit using microsieve emulsification. The surface morphology of the microspheres formed depends on the ratios of the two polymers and was further investigated after removal of the Eudragit shell from the microspheres by treatment at alkaline pH. The structures were investigated with optical, electron and atomic force microscopy. A mechanism is given for the formation of the different morphologies of the microspheres.

**In Chapter 6** a general discussion of the research that is described in the previous chapters is presented. A relation between the aim and outcome of the research is elaborated. Additionally, scope, perspective and recommendations for future research are proposed.

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# Chapter 2

## *High throughput vegetable oil-in-water emulsification with a microsieve*


## Abstract

Emulsification with high-porosity micro-engineered membranes leads to stable emulsions with a low droplet span when besides a surfactant in the continuous phase an additional, suitable surfactant is used in the dispersed phase. This surfactant should exhibit relatively fast adsorption dynamics, which is more critical when the surfactant in the continuous phase has slower dynamics. Dispersed-phase fluxes of up to $92.5 \times 10^{-6}$ $m^3/m^2s$ could be achieved, which is an order of magnitude higher than previously reported for SPG membrane-based cross-flow emulsification.



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## Introduction

Emulsions are widely used in the food, cosmetic and pharmaceutical industries. Most of the emulsions produced by conventional emulsification techniques (stirring and homogenization) are however polydisperse and their preparation is energy intensive. Several new techniques such as emulsification with microchannels *[1]*, microcapillaries *[2]*, and other microfluidic devices *[3-7]* have been investigated for the production of monodisperse emulsions and microparticles with lower energy consumption. However, upscaling to practical product volumes is a major issue with these techniques. Membrane emulsification is one of the techniques that has potential for upscaling the production of emulsions with droplets of well-defined size *[8]*. Different types of membrane are available for emulsification. Shirasu porous glass (SPG) membranes have been used for the emulsification of rapeseed oil as o/w and w/o/w double emulsions *[9]*. The droplet size and size distribution is dependent on the diameter of the pores in the membrane and process parameters like transmembrane pressure and cross-flow velocity. Nano- and microengineered silicon nitride membranes fabricated with photolithographic techniques with well-defined pore size and geometry are interesting for use in emulsification due to their very high transmembrane fluxes at low transmembrane pressures *[10,11]*.

In cross-flow membrane emulsification the phase to be dispersed, e.g. vegetable oil, is pressed through the membrane; the continuous phase, e.g. water, flows across it and induces the detachment of the droplets at the mouths of the pores. The size of the droplets can be tuned by applying different shear rates and transmembrane pressures *[12]*. To make the process commercially attractive the productivity of the system needs to be high ($> 30 \times 10^{-6}$ $m^3/m^2s$). This may be achieved by the use of high porosity membranes with uniform pore size and regular spacing, but this will increase the risk of coalescence between adjacent growing droplets from neighboring pores *[13]*. Thus, fast stabilization of the forming droplets is important.

Surfactants with a low hydrophilic-lipophilic balance (HLB) are more lipophilic and are normally used to make W/O emulsions, while those with a high HLB are more hydrophilic and better for making an O/W emulsion. Therefore, with conventional emulsification, the type of emulsion created and its stability basically depend on the HLB value of the surfactant and liquid-liquid interactions. In addition, the surfactant system helps to keep the surface of the membrane wetted by the continuous phase *[14-16]*. Thus, a water-continuous system will

benefit from a high HLB surfactant system, and an oil-continuous system from a low HLB surfactant.

A complication of cross-flow membrane emulsification is the fact that a fresh oil-water interface is continuously generated with a high rate at the membrane surface. This implies that surfactant is continuously depleted from the liquid near the membrane. To ensure sufficient stabilization of the droplets that are forming, one generally uses very high bulk surfactant concentrations ensuring high enough transport towards the interfaces.

Even when a surfactant supplied in the dispersed phase will typically have an HLB value that is not appropriate for good stabilization, it does ensure that surfactant is available at the forming interfaces, as it is transferred with the dispersed phase itself. Thus, one may expect that surfactants in the dispersed phase may have a strong effect on the dynamics of the emulsification process.

In this article we present and discuss the results of emulsification process studies with a high porosity micro-engineered membrane. Focus is on the influence of surfactants supplied both via the continuous and the dispersed phase and on the interactions between dispersed and continuous phase and membrane surface.

## Materials and Methods

### *Materials*

Tween 20 (polyoxyethylene sorbitan monolaurate, Merck), DTAB (dioctyl triethyl ammonium bromide, Aldrich), and sodium dodecyl sulphate (SDS, Aldrich) in demineralized water were used as surfactant in the continuous phase. Span 80 (Merck), Brij 30 (ACROS Organics), Brij 97 (ACROS Organics), polypropylene glycol P400 (Fluka, Sigma Aldrich), Pluronic L121(BASF) and soybean lecithin (BDH, VWR International Ltd. England; HLB value 8.00) were used as cosurfactants in sunflower oil (purchased from local supermarket) as the dispersed phase.

### *Microsieve and module*

Micro-engineered membranes were obtained as a kind gift of Aquamarijn BV. The emulsification module and cross flow emulsification setup (figure 2) were provided by Nanomi Monosphere Technology. The 5x5 mm silicon nitride membrane has an effective area of 3x3 mm. The membrane was 1 µm thick, and contained 5 µm diameter pores (figure 1), with distance between pores of 10 µm, yielding a porosity of 30 %. The microsieves were

treated with air plasma to obtain a hydrophilic surface. Then they were fixed into the membrane holder with an epoxy glue. The membrane holder was then placed on top of and in the middle of the emulsification module consisting essentially of a cross flow channel with height, width and length dimensions of 600 μm, 0.65 cm and 13.4 cm, respectively.

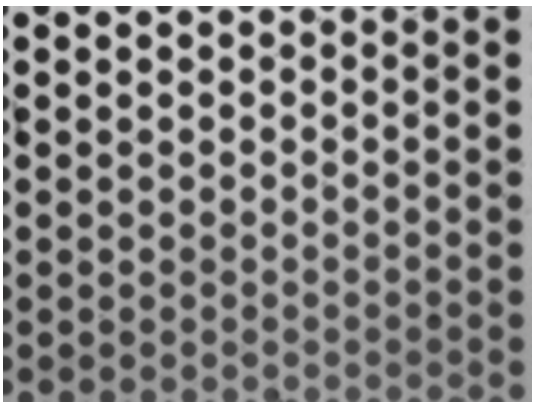

*Figure 1. Optical micrograph of a micro-engineered membrane with a uniform 5 μm pore size and a porosity of 30 %.*

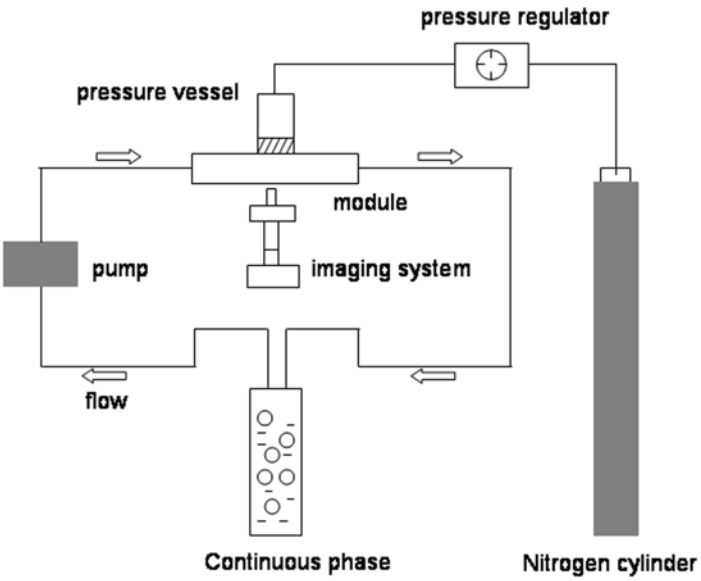


*Figure 2. Block diagram of the cross-flow emulsification setup.*

***The experimental setup***

The experimental setup is shown in figure 2; the dispersed phase was injected by applying a nitrogen pressure on the liquid from a nitrogen cylinder. To maintain an accurate pressure

inside the vessel it was attached to a pressure regulator with a portable pneumatic calibrator (Wallace and Tiernan SERIES 65-120). A Verder gear pump (VG 1000 DIGIT) was used for recirculation of the continuous phase via a 1000 ml container and polyurethane-polyether tubing connections.

***Cleaning of the module and microsieve***

Before each experiment the module was thoroughly cleaned by circulating first 300 mL of demineralized water and then 300 mL of an aqueous solution of the continuous phase surfactant, like 4% Tween 20. This solution was circulated through the tubing for one hour. Immediately after the experiments were terminated the membrane along with the holder was cleaned by extensive flushing with 5 mL of ethanol and 5 mL of hexane and subsequently dried under a nitrogen flow. The cleaning of the membrane was confirmed by optical microscopic inspection of the membrane and measuring the contact angle (KRUSS DSA 100) of the membrane.

***Emulsification methods and process conditions***

For each experiment 1 mL of disperse oil phase was used and 300 mL of continuous aqueous phase, and this composition was kept constant for all the emulsification experiments with different surfactants. All these emulsifications were carried out at the same cross flow velocity with an applied shear stress of 0.709 Pa, since the use of a gear pump influences the droplet size with increase in velocity of the continuous phase flow. The experiments were carried out at room temperature (about 20 °C). Initially, only a cross flow through the module was applied and then the pressure over the membrane was gently increased to start the emulsification. It was checked that the pump and flow in the cross-flow loop did not significantly alter the droplet size and size distribution. At higher flows, some influence was seen; wherever this was the case, the data were left out of the analysis. The emulsification experiments lasted typically 30 minutes. During this time no change in throughput and droplet size or change in membrane properties were observed.

***Visualization of droplet formation and droplet size measurements***

Droplet formation was observed by placing a microscope (Optic and Technology) under the cross-flow channel module. The microscope was equipped with a Moticam 2000 camera and the images were retrieved and stored with the Motic Image Plus program. The droplet size

distribution was determined by a Malvern mastersizer 2000. Pictures of the prepared emulsions were taken through an Olympus BH2 microscope. The average droplet size was obtained from the mastersizer as d(0.5). The droplet size spans are calculated as ($d_{90}$ - $d_{10}$)/$d_{50}$, based on droplet volumes, where $d_{90}$, $d_{10}$ and $d_{50}$ are the particle sizes at which 90%, 10% and 50% of the distribution lies below the cumulative size. A span value between 0.3-0.8 indicates a narrow size distribution.

***Measurement of static interfacial tension***

The Wilhelmy plate technique was used to measure the equilibrium interfacial tension. A beaker containing a two-layer system of the oil phase with different concentrations of Span 80 and the water phase containing 4 % (w/v) Tween 20 was placed under the microbalance (METTLER AE50) attached to the Wilhelmy plate. The plate was cleaned before use and the mass on the plate due to wetting was measured with the microbalance and the interfacial tension was calculated with the equation:

$\gamma = m\, g / 2\, l$

where $\gamma$ is the interfacial tension, $m$ the measured weight increase due to wetting of plate, $g$ the gravitational constant and $l$ the length of the plate.

## Results and discussion

***Membrane surface properties***

For preparation of oil in water emulsions, one needs a membrane that is strongly hydrophilic. The required hydrophilic surface was obtained by treating the silicon nitride membrane with air plasma. The surface hydrophilicity may however be altered by adsorption of surfactants from the dispersed or the continuous phase. The proper choice of surfactants in membrane emulsification varies for different systems and is not straightforward. The system should stabilize the oil-water interface but should not change the wetting properties of the membrane. At the same time, the surfactant supply should match the rate of interfacial expansion, such that the droplets can be stabilized quickly enough. Therefore, the effects of different surfactants with different HLB values and different charges on the emulsification process were studied, either in one phase or in both phases.

***Effect of a high HLB value surfactant in the water phase***

Emulsification of sunflower oil in a 4% (w/v) Tween 20 aqueous solution with a silicon nitride microsieve resulted in slight spreading of oil on the membrane surface, even though Tween 20 is a nonionic surfactant with good oil-water emulsification properties (HLB = 16.9). The high pore density of the membrane may have caused droplet coalescence, because with low porosity membranes good emulsification results can be achieved *[17]*. A second factor is local wetting of the membrane. Since the pores are very close to each other a slight spreading of oil on the membrane surface outside the pore will already lead to an interconnection of the oil-wetted pores, leading to coalescence of oil droplets from different pores *[13]*. Indeed, oil droplets were found to be sticking to the surface of the membrane.

In a previous study on microchannel emulsification of soybean oil in water, it was found that while SDS-stabilized emulsion droplets detach easily from the membrane surface, Tween 80-stabilized droplets were found to stick to the membrane surface *[18]*. It was hypothesized that this was caused by the strong electrostatic repulsion between the negative surface potential of the silicon/silicon oxide membrane and the anionic SDS. Therefore, we also used SDS as an anionic surfactant with a HLB value of 22 in the continuous water phase. Surprisingly, upon using 1% SDS in the continuous phase, wetting of the membrane by sunflower oil was still observed. This may be well related to the rate of transfer of the surfactant to the membrane surface, which may be too low compared to the rate of interfacial expansion. This would lead to local depletion of the surfactant near the forming droplets, local coalescence and subsequent local wetting of the membrane by the dispersed phase.

***Effect of nonionic surfactants in both oil and water phase***

If indeed the rate of supply of surfactant is limiting, one can expect that supplying a surfactant through the dispersed phase would be beneficial. Since the droplets are small, the diffusion distance for adsorption is small, and the supply of the surfactant therefore fast. In addition, a high throughput of dispersed phase is accompanied by a proportionally increased supply of surfactant. Thus, the dynamics of the supply of surfactants from the dispersed phase is intrinsically better suited for high throughput emulsification. Even though an oil-soluble surfactant with a low HLB alone will not stabilize the droplets very well, together with the high HLB surfactant in the continuous phase, it may be sufficient to avoid coalescence during the short time of droplet formation and snap-off.

We therefore applied Span 80 (HLB = 4.3) as a surfactant in oil and Tween-20 in water, both of which are non-ionic. This resulted in a good and smooth droplet formation in high porosity microsieve emulsification. It resulted also in an emulsion with a narrow size distribution, of which figure 3 gives a typical result (1% Span 80 in oil and 4% Tween 20 in water). The average droplet diameter d(0.5) was 33 μm with a span of 0.73.

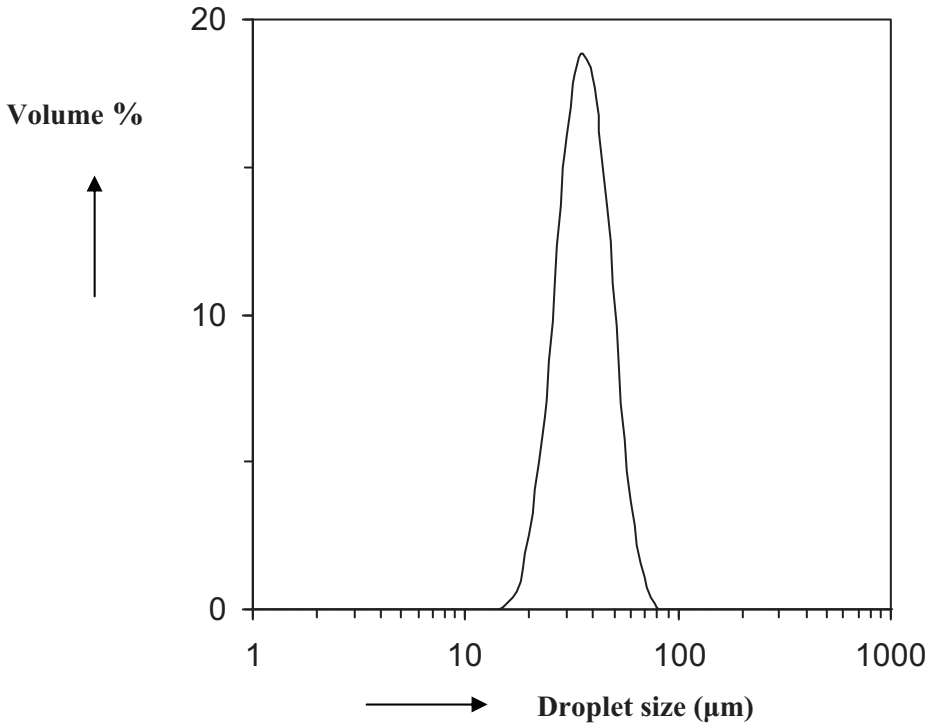


*Figure 3. The droplet size distribution of an emulsion prepared with a high porosity microsieve in the presence of 1% Span 80 in oil and 4% Tween 20 in water at an applied pressure of 38 mbar and a shear stress of 0.709 Pa.*

The difference in surface wetting behavior due to the presence of Span 80 in oil was clear from contact angle measurements. For these experiments a plasma treated, hydrophilic silicon nitride surface was placed in a cuvette, and an oil droplet was deposited on the surface. Upon addition of the aqueous Tween 20 solution the oil droplet remained on the surface. Using Span 80 in the oil, the droplet disintegrated in many tiny droplets and was readily flushed away from the surface upon adding the aqueous Tween 20 solution. However, during actual membrane emulsification the droplet size remained the same and no further disintegration of the droplets was observed. A reason for the different behavior could be that during

emulsification a fresh oil-interface is continuously generated at micrometer scale, therefore actual disintegration of the droplets is not observed during the emulsification process.

Several phenomena can play a role in the droplet formation process. First, in the presence of Span 80 in oil and Tween 20 in water the interfacial tension of the oil and water to the surface is very low (see figure 4), therefore oil droplets easily detach from the surface. Secondly, the interaction between the surfactants may induce the transport of the surfactant in the dispersed phase towards the droplet interface. Co-transport of some of the dispersed phase with the surfactant will lead to spontaneous disintegration of the oil droplet into small droplets *[19,20]*. It is difficult to say which phenomenon is dominant here; however, it is clear that the presence of Span 80 in oil promotes droplet formation and stabilization during membrane emulsification.

**Table 1.** Emulsification performance using different surfactant combinations

| **Surfactant in dispersed sunflower oil phase (w/v)** | | **Surfactant in continuous aqueous phase (w/v)** | | **Emulsification performance** |
|---|---|---|---|---|
| **Type** | **HLB** | **Type** | **HLB** | |
| 4% Span 80 | 4.3 | no surfactant | - | wetting / coalescence |
| no surfactant | - | 4 % Tween 20 | 16.7 | wetting / coalescence |
| 4% Span 80 | 4.3 | 4 % Tween 20 | 16.7 | good droplet formation |
| 4% Lecithin Soya. | 8.0 | 4 % Tween 20 | 16.7 | strong wetting / coalescence |
| 4% Brij 30 | 9.7 | 4 % Tween 20 | 16.7 | wetting / coalescence |
| 4% PPG 400 | 9.7 | 4 % Tween 20 | 16.7 | coalescence |
| 4% Pluronic L121 | 0.5 | 4 % Tween 20 | 16.7 | coalescence |
| no surfactant | - | 1 % SDS | 22.0 | wetting / coalescence |
| 4% Span 80 | 4.3 | 1 % SDS | 22.0 | good droplet formation |
| 4% Lecithin Soya. | 8.0 | 1 % SDS | 22.0 | wetting / coalescence |
| 4% Brij 30 | 9.7 | 1 % SDS | 22.0 | good droplet formation |
| 4% Brij 76 | 12.4 | 1 % SDS | 22.0 | good droplet formation |
| 4% Span 80 | 4.3 | 1 % DTAB | 23.3 | wetting |

Beside the combination of Span 80 and Tween 20, other nonionic surfactant combinations for emulsification were studied. The use of Brij 30 (HLB 9.7) resulted in wetting of the membrane and coalescence of droplets. This is due to the low solubility of Brij 30 in oil, even though it has a stronger interaction with the continuous phase than Span 80. Also the addition of polymeric surfactants such as polypropylene glycol P400 (HLB 9.7) and pluronic L121 (HLB 0.5), resulted in coalescence of the droplets (see Table 1). It is evident that these polymeric surfactants have slower adsorption dynamics to the freshly formed interface. In

short, the surface coverage by surfactant needs to be faster than the creation of the droplet if coalescence and wetting is to be prevented. Surfactants with a high HLB or which have a high molecular weight will diffuse more slowly, and thus do not give the fast interface coverage that is needed during droplet formation.

***Effect of a cationic surfactant in the water phase***

Oil-in-water membrane emulsification with use of 1% cationic surfactant dodecyl trimethyl ammonium bromide (DTAB) in water resulted in strong wetting of the membrane surface resulting in excessive droplet coalescence (Table 1). It has already been reported *[18]* that the presence of a cationic surfactant in the continuous aqueous phase leads to wetting of a plasma-treated membrane surface during oil in water emulsification.

Emulsification with Span 80 in oil and 1% cationic surfactant DTAB in water also results in slight wetting of the membrane by disperse oil phase; it was observed that a small number of oil droplets were sticking to the membrane surface. However, most of the droplets did form without coalescing, and once formed they are stable. The positively charged surfactant in water has a strong interaction with the negatively charged membrane surface and will form multilayers on the membrane *[14]*, leading to loss of hydrophilicity of the membrane. This will lead to wetting of the membrane by the dispersed phase. The observations as reported in table 1 show that cationics indeed render the surface hydrophobic, resulting in an enhanced wetting by oil. This indicates that the stabilization of the continuous phase is essential, and that the role of the surfactant in the dispersed phase is probably only important for the first period during and after droplet formation.

These results are supported by the results obtained with lecithin (HLB 8) in the dispersed phase. This is a surfactant mixture with zwitterionic properties, which may strongly adsorb to the membrane surface, rendering it less hydrophilic. The dynamics of adsorption of lecithin will also be much slower than that of nonionic surfactants, as it is not molecularly dissolved, but is present in the form of lamellar aggregates at the interface.

***Effect of a nonionic surfactant in the oil phase and an anionic surfactant in the water phase***

As discussed previously, the combination of a nonionic surfactant in the continuous phase only led to good emulsification when combined with a surfactant like Span 80 in the dispersed phase. The anionic surfactant SDS has faster dynamics *[21]* compared to Tween 20, and may yield stronger stabilization because of the additional electrostatic repulsion. However, use of

SDS alone does not lead to good emulsification, but yields wetting of the membrane by the dispersed phase. Apparently, the same arguments apply as for Tween 20 in the continuous phase. One would expect good wetting by the continuous phase, due to the strongly negatively charged membrane surface, and the negative oil-water interface resulting from the use of SDS. The fact that the membrane was wetted by the dispersed phase is a direct indication of the insufficient dynamics of the surfactant during droplet formation.

Systems containing SDS in the aqueous phase and Span 80 or Brij 30 or Brij 76 in the dispersed phase resulted in good droplet formation. The fact that Brij 30 and Brij 76 give good results in these systems is likely due to the faster dynamics of SDS compared to Tween 20 *[21]*, which makes the dynamics of the surfactant in the dispersed phase less critical.

As mentioned before, lecithin, being a mixture of zwitterionic phospholipids, is not molecularly dissolved in the oil but probably present in the form of lamellar aggregates, and will thus show very slow adsorption. Even though in this system with SDS in the aqueous phase membrane wetting occurred, it was substantially reduced compared to the system with Tween 20 in the continuous phase. Once more, this is likely due to the faster and more effective stabilization by SDS than by Tween 20.

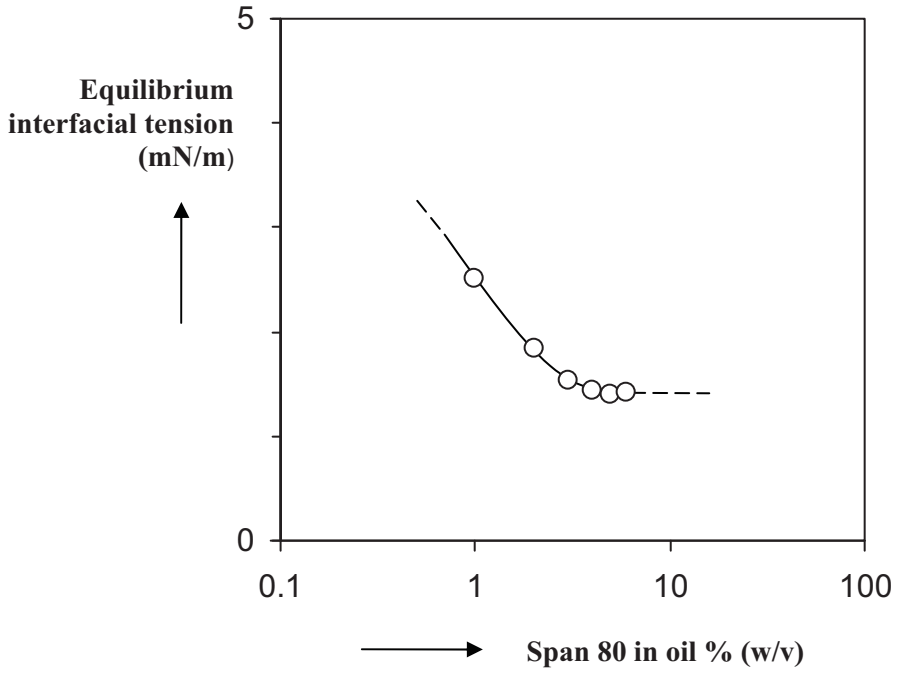


*Figure 4. Equilibrium interfacial tension at the oil-water interface, using different concentrations of Span 80 in oil and a fixed amount of 4% (w/v) Tween 20 in water.*

***Influence of process parameters on the emulsification***

In this section the effect of various process parameters on the performance of the emulsification using surfactant combinations that work well is discussed. For systems with Span 80 in the oil phase and 4% Tween 20 in the water phase the influence of increasing amounts of Span 80 on the interfacial tension were determined. Addition of more Span 80 to the oil phase rapidly decreases the interfacial tension to low values (figure 4). However, these values were obtained under static conditions, which means that they cannot be directly translated to the conditions during droplet formation. The equilibrium interfacial tension of sunflower oil with 4 % (w/v) Tween 20 was found to be 9.1 mN/m.

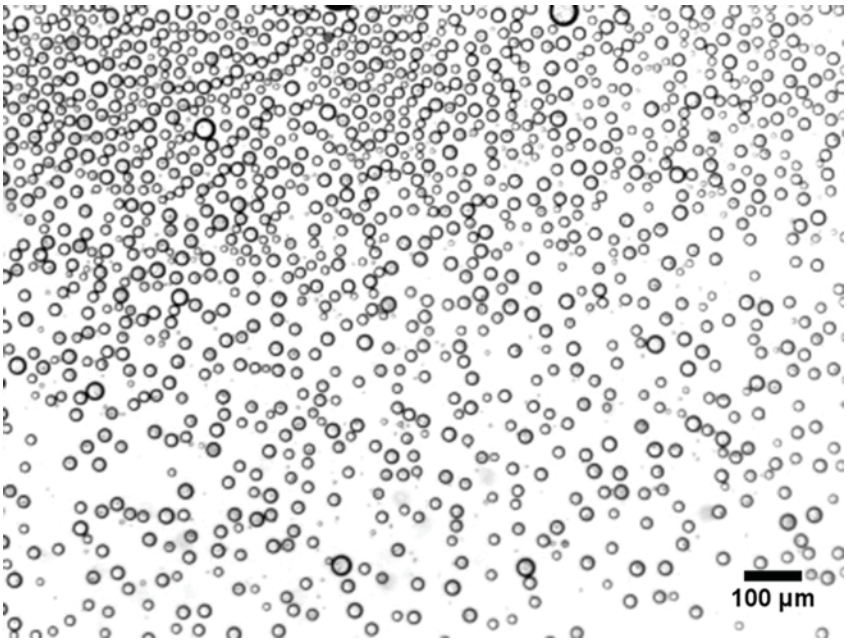


*Figure 5. Optical micrograph of droplets obtained upon microsieve emulsification of sunflower oil containing 4% Span 80 and in 4% Tween 20 in water, at a pressure of 20 mbar. The applied shear stress was 0.709 Pa.*

Figure 5 shows a representative optical microscopy picture of droplets prepared with 4 % (w/v) of Span 80 in the oil phase and 4 % (w/v) Tween 20 in the water phase. The applied shear rate was 0.709 Pa at the dispersed phase pressure of 20 mbar. For this system, the average droplet size (20 µm; span 0.9) was found to be only slightly dependent on the concentration of Span 80 in the oil.

The effect of using different concentrations of Span 80 on the droplet size and span is shown in Figure 6. The droplet size decreases with increase in Span 80 concentration in oil, where as the span remains almost constant. Since all experiments were carried out at the critical pressure of emulsification (40, 25, 21 and 20 mbar for 1, 2, 3 and 4% of Span 80 in oil respectively) and at a constant applied shear stress, the decrease in droplet size could be caused by the enhanced dynamics of the surfactant with the increase of Span 80 concentration in oil. The droplet to pore diameter ratio was in the range of 3 to 7, which is also expected for single pore emulsification *[13]*. This indicates that the emulsification is very stable against coalescence for all concentrations of Span 80 used in oil. Even upon six days storage of the prepared emulsions no change in the droplet size and span was observed. It means that the presence of suitable surfactants, i.e. Span 80 in oil and Tween 20 in water, improves the emulsion stability due to steric repulsion between both surfactants. Even though a span of 0.8 can be considered as a good size distribution, a much narrower size distribution has been obtained with SPG-based membrane emulsification, however under different process conditions *[9]*.

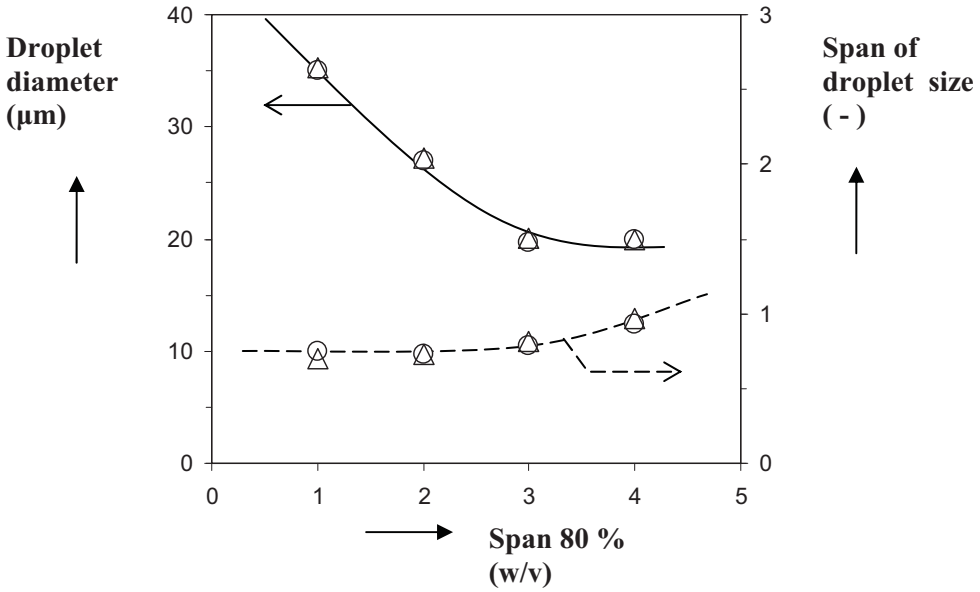


*Figure 6. Average droplet diameter and span upon emulsification with different concentrations of Span 80 in the dispersed (oil) phase, at the critical pressure of emulsification and a shear stress of 0.709 Pa. Solid line is droplet diameter; dotted line is span. Circles represents immediately after preparation and triangles after storage for 6 days.*

In a computational fluid dynamic study *[22]* it was shown that for cylindrical pores of 7 µm, the resulting droplets have a diameter of about 33 µm (i.e., a ratio of 4.7). To avoid coalescence between two neighboring droplets, the distance between two adjacent pores should therefore be at least 5 times the droplet size. However the droplet will get deformed in the direction of flow during the detachment process. Therefore, considering this deformation, the distance between the pores should be 7 times the pore diameter. In this way it is only possible to design and use a microsieve with a maximum porosity of 1.5 %. That a well-defined emulsion was obtained with the use of a high porosity microsieve, was due to the fact that in the present system the droplets are quickly protected from coalescence and thus remain stable, even though they may well press against each other. With the use of a second surfactant in the dispersed phase the Laplace pressure can be lowered due to a decrease in the interfacial tension. Therefore, this results in more active pores at corresponding pressures, yielding higher fluxes.

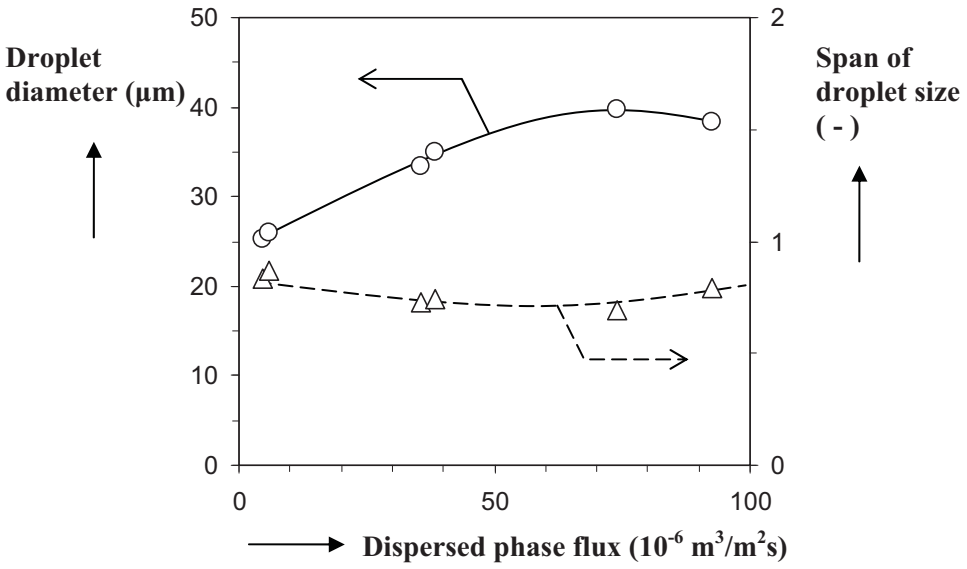


*Figure 7. Effect of disperse phase flux on the droplet size (–○–) and span (--Δ--) of the droplets. The disperse phase was 1% Span 80 and the continuous phase was 4% Tween 20. The applied shear stress was 0.709 Pa.*

The disperse phase flux can be further increased by increasing the pressure over the membrane. In figure 7, the average droplet size and its span are plotted versus the disperse phase flux for 1% Span 80 in oil and 4% Tween 20 in water. With increase in flux, the droplet size increases gradually and no significant change occurs above the pore activation pressure

(38 mbar). The force or torque balance models (e.g. Peng and Williams *[23]*) would indicate that the dispersed phase flux (or the transmembrane pressure) is not important for the droplet size obtained. However, these models do not take the dynamics of droplet formation into account. Van der Graaf et al. *[24]* showed that for a T-shaped microchannel, the flow rate of the dispersed phased is important, since the droplet detachment takes some time. They showed that the smallest droplets are produced at low dispersed phase flow rates. At higher dispersed phase flow rates, the frequency of droplet formation from a single pore increases, and the time necessary for necking and snap off becomes significant compared to the total droplet formation time. They also found that the droplet volume could be described by a critical volume, plus a necking contribution that was more or less proportional to the dispersed phase flow rate. Examination of figure 7 shows that this description could also apply in our system.

At the pore activation pressure (the Laplace pressure at which emulsification starts), the flux was 6 x $10^{-6}$ $m^3/m^2s$. It was possible to increase the flux of the oil phase up to 92.5 x $10^{-6}$ $m^3/m^2s$ without significantly changing the span of droplet.

Due to the presence of the large number of pores in the microsieve, it was difficult to observe or estimate the number of active pores during the emulsification process. Dispersed-phase fluxes of up to 92.5 x $10^{-6}$ $m^3/m^2s$ could however be achieved, which is considerably higher than the value of 6.94 x $10^{-6}$ $m^3/m^2s$ reported earlier for SPG-based cross-flow emulsification *[25]*.

## Conclusions

Coalescence of droplets and wetting of high-porosity silicon nitride microsieve membranes by the dispersed phase during oil droplet formation, was prevented by adding a suitable surfactant to the dispersed phase. This leads to stable and narrow size distribution emulsions. The surfactant in the dispersed phase should exhibit relatively fast adsorption dynamics, which is more critical when the surfactant in the aqueous continuous phase has slower dynamics (e.g., Tween 20 compared to SDS). The flux of the disperse phase could be increased an order of magnitude compared to previous methods, without loss of low span of the droplets. Thus, use of a high-porosity membrane, in combination with suitable surfactants in both the dispersed and continuous phases led to a much more effective and efficient emulsification process.

## Acknowledgments

This research is carried out within the work package Microengineering of Supramolecular Structures of the Dutch MicroNed program is also thanked for financial support. The authors thank Gert Veldhuis of Nanomi for valuable advice regarding the use of the emulsification module and setup. Aquamarijn is thanked for providing the microsieves. Jos Sewalt from the Food Process Engineering group, Wageningen, is thanked for technical information regarding the use of the Malvern mastersizer and Karin Schroën for helpful discussions.

## Supplementary information

A movie of edible oil in water emulsification with a high porosity microsieve, in the presence of 4% (w/v) Span 80 in oil and 4% (w/v) Tween 20 in water is available online in the publication: *Journal of Membrane Science,* **2010**, 347, 1-7

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# Chapter 3

## *Porous microcapsule formation with microsieve emulsification*


## Abstract

A simple route is presented to prepare core-shell Eudragit microcapsules through a solvent extraction method with the use of microsieve emulsification. Droplets from a solution of Eudragit FS 30D (a commercial copolymer of poly(methyl acrylate-co-methyl methacrylate-co-methacrylic acid) 7:3:1) and hexadecane in dichloromethane are dispersed into water, using a micro-engineered membrane with well-defined pores, in a cross-flow setting. The dichloromethane is extracted from the droplets, which induces demixing in the droplets, leading to a hexadecane-rich core, and an Eudragit-rich shell. The obtained microcapsules have a narrow size distribution due to the microsieve emulsification process. The capsules have a porous shell as shown by SEM and AFM measurements. Their porosity and pore size is dependent on the ratios of Eudragit and hexadecane in the dispersed phase. At pH 7.1 and above Eudragit (FS 30D) dissolves in water; this pH change is used to release the contents of the microcapsule.



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## Introduction

Sensitive, volatile or reactive additives such as drugs, biocultures, flavors and vitamins can be turned into stable functional ingredients through microencapsulation. With careful fine-tuning *[1-3]* of the microcapsules, new ingredients can be developed with a large variety of properties and wide applicability. The oral delivery of components that are susceptible to degradation *[4-7]*, such as peptides, nanovectors, aptamers, enzymes, living cells and probiotics in microcapsules has substantially increased in the past decades. Several strategies have been developed to counter-balance the digestive influence of the stomach (pH 2-3) and bile salts in the duodenum (pH 6-6.5) by increasing the stability and activity of the encapsulated ingredients. Especially the lower gastrointestinal (GI) tract from the small intestine (pH 6.5-7.0) to the colon (pH 7.0-8.0) has been used as site to target these agents.

Microcapsules are built up of a core and a shell, and the release of the core material is co-determined by the permeability *[3, 8]* of the capsule wall. Since the size of the capsule is important for the rate of release, control over the size and size distribution of microcapsules is a crucial factor. Various studies have been performed on membrane emulsification for production of emulsions, particles and microcapsules *[9-13]*. We recently investigated conditions for high throughput production of well-controlled oil-water emulsions using cross-flow membrane emulsification with high porosity micro-engineered microsieves *[14]*. Microsieve emulsification has the additional advantage that microcapsules can be prepared with a minimum of energy consumption.

Different approaches have been employed in the past to prepare microcapsules with tunable size, permeability and mechanical strength *[2, 3, 15, 16]*. Phase separation is an approach to prepare oil-core polymer-shell capsules *[17-26]*, in which a non-volatile poor solvent (alkane) is added to a polymer solution in a volatile organic solvent, which is then emulsified in an aqueous phase to form an oil-water emulsion. The solvent diffuses out of the droplets, through the continuous phase, and evaporates at the surface of the bath. The extraction of the solvent from the droplet induces instability in the droplet. An inner core droplet of the poor solvent is formed, while the solution around this inner core becomes even more highly concentrated in the polymer. Thus a polymeric shell is created around the inner droplet which ultimately solidifies by gelation, crystallization or glassification. Although fabrication of core-shell microcapsules by phase separation is well known, capsule formation with porous shell membranes is not very well understood. Because the capsule formation process starts with an emulsion droplet, precise control over the emulsion droplet size should lead to well-defined

microcapsules with a narrow size distribution and thus similar properties. In this study cross-flow microsieve emulsification is used to generate an oil-water emulsion with a narrow size distribution. The emulsified droplets consist of Eudragit as a hydrophilic polymer, hexadecane as oil and dichloromethane as solvent. Eudragit FS 30D is a poly(methyl acrylate-co-methyl methacrylate-co-methacrylic acid) copolymer with monomer ratios of 7:3:1. It is insoluble in water below pH 7 and dissolves above pH 7. Since Eudragit is relatively hydrophilic, a porous shell is formed. The influence of polymer and oil concentration on the phase separation process that leads to the core-shell microcapsules is investigated. Furthermore, the pH-triggered dissolution process of the polymer shell, releasing the inner oil droplets, was studied. The results of this model study can lead to the development of microcapsules with pH-triggered release under physiologically relevant conditions.

## Materials and Methods

### *Materials*

An aqueous dispersion of Eudragit FS 30 D (ED) was obtained as a gift from Evonik Industries. This dispersion was freeze-dried to remove the water. The resulting powder was then used to prepare the capsules. Dichloromethane (DCM; Merck) was used as volatile organic solvent to dissolve the polymer and hexadecane (Merck), which was also used as a poor solvent for the polymer. A 1 % aqueous sodium dodecyl sulfate (SDS; Fluka) solution (pH 5) was used as continuous phase for the emulsification.

### *Microengineered membrane, emulsification device and microcapsule formation*

Details of the membrane and emulsification set-up used have already been published *[14]*. The membrane used was a 5x5 mm microchip with a silicon nitride membrane with an effective area of 3x3 mm. The thickness of membrane was 1 μm, and contained 5 μm diameter pores with distances between the pores of 10 μm, yielding a porosity of 30 %. The microsieves were treated with air plasma to obtain a hydrophilic surface. Then they were fixed into the membrane holder with an epoxy glue. The disperse phase was a solution of Eudragit in DCM containing hexadecane; 1% SDS in water was used as continuous phase. All experiments were carried out at a pressure of 30 mbar (capillary pressure is 23 mbar) and a shear stress of 0.71 Pa imposed by the cross-flow of the continuous phase. At these conditions control over the disperse phase flux and resulting droplet formation was good. At higher

pressures it becomes difficult to control the droplet formation. The Eudragit polymer was first dissolved in DCM. To this solution the poor solvent hexadecane was added. This mixture was then pressed through a high porosity microsieve into a 1% aqueous SDS solution to form an oil in water emulsion by the method as described before *[14]*.

***Characterization of the microcapsules***

Scanning electron microscopy: The aqueous dispersions of the capsules were filtered through a Nuclepore polycarbonate membrane and then dried at ambient conditions. Subsequently, the capsules were sputter-coated with gold/palladium and visualized along with the filter membrane by a field emission scanning electron microscope (JEOL 6300 F, Tokyo, Japan) at a working distance of 8 mm, with SE detection at 5 kV. All images were recorded digitally and were optimized and resized by Adobe Photoshop 7.0.

Confocal laser scanning microscopy: The cavity for the sample was prepared by cutting a rectangular hole in a piece of parafilm and putting it on a glass slide. This was then covered with a glass cover slip fixed firmly to the glass slide by applying nail polish. Then the capsule dispersion was added from the side opening with the use of a micropipette. The sample was then investigated with a confocal laser scanning microscope (Zeiss LSM 510 Meta); green fluorescence protein was excited at 488 nm and Nile red at 633 nm.

Atomic force microscope: The aqueous dispersions of the capsules were filtered through a regenerated cellulose polymeric filter membrane and subsequently dried in air. Then the surface of the capsule was scanned by tapping-mode atomic force microscopy (AFM; Asylum Research MFP-3D SA AFM). Height images were obtained in AC mode in air using NSC35/AIBS ultra sharp cantilevers (MikroMasch Europe).

## Results and discussion

***Microcapsule formation and size determination***

The average size of the droplet formed immediately after microsieve emulsification is 53 μm with a coefficient of variation of 12% as estimated from movie pictures made of the emulsification. During the microcapsule formation, at the low concentrations used, the DCM is miscible with water. Therefore, it migrates from the droplets towards the external water phase. As hexadecane is neither compatible with water nor polymer and causes liquid-liquid demixing between polymer and hexadecane, the oil tends to nucleate in the middle of the

capsule and the polymer starts to gelate around the oil droplet. This subsequently results in the formation of hexadecane core/polymer shell microcapsules. The remaining DCM in the water phase is slowly evaporated by stirring in contact with air. The obtained microcapsules (figure 1) have a relatively narrow size distribution (figure 2), with an average diameter of 30 μm and a coefficient of variation of 15%. The diameter is larger than would be expected based on the initial concentrations of Eudragit and hexadecane. The main reason for this is that the Eudragit is hydrophilic and becomes swollen in the aqueous environment. An additional reason could be that as soon as the droplet starts forming from the microsieve pore, DCM already starts to go to the aqueous phase, thus increasing the concentrations of Eudragit and hexadecane in the initial DCM droplet.

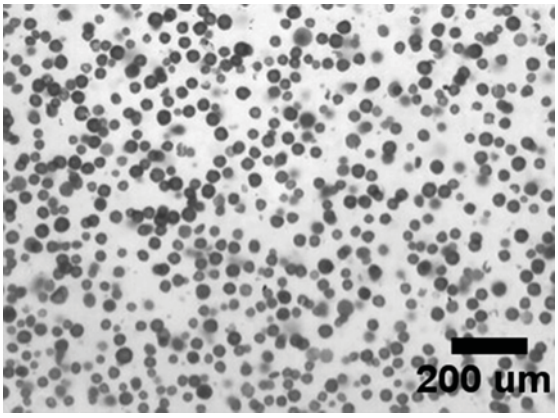



*Figure 1. Optical microscope picture of Eudragit capsules prepared at a 2% polymer and 2% hexadecane initial concentration.*

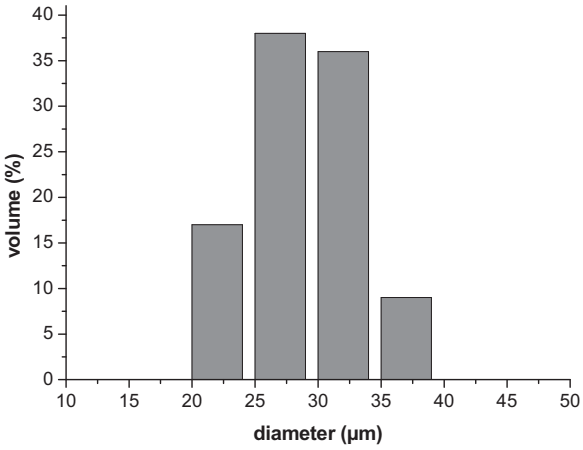
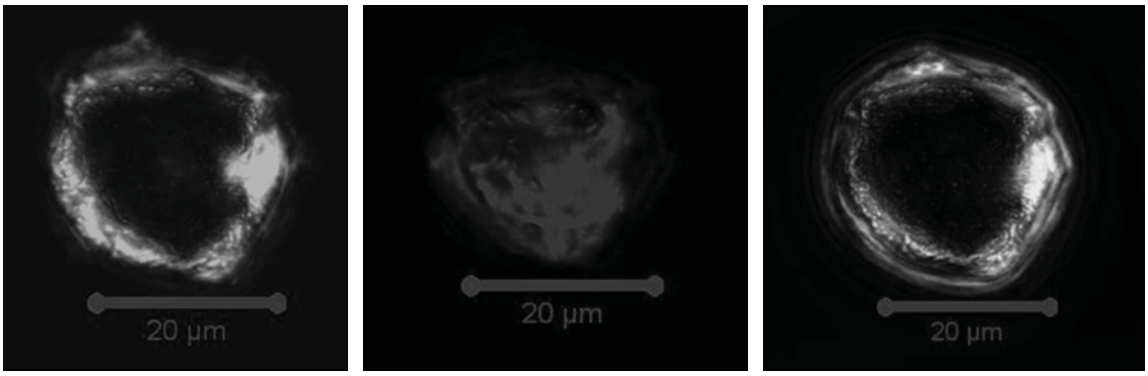



*Figure 2. Size distribution of Eudragit microcapsules determined from optical microscopy; capsules were prepared at a 2% polymer and 2% hexadecane initial concentration.*

***Phase separation studies with CLSM***

Figure 3 shows confocal microscopy pictures of a capsule, in which the hexadecane is stained with hydrophobic Nile Red and the Eudragit with green fluorescent protein (GFP). As is clearly seen, the hexadecane is mainly present in the centre of the capsules, while the Eudragit forms a shell around it; however, it can be seen that some hexadecane is also present in the shell. Observation of these microcapsules with optical microscopy (figure 1) does not provide information on the presence of oil in the shell, however investigation with CLSM confirms the presence of oil in the shell, probably in the form of tiny droplets. The phase separation process starts at the surface of the droplets. Since the concentrations of both Eudragit and hexadecane increase, both components start to phase separate from the solution, and hexadecane droplets can get trapped in the developing Eudragit shell. The number and size of these droplets will depend on the ratios and initial concentrations of both Eudragit and hexadecane. An implication of the presence of hexadecane droplets in the shell on the formation of a porous shell of the capsule is discussed in the next section.

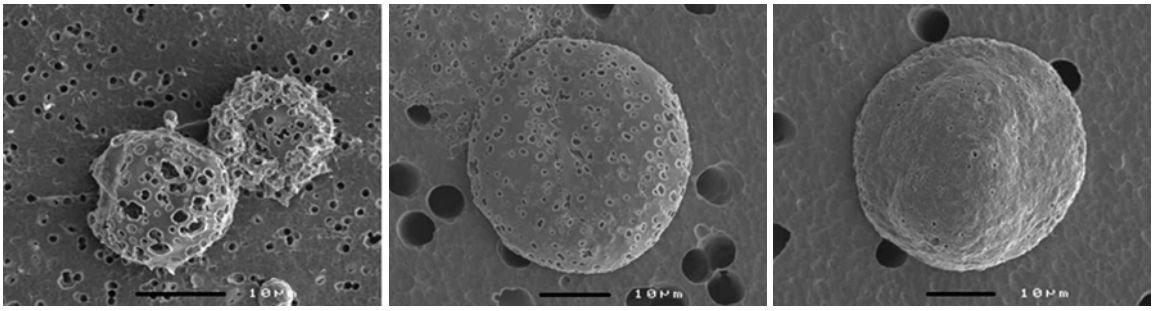



a) b) c)

*Figure 3. Microcapsules prepared from a 4% polymer and 8% hexadecane solution in DCM a) excitation of the green fluorescence protein b) excitation of the Nile red c) excitation of both Nile red and green fluorescent protein. (For color picture see Appendix on page number 101).*

***Influence of initial composition on microcapsule morphology as studied with SEM and AFM***

The phase separation process results in microcapsules with either a porous or a dense membrane depending on the conditions used. This is clear from scanning electron microscopy (SEM) pictures of the microcapsules.



a) b) c)

*Figure 4. SEM images of microcapsules prepared by phase separation at different initial polymer and hexadecane concentrations, a) 2% polymer and 15% hexadecane, b) 3% polymer and 15% hexadecane, c) 4% polymer and 8% hexadecane. Scale bar is 10 µm.*

Figure 4 shows SEM images of microcapsules prepared at different polymer and hexadecane initial concentrations. It is seen that using a small ratio of polymer over hexadecane results in porous shells, which seem relatively weak (figure 4a). This could be caused by coalescence of the small trapped hexadecane droplets in the relative thin Eudragit shell giving larger pores, or by channels formed in the shell by DCM diffusing out of the core or a combination of these processes. Using more polymer relative to hexadecane results in a lower porosity and smaller pores (figure 4b), and almost complete disappearance of the pores at sufficiently high polymer concentrations. Earlier studies indicate that pore formation can be caused either by in-diffusion of water and/or out-diffusion of DCM *[18, 27]* during the capsule formation process. It is also possible that the pores in the capsule shell are formed by residual amounts of hexadecane which did not have time enough to diffuse into the core of the droplet during the phase separation process *[28]*. This is supported by the fact that an increase in concentration of hexadecane, relative to the polymer concentration, leads to more pores (which would therefore be made up of hexadecane droplets). Therefore, upon increasing the polymer to hexadecane ratio (in figure 4 going from a) to c)) the shell surface morphology changes from porous to dense. An increase in hexadecane concentration at the periphery of the shell may result in coalescence of several hexadecane droplets, and thus bigger pores upon increasing the hexadecane concentration. The overlap image of the confocal laser scanning microscopy in figure 3c indeed shows the presence of small amounts of hexadecane at the edge of the shell.

The surface of the microcapsule prepared at an initial 4% polymer and 8% hexadecane (compare Figure 4c) was also investigated with Atomic Force Microscopy (AFM). Since the size of microcapsules is quite big (30 µm) for AFM, only a part of the capsule surface could

be scanned. Figure 5 shows an AFM image of the capsule surface, which clearly shows the curved surface having pores with sizes of around 100 nanometers, which corresponds nicely with the electron microscopy results (Figure 4c).

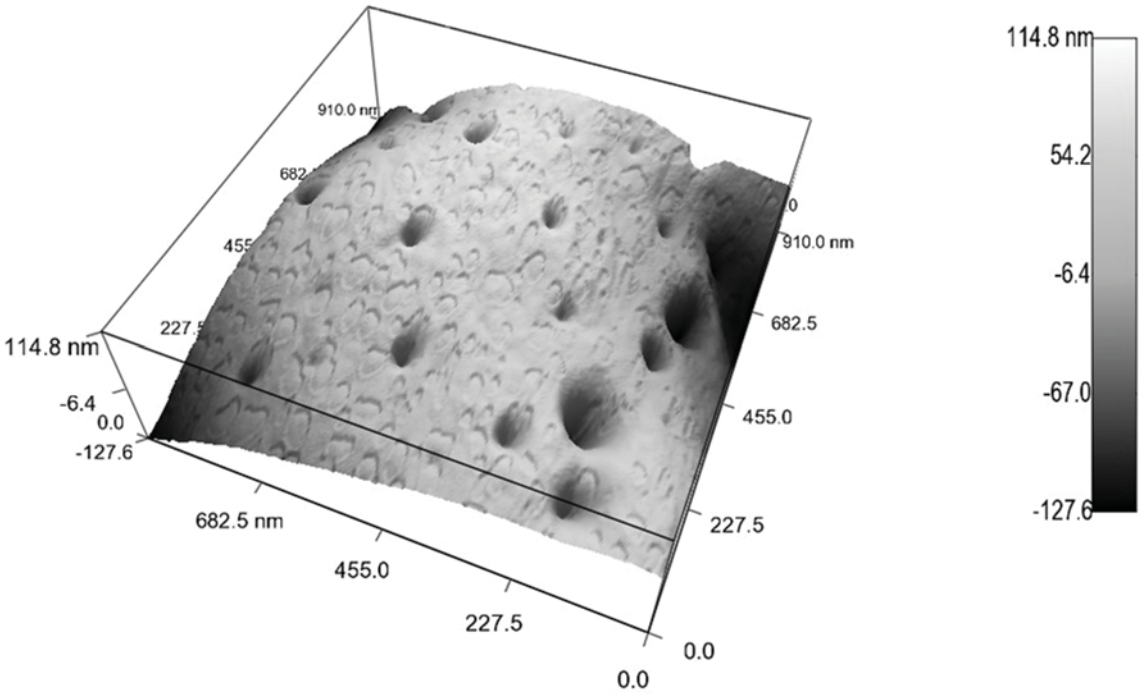



*Figure 5. AFM image of a capsule surface prepared from an initial 4% polymer and 8% hexadecane solution in DCM.*

***Dissolution studies of microcapsule shells at different pH***

An interesting property of the Eudragit FS 30D used in this study is that it is insoluble in water below pH 7 and readily dissolves above pH 7. Therefore, it can potentially be used for oral delivery to the extreme lower part of the gastrointestinal tract. The pH-dependent behavior of microcapsules prepared from an initial 4% Eudragit and 8% hexadecane solution in DCM was investigated by optical microscopy.

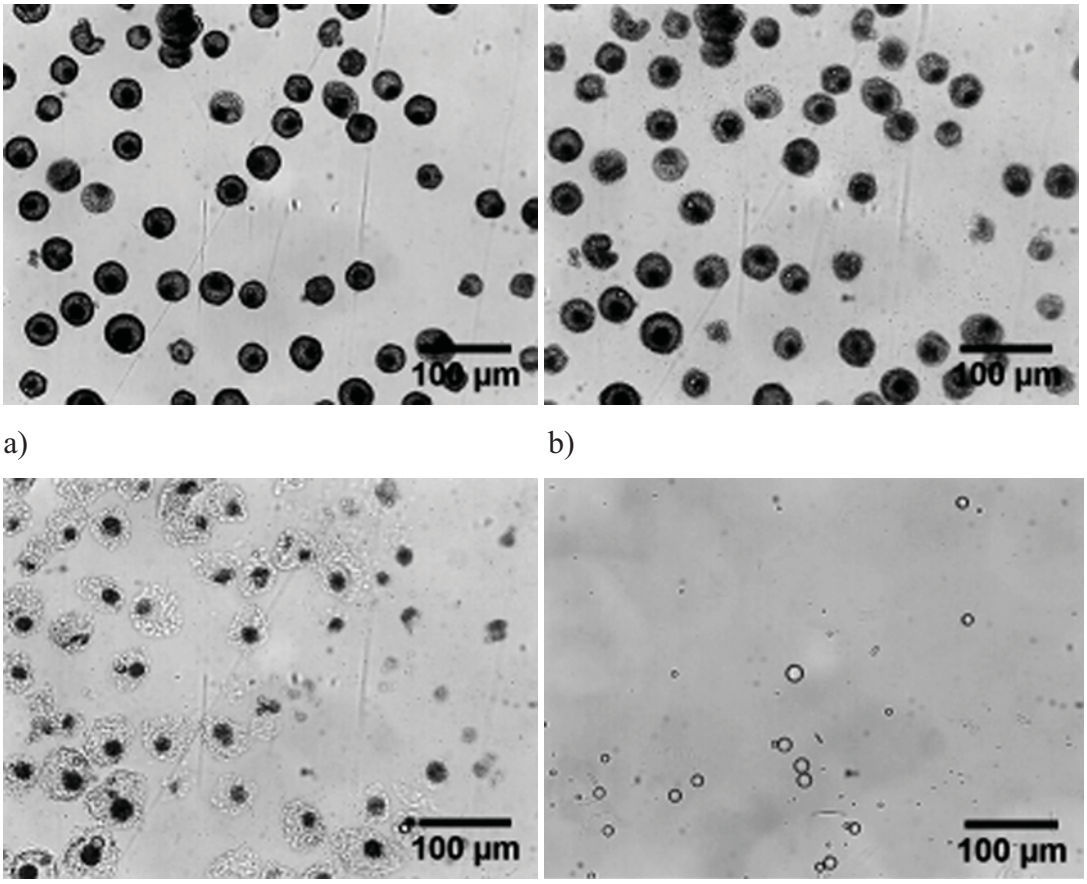



*Figure 6. Dissolution of the Eudragit shell of microcapsules prepared from a solution of 4% polymer and 8% hexadecane in DCM at pH 7.1 as function of time: a) 2 minutes b) 5 minutes c) 17 minutes d) 30 minutes after increasing the pH from ~5 to 7.1.*

The microcapsules are stable below pH 7. At pH 7.1 (figure 6) the Eudragit shell of the microcapsules slowly starts to swell. Since water can in-diffuse in the microcapsule shell, the contrast under the light microscope becomes less, due to the reduction of the refractive index. After about 30 minutes at pH 7.1 the shell is more or less dissolved in water. Bare hexadecane droplets are now seen (figure 6d). The size of these droplets is slightly smaller than expected on the basis of the initial concentrations of hexadecane. This is probably caused by the loss of the tiny hexadecane droplets that were initially trapped in the Eudragit shell. At pH 8.0 the microcapsules shown a very quick pH response, they get swollen rapidly (movie in supporting information) and within a minute Eudragit is dissolved with release of the hexadecane droplets. This indicates that the capsules are stable at pH below 7 and will quickly release their contents above pH 7. The microcapsule preparation method described here may be

interesting for encapsulation and controlled release of oil-soluble active ingredients for delivery to lower gastrointestinal tract.

## Conclusions

Microcapsules with a narrow size distribution of around 30 micron were prepared from Eudragit and hexadecane dissolved in DCM, using cross-flow emulsification with a micro-engineered microsieve membrane. Due to a phase-separation process by which the DCM is removed, a hexadecane core is formed in the microcapsules, surrounded by an Eudragit-rich shell. At polymer concentrations which are low relative to the concentration of hexadecane, the shells were found to be porous. Increasing the polymer concentration, relative to the oil concentration, resulted in a reduction of the porosity and pore size. The capsules are stable at pH lower than 7, whereas the oil core was released in half an hour at pH 7.1 and within a minute at pH 8.0 due to dissolution of Eudragit shell.

The present study indicates that with a careful choice of polymer, oil and solvent, and their relative concentrations, microcapsules can be obtained with a well-defined core-shell morphology. Depending on the concentrations of both polymer and oil, the phase separation processes differ. At higher concentrations of polymer, the tiny oil droplets that are captured in the forming Eudragit shell cannot coalesce anymore and therefore lead to small pores. The present study shows that core-shell microcapsules can be made with a porous shell of which pore size and porosity can be easily tuned. This may influence the permeability properties of the shell. Combined with the microsieve emulsification process and the pH-triggered dissolution of the Eudragit shell, this research may lead to the development of microcapsules with special release properties.

## Acknowledgments

The Dutch MicroNed program is thanked for financial support. Aquamarijn is thanked for providing the microsieves and Evonik Pharmacy Inc. for the Eudragit FS 30D sample. Adriaan van Aelst for SEM measurements and Marcel Giesbers for AFM measurements.

## Supporting information

A movie of the dissolution of the Eudragit shell at pH 8 for microcapsules prepared from an initial 4% Eudragit and 8% hexadecane solution in DCM is available online in the publication: *Journal of Colloid and Interface Science*, **2011**, *355*, 453-457.

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# Chapter 4

## *Hollow capsules: influence of the encapsulated oil on the capsule morphology*


## Abstract

Microcapsules were prepared by microsieve membrane cross flow emulsification of Eudragit FS 30D/dichloromethane/edible oil mixtures in water, and subsequent phase separation induced by extraction of the dichloromethane through an aqueous phase. For long-chain triglycerides and jojoba oil, core-shell particles were obtained with the oil as core, surrounded by a shell of Eudragit. Medium chain triglyceride (MCT oil) was encapsulated as relatively small droplets in the Eudragit matrix. The morphology of the formed capsules was investigated with optical and SEM microscopy. Extraction of the oil from the core-shell capsules with hexane resulted in hollow Eudragit capsules with porous shells. It was shown that the differences are related to the compatibility of the oils with the shell-forming Eudragit. An oil with poor compatibility yields microcapsules with a dense Eudragit shell on a single oil droplet as the core; oils having better compatibility yield porous Eudragit spheres with several oil droplets trapped inside.



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## Introduction

Microcapsules are useful ingredients of many products in daily life such as cosmetics, functional foods, and pharmaceuticals *[1]*. Microcapsules with a polymeric shell surrounding a core filled with oil *[2-4]* are interesting for encapsulation and delivery of oil-soluble components. Many flavors and fragrances, but also antioxidants, vitamins and pharmaceuticals are oil-soluble and sensitive to the acidic conditions in the stomach which limits their applicability for oral delivery *[5-7]*. Therefore, providing the microcapsules with a pH-sensitive polymer shell could improve the delivery and bioavailability of these substances *[7-9]*.

Microcapsules can be prepared by a wide range of methods, including spray drying or chilling, extrusion and phase separation. The last method allows for the preparation of very small microcapsules that should not influence the taste of the product, while still ensuring a good polymeric shell around the encapsulated component. In phase separation, a polymer solution is emulsified in a non-solvent. The solvent inside the droplet is then slowly extracted. The to-be-encapsulated component forms a separate phase at some point in the process, usually in the middle of the solidifying droplet. The morphologies and properties of the resulting capsules depend on factors like the extraction rate of the solvent, demixing time and the phase behavior of the system *[10]*.

Several strategies have been employed in the past to prepare capsules by changing one or more components of the system. The use of an amorphous polymer results in slow solidification and transition into a glassy, dense shell; a semi-crystalline polymer may lead to faster solidification and to porous shells. If the non-solvent surrounding the droplets is more compatible with the solvent, i.e. has a better solubility, the solvent is extracted more quickly, which results in faster demixing and this can possibly influence the morphology of the resulting capsules *[11, 12]*.

The removal rate or extraction of the solvent is a very important parameter for microcapsule formation. This is not only influenced by the non-solvent but also by the type of encapsulated component (oil) that is used. Depending on the compatibility of the polymer and the oil, the demixing rates vary, which leads to different shell morphologies, for example dense with a single oil core, or a porous shell that gradually opens up to a porous core *[13-15]*. Finally, oils that are relatively well compatible with the shell-forming polymer will swell the material, which will influence the physical properties of the polymer phase, such as its thermal and mechanical properties *[14]*.

Encapsulation of materials within a shell of stimuli-responsive polymers (e.g. by pH or temperature) have gained specific interest for controlled release applications *[16-18]*. Eudragit FS 30D is a commercial random copolymer of poly (methyl acrylate-co-methyl methacrylate-co-methacrylic acid) 7:3:1, which is used extensively for enteric coating applications. The polymer contains a certain percentage of carboxylate groups, which makes them water-soluble above pH 7.0. These properties have been used to obtain sustained delivery of vitamin C by encapsulating in an Eudragit shell with slightly different properties (Eudragit RL) *[19]*. Control over the permeability properties is important and this has been achieved with a variety of routes, e.g. by adsorption of colloidal Eudragit particles on an emulsion droplet *[20]*.

Phase separation is a simpler route to prepare porous microcapsules of Eudragit. Eudragit is dissolved in a low concentration in dichloromethane, together a certain amount of the to-be-encapsulated oil. At these low concentrations, these two components are both dissolved in the dichloromethane. This solution is then emulsified in water. The dichloromethane dissolves in low concentrations in the water, thus slow diffusion from the droplet occurs into the aqueous phase and finally the dichloromethane can evaporate at the air-water surface of the system. As the droplets slowly lose solvent they become more concentrated in Eudragit and oil. At some point, the Eudragit and oil are not miscible anymore, and under the right conditions will demix into a shell of Eudragit, and a core consisting of oil.

Since Eudragit protects the encapsulated oil from an acidic environment and dissolves at a pH above 7.0, it is a potential candidate for use in oral delivery of oil-soluble or oil-dispersible components to the extreme lower part of the gastrointestinal (GI) tract. Since the final morphology depends on the miscibility of Eudragit and oil, it is important to chart the response of the formation process to different oils. This process can also be used to encapsulate lipophilic components inside the oil core and influence their delivery *[21, 22]*. Once the capsules are formed, removal of the oil core by solvent extraction results in hollow capsules. These hollow capsules are interesting candidates for ultrasound-mediated delivery systems, due to the presence of gas inside these capsules. Use of different oils is expected to result in different morphologies of the hollow capsules *[13, 14, 23-26]*. In this paper we discuss the preparation of Eudragit capsules with different types of oils starting with the formation of the primary droplets using microsieve emulsification. This method yields relatively narrow size-dispersed microcapsules under mild conditions *[15]*. Based on the results with different types of oil, we gain insight into the relation between oil properties and

the resulting microcapsules and thus obtain understanding about which functional oils can successfully be encapsulated in Eudragit capsules and what type of morphologies are obtained. Furthermore, extraction of the encapsulated oil from the microcapsule with a suitable solvent results in hollow pH-sensitive microcapsules. Conditions are described for obtaining these hollow and multi-compartment microcapsules of Eudragit.

## Materials and methods

### *Materials*

An aqueous dispersion of Eudragit FS 30 D (ED) was obtained as a gift from Evonik Industries. This dispersion was freeze-dried to remove the water. The resulting polymer powder (Eudragit) was then used to prepare the capsules. Dichloromethane (DCM; Merck) was used as volatile organic solvent to dissolve the Eudragit and the different oils. The dispersed phase for the emulsification was prepared by first dissolving the Eudragit polymer in DCM and then adding the poor solvent (oil). The final concentrations of both Eudragit and the oil in DCM were 3 % (w/v).

Medium chain triglyceride (MCT oil; Migylol 812 N; SASOL GmbH), olive oil (Fluka), jojoba oil (Sigma Aldrich), Sunflower oil (obtained from a local super market) and coconut oil (Sigma Aldrich) were used as obtained. A 1 % aqueous sodium dodecyl sulfate (SDS; Fluka) solution was used as the continuous phase for the emulsification.

### *Microengineered membrane emulsification and encapsulation*

The details of the membrane and emulsification set-up used have already been published *[27]*. All emulsification experiments were carried out at 25 °C and at a disperse phase pressure of 30 mbar and a shear rate 0.71 Pa of the continuous phase. This dispersed phase mixture was pressed through a 5 μm high-porosity microsieve into a 1 % aqueous SDS solution to form an oil-in-water emulsion. The obtained dispersion was slowly stirred overnight in an open container to evaporate the remaining DCM.

### *Preparation of hollow capsules*

The microcapsules were filtered and washed several times with water to remove the SDS. After drying in contact with air, the capsules were suspended in hexane and left standing overnight and shaken manually a few times. Then hexane was removed by pipette and

replaced several times with fresh hexane. After removal of the hexane, the capsules were dried in air at ambient conditions.

***Characterization of the capsules***

Optical microscopy: An Olympus BH2 microscope was used to obtain optical images. For this, aqueous suspensions of the capsules were put on a glass slide. The microscope was equipped with a Moticam 2000 camera and the images were retrieved and stored with the Motic Image Plus program. The scale bar was introduced with the image J program.

Scanning electron microscope of microcapsules: The hollow capsules were placed on a cellulose acetate filter membrane after which the membranes were sputter-coated with 3 nm gold and mounted in the sample holder without glue. Samples were analyzed at 2 kV at room temperature in a field emission Auger microprobe (JEOL, JAMP-9500F). To observe the internal structure; capsules were glued between two adhesion tapes after which the two tapes were separated to fracture some of the capsules.

## Results and discussion

The phase separation will depend on the mutual interactions between the polymer and the oil *[28]*. Therefore, different oils were chosen with different chain lengths and polarities to see how these factors influence the interaction between polymer and oil. All capsules were prepared using the same concentrations of polymer and oil (3%), so that only the properties of the oil influence the final structure of the microcapsules rather than a variation in concentrations. For possible use of these microcapsules as vehicles for delivery to the lower intestinal tract the pH-sensitive Eudragit and digestible oils were chosen as model compounds. The triglycerides all have three ester groups, but differ in molecular weight (about 400-500 for MCT oil and 900-1000 for sunflower, coconut or olive oil). Jojoba oil is a mixture of monoesters with a molecular weight of about 550-650. Because it is a monoester it is more apolar than the triglycerides.

Figure 1 shows optical microscopy images of the microcapsules prepared with the different oils. As observed before *[15],* microcapsules with an average size of about 30 μm and a coefficient of variation of 15% are formed under these conditions. The variation in size seems limited. The capsules prepared with MCT (figure 1a) shows several tiny MCT oil droplets enclosed within the polymer shell, which are not clearly visible through the polymer shell.

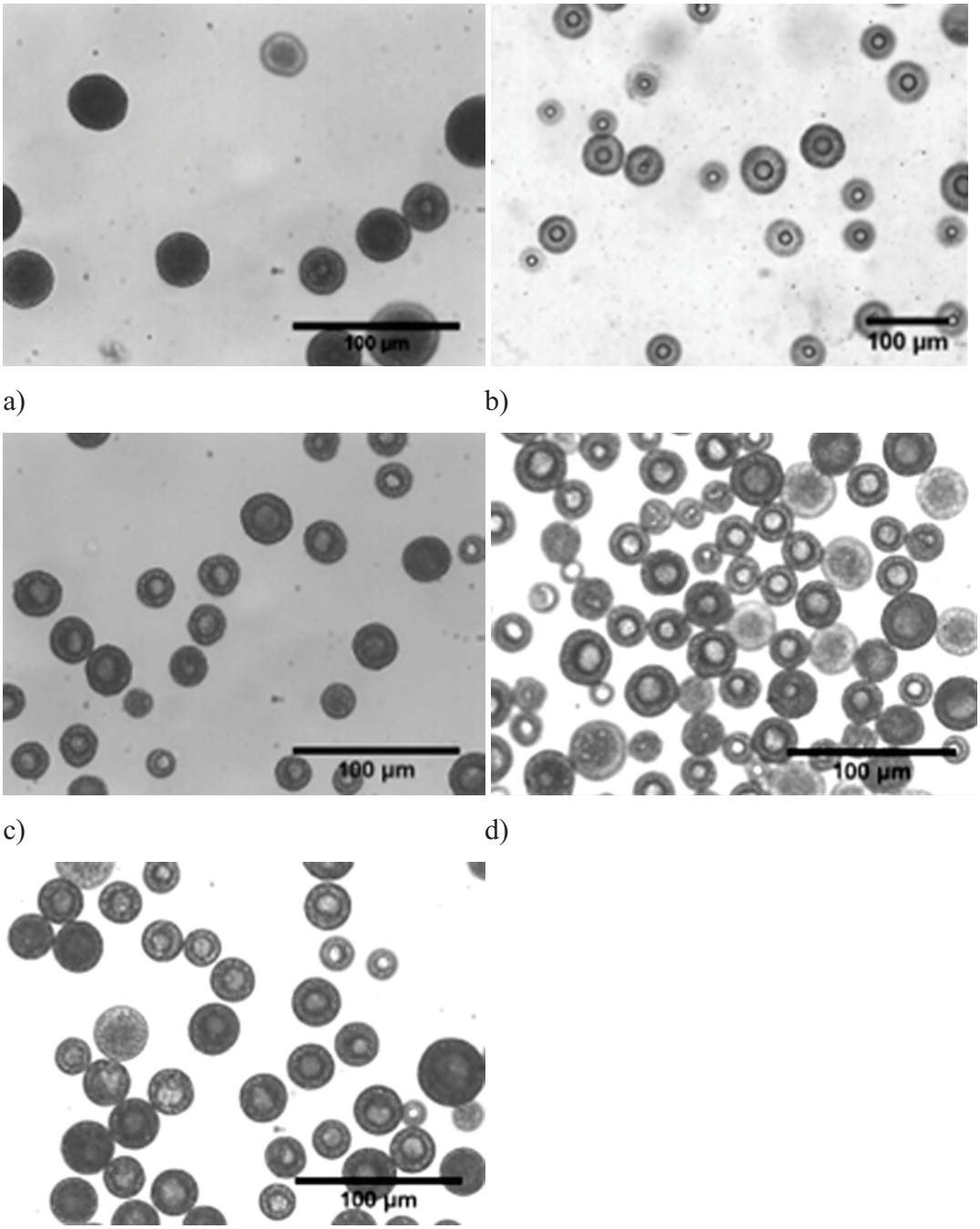
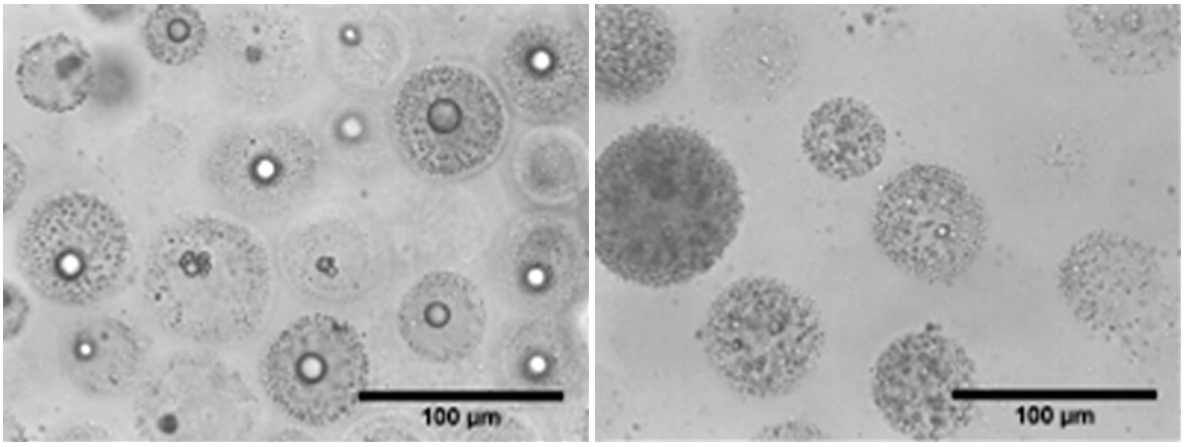



*Figure 1. Optical micrographs of microcapsules prepared from 3% Eudragit and 3% oil a) MCT oil; b) sunflower oil; c) olive oil; d) coconut oil; e) jojoba oil.*

Since the Eudragit matrix dissolves at pH 8 within a minute *[15],* its easier to see the entrapped droplets when they get released after the dissolution process. Indeed, figure 2b shows that tiny droplets are released during the Eudragit dissolution process. These observations suggest that in the microcapsule formation process with MCT oil, small oil

droplets get entrapped in the solidifying Eudragit matrix, instead of forming a single oil droplet at the center.

Microcapsules prepared with longer chain triglyceride oils such as sunflower oil, olive oil and coconut oil and also with jojoba oil, show a core-shell structure with a single oil droplet as core (figure 1b-e); this is also visible after dissolution of the Eudragit at higher pH (figure 2a); however, small amounts are also entrapped as small droplets in the Eudragit shell. Similar results were obtained with the other long chain triglycerides and also with Jojoba oil. Upon dissolution of MCT oil no large droplets in the core were observed (figure 2b).




a) b)

*Figure 2. Optical micrographs of microcapsules, recorded approximately 2 minutes after exposing them to pH 8, i.e. after dissolution of the Eudragit matrix a) sunflower oil b) MCT oil.*

During the microcapsule formation in which water diffuses in and DCM diffuses out of the droplet, the concentrations of the oil and polymer change continuously. As DCM is removed, the concentrations of both Eudragit and oil increase. At a certain concentration the Eudragit will solidify by gelation or glassification. If phase separation of the oil occurs far before this point, the solution still has a low viscosity and diffusion of the oil through the matrix is still possible. Either by the formation of a single nucleus, or by ripening or coalescence of multiple ones, a larger droplet results in the middle of the microcapsule. If the oil phase separates after the Eudragit solidifies, the small droplets cannot coagulate anymore and separate droplets will be entrapped in the polymer matrix. In general, the solubility of molecules decreases with increasing molecular weight and thus phase separation for higher molecular weight molecules will already start at a lower concentration *[29, 30]*. This explains the difference in behavior

between the long chain triglycerides and MCT oil. Although jojoba oil has a similar molecular weight as MCT oil, it is expected to phase separate earlier due to its more apolar nature.
For microsphere formation with suspension polymerization in the presence of different oils, it is known that the phase separation process can be related to the degree of incompatibility or immiscibility of the different oils with the shell-forming polymer and this determines the structure and morphology of the formed microcapsules *[31]*. The degree of incompatibility depends on the molecular weight of the oil: the higher the molecular weight the more incompatible, and the polarity: a more apolar oil is more incompatible. Here we observe similar behavior for the solvent extraction-induced phase separation. With long chain vegetable oils like sunflower or olive oil, the phase separation is occurring earlier as compared to medium chain triglyceride oil. Jojoba oil, which has only one ester group, is more apolar than triglycerides and therefore is less compatible with Eudragit and forms a single core-shell structure, despite having a lower molecular weight than the long chain triglycerides.

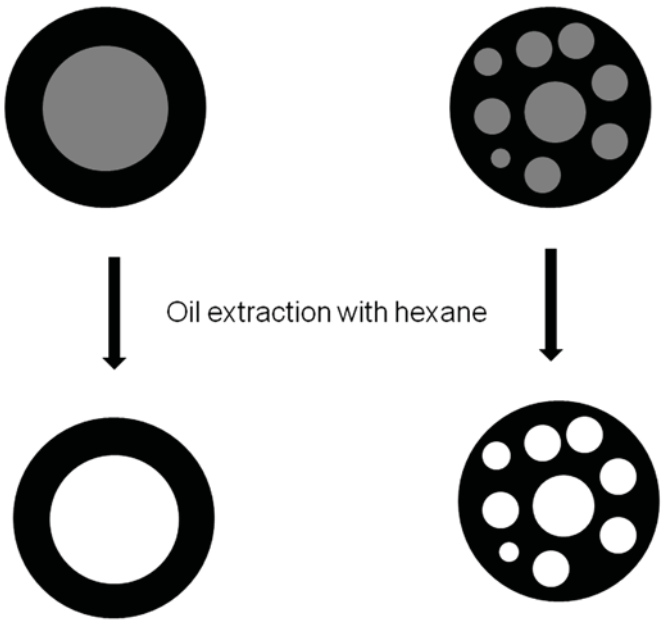


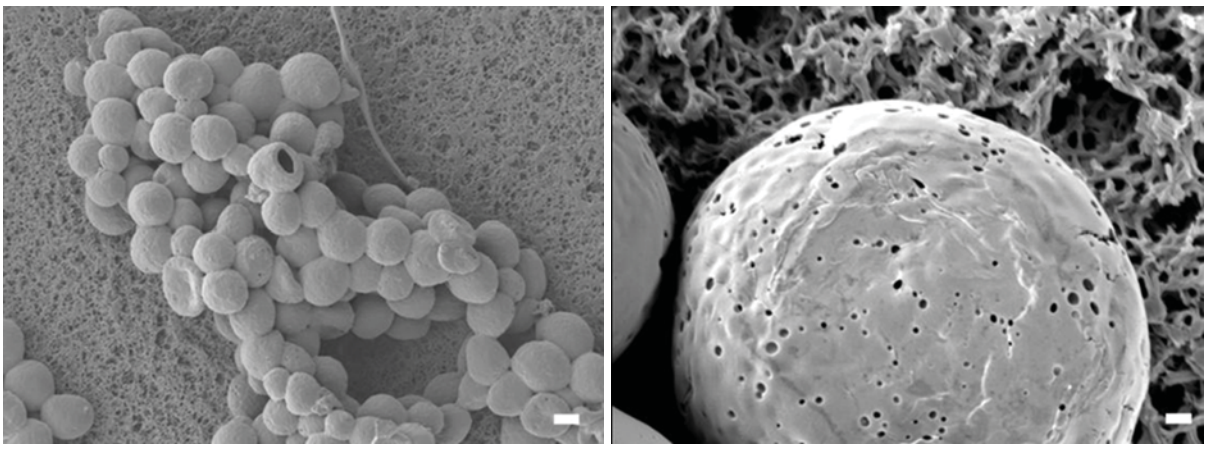

*Figure 3. Schematic representation of the preparation of empty microcapsules with different structures formed in Eudragit microcapsules with use of different oils.*

During the phase separation-induced capsule formation, oil-rich and Eudragit-rich phases will start to separate after bringing the solution in contact with water. The polar polymer-rich

phase will start to migrate towards the external aqueous phase, whereas the apolar oil-rich phase will tend to move towards the core of the particle. This induces concentration gradients in the particles, that also change with time. This means that depending on the distance to the surface the phase separation process results in different morphologies. If the molecular weight of the triglyceride vegetable oil is high enough or if it is apolar enough as in the case of jojoba oil, phase separation occurs relatively early and most of the forming droplets have ample time to nucleate in the center. For the more polar low molecular weight oil, phase separation occurs later in the extraction process where the polymer already has started to solidify, thus the coagulation of the forming oil droplets is less complete in this case (Figure 3).

Since these processes are also dependent on the relative concentrations of the two components and time, the resulting internal morphology is dependent on the distance to the surface of the microcapsule. This is the reason that even for the high molecular weight oils some small oil droplets become entrapped in the Eudragit shell. It is to be expected that an oil that is even less compatible with the Eudragit than the long-chain oils, will show less or even no droplets in the shell.

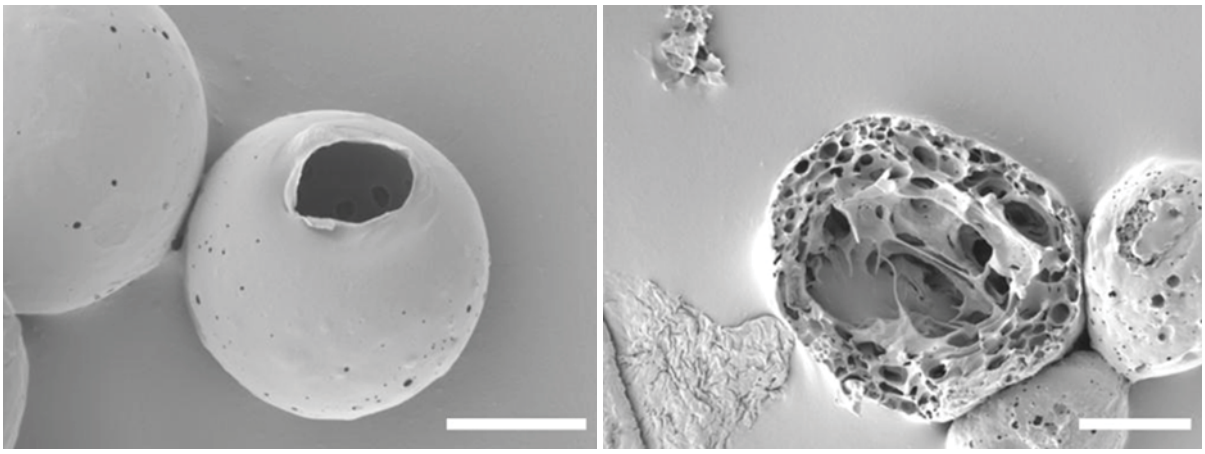
Removal of the oil template could be accomplished by a simple process of exposing the dried capsules to a solvent that can extract the oil without influencing the polymer shell morphology (Figure 3). This is possible by exposing the capsules to hexane to extract the oil from the capsules. This process results in the formation of hollow capsules which can be clearly imaged with scanning electron microscopy.



a) b)

*Figure 4. SEM images of hollow microcapsules a) Microcapsules prepared with jojoba oil scale bar (10 µm) b) Surface morphology of microcapsule prepared with sunflower oil scale bar (1µm).*

Figure 4a shows a SEM image of hollow capsules after extraction of jojoba oil. Most of them have an intact shell, even after exposure to vacuum. A few capsules have a broken shell, indicating that the capsules are indeed hollow. Other capsules look inflated, which is also an indication that they are hollow. Figure 4b shows a magnified image of a hollow capsule after extraction of sunflower oil. A porous surface is seen with some holes. We expect that these holes are related to the slight porosity of the shell as was noted earlier *[15]*. The presence of holes in the initial shell of the capsule also allows the hexane to diffuse in the microcapsules and to extract the oil from the core thus leaving a cavity in the center of the microcapsule *[32]*.



a) b)

*Figure 5. SEM images of broken hollow microcapsules, scale bar (10 µm) a) Microcapsules prepared with sunflower oil b) Microcapsules prepared with MCT oil.*

Based on the optical microscopy images it is expected that microcapsules derived from high molecular weight oils are hollow, whereas microcapsules with multiple compartments are expected from particles derived from the lower molecular weight MCT oil. Pulling apart two adhesive tapes that have microcapsules between them results in breaking of some of them. Figure 5a shows a SEM image of capsules after removal of sunflower oil. The depicted capsule has a single large void. Figure 5b shows capsules after removal of MCT oil. In this case multi-compartment capsules are formed. These SEM images confirm the findings with optical microscopy. Treatment of the capsules with adhesive tape in order to break them resulted only in a few capsules that actually broke. This indicates that the capsules are not very fragile and can withstand some physical stress.

The internal structure of these hollow and multi-compartment capsules by extraction of the oil core is completely dependent on the initial morphology of these microcapsules. Therefore, by designing the desired initial structure inside the capsule with the use of different types of oil allows for the precise fabrication of desired hollow and multi-compartment capsules.

## Conclusions

Eudragit microcapsules were prepared by emulsifying droplets of a dilute solution of a shell-forming polymer (Eudragit) and an oil that was to be encapsulated in water. By doing so, the solvent (dichloromethane) slowly extracted from the droplets, which induced demixing between oil and polymer, creating a solid shell of Eudragit around one or more oil droplets. For the droplet preparation, microsieve cross-flow membrane emulsification was used, since this yielded narrowly-dispersed microcapsules with an average size of about 30 μm.

The morphology of the microcapsules depends on the compatibility between the oil that is encapsulated, and the Eudragit polymer. Use of long chain length oils such as sunflower oil, olive oil and coconut oil, which have poor compatibility with the Eudragit, mainly yield microcapsules with a single encapsulated oil droplet covered with an Eudragit-rich shell. On the other hand, microcapsules prepared with a relatively short chain length oil such as medium chain triglyceride oil, which has a better compatibility with the Eudragit, results in capsules with many small oil droplets encapsulated in an Eudragit-matrix. Jojoba oil, which is more apolar than triglycerides, also gave mainly single oil droplet microcapsules.

The final microcapsule morphology was thus shown to be dependent on the interaction between oil and polymer. Poor interaction will already induce demixing between oil and polymer at low polymer concentrations, allowing for the formation of one single oil droplet at the centre. Good interaction will delay the demixing to the stage at which the polymer concentration is already quite high, which will inhibit the transport of the oil to a single droplet and this leads to the formation of many small droplet dispersed in an Eudragit matrix.

Optical microscopy of the microcapsules and SEM investigations of microcapsules from which the oil was removed confirm this hypothesis.

In summary, a method is presented for microencapsulation of food grade oils in well-defined microcapsules, and a rationale is given of the microcapsule morphology as function of the compatibility of the materials. We believe that this research may lead to the development of rational design of hollow or filled microcapsules that are useful for encapsulating active ingredients and releasing them by a pH trigger *[32-34]*.

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# Chapter 5

## *Structured microspheres from polymer blends*


## Abstract

Microspheres from PMMA (polymethyl methacrylate) and Eudragit FS 30D (a commercial copolymer of poly(methyl acrylate-*co*-methyl methacrylate-*co*-methacrylic acid) 7:3:1) were prepared using microsieve emulsification. A mixture of these polymers in dichloromethane was dispersed into water, leading to extraction of DCM in water and the formation of microspheres with a PMMA core and a partially demixed Eudragit shell. With a higher ratio of Eudragit to PMMA, more and bigger pores can be seen on the surface of the microspheres. Eudragit can be removed from the microspheres under alkaline conditions. Depending on the initial Eudragit to PMMA ratio, PMMA microspheres with different surface morphologies are obtained. At low Eudragit concentrations microspheres with a crumpled surface are formed, while at higher Eudragit concentrations microspheres are formed with a core to which dendritic PMMA structures are attached. At even higher Eudragit concentrations the microspheres obtained after dissolving the Eudragit show a nanorough surface.



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## Introduction

Microspheres are considered for use in separation processes, biorecognition *[1]*, for immobilization of catalysts *[2-4]* and as potential vehicles in drug delivery applications *[5]*. Since their properties are strongly dependent on their size, shape and morphology, precise control over these aspects is of major concern *[6]*. Additionally, microspheres have been prepared with a wide range of polymers and polymer blends. The nature of these polymers and the morphologies of the microspheres have a significant impact on the release rates and profiles. The material properties can for example be modified by blending with other polymeric materials *[7]*. The type of polymer and its interaction with solvent and non-solvent determines the formed morphologies of membranes *[8, 9]*. This concept can also be applied in microsphere formation. If during formation of microspheres, phase separation between the two polymers occurs in such a way that one polymer completely spreads over the other, double-walled microspheres are formed *[10]*. With a careful choice and use of enthalpic interactions, chain lengths, polymer surface tension and spreading coefficients, well-defined, double-walled microspheres can be designed and obtained *[11]*. Several combinations of polymers have been used in literature to prepare double-walled microspheres. For example, polylactide-*co*-glycolide (PLG) / polyorthoester (POE) mixtures have been used for preparation of microspheres with a PLG core and a POE shell *[12]*. Double-walled microspheres were also obtained from polylactic acid / poly[1,3-bis(*p*-carboxyphenoxy-propane)-*co*-sebacic anhydride] mixtures, where complete phase separation occurred. However, for polylactic acid / POE mixtures, hybrid microspheres were obtained, in which the POE did not completely encapsulate the core and with polylactic acid / polystyrene mixtures microspheres were obtained that did not show any coverage of the core *[10]*. A more detailed study was performed with PLG / poly[1,6-bis(*p*-carboxyphenoxyhexane)] mixtures, where different ratios of core and shell-forming polymers were used. For this system it was found that phase separation between the two polymers and the formation of double-walled microspheres was incomplete when a low amount of shell-forming polymer was used *[13]*. Though fabrication of double-walled microspheres through the use of mixtures of different polymers is known, there is still no complete understanding of the phase separation process that determines the shape and morphology of the obtained double-walled microspheres. Especially, the shape and morphology of the core, obtained after dissolution of the outer shell polymer still requires investigation. This study aims at exploring the morphology of core-shell microspheres using blends of two different polymers in different ratios.

Blends of enteric polymers like Eudragit and non-enteric polymers are interesting as materials for controlled release delivery systems *[7, 14]*. Eudragit is a class of (meth)acrylate-based copolymers that is used extensively for enteric coatings. Since they contain a certain percentage of carboxylic acid groups, they are insoluble at low pH (<7), but soluble at higher pH. For diffusion-controlled release, properties like shell thickness and porosity are important. A recent technique to obtain porous films with a co-continuous structure uses a blend of two polymers from which one polymer is later selectively leached out *[15]*. This allows one to prepare membranes with highly interconnected pores with control over porosity, pore size and surface morphology *[16-19]*. In this paper we describe the use of this technique for the preparation of microspheres from poly methyl methacrylate (PMMA) and Eudragit FS 30 D blends by microsieve emulsification, since microsieve emulsification is a low-energy consuming process that yields microspheres with a rather narrow size distribution and this process can easily be upscaled. Eudragit is not miscible with PMMA; therefore phase separation between PMMA and Eudragit is expected after bringing droplets of this solution in contact with a non-solvent. The internal structures obtained upon phase separation of the mixture will depend on the ratio of two polymers. The Eudragit used is soluble in water above pH 7.0 *[20]*, and it is therefore selectively leached out of the initial microspheres under alkaline conditions, yielding PMMA microspheres with different surface morphologies that are investigated with different microscopic techniques.

## Materials and Methods

### *Materials*

An aqueous dispersion of Eudragit FS 30 D (ED; MW 220,000; Tg 40 °C) was obtained as a gift from Evonik Industries. This dispersion was freeze-dried to remove the water. The resulting polymer was then used to prepare the microspheres. Poly(methyl methacrylate (PMMA; MW 120,000; Tg 105 °C) was obtained from Aldrich. Dichloromethane (DCM; Merck) was used as a volatile organic solvent. A 1 % aqueous sodium dodecyl sulfate (SDS; Fluka) solution was used as continuous phase for the emulsification.

### *Microengineered membrane, emulsification device and microsphere preparation*

Details of the microsieve membrane and the emulsification set-up used were already published *[21]*. Eudragit and PMMA were dissolved in DCM separately in different

concentrations and then mixed to obtain the disperse phase with the desired concentrations and ratios. This polymer mixture in DCM was then pressed through a 5 μm high porosity microsieve into the continuous phase consisting of a 1% SDS solution in water at pH 6.5 to form an oil in water emulsion. All experiments were carried out at a disperse phase pressure of 30 mbar and a continuous phase shear rate of 0.71 Pa. Since the amount of water is large as compared to DCM, DCM will be extracted to the water phase and evaporates into air at the water surface. The phase separation process between PMMA and Eudragit occurs by liquid-liquid demixing and gelation, to form a PMMA core and an Eudragit-rich shell (Figure 1). Once the microspheres have been formed they are analyzed at pH 6.5 (preparation condition). For the removal of the Eudragit polymers, the microspheres were first washed with water and then brought to pH 8.0 and kept at this pH for at least 1 day. Before visualization, the buffer containing the dissolved Eudragit was washed away with water.

***Characterization of the microspheres***

Optical microscopy: The suspension of microspheres was spread on a glass slide and observed visually. Images of the microspheres were made using an Olympus BH2 microscope.

Scanning electron microscope: A drop of an aqueous suspension of the microspheres was put on a Nuclepore polycarbonate membrane (Costar) with 5 μm holes. These membranes were placed on filter paper to remove the fluid through the membrane, leaving the spheres on the top side of the membrane. After air drying the membrane was glued onto a sample holder by carbon adhesive tabs (EMS, Washington, USA) and subsequently sputter-coated with 3 nm tungsten (MED 020, Leica, Vienna, Austria). Samples were analyzed at 3 kV at room temperature in a field emission scanning electron microscope (Magellan 400, FEI, Eindhoven, the Netherlands). Images were digitally recorded.

Atomic force microscope: The aqueous dispersions of the microspheres were filtered through a regenerated cellulose polymeric filter membrane (Whatman, RC55, 0.45 μm pore size) and subsequently dried in air. Then the surface of the capsule was scanned by tapping-mode atomic force microscopy (Asylum Research MFP-3D SA AFM). Height images were obtained with Tap 150 DLC silicon cantilevers (Budgetsensors) in AC mode in air.

DSC: The thermograms were obtained using a TA Q1000 instrument with a TZero technology system.

## Results and Discussion

***Microsphere formation***

The two polymers (PMMA and Eudragit) are expected to be immiscible due to their difference in chemical nature. To confirm this, the glass transition temperature of a polymer film prepared by evaporating DCM from a mixture of 1.5% PMMA and 1.5% Eudragit solutions was measured by DSC. Two different Tg-values were obtained, one around 40 °C for Eudragit and one around 105 °C for PMMA, which are the values expected for the pure polymers. This indicates that these polymers are indeed molecularly immiscible *[17]*. As the microsphere preparation starts with a low concentrations of both polymers in DCM (typically 5 wt% or lower in total), we start with a homogeneous solution. However, after bringing this polymer mixture solution in contact with a nonsolvent (water), the DCM is extracted from the droplets, the polymer concentrations rise, and phase separation between the two polymers will occur, which will ultimately be stopped by gelation and glassification of an amorphous polymer (figure 1) *[20]*. The microspheres resulting from the microsieve emulsification had an average size of about 20 μm and a coefficient of variation of about 20%, which is slightly higher than previously reported for a similar system *[20]*; this is probably caused by slight wetting of the microsieve surface during the emulsification process. Interestingly, the average size did not change much when the Eudragit concentration was increased from 1.5 to 3.5%. Upon removal of the Eudragit shell from the obtained microspheres at pH 8, the average sizes of the resulting cores decreased as expected. The sizes of the cores became smaller when the initial Eudragit concentrations were higher.

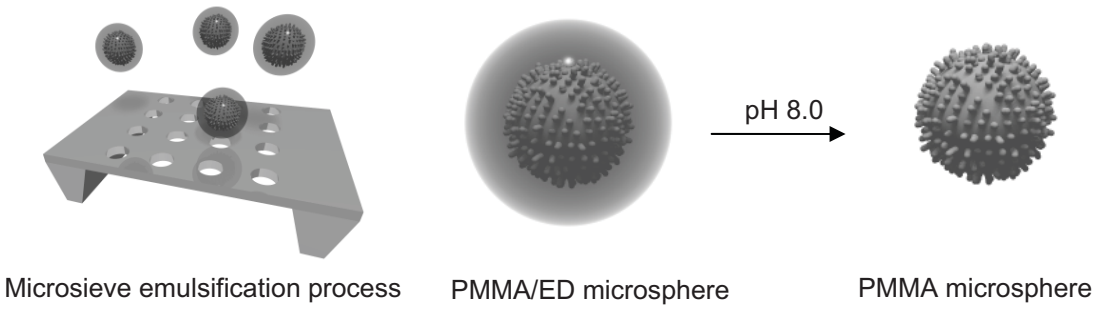
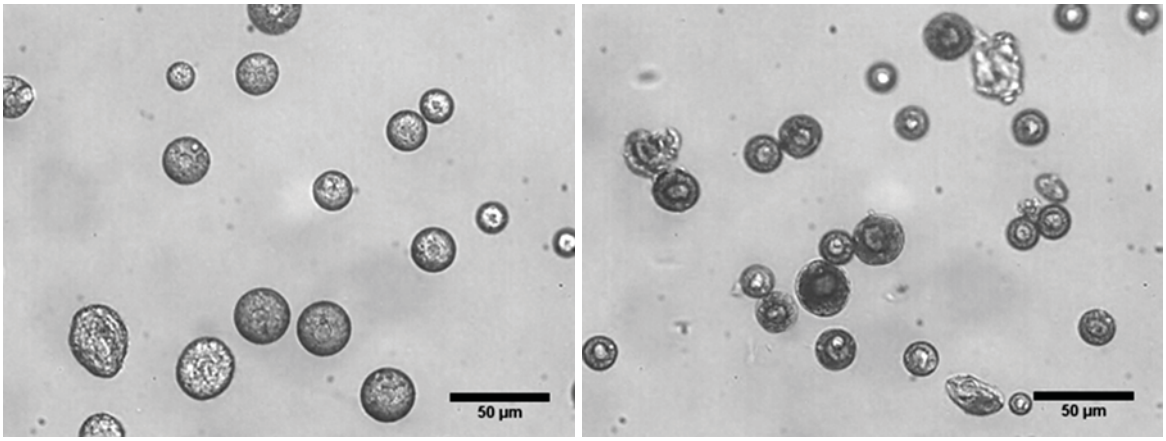



*Figure 1. Microsphere preparation using microsieve emulsification. The Eudragit (ED) shell of the resulting PMMA core / Eudragit shell double-walled microspheres dissolves upon bringing the pH to 8.0, resulting in PMMA microspheres with different surface morphologies. (For color picture see Appendix on page number 101).*

***Studies with optical microscopy***

Figure 2 a) shows an optical microscopy image of microspheres prepared from a mixture of 1.5 wt% PMMA and 1.5 wt% Eudragit before leaching out Eudragit. No clear core shell structure could be seen. However, upon increasing the amount of Eudragit to 3.5% in the initial mixture the encapsulated PMMA spheres within the Eudragit shells became clearly visible (figure 2b). This shows that the composition of PMMA and Eudragit in the initial mixture influences the formation of the core-shell morphology.




a) b)

*Figure 2. Optical microscopy images of microspheres at pH 6.5 (before leaching out Eudragit) a) PMMA 1.5% and Eudragit 1.5% b) PMMA 1.5% and Eudragit 3.5% .*

Figure 3 shows optical microscopy pictures of microspheres at pH 8.0 (after leaching out Eudragit) obtained from different ratios of PMMA and Eudragit in the initial mixture. Microspheres prepared from 1.5% PMMA and 1.5% Eudragit (figure 3a) show a slightly crumpled morphology (see discussion of SEM images in later section). With an increase in amount of Eudragit to 2.5-3% (figure 3b and 3c) the microspheres show a distinct core, but also a highly swollen outer shell. At 3.5% of Eudragit in the mixture, no swollen corona is seen anymore and only a small core is left (figure 3d).

A reason that the shell did not completely dissolve for the microspheres prepared from 2.5% and 3% of Eudragit, may be due to the fact that with larger amounts of Eudragit, some of the PMMA will be initially solubilized in the forming Eudragit shell, and will later on in the formation process precipitate in the form of small but physically connected PMMA domains in the Eudragit shell. After dissolution of the Eudragit at pH 8.0, the PMMA domains form dendritic structures on the surface of the core and in contact with water the shell swells. These observations with optical microscopy were confirmed with SEM and AFM and will be

discussed in next section. Also the effect of composition on polymer demixing in the microsphere will be discussed.

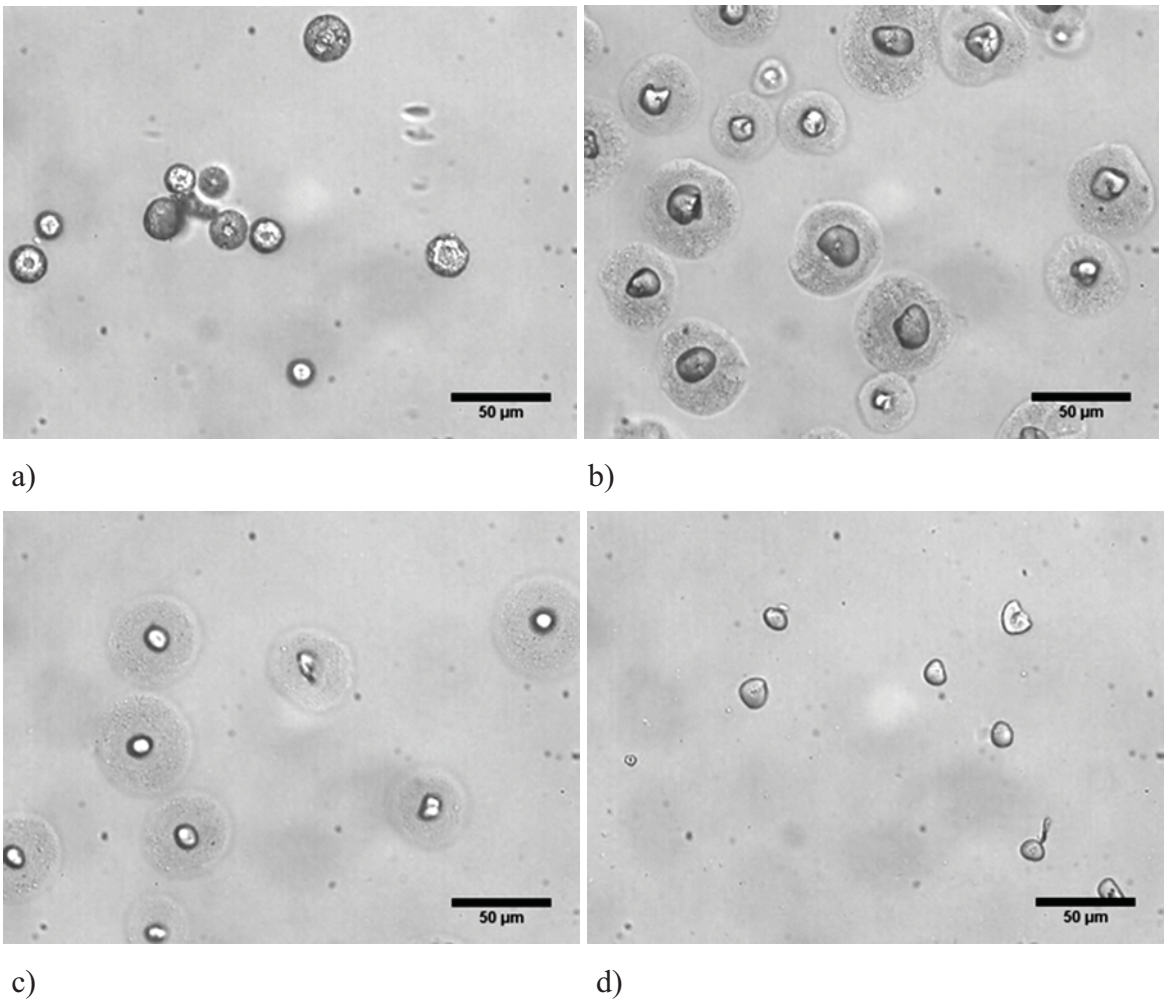
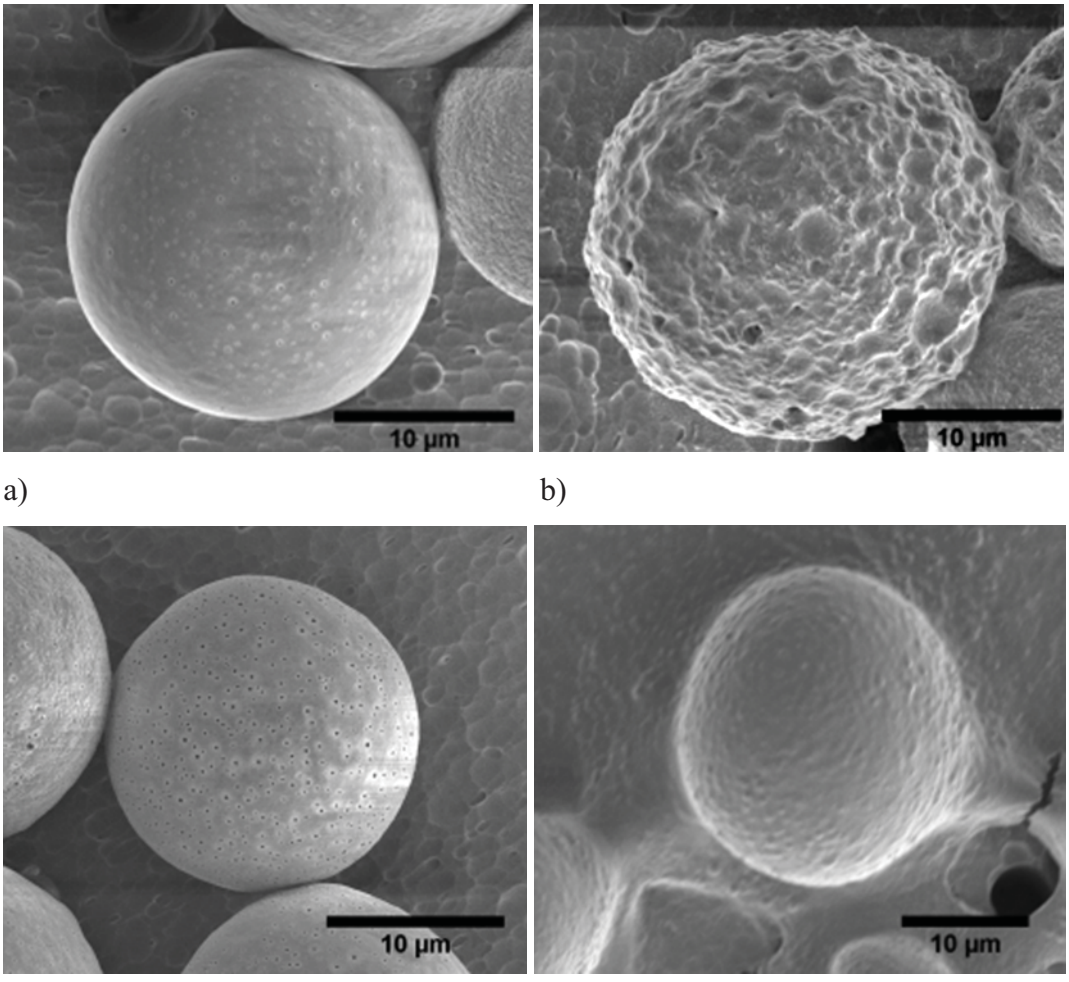



*Figure 3. Optical microscopy pictures of microspheres at pH 8.0. Microspheres prepared from a) 1.5% PMMA and 1.5% Eudragit, b) 1.5% PMMA and 2.5% Eudragit, c) 1.5% PMMA and 3% Eudragit, d) 1.5% PMMA and 3.5% Eudragit.*

***SEM-study of effect of composition on microsphere morphology***

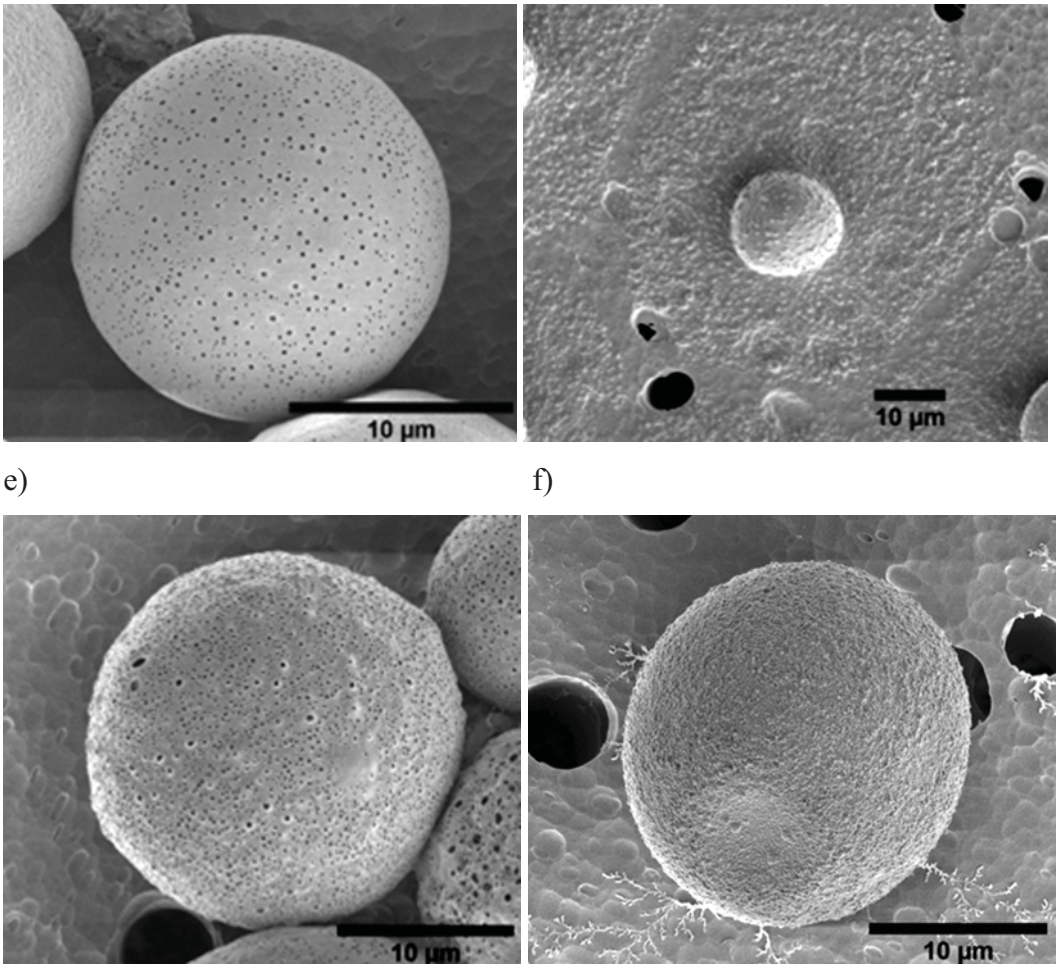
Figure 4 shows SEM images of the dried microspheres obtained from different ratios of PMMA and Eudragit in the initial DCM solution at pH 6.5 and at pH 8.0 (after dissolving the Eudragit). The shells of the microspheres prepared at pH 6.5 appear porous at all concentrations of Eudragit (figure 4 a, c, e and g). Upon increasing the amount of Eudragit it is seen that the number and size of the pores at the surface increases. For membrane formation by non-solvent induced phase separation the enhanced interaction of polymer with non-solvent increases the porosity of the outer surface *[22]*. During the formation of the

microspheres a phase separation process occurs, resulting in the formation of a core rich in PMMA and a shell rich in Eudragit. The dissolution of DCM in water at the surface of the droplets causes the PMMA to move to the core of the droplet that is still rich in DCM and the hydrophilic Eudragit to move to the surface of the droplet where it phase separates. Water diffuses into the droplet due to the favorable interaction with the hydrophilic Eudragit. On the other hand, the DCM that is still present in the droplet needs to diffuse out through the forming Eudragit shell. This will lead to the formation of DCM-rich channels *[20]* through the Eudragit layer, that may initially still contain some PMMA. In the later stages of the microsphere formation, the polymers in these channels will phase separate from the disappearing DCM solvent into an Eudragit matrix containing small trapped nodules of PMMA. Similar behavior was observed during the formation of double-walled microspheres with poly(L-lactic acid) (PLLA) shells and poly(D,L-lactic-*co*-glycolic acid) (PLGA) cores *[23]*.




a) b)

c) d)




e) f)

g) h)

*Figure 4. SEM images of microspheres a) 1.5% PMMA and 1.5% Eudragit, pH 6.5 b) 1.5% PMMA and 1.5% Eudragit pH 8.0 c)1.5% PMMA and 2.5% Eudragit pH 6.5 d) 1.5% PMMA and 2.5% Eudragit, pH 8.0 e) 1.5% PMMA and 3% Eudragit, pH 6.5 f) 1.5% PMMA and 3% Eudragit, pH 8 g) 1.5% PMMA and 3.5% Eudragit, pH 6.5 h) 1.5% PMMA and 3.5% Eudragit, pH 8.0. The magnification in Figure f) is lower to show the extended corona of the microsphere.*

When phase separation between the two polymers occurs due to disappearance of the solvent, domains are formed in the shell that are rich in Eudragit and leaner in PMMA. When this happens close to the critical point, i.e. the point at which the initial phase separation occurs, the phase separation between the two polymers is relatively incomplete *[24]*. Therefore, there is still a significant concentration of PMMA in the domains that are rich in Eudragit. Upon progress of the extraction process, in which DCM is removed (and replaced by water), the low concentrations of PMMA in the domains of Eudragit become unstable and precipitate in the

form of small trapped spheres. This behavior is also known in other fields (e.g. membrane formation by phase separation) *[25]*.

These complex phase separation processes will lead to different domains in the shell; some containing almost pure but water-rich Eudragit and some containing Eudragit with trapped PMMA spheres. During drying the water-rich Eudragit domains will shrink and leave holes or pores that are visible with SEM and also with AFM as will be discussed later.

Upon increasing the pH to 8.0, the surface morphology of the microspheres changes significantly due to the dissolution of the Eudragit. In control experiments it was seen that Eudragit rapidly dissolves at pH 8.0. Typically, pure Eudragit microspheres of this size completely dissolve within tens of seconds at this pH. The core-shell microspheres investigated here, were kept at pH 8.0 for at least one day before microscopic investigation.

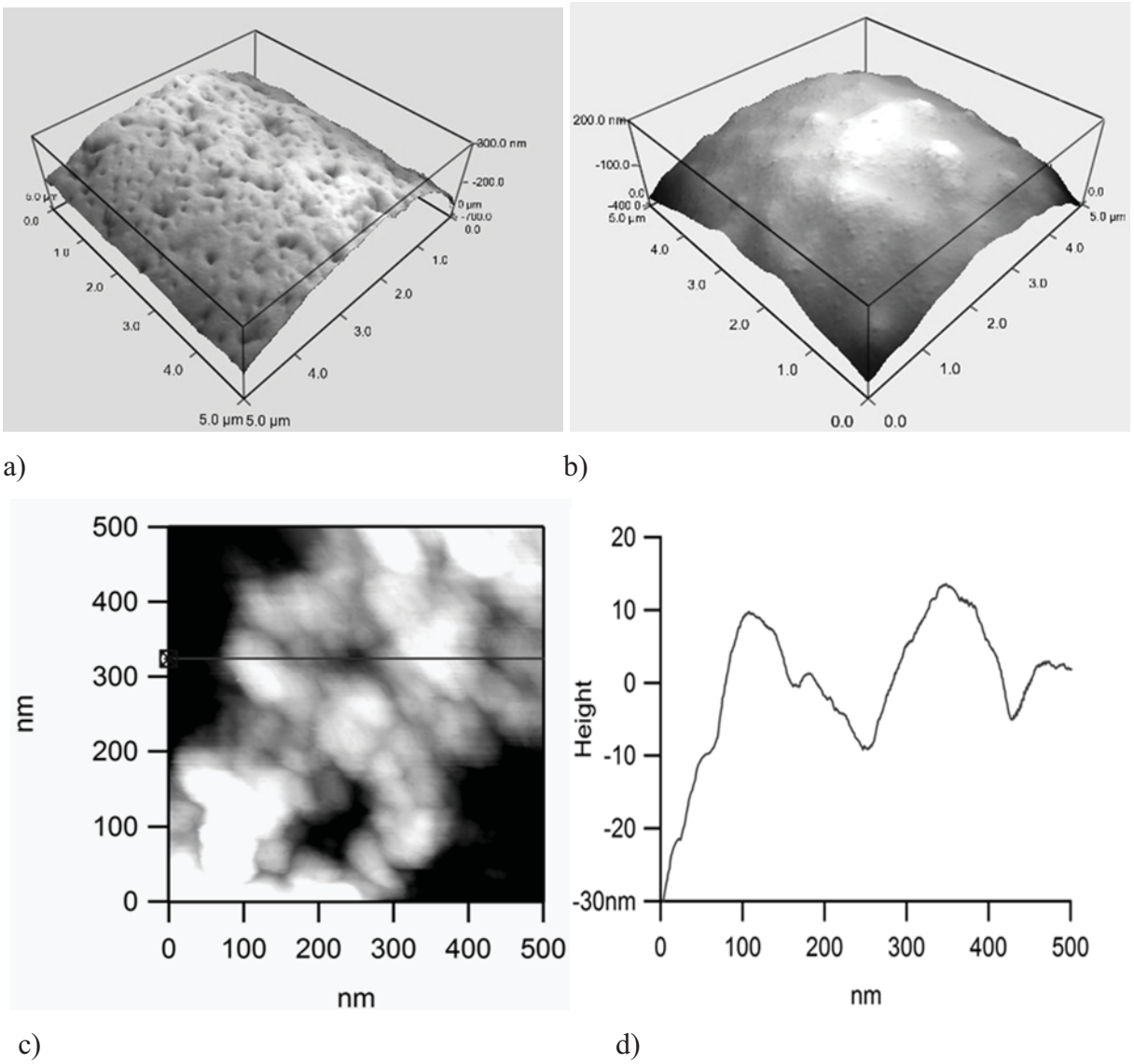
A dried microsphere from 1.5% PMMA and 1.5% Eudragit, obtained after dissolution of the Eudragit (Figure 4b) has a crumpled surface with indentations. We expect that the initial shell of these microspheres still contained relatively large PMMA domains, mostly connected to the core structure, since they were the channels through which the DCM diffused out of the core. Removal of the Eudragit domains leaves empty pockets resulting in an irregular surface of the remaining microspheres.

Microspheres obtained from initial mixtures containing 2.5% or 3% Eudragit show cores upon dissolution of the Eudragit, whose surfaces are covered with a swollen corona, as clearly seen with optical microscopy (Figures 3b and c). Also the SEM pictures show microspheres with a core that is surrounded by an extended corona of less dense fiber-like material (figures 4d and 4f). The fact that these microspheres have a extended corona even after exposure to pH 8, indicates that this shell consists of PMMA. During the dissolution process the Eudragit is removed from the microsphere. The PMMA in the shell was presumably mostly present in the channels that served to diffuse out the DCM as explained before. Upon dissolution at pH 8.0, the pure Eudragit domains and the Eudragit in the mixed domains were removed and the PMMA spheres became physically aggregated into fibrous structures connected to the core, which give the microspheres a swollen appearance. At present it is unclear whether the physical aggregation was already present or occurs during the dissolution process.

***AFM-studies on the core-shell morphology of the microspheres***

Optical microscopy (figure 3c) and SEM (figure 4f) already showed that microspheres obtained from an initial concentration of 1.5% PMMA and 3% Eudragit, after bringing them

to pH 8.0, have a PMMA core surrounded by an open extended corona. For comparison with the SEM pictures, additional AFM studies were performed on these microspheres.




a) b)

c) d)

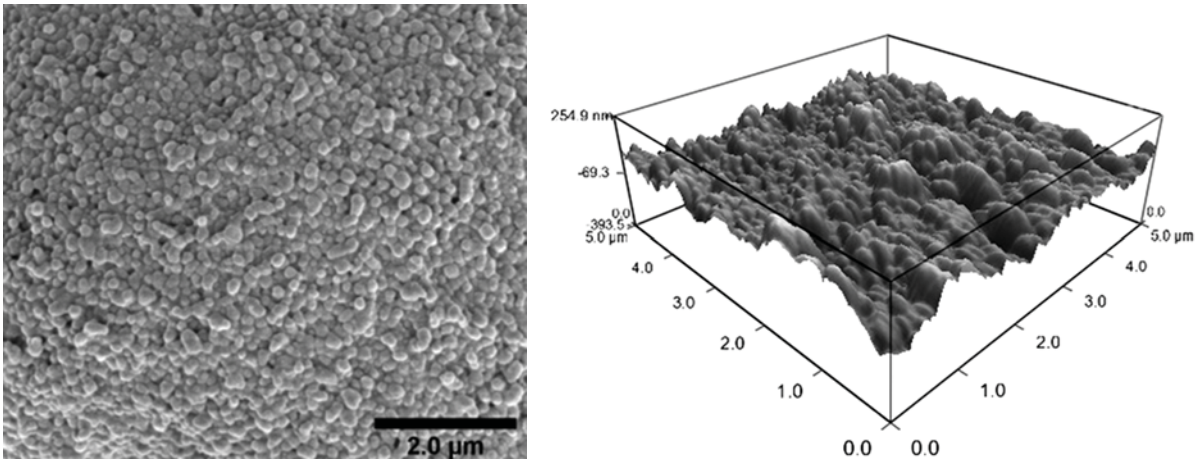
*Figure 5. AFM images of a microsphere prepared from 1.5% PMMA and 3% Eudragit a) 3D height image of a microsphere surface at pH 6.5 b) 3D height image of a microsphere core at pH 8.0 c) 2D height image of a microsphere corona (nodules loosely packed around PMMA core) at pH 8.0 d) section profile of c).*

Figure 5a shows a 3D AFM height image of a microsphere at pH 6.5, i.e. before dissolving the Eudragit, which indeed shows a porous surface with pore sizes of about 100 nm, without any nodular structure. Figure 5b shows the 3D AFM height image of the core of the microsphere at pH 8.0, i.e. after dissolving the Eudragit. This picture shows that the core of

the microsphere is rather smooth with hardly any structure. Figure 5c shows a zoomed-in image of the material of the corona of the microsphere. It is seen that the corona consists of aggregates of small nodular particles of about 50 nm with a roughness of around 20 nm (figure 5d). These are small particles formed by the phase separation between the two polymers and will be discussed in the next section in detail.

***SEM and AFM studies on the nodular structure formation on the microspheres***

The microspheres, prepared from an initial concentration of 1.5% PMMA and 3.5% Eudragit, have an increased porosity (figure 4g) at pH 6.5, due to the higher amount of Eudragit. After exposure of the microspheres to pH 8.0, the Eudragit shell material was removed (figure 4h) and no core-shell morphology is observed anymore (figure 3d) and some small particles are visible on the microsphere surface. Figure 6a is a zoomed-in image of figure 4h, which clearly shows the presence of densely packed polymeric nodules on the microsphere surface with a size of about 100 nm. Due to the low concentration of PMMA in this system, it can be expected that the residual PMMA in the channels through which the DCM diffused out only very small isolated spheres formed. During the dissolution process of the Eudragit these small PMMA spheres partly precipitated on the core surface and became physically attached there.




a) b)

*Figure 6. a) SEM image and b) 3D topographical AFM image of a microsphere surface at 1.5% PMMA and 3.5% Eudragit, pH 8.0 (i.e. after dissolving Eudragit).*

Similar nodular structures have also been observed during the preparation of polyethersulfone membranes in the presence of hydrophilic polyvinylpyrrolidone additives *[25]*, but there has

been a long standing debate on whether they are real, or whether they represent an artifact created by the sample preparation procedure for SEM. Detailed information about the surface structures can also be obtained from AFM. An advantage of AFM as compared to SEM is that additional sample preparation, such as a complete removal of water and the use of a contrast-enhancing metal coating is not necessary. Furthermore, the measurements can be carried out at ambient conditions, which can be essential for retaining the original information about the surface morphology *[26]*. Figure 6b is a 3D-height image of the surface of a microsphere prepared at an initial concentration of 1.5% PMMA and 3.5% Eudragit after leaching out the Eudragit at pH 8. A nodular structure can clearly be seen, even without sputtering. The fact that we also see particles with a size of around 100 nm is an indication that the nodules aggregate on the surface of microsphere. Even some bigger aggregates of around 0.5 - 1 μm can be seen, which might be supernodular aggregates. This is more clearly seen in the AFM image than in the SEM image. Fujh et al. *[27]* observed similar nodular structures and nodular aggregate structures for commercial PMMA hollow fiber membranes. A mechanism was proposed by Khulbe et al. for nodular structure formation of poly(2,6-dimethyl-1,4-phenylene oxide) (PPO) membranes *[28]*. They suggested that a nodule is made up of a combination of several macromolecules *[29]* and that combination of several nodules results in nodular aggregates *[30]*. Further combinations of these nodular aggregates then in turn result in supernodular aggregate structures. The observation with SEM and AFM of similar structures on the surface of microspheres at pH 8, prepared from an initial concentration of 1.5% PMMA and 3.5% Eudragit, suggest a similar mechanism. The free space between the nodules may have beneficial permeation properties for components to be encapsulated, such as drugs. It was shown that for a PPO ultrafiltration membrane, the permeability of gasses through the membrane *[31]* depended on the surface roughness.

Blending of PMMA and Eudragit results in microspheres with different core-shell structures which depend on the initial composition of the mixture. These microspheres are stable in aqueous solution below pH 7.0. After bringing these microspheres to pH 8.0 the Eudragit dissolves to leave PMMA microspheres which show different surface structures, depending on the initial composition. This type of structures or morphologies of microspheres may be used for a wide range of applications in controlled release. Most likely they can also be obtained when using different polymer blends and different relative compositions.

## Conclusions

Microspheres were prepared from blends of PMMA and Eudragit by the use of microsieve emulsification. These microspheres consist of a PMMA core inside an Eudragit-rich shell. Variation in the initial composition of PMMA and Eudragit leads to variation in the core and shell size. After selectively removing the Eudragit at pH 8, PMMA-rich microspheres were formed. The surface morphology of the obtained PMMA microspheres was strongly influenced by the composition of the initial PMMA and Eudragit mixtures.

These microspheres, prepared from PMMA and Eudragit mixtures, are porous due to diffusion of water into the Eudragit shell. As the amount of Eudragit is increased, a thicker and more porous outer shell is formed due to the enhanced interaction of water with Eudragit. After dissolution and removal of the Eudragit, different structures of the core surface are formed, such as a crumpled irregular surface, a fiber-like swollen corona and a surface covered with nodular structures, simply by changing the amounts of PMMA and Eudragit in the initial mixture. These structures are formed as a result of phase separation processes, during demixing of the two polymers. Therefore, by combining microsieve emulsification with a solvent extraction induced demixing in polymers, microspheres can be produced with well-determined size, shape and surface morphology.

## Acknowledgments

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# Chapter 6

## *Discussion and outlook*


## Abstract

This chapter starts with a discussion of the results of the experimental chapters. After that, each topic covered in this thesis is discussed in general terms by comparison with existing literature. In the last part of this chapter an outlook of the research is presented. Further improvements in the emulsification process and material combinations for obtaining desired microcapsules and microspheres are described. Some possible industrial applications are mentioned.

The objective of the research reported in this thesis was to determine the conditions for the preparation of microcapsules and microspheres for oral delivery of active ingredients that are sensitive to acidic conditions. The microcapsules or microspheres for such applications require a shell having specific properties for targeted delivery to the lower gastrointestinal tract. Besides the material properties of the shell, the uniformity and shell thickness are major determinants for the controlled release properties.

The microsieve emulsification technique was expected to yield microcapsules and microspheres with narrow size distributions. Phase separation is an elegant method to prepare microcapsules and microspheres with controlled shell morphologies. Therefore, a combination of phase separation processes and microsieve emulsification was thought to be a good approach to obtain structured microcapsules and microspheres with desired size and surface morphology.

**In Chapter 2** emulsification with a high-porosity silicon nitride micro-engineered membrane is described. Coalescence of droplets and wetting of high-porosity silicon nitride microsieve membranes by the dispersed phase during oil droplet formation was prevented by adding a suitable surfactant to the dispersed phase. This leads to stable and narrow size distribution emulsions. The surfactant in the dispersed phase should exhibit relatively fast adsorption dynamics, which is more critical when the surfactant in the aqueous continuous phase has slower dynamics (e.g. Tween 20 compared to SDS). The flux of the disperse phase could be increased an order of magnitude compared to previous methods, without loss of low span of the droplets. Thus, use of a high-porosity membrane, in combination with suitable surfactants in both the dispersed and continuous phases led to a much more effective and efficient emulsification process.

The results presented in this chapter are the basis for the emulsification processes used in the remaining three chapters, on the preparation of a variety of microcapsules and microspheres.

**In Chapter 3** the preparation of microcapsules with a narrow size distribution of around 30 micron from Eudragit and hexadecane dissolved in dichloromethane (DCM) is described, using cross-flow emulsification with a micro-engineered microsieve membrane. Eudragit is insoluble in water below pH 7, but becomes soluble above this pH. Due to gradual extraction of DCM through the continuous aqueous phase, phase separation between Eudragit and hexadecane occurred, which yielded microcapsules with a hexadecane core, surrounded by an

Eudragit-rich shell. The capsules were stable at pH values below 7.0, whereas the hexadecane was released in half an hour at pH 7.1 and within a minute at pH 8.0 due to dissolution of the Eudragit shell.

At polymer concentrations which were low relative to the concentration of hexadecane, the shells were found to be porous. Increasing the polymer concentration relative to the hexadecane concentration, resulted in a reduction of the porosity and pore size. Insight in the phase separation process explains these findings. At higher concentrations of polymer, the tiny hexadecane droplets that were captured in the forming Eudragit shell were unable to coalesce anymore and therefore lead to small pores.

It was shown that core-shell microcapsules could be prepared with a porous shell of which pore size and porosity can be easily tuned. This could influence the permeability of the shell. Combined with the microsieve emulsification process and the pH-triggered dissolution of the Eudragit shell, this may result in the development of microcapsules with tuned release properties.

**In Chapter 4** the preparation of Eudragit microcapsules with an average size of about 30 μm was investigated, once more using cross flow membrane emulsification as basis. Several vegetable oils with different chain lengths and polarities were encapsulated. The encapsulation of the oil and the morphology of the resulting microcapsules depends on the interactions between the polymer and the type of oil used. Microcapsule formation using long chain length oils such as sunflower oil, olive oil and coconut oil resulted in microcapsules with a single encapsulated oil droplet in the core, surrounded by a relatively dense Eudragit shell. On the other hand, capsules prepared with a relatively short chain length oil such as medium chain triglyceride (MCT) oil resulted in microcapsules with several small oil droplets inside an Eudragit-matrix.

This was found to be related to the interaction between the oil and the Eudragit. Oils with higher molecular weight generally are less compatible with Eudragit, which is a relatively hydrophilic polymer. Extraction of the solvent (DCM) from the droplet resulted in phase separation already at low polymer concentration, allowing for fusion of the small initially formed oil droplets into one larger single droplet. The use of oils that have a better compatibility with Eudragit will lead to a situation in which phase separation occurs at higher polymer concentrations, at which the Eudragit matrix has already more or less solidified. Extraction of the oil from the microcapsules with hexane resulted in the formation of hollow

porous shells. Investigation of these hollow capsules with SEM confirmed the conclusions reached by optical microscopy about the phase separation process.

**In Chapter 5** the preparation of microspheres from blends of poly(methyl methacrylate) (PMMA) and Eudragit was investigated. Once more, the primary emulsion droplets were formed with microsieve emulsification. The microspheres were found to consist of a PMMA core inside an Eudragit-rich shell. Variation in the initial composition of PMMA and Eudragit led to variation in the core and shell sizes. After selectively removing the Eudragit shell at pH 8.0, PMMA microspheres remained. The surface morphology of the obtained PMMA microspheres was strongly influenced by the composition of the initial PMMA and Eudragit mixtures.

These microspheres, prepared from PMMA and Eudragit mixtures, were porous due to diffusion of water into the Eudragit shell. As the amount of Eudragit was increased, a thicker and more porous outer shell was formed due to the enhanced interaction of water with Eudragit. After dissolution and removal of the Eudragit, different core surface structures were found, such as a highly irregular, crumpled surface, or a surface covered with nodular structures. In some cases, a fiber-like, swollen corona was found to surround the core, with demixed PMMA attached to the core in a highly swollen state.

The four experimental Chapters of this thesis are all related to the goal of developing suitable vehicles for encapsulation and controlled release, i.e. investigating microsieve emulsification and phase separation to obtain microcapsules, hollow microcapsules and double-walled microspheres. A general discussion of each of these points is given below.

***Microsieve emulsification***

Currently, microsieve emulsification seems to have much potential for obtaining narrowly dispersed emulsions, microcapsules and microspheres *[1-3]*. The main benefits of using microsieves are the freedom in choice of pore size, shape, porosity and thickness. These engineered microsieves typically have a thickness that is smaller than their pore size; therefore they allow for preparation of emulsions at a lower transmembrane pressure than other more conventional membrane emulsifications (e.g. with an SPG membrane).

A major challenge of microsieve emulsification is to increase the production rate of oil droplets. For this the membrane should remain wetted by the continuous phase. If the oil

(dispersed phase) wets the surface of the microsieve even slightly, the emulsification process may easily fail to produce narrowly dispersed droplets *[4]*. For relatively long term emulsification of oil by a silicon nitride microsieve, wetting by the oil should be minimal. Droplet coalescence can be avoided by the use of proper surfactants; however, the dynamics of surfactant adsorption at the oil-water interface have to be faster than the dynamics of the droplet formation *[4]*. The effect of interfacial tension on the dynamics of droplet formation has already been extensively studied with single pore membranes *[5]*. A simple rule was found that states that the dynamics of surfactant adsorption should be faster than the rate of droplet formation.

Improving the flux of the to-be-dispersed phase is quite important for membrane emulsification processes and several reports have shown possibilities for this, obtained from basic understanding of the droplet formation process. This understanding was mainly obtained from extensive studies with single pores. A study from Dijke et al. *[6]* showed that with use of terrace-based micro-channels many droplets can be produced simultaneously from a single channel. This may be applied in a microsieve-based system.

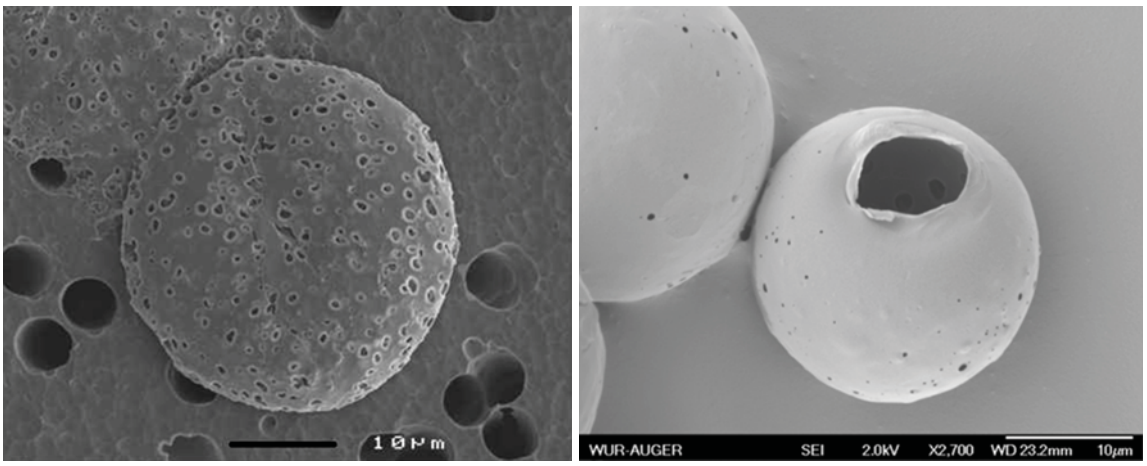
The flux reported in this thesis ($> 90 \cdot 10^{-6}$ $m^3/m^2s$) for production of 25 μm-sized particles with the use of 5 μm round pores is attractive for applications. Even at this flux the operating pressure was still quite low (~ 25 mbar), so that narrowly size-dispersed droplets can be made under controlled dispersed phase flux flow. It was also found that not all pores in the microsieve were active and contributed to the emulsification process. In order to achieve even higher dispersed phase fluxes, it is quite important to make all the pores active. No clear solution for this problem is available at this moment.

***Microcapsules***

Although preparation procedures of microcapsules are already known for several decades, there is still interest in obtaining microcapsules with tailor-made properties and understanding their formation processes. An interesting option is to combine new materials and processes to achieve these designed microcapsules.

For uniform release of the encapsulated material, the size and uniformity of the capsules is important. Therefore, microsieve emulsification was used to yield the narrow size-dispersed capsules. The specific size of the capsules can be fine-tuned by varying process parameters like transmembrane pressure and cross-flow velocity, and membrane pore size and shape.

For preparation of the core-shell microcapsules a phase separation method was used. Phase separation is traditionally used for the preparation of special or porous polymeric films. Recently, Sawalha et al. *[7, 8]* investigated phase separation for the preparation of poly-lactide capsules and used polymer films as a model system. The combination of the use of the pH-sensitive polymer Eudragit and different oils to be encapsulated is new. The additional combination with microsieve emulsification resulted in narrow size-dispersed capsules.




a) b)

*Figure 1. SEM images of capsules prepared in the present research a) Hexadecane core-Eudragit shell capsule b) Hollow capsule obtained after extraction of the oil in the core.*

During the capsule formation the solvent removal rate is very important and this ultimately effects the morphologies of the formed capsules. It can be influenced by several parameters such as change in temperature, non-solvent properties, oil type and the solvent itself. In this study only the oil type (core) was changed, which gives better insight in their effect on the morphological properties of the capsules. Upon increasing the molecular weight of the oil the morphology of the microcapsules changed from a multicompartment, porous microcapsule into a core-shell microcapsule. Capsules prepared with Eudragit as a shell are somewhat porous due to the hydrophilic nature of this polymer.

The surface morphology and internal structure of the capsules can be controlled by changing the type and relative amounts of core material. For microcapsules with hexadecane and Eudragit the pore size can be influenced by just changing the amount of Eudragit and hexadecane *[3]*. Such porous microcapsules with different structures can be useful for potential applications in drug delivery, tissue engineering or regenerative medicine *[9]*.

***Hollow microcapsules***

Hollow microcapsules or particles are gaining significant interest for encapsulation of active ingredients *[10, 11]*. In addition, hollow capsules with a single void compartment can be used as micro-reactors, whereas multi-compartment hollow capsules can be used as multi-compartment micro-reactors, e.g. in enzyme-catalyzed reactions or simultaneous multiple drug delivery in bio-medicine applications; however, for the latter case production by different techniques is required *[12]*.

Different methods are known for the preparation of hollow capsules. Colloidosomes in which oil is encapsulated by the adsorption of colloidal particles at the surface yields hollow porous shells after removal of the oil template *[13]*. This route can also be combined with microsieve or membrane emulsification to control the capsule size *[14]*. A practical limitation is the effective adsorption of colloidal particles at the oil-water interface.

Layer-by-layer adsorption is another route by which the capsule shell is assembled on an oil template by electrostatic deposition of thin alternating layers of e.g. proteins and polysaccharides *[15]*. After removal of the oil template e.g. by freeze drying, hollow capsules can be formed.

Serious limitations of this system are that many adsorption cycles are required and that it can only be performed with charged polymers. A recently described method uses a combination of the colloidosomes route with the layer-by-layer technique to control the porous structure of hollow capsules *[16]*.

The phase separation method is an elegant approach to prepare core-shell capsules in a single step, which can in principle be used for a wide range of polymers and oils *[7]*. The removal of the core by extraction or freeze drying, results in hollow capsules. The morphology of these capsules is controlled by the initial phase separation process during formation.

A microcapsule with an oil core encapsulated as a single droplet yields a hollow shell after extraction of the oil. On the other hand, a microcapsule with several oil droplets (multi-compartment core) yields a multi-compartment structured hollow capsule after extraction of the oil. Again, the formation of these structures can be related to the solvent removal rate during the phase separation process as discussed in the previous section. An oil that shows poor compatibility with the shell-forming polymer will induce phase separation already at low polymer concentrations, which will allow fusion of the oil into one single core droplet. Using an oil that has better compatibility with the shell-forming polymer will lead to phase separation only when much more of the solvent has been extracted. The polymer

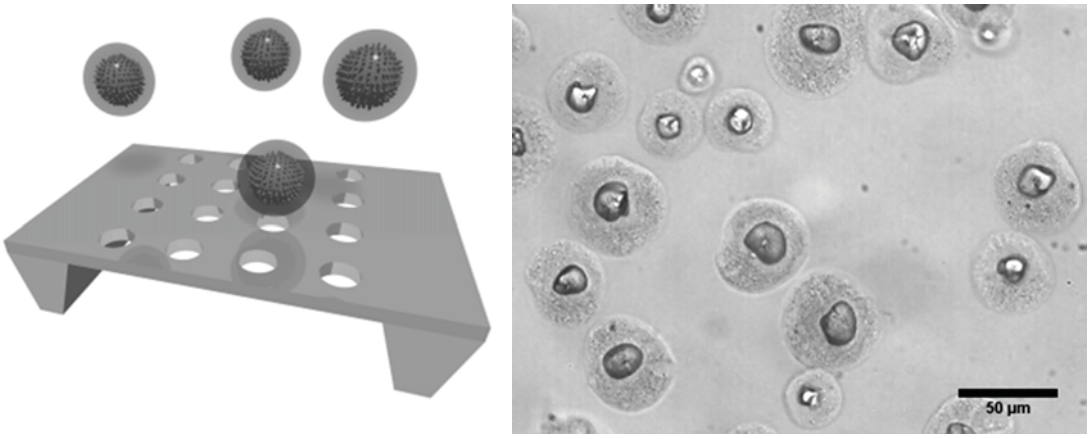
concentration is then so high, that the fusion of oil droplets is severely hindered, leading to multiple, very small oil droplets. This was demonstrated in this thesis with the system Eudragit-DCM-water with triglyceride oils having different chain lengths. These findings are related to observations in other systems e.g. suspension polymerization of divinylbenzene in the presence of different MW oils yields poly divinylbenzene microspheres with different structures *[17]*, which shows the general applicability of the principle.

***Double-walled microspheres***

Double-walled microspheres may find application as triggered release systems, in which the outer wall protects the active ingredient from a specific environment (e.g. acid in the stomach), while the second wall may allow for release by swelling upon uptake of water, giving a quick release of the contents *[18]*.

Double-walled capsules can be made by spray-drying followed by fluidized bed coating, but this necessarily leads to large capsules. For many products one would prefer microcapsules with dimensions smaller than e.g. 10 μm. This is possible with microsieve emulsification and phase separation using a polymer blend.

The phase separation between the polymers determines the final morphology of the double-walled microspheres. In one literature report the overall microsphere size and the core size-shell thickness ratio were controlled by droplet formation via two coaxial nozzles. The flow rates of the two polymer solution feeds through these nozzles were varied, resulting in different core sizes and shell thicknesses *[19]*. However, the scalability of such a method to industrial scale is questionable. The microsieve emulsification developed in this thesis is feasible to scale up while retaining the microsphere size, and the proposal for industrial scale production will be discussed in the last section of this chapter. In this thesis, this principle was demonstrated by using a blend of Eudragit and PMMA. The external and internal structures of the microspheres depend on the initial concentrations of PMMA and Eudragit. With increasing amounts of Eudragit, a more porous outer shell was formed due to enhanced interaction with water. On the other hand, the internal structure (i.e. observed after dissolving the Eudragit) also varies with the composition ratio of the two polymers. At equal amounts of PMMA and Eudragit, the PMMA microspheres obtained after dissolution of the Eudragit shell had a crumpled surface with indentations.




a) b)

*Figure 2. a) Double-walled microsphere formation with microsieve emulsification b) Optical microscopic image of double-walled microspheres at pH 8; the core-shell morphology is due to a partially dissolved shell (Eudragit in the shell is dissolved, leaving the PMMA domains as a fibrous shell).*

With a further increase in Eudragit concentration, the microspheres seen upon dissolution of the Eudragit at pH 8.0, contained fibrous structures radiating from, but still connected to the core, probably due to PMMA spheres in the shell that became physically aggregated during the dissolution of Eudragit. At an even higher concentration of Eudragit in the mixture, the residual PMMA in the Eudragit formed only very small spheres that were not physically connected to one another. During the dissolution process of the Eudragit these small spheres precipitate on the core surface and become physically attached there. Therefore microspheres with a nodular surface morphology are formed in that case.

**Table 1.** Summarizing the results (Microcapsules)

| Structure (morphology) | Polymer concentration | Oil concentration | Polymer-oil compatibility |
|---|---|---|---|
| Single-core microcapsules | Low | Low | Poor |
| Multiple-core, porous particles | Moderate | Moderate | Good |
| Defect-less shell | Higher | Moderate | - |
| Hollow capsules[a] | Low | Low | Poor |
| Multi-hollow capsules[a] | Moderate | Moderate | Good |

[a]after extraction of oil from capsules

**Table 2.** Summarizing the results (Microspheres after extraction of Eudragit)

| Structure (morphology) | PMMA concentration | Eudragit concentration |
|---|---|---|
| Crumpled microspheres | Moderate | Moderate |
| Swollen microspheres with corona | Moderate | Higher |
| Nanorough covered microspheres | Low | Even higher |

## Outlook of research and possible industrial applications

***Microsieve emulsification***

Although microsieve emulsification lends itself in principle to large scale production, the industrial application of this process is restricted to high added value products due to the relatively high costs necessary for a high production volume. The transmembrane flux reported in this thesis (> $90 \times 10^{-6}$ $m^3/m^2s$ for ~ 25 μm droplets) is an interesting step forward to make the process commercially attractive. For the production of 1 ton encapsulates per hour with the systems discussed in this thesis (using 3 wt% polymer, and 3% oil), one would need to emulsify roughly 17 tons of solution per hour. With the fluxes found here, this would require roughly 40 $m^2$ microsieve surface area (assuming a density of 1330 $kg/m^3$ of the solution), which is well within practical range.

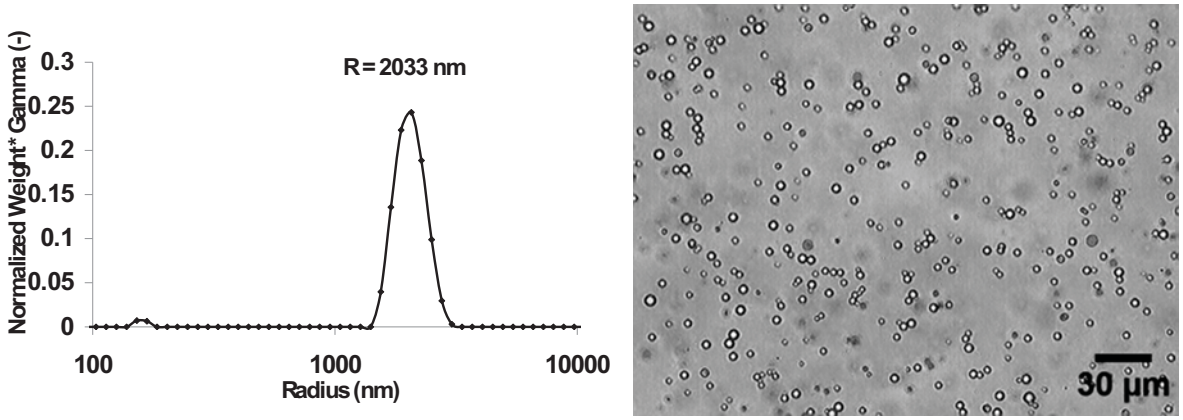



*Figure 3. Sunflower oil-water emulsion with an average size of 4 μm prepared using a 0.8 μm high porosity Aquamarijn microsieve a) DLS graph of the emulsion b) optical microscope image. Conditions: 4% Span 80 in oil and 1% SDS in water; operating pressure was 124 mbar.*

In food applications the size of capsules typically has to be smaller than 20 μm, otherwise it will influence the sensory properties of the product. For injectable drug delivery systems a microsphere size of around 10 μm is desired *[2]*. The size of the emulsion droplets can be reduced by using a smaller pore size of the microsieve. With microsieves having a pore size of 0.8 μm, a droplet size of 4 μm is produced (figure 3). This preliminary experiment demonstrates the feasibility of preparing small droplets with this technique.

A major problem associated with long term processing using microsieve emulsification is fouling. Surface modification of these sieves with organically coated monolayers or polymeric brushes could help to prevent this problem. However, it is difficult to design a single coating that can repel all components during emulsification. Recent work has shown that zwitterionic polymer-based coatings can significantly repel protein adsorption to the surface *[20]*. The repulsion efficiency varies with the type of protein. Therefore, such a modification may not always guarantee stable emulsification performance in the presence of different polymers and oil mixtures. On the other hand, organic or polymer coatings could give ample opportunities for optimization of the surface properties.

***Microcapsules***

Although microcapsule preparation is considered a well-established field, for each application new combinations of new materials and processes are required. The design of microcapsules starts first by considering the application for which the capsules are aimed. Specific material properties need to be identified and then this can be translated into suitable processes. Therefore, by identifying the right materials and processes a functional encapsulate can be prepared. Preparing the capsules by a phase separation process is a simple and robust method, that can be used for a wide range of polymers.

Different oil-soluble or oil-dispersible ingredients can for example be encapsulated in Eudragit capsules using the phase separation process. These porous microcapsules are especially interesting materials for cell immobilization *[21]*, since the porous matrix will allow essential micronutrients to diffuse into the capsules. However, the DCM used in the present studies for the preparation of the capsules is probably not very compatible with the cells. Therefore, it may need to be replaced with other less toxic solvents. However, this will also affect the phase separation process which is dependent on the solvent removal rate. Furthermore, this general phase separation process may in principle also be carried out with other more biocompatible polymers like polycaprolactone, polylactide, ethyl cellulose or

hydroxypropyl methylcellulose. It is clear that determining the optimal conditions for the preparation of microcapsules with other polymers and solvents requires additional research.

***Hollow microcapsules***

The hollow capsules presented in this thesis could be an adequate product for loading active ingredients. Hollow capsules prepared with different types of internal structures can also act as microreactors *[12]*. The hollow capsules may be used to load them with specific enzymes. On demand release of these enzymes will then initiate enzymatic reactions. A possible application may be in microbially-enhanced oil recovery *[22]*, in which microbes are loaded in microparticles and subsequently released in the medium for recovering oil from a crude mixture.

Additionally, hollow capsules are used for ultrasound-mediated drug delivery systems *[23]* in which the drug-loaded particles are exposed to an acoustic medium to release the active component. For this application, the capsules need to be smaller and more uniform than the present capsules; this may be achieved by using smaller pore size membranes as mentioned in the emulsification section. With the same process, hollow capsules may be obtained with other biocompatible polymers such as polycaprolactone, polylactide, ethyl cellulose, or hydroxypropyl methylcellulose.

***Double-walled microspheres***

Double-walled microspheres prepared by blending of two polymers results in microspheres with different core-shell structures. Eudragit dissolves at alkaline pH to leave the encapsulated microspheres with different surface structures, depending on the initial composition. Microspheres from blends of two polymers may thus be used to obtain both pH-dependent and pH-independent release of encapsulated ingredients. The PMMA core material used in the present study may also be replaced with biodegradable polymers like polycaprolactone, polylactide, ethyl cellulose or hydroxypropyl methylcellulose can potentially lead to useful materials for oral delivery systems.

A major advantage of double-walled microspheres is that active ingredients can be located in the core and both the core and shell can be engineered to achieve a tailored release profile. Since some core material may be expected to remain in the shell during the phase separation process, it will be released immediately upon dissolution of Eudragit and the remaining active

ingredient, located in the core (e.g. consisting of polylactide) will be released during a longer period by diffusion through the matrix.

Complex structures formed with phase separation are expected to strongly influence release properties. Therefore, release studies with model drug compounds are very interesting for future investigations. More delicate materials like probiotics can be initially entrapped in oil shells and further coated with Eudragit for pH-triggered release. These encapsulated probiotics may then be further protected from the low pH in the stomach by an oil barrier in an Eudragit shell. When the capsules reach the colon, the Eudragit shell is expected to dissolve while releasing the probiotics. This concept may also be applied in medicinal foods, since addition of probiotics has been claimed to have several health benefits like prevention of colon cancer *[24]*. The dual coating of oil and Eudragit may enhance the viability of probiotics during their passage through the upper gastrointestinal (GI) tract. This general concept can also be applied to food grade biopolymers with similar properties such as shellac, ethyl cellulose or hydroxypropyl methylcellulose and other less toxic solvents such as ethyl acetate or alcohols.

## Proposal

***Towards industrial scale production of microspheres and capsules***

A serious hurdle towards upscaling the production of microcapsules is the use of dilute solutions. Preparing 1 ton of microcapsules from a solution of 3 wt% Eudragit and 3 wt% oil in DCM would require the use of about 17 tons of DCM, which all has to evaporate. The aqueous phase normally is a number of times larger than the dispersed phase (e.g. 300 tons or more). Since the evaporation times for DCM are in the range of several hours, one would need very large reactor volumes. For 1 ton per hour production and 6 hours evaporation time, one would need 330 tons / h x 6 = 1980 $m^3$ reactor volume. This shows that there are still important challenges for industrialization. An increase of the concentrations used will help to dramatically reduce these numbers (Table 3). It is clear that this will have a major influence on the phase separation process, which therefore needs to be further investigated and optimized for lower volumes of DCM and continuous water phase.

**Table 3.** Amount of DCM required to prepare 1 ton of capsules

| Amount of Eudragit % (w/v) | Amount of oil % (w/v) | Amount of DCM required ton |
|---|---|---|
| 3 | 3 | 17 |
| 5 | 5 | 10 |
| 7 | 7 | 7 |
| 10 | 10 | 5 |

New techniques using dead-end emulsification as e.g. developed by Nanomi (**www.nanomi.com**) will also dramatically lower the required volume of the continuous phase. A much higher concentration (e.g. a ratio of dispersed phase to continuous phase of up to 10-20%) is well possible. A method developed by Hennink et al. *[25]* for the preparation of solvent free microcapsules can also be an alternative. However, this can most probably only be performed with aqueous phase separated polymers in water-water type of emulsion. Nevertheless, it is clear that suitable combinations of materials and processes can be designed for different applications.

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# Summary

Encapsulation and use of capsules for controlled release has several applications in pharmaceuticals, foods, cosmetics, detergents and many other products for consumers. It can contribute to sustainability, since it allows an efficient use of active materials, delivery at the required site and possibly a longer shelf life of the products. Many encapsulation systems are basically very thin shells (10 nm – 10 μm) around microscopic reservoirs (100 nm – 100 μm), in which active ingredients are trapped. The release properties are strongly dependent on the material properties of the shell, but also on their size and uniformity.

The overall objective of this research is to understand the formation process of microcapsules and microspheres by using phase separation in well-defined droplets of a polymeric solution. The primary droplets were produced with microsieve emulsification; the polymer used was Eudragit FS 30D (a commercial copolymer of poly (methyl acrylate-co-methyl methacrylate-co-methacrylic acid) 7:3:1). These charged carboxylate groups make the polymer water-soluble at higher pH (>7), allowing for release by a change in pH.

Chapter 2 presents results that give more insight into microsieve emulsification with high porosity micro-engineered membranes. The droplet formation was strongly influenced by the dynamics of surfactant adsorption. The presence of suitable surfactants in both phases prevents the coalescence of droplets and wetting of microsieve membranes by the dispersed phase during oil droplet formation. This resulted in the formation of stable emulsions of droplets with a narrow size distribution. The flux of the dispersed phase could be increased an order of magnitude compared to previous methods, without loss of size-distribution of the droplets. Thus, use of a high-porosity membrane, in combination with suitable surfactants in both the dispersed and continuous phases resulted to a much more effective and efficient emulsification process.

In Chapter 3 crossflow microsieve emulsification was used to prepare porous microcapsules with an average size of about 30 μm. A mixture of Eudragit and hexadecane in DCM was emulsified in water. Being a poor solvent for this polymer, demixing of the droplet into a polymeric shell and a hexadecane-rich core occurred upon extraction of the DCM into the water phase. At a low ratio of concentrations of polymer and hexadecane, the shells were found to be porous. Increasing this ratio resulted in a reduction of the porosity and pore size of the shell. The Eudragit has a pH-dependent solubility. It is insoluble at acidic conditions and rapidly dissolves at alkaline conditions. The capsules were found to be stable at a pH lower

than 7.0, whereas the oil core was released within half an hour at pH 7.1 and within a minute at pH 8.0. The morphology of the microcapsules can be adapted with a careful choice of the concentrations of polymer, hexadecane and solvent. At higher concentrations of polymer, the tiny oil droplets that were captured in the forming Eudragit shell were unable to coalesce completely and small, isolated pores were formed within the shell matrix. This could influence the permeability properties of the shell.

The potential for new microcapsule morphologies was further explored in Chapter 4 where the formation of Eudragit capsules with other oils instead of hexadecane was studied, and in Chapter 5 where a blend of PMMA and Eudragit was used.

In Chapter 4 the effects of chain lengths of vegetable oils on the formation of porous microcapsules with hollow and multi-compartment structures is discussed. The encapsulation of oil and the morphology of the resulting microcapsules depend on the interaction between the Eudragit polymer and the type of oil that was used. Microcapsule formation using long chain length oils such as sunflower oil, olive oil and coconut oil resulted in well-defined microcapsules with a single encapsulated oil droplet, covered with a Eudragit-rich shell. On the other hand, capsules prepared with relatively short chain length oils, such as medium chain triglyceride oil, resulted in capsules with many individual small oil droplets encapsulated in an Eudragit matrix. This is thought to stem from different rates of phase separation. Medium chain length oil (MCT oil, a low MW oil) is relatively well soluble. Thus, the solvent may diffuse out for a significant time, without phase separation setting in. Only when the polymer concentration has already become rather high, phase separation occurs and, the MCT oil droplets get trapped in the Eudragit matrix. Long chain length oils are less soluble, and phase separation between the oil and polymer will set in at an earlier stage, before much solvent has diffused out, and the polymer concentration is still relatively low. Thus the initial small oil droplets merge into one single core. Extraction of the oil from the microcapsules with hexane results in the formation of hollow porous shells as was investigated with optical microscopy and SEM. These structures are formed during microcapsule formation due to the complex phase separation processes in the Eudragit-water-oil-DCM quaternary system.

In Chapter 5 the formation of microcapsules is further explored by using a blend of poly(methyl methacrylate) (PMMA) and Eudragit. Microspheres formed with this blend were found to consist of a PMMA core inside an Eudragit-rich shell, which tends to be porous. As the amount of Eudragit is increased, a thicker and more porous outer shell is formed due to the

enhanced interaction of water with Eudragit. After dissolution of the Eudragit at high pH, different core surface structures resulted, from irregular surfaces to microspheres with a fiber-like, swollen corona around it, and to a surface covered with small nodular structures, dependent on the concentrations of PMMA and Eudragit in the initial mixture. As already indicated above, these structures are formed as a result of complex phase separation processes between polymers and (non)solvents, and between the two polymers.

In Chapter 6 the results described in this thesis were compared with existing literature, yielding an outlook on the field of microencapsulation through phase separation. Microsieve emulsification is feasible for the production of emulsions with a throughput from millilitres to tons of volume. The microcapsules developed here can be used for encapsulation of oil-soluble active ingredients and release by a pH trigger. The hollow capsules can possibly be interesting materials as micro-reactors, e.g. by loading with enzyme and performing enzymatic reactions on demand. The complex structures formed upon phase separation of two polymers can be employed for obtaining complex release profiles. A general concept is discussed on how to obtain various interesting complex structures with phase separation combined with microsieve emulsification. Finally, a conceptual process design is discussed for industrial scale production of microcapsules and microspheres with use of microsieve emulsification.

This thesis has yielded insight in the formation of a range of microcapsule morphologies by investigating a range of new production methods (microsieves and demixing conditions) and formulations (different concentrations, oils and using one polymer or a blend), and through this provides better insight into the mechanisms of microcapsule formation. While some of the structures may be directly used for microcapsule formation, some other structures may well have potential for other applications.

# Samenvatting

Het opnemen van stoffen in en de toepassing van capsules voor gecontroleerde afgifte van de ingesloten componenten vindt toepassing in diverse farmaceutische producten, voedingsmiddelen, cosmetica en vele andere consumentenproducten. Het kan bijdragen aan duurzaamheid, aangezien er efficiënt gebruikt gemaakt wordt van actieve componenten, er afgifte op de gewenste locatie plaatsvindt en het kan leiden tot een langere houdbaarheid van de producten. Vele microcapsules zijn in principe microscopische reservoirs (100 nm – 100 µm doorsnede), waarin actieve componenten opgesloten zitten, met daaromheen een dunne schil (10 nm – 10 µm). De afgifte-eigenschappen zijn sterk afhankelijk van de materiaaleigenschappen van de schil, maar ook van de grootte en de gelijkvormigheid.

De algemene doelstelling van dit onderzoek is om inzicht te krijgen in het vormingsproces van microcapsules en microbolletjes door middel van fasescheiding in goed gedefinieerde druppels van een polymeer oplossing. De primaire druppels worden geproduceerd met behulp van membraanemulsificatie van een Eudragit FS 30D polymeerolossing (een commercieel poly(methyl acrylaat-co-methyl methacrylaat-co-methacrylzuur) 7:3:1 copolymeer). De geladen carboxylaat groepen maken het polymeer oplosbaar bij een pH >7, waardoor afgifte door verandering van pH mogelijk is.

Hoofdstuk 2 beschrijft resultaten die meer inzicht geven in het emulsificatieproces van olie in water met behulp van microzeefmembranen die zo ontworpen zijn dat ze een hoge porositeit hebben. De aanwezigheid van geschikte surfactanten in beide fasen voorkomt het samensmelten van druppels na vorming en tevens het hechten van de oliedruppels aan het membraan tijdens de vorming. De druppelvorming wordt sterk beïnvloed door de dynamiek van surfactantadsorptie. Het gebruik van geschikte surfactanten resulteerde in stabiele emulsies van druppels met een smalle grootteverdeling. De flux van de gedispergeerde fase was een grootteorde hoger in vergelijking met andere methoden, zonder dat dit invloed had op de grootteverdeling van de druppels. Het gebruik van hoogporeuze membranen in combinatie met geschikte surfactanten in beide fasen resulteert dus in een veel effectiever en efficiënter emulsificatieproces.

In hoofdstuk 3 werd hetzelfde emulsificatieproces met microzeven gebruikt om poreuze microcapsules te maken met een gemiddelde grootte van circa 30 µm. Een mengsel van Eudragit en hexadecaan in dichloormethaan werd in water geëmulgeerd. Omdat hexadecaan een slecht oplosmiddel is voor dit polymeer en omdat het dichloormethaan werd geëxtraheerd

naar de waterfase vond er ontmenging in de druppels plaats waarbij een polymere schil rond een hexadecaan-rijke kern ontstond. Bij een lage verhouding in concentraties van polymeer tot hexadecaan bleken de polymeerschillen poreus te zijn. Verhogen van deze verhouding resulteerde in een minder poreuze schil met kleinere poriegroottes. De oplosbaarheid van Eudragit in water is pH-afhankelijk. Het is onoplosbaar in zuur maar lost snel op onder basische omstandigheden. De capsules bleken stabiel te zijn bij een pH lager dan 7,0; bij een pH van 7,1 kwam de oliekern in een half uur vrij en bij pH 8,0 zelfs binnen één minuut. Door een zorgvuldige keuze van de concentraties polymeer, hexadecaan en oplosmiddel kan de uiteindelijke morfologie van de microcapsules bepaald worden. Bij hogere concentraties aan polymeer werden kleine oliedruppels gevangen in de vormende Eudragit schil en deze waren daardoor niet meer in staat om volledig met elkaar te versmelten waardoor kleine poriën in de polymeermatrix van de schil ontstonden. Dit kan de permeabiliteitseigenschappen van de schil beïnvloeden. De mogelijkheid voor vorming van microcapsules met een andere morfologie werd verder onderzocht in hoofdstuk 4, waarbij andere oliën in plaats van hexadecaan werden gebruikt, en in hoofdstuk 5, waar een mengsel van PMMA en Eudragit werd gebruikt.

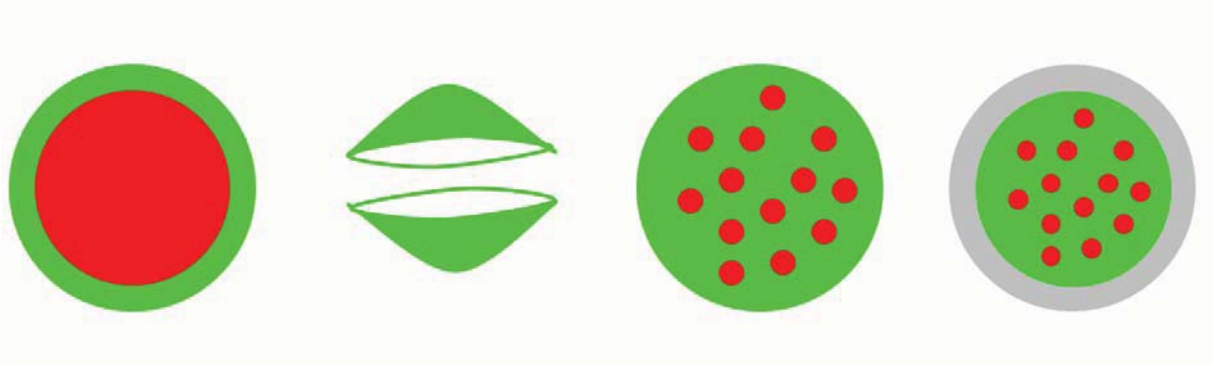
In hoofdstuk 4 worden de effecten van ketenlengte van plantaardige oliën op de vorming van poreuze microcapsules besproken. De encapsulatie van olie en de morfologie van de ontstane microcapsules zijn afhankelijk van de interactie tussen het Eudragit polymeer en het gebruikte type olie. Emulsificatie met oliën met lange vetzuurketens, zoals zonnebloemolie, olijfolie en kokosolie, resulteerde in goed gedefinieerde microcapsules waarin een enkele druppel olie de kern vormde, omringd door een Eudragit-rijke schil. Anderzijds, indien oliën werden gebruikt met relatief korte vetzuurketens, resulteerde dit in capsules met veel kleine individuele oliedruppeltjes verspreid in een Eudragit matrix. Dit is waarschijnlijk het gevolg van verschillende snelheden waarmee de fasescheiding plaatsvindt. Een dergelijke olie met een laag moleculair gewicht (MCT olie) is relatief goed oplosbaar. Hierdoor kan het oplosmiddel er langer uit diffunderen voordat er fasescheiding plaatsvindt. Pas als de polymeerconcentratie erg hoog is geworden treed er fasescheiding op en worden de MCT oliedruppeltjes opgesloten in de Eudragit matrix. Oliën met lange vetzuurketens zijn minder goed oplosbaar waardoor fasescheiding tussen de olie en het polymeer in een eerder stadium plaatsvindt, voordat veel oplosmiddel naar buiten is gediffundeerd en de concentratie van het polymeer nog relatief laag is. Hierdoor kunnen de initieel gevormde kleine oliedruppeltjes nog samensmelten tot één enkele kern. Extractie van de olie uit de microcapsules met behulp van hexaan resulteerde in holle poreuze capsules zoals optische microscopie en SEM-opnamen lieten zien. Deze

structuren zijn ontstaan tijdens de vorming van de microcapsule door de complexe fasescheidingsprocessen in het Eudragit-water-olie-dichloormethaan systeem.

In hoofdstuk 5 is de vorming van microcapsules verder onderzocht voor mengsels van poly(methyl methacrylaat; PMMA) en Eudragit. De microdruppels die gevormd werden met dit mengsel bleken een kern te hebben van PMMA, waaromheen een poreuze Eudragit-rijke schil zat. Wanneer er meer Eudragit wordt gebruikt, wordt er een dikkere en meer poreuze Eudragit schil gevormd door een sterkere interactie met water. Nadat Eudragit is opgelost bij hoge pH ontstaan er verschillende oppervlaktestructuren van de overgebleven kern. Dit varieert van onregelmatige oppervlakken tot microdruppels omgeven door een fiber-achtige gezwollen corona, tot een oppervlak bedekt met kleine knobbelachtige structuren, afhankelijk van de concentraties van PMMA en Eudragit in het oorspronkelijke mengsel. Zoals hierboven reeds beschreven is worden deze structuren gevormd als gevolg van de complexe fasescheidingsprocessen tussen de twee polymeren en de twee oplosmiddelen.

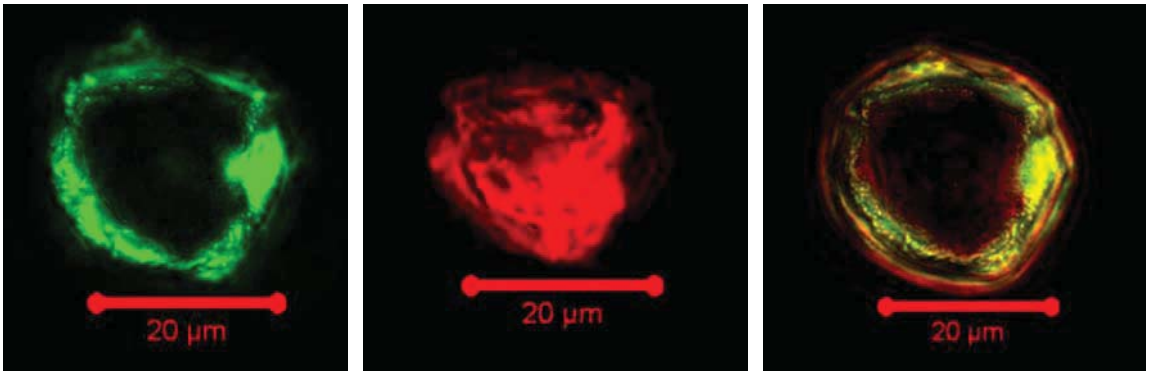
In Hoofdstuk 6 worden de resultaten uit dit proefschrift vergeleken met bestaande literatuur, waardoor een betere visie op het gebied van micro-encapsulatie met behulp van fasescheiding wordt verkregen. Met behulp van microzeefemulsificatie zijn productievolumes van milliliters tot tonnen emulsies haalbaar. De microcapsules die hier ontwikkeld zijn kunnen worden gebruikt om olie-oplosbare actieve bestanddelen te encapsuleren, welke kunnen vrijkomen door een pH-schakelaar. De holle capsules kunnen mogelijk interessant zijn als micro-reactor, bijvoorbeeld door er een enzym in te plaatsen en op afroep enzymatische reacties te laten plaatsvinden. De complexe structuren, welke gevormd werden na fasescheiding van twee polymeren, kunnen gebruikt worden om complexe afgifteprofielen te verkrijgen. Er is een algemeen concept beschreven hoe verschillende interessante complexe structuren kunnen worden verkregen met behulp van fasescheiding in combinatie met microzeefemulsificatie. Ten slotte wordt een concept voor een procesontwerp besproken voor productie van microcapsules en microbolletjes op industriële schaal met behulp van microzeefemulsificatie.

Dit proefschrift heeft inzicht gegeven in de vorming van microcapsules met verschillende morfologieën door een reeks van nieuwe productiemethoden (microzeven en fasescheidingscondities) en formuleringen (verschillende concentraties, oliën en het gebruik van één polymeer of een polymeermengsel) te onderzoeken en geeft daarmee een beter inzicht in de mechanismen van microcapsulevorming. Sommige van de onderzochte structuren kunnen direct toegepast worden voor de vorming van microcapsules, terwijl sommige andere verkregen structuren veelbelovend zijn voor andere toepassingen.



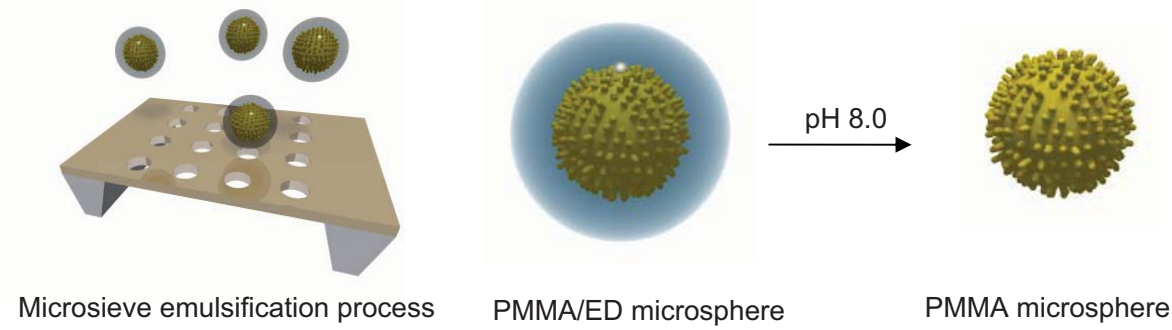
a) b) c) d)

*Chapter 1, figure 1, page 3*




a) b) c)

*Chapter 3, figure 3, page 40*




*Chapter 5, figure 1, page 65*

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***Nagesh***

# Curriculum vitae

Nagesh A. Wagdare was born on 19th June, 1983 in Gholsgaon, a small village near Akkalkot in Maharashtra, India. He went to high school (1998-2000) at Maharashtra State Technical College, Solapur. Subsequently, he studied for BSc in Chemistry (2000-2003) at Shivaji University.

On completion of his bachelor studies, he continued to pursue his MSc in Polymer Chemistry (2003-2005) at Shivaji University. As part of his MSc studies, he worked on the thesis project: "Synthesis of aliphatic polyesters for controlled release", under supervision of Prof. S. V. Lonikar at the Department of Chemistry, Solapur. From September 2005 to May 2007, he worked on an industrial project: "Microcapsules for fabric care", in the group of Dr. Parashuram G. Shukla, Polymer Science and Engineering Division, National Chemical Laboratory, Pune.

From June 2007 to May 2011, he worked on the PhD project: "Structuring Microspheres", at Wageningen University, The Netherlands. He was supervised by Dr. Antonius Marcelis and Prof. Cees van Rijn from the Laboratory of Organic Chemistry, and Prof. Remko Boom of the Food Process Engineering Group. Most of the research performed in the last four years is described in this thesis. From June 2011, Nagesh is working as a Scientist for Aquamarijn Micro Filtration BV, The Netherlands.

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# Publication list

## Peer Reviewed Journals

Nagesh A. Wagdare, Antonius T.M. Marcelis, O. Boen Ho, Remko M. Boom and Cees J.M. van Rijn, High throughput vegetable oil-in-water emulsification with a high porosity micro-engineered membrane, *Journal of Membrane Science*, **2010**, *347*, 1-7

Nagesh A. Wagdare, Antonius T.M. Marcelis, Remko M. Boom and Cees J.M. van Rijn, Porous microcapsule formation with microsieve emulsification, *Journal of Colloid and Interface Science*, **2011**, *355*, 453-457

Nagesh A. Wagdare, Antonius T.M. Marcelis, Remko M. Boom and Cees J.M. van Rijn, Microcapsules with a pH responsive polymer: influence of the encapsulated oil on capsule morphology*, Colloids and surfaces B: Biointerfaces*, **2011**, Article in press.

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## Conference Proceedings

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Nagesh A. Wagdare, Antonius T.M. Marcelis and Cees J.M. van Rijn, Fabrication of edible oil-water emulsions and double emulsions with high porosity microsieves, *International Conference on Food Rheology and Structure*, Zürich, Switzerland, **2009**

Nagesh A. Wagdare, Antonius T.M. Marcelis, Remko M. Boom and Cees J.M. van Rijn, Phase separation-induced pH-sensitive microcapsule formation, *World Congress on Emulsions*, Lyon, France, **2010**

## Overview of completed training activities

***Discipline specific activities***

*Courses*

Polysaccharides as Food Colloids, Wageningen, 2007

Advanced Organic Chemistry, Wageningen, 2009-2011

Early stage researcher training in bioencapsulation, Anzre, Switzerland, 2009

*Conferences*

*International conference on bioencapsulation, Dublin, Ireland 2008

*International conference on food rheology and structure, Zürich, Switzerland, 2009

Annual NWO conference (Organic Chemistry), Lunteren, 2007 and 2008

Mini-symposium on membrane emulsification, Wageningen, 2009

Mini-symposium on dynamics of soft matter, Wageningen, 2009

World emulsion congress, Lyon, France, 2010

Annual NPS conference, Veldhoven, Netherlands, 2010

Nanoned/Microned symposium, Netherlands, 2007-2010

Food Process Engineering annual meeting, 2009 and 2010

Dutch Polymer Days, Veldhoven, Netherlands, 2010

* Represents oral presentations. At the other Conferences posters were presented

***General courses***

VLAG Ph.D. week, 2008

Techniques for scientific writing and presenting papers, 2010, Wageningen

***Optionals***

Preparation Ph.D. research proposal

Group meetings Laboratory of Organic Chemistry, 2007-2009,

Ph.D. study trip, Laboratory of Organic Chemistry, China, 2009

Ph.D. study trip, Food Process Engineering Group, USA, 2010

Literature study, Laboratory of Organic Chemistry, 2007-2008

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