Production of microcapsules by templating pickering emulsion droplets : mechanism and practical applications

Production of Microcapsules by Templating Pickering Emulsion Droplets: Mechanism and Practical Applications

PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de Technische Universiteit Eindhoven, op gezag van de rector magnificus prof.dr.ir. C.J. van Duijn, voor een commissie aangewezen door het College voor Promoties, in het openbaar te verdedigen op maandag 8 september 2014 om 16:00 uur

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Those three things

Autonomy

Complexity

And a connection between

Effort and Reward

Are, most people agree,

The three qualities

That **work** HAS to have if it is to be

Satisfying

It is not how much money we make

That ultimately makes us happy between nine and five

It's whether our work

Fulfills us

"Malcolm Gladwell"

Production of Microcapsules by Templating Pickering Emulsion Droplets: Mechanism and Practical Applications

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Summary

In the field of microencapsulation there is an ambition to precisely control the architecture of microcapsules for protection or/and controlled release applications. Besides that, the encapsulated species should preserve their original properties during and after the encapsulation procedure. The microencapsulation procedures in this thesis were inspired by these two imperative demands. Therefore, Pickering stabilized emulsion droplets were found to be versatile and suitable templates for microcapsules. Microcapsules were produced by interconnecting the stabilizing particles of the Pickering emulsion or by an interfacial shell-forming reaction.

Chapter 1 outlines the aim of this thesis is given and a general description of microencapsulation. Subsequently, a variety of microencapsulation procedures and materials are briefly discussed. Chapter 1 continues with a description of Pickering emulsions and why these types of micron-sized droplets are suitable templates for microcapsules.

Chapter 2 describes multiple methods to produce microcapsules by templating Pickering emulsion droplets. Initially poly(methyl methacrylate) or silica microparticles were used to stabilize the Pickering emulsion. Microcapsules were produced by growth of the stabilizing micro particles at the surface of the aqueous emulsion droplets, which as a consequence leads to overlap, thus resulting in a microcapsule. Microcapsules were also produced by placing reactants or/and catalyst in the opposite phases, *i.e.* the continuous and the dispersed phase of the initial Pickering emulsion. So the necessary reactants for solid formation only meet at the liquid/liquid interface and a microcapsule is produced. In addition, the importance of the shell toughness is discussed.

Chapter 3 explains the synthesis and characterization of poly(methyl methacrylate)-silica microcapsules. The water-in-oil Pickering emulsion is originally stabilized by poly(methyl methacrylate) microparticles. The capsules were produced by the formation of silica at the liquid/liquid interface. The reaction is directed to that interface by a catalyst with amphiphilic properties. Tetraethyl orthosilicate was used as silica precursor, which is initially oil-soluble. The catalyst used is *n*-hexylamine. The thickness of the shell could be controlled by adjustment of the amount of silica precursor. The Pickering emulsion and the microcapsules were characterized by light microscopy and scanning electron microscopy. The kinetics were investigated by gas chromatography.

Chapter 4 describes the synthesis, characterization and mechanism of formation of silica microcapsules by templating Pickering emulsion droplets. The water-in-oil Pickering emulsion is primarily stabilized by the synergistic interactions of silica microparticles and *n*-hexylamine. Similar to Chapter 3,

tetraethyl orthosilicate is used as silica precursor. The locus of reaction as well as the direction of silica growth were determined by confocal fluorescence microscopy using silica precursors labeled with two dyes, *i.e.* rhodamine and fluorescein. The order of addition of these dyes during the shell formation reaction revealed in which direction the shell is growing, specifically from the inside to the outside. Based on all the characterization results, a comprehensive explanation about the mechanism of formation is presented in this chapter.

The formation of hybrid poly(styrene-*co*-maleic anhydride) – silica microcapsules is described in Chapter 5. This is accomplished by interconnection of the stabilizing microparticles of a Pickering emulsion droplet. The silica microparticles that stabilize the initial water-in-oil Pickering emulsion were functionalized with reactive amine groups. These amine groups were subsequently reacted with poly(styrene-*co*-maleic anhydride), resulting in polymer interconnected silica microparticles. The microcapsules were visualized by light microscopy, scanning electron microscopy and confocal fluorescence microscopy. For application of confocal fluorescence microscopy, the silica microparticles and the polymer were initially labeled with a red and a green dye, rhodamine and fluorescein respectively.

Chapter 6 deals with the compartmentalization of bacteria in microcapsules. Specifically, a *lactobacillus plantarum* strain 423 was encapsulated in poly(organosiloxane) microcapsules. In this case, the water-in-oil Pickering emulsion is stabilized by hydrophobized silica microparticles. The bacteria were present inside the aqueous emulsion droplets. Alkylchlorosilanes were added, which were also used for hydrophobization of the Si-microparticles. Alkylchlorosilanes reacted with water at the interface and the hydroxyl groups of the stabilizing microparticles, resulting in a microcapsule. The viability of the bacteria was confirmed during and after the encapsulation procedure by using 4',6-diamidino-2-phenylindole (DAPI) and also LIVE/DEAD® BacLight™ in combination with confocal fluorescence microscopy.

In conclusion, microcapsules with minimal contamination of the encapsulated aqueous core have been produced by templating Pickering emulsion droplets followed by an interfacial reaction or by interconnecting the stabilizing microparticles.

Samenvatting

Het inkapselen van bijvoorbeeld geneesmiddelen, enzymen, en micro-organismen kan succesvol worden uitgevoerd door het toepassen van Pickering-gestabiliseerde emulsies als sjabloon. De uitdaging is om één of meerdere robuuste procedures te ontwerpen voor het maken van microcapsules met vooraf vastgestelde eigenschappen. De moleculen of materialen die moeten worden ingekapseld mogen tijdens het hele proces niet veranderen. De wand van de microcapsules moet in het algemeen doorlaatbaar zijn voor kleine moleculen. Bij gecontroleerde afgifte van geneesmiddelen moet de wand door een externe stimulus doorlaatbaar worden voor de betreffende farmacologisch actieve component. Het onderzoek waarvan de resultaten zijn samengevat in het voorliggende proefschrift is gericht op het ontwerp van synthese routes van microcapsules met de eerdergenoemde eigenschappen. De rode draad in het werk is het aan elkaar verbinden van de vaste deeltjes die zorg dragen voor de stabilisatie van de water-in-olie emulsies en het bewerkstellen van een reactie aan het grensvlak van de emulsiedruppels.

Hoofdstuk 1 geeft het doel van het in dit proefschrift beschreven onderzoek. Verder wordt een algemene beschrijving van inkapselen op microschaal gegeven tezamen met de verschillende mogelijkheden om het inkapselingsproces uit te voeren. Hoofdstuk 1 gaat daarna verder met een beschrijving van Pickering emulsies en er wordt uitgelegd waarom dit type emulsie zo geschikt is als basisvorm voor microcapsules.

Hoofdstuk 2 beschrijft verschillende procedures om microcapsules te produceren door Pickering emulsiedruppels te gebruiken als sjabloon voor de capsules. In eerste instantie worden poly(methyl methacrylaat) deeltjes of silicadeeltjes gebruikt om de druppels te stabiliseren. Microcapsules worden uit de Pickering emulsie gevormd door de stabiliserende deeltjes aan het olie-water grensvlak te laten groeien. Omdat de deeltjes vastzitten aan het grensvlak resulteert dit op een bepaald moment in overlap van de groeiende deeltjes. Op dit moment is een microcapsule gevormd. Microcapsules werden ook geproduceerd door reactanten en/of de katalysator in “tegenovergestelde fasen”, oftewel de continue en de gedispergeerde fase van de Pickering emulsie te plaatsten. Als gevolg hiervan komen alle benodigde componenten voor de reactie waarmee de vaste stof wordt gevormd, alleen met elkaar in contact aan het fasen grensvlak. Vorming van microcapsules is door deze vastliggende reactiezone goed regelbaar. Tot slot is het belang van de schileigenschappen voor de beoogde toepassingen bediscussieerd.

Hoofdstuk 3 beschrijft de synthese- en karakteriseringprocedures van poly(methyl methacrylaat)-silica microcapsules. De water-in-olie Pickering emulsiedruppels (water-in-*n*-heptaan) zijn in eerste instantie gestabiliseerd

door poly(methyl methacrylaat) microdeeltjes. De capsules worden vervolgens geproduceerd door de vorming van silica op het vloeistof/vloeistof fasen grensvlak. De reactie wordt naar het fasen grensvlak geleid door *n*-hexylamine. Deze katalysator heeft amfifiele eigenschappen. Tetraethyl orthosilicaat werd gebruikt als silica precursor en is aanvankelijk oplosbaar in de oliefase. De dikte van de schil kan worden ingesteld door de hoeveelheid silica precursor die wordt gebruikt. De Pickering emulsies en de microcapsules werden gekarakteriseerd door licht-microscopie en elektronenmicroscopie. De kinetiek van de silicavorming werd onderzocht door middel van gaschromatografie.

Hoofdstuk 4 beschrijft de synthese, de karakterisering en het mechanisme van de vorming van silica microcapsules, geproduceerd door silicadeeltjes gestabiliseerde Pickering emulsie druppels als sjablonen te gebruiken. De water-in-olie Pickering emulsie werd in eerste instantie gestabiliseerd door de coöperatieve interacties van silica microdeeltjes en *n*-hexylamine. Net als beschreven in hoofdstuk 3 werd tetraethyl orthosilicaat als silica precursor gebruikt. De locus van de reactie in combinatie met de groei- richting van silica werd bepaald met behulp van confocale fluorescentie microscopie en het merken van de silica reactanten met 2 verschillende kleurstoffen, namelijk rhodamine en fluoresceïne. De volgorde van toevoegen van de kleurstoffen en de volgorde waarin ze werden waargenomen in de schil geeft de groeirichting aan, om precies te zijn van binnen naar buiten. Gebaseerd op alle karakteriseringresultaten is een uitvoerige uitleg over het mechanisme van de van de vorming van de schil gepresenteerd.

De vorming van hybride poly(styreen-co-maleïnezuuranhydride)-silica microcapsules is beschreven in hoofdstuk 5. De vorming van deze hybride microcapsules werd gerealiseerd door het onderling verbinden van de stabiliserende microdeeltjes van de emulsiedruppel. De silica deeltjes die de emulsie initieel stabiliseren werden voorzien van reactieve amine groepen. Deze amine groepen reageerden hierna met poly(styreen-co-maleïnezuuranhydride), resulterend in door polymeer verbonden silica microdeeltjes. De microcapsules werden zichtbaar gemaakt met lichtmicroscopie, elektronenmicroscopie en confocale fluorescentie microscopie. Voor het gebruik van de confocale fluorescentie microscoop werden de silica deeltjes gelabeld met de rode kleurstof rhodamine. Het polymeer werd gelabeld met de groene kleurstof fluoresceïne.

Hoofdstuk 6 beschrijft de compartimentalisatie van bacteriën in microcapsules. Om precies te zijn: de *lactobacillus plantarum* stam 423 werden ingekapseld in poly(organosiloxaan) microcapsules. De initiële Pickering emulsie was gestabiliseerd met hydrofobe silicadeeltjes. De bacteriën waren aanwezig in de waterige emulsiedruppels. Aan de Pickering emulsie werden alkylchlorosilanen toegevoegd. Deze chlorosilanen reageren met water aan het grensvlak van de emulsiedruppels en met de reactieve

groepen op het oppervlak van de stabiliserende silica deeltjes, resulterend in microcapsules. De levensvatbaarheid van de bacteriën na het inkapselen werd vastgesteld door middel van confocale fluorescentie microscopie, door gebruik te maken van 4',6-diamidino-2-phenylindole (DAPI) en ook LIVE/DEAD® BacLight™.

Kortom, microcapsules met zo gering mogelijke contaminatie van de waterige kern zijn geproduceerd door water-in-olie Pickering emulsies als sjablonen toe te passen. De eigenlijke microcapsules werden gevormd door een grensvlakreactie of door het met elkaar verbinden van de stabiliserende microdeeltjes.

Contents

1. Introduction

1.1 Microencapsulation

In microencapsulation a micro-sized droplet is being surrounded by a solid layer. The IUPAC definition of a microcapsule is: *Hollow microparticle composed of a solid shell surrounding a core-forming space available, to permanently or temporarily, entrapped substances.*[1] These entrapped substances are also referred to as active ingredients or encapsulant.[2] The purpose of microencapsulating active ingredients is to protect them from the environment or to enable their controlled release into the environment. Examples of active ingredients that are encapsulated to protect them from the environment are phase change materials[3–8], probiotic bacteria[9,10] (increasing the shelf-life) or of vitamins (against deteriorating by exclusion of oxygen).[11] Examples of controlled release applications are: the release of drugs inside the body[12,13] or from a wound dressing[14], and release of a selected reactant in chemical reactions[15,16]. The combined function of the microcapsules for protection and controlled release is also possible, namely for the protection of bacteria, and simultaneously for the controlled release of their produced antibiotics.[14,17–19] Microencapsulation is also used to mask the odor of active ingredients[20], or to convert liquids into powders[21,22], or to prevent evaporation of volatiles[23,24] and also to protect the environment from toxic active ingredients, *e.g.* pesticides.[25,26]
Logically, the various applications ask for different types of microcapsules. Microcapsules that are used for protection applications should seal the active ingredients from the environment. If the microcapsules are used for controlled release purposes they should have a certain, controllable permeability. Another possibility is the *in situ* release of the active ingredient. In that case the microcapsules should be responsive to external stimuli, so that the shell becomes porous upon a certain stimulus such as temperature or pH.[16,27,28] Also biodegradable microcapsules are suitable for controlled release applications, as release will increase with the shell degradation.[29,30] Besides the shell properties, different active ingredients require different encapsulation procedures. For instance, delicate material, *e.g.* enzymes and bacteria, are sensitive towards changes in temperature and pH and/or they are sensitive to certain chemicals. Due to the various applications and the challenges encountered with the synthesis of microcapsules with the right properties there has been a growing interest in the topic. This growing interest resulted in a large number of publications that increases almost year by year, see Figure 1.

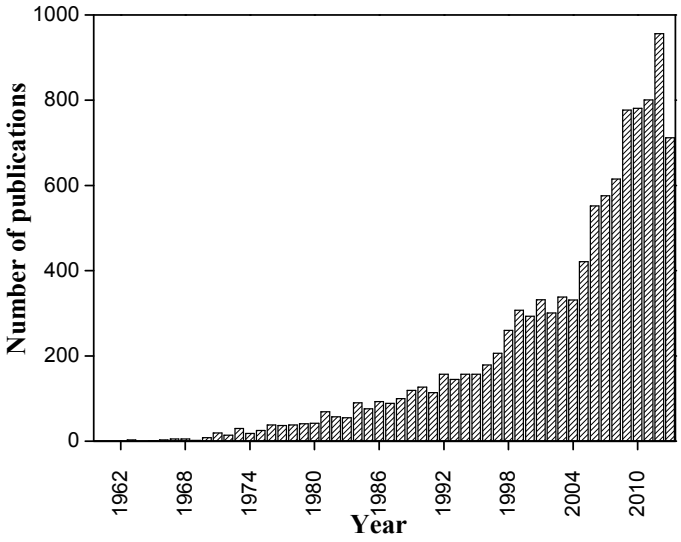


Figure 1. The number of publication per year on microencapsulation from the Chemical Abstract Database.

1.2 Microencapsulation procedures and capsule material

Microencapsulation is the formation of a solid shell around a microdroplet. The droplets are often referred to as templates for the microcapsules. This means that in order to produce microcapsules, first the micro-sized droplets need to be formed. The stability of the template droplets against coalescence or deformation during the shell formation is a prerequisite for successful encapsulation.[31] The droplets that are most widely employed in literature as templates for microcapsules are produced using microfluidics[32–35], also stabilized by surfactants[36,37] and/or stabilized by solid particles. Emulsions stabilized by solid particles are Pickering emulsions,[38] which will be discussed in more detail in Section 1.4. After the formation of stable droplets, a solid shell can be produced by an interfacial reaction, most commonly via positioning the components necessary for the reaction in "opposite" liquid phases, *e.g.* reactant 1 in the organic phase and reactant 2 in the water phase.[39–41] Alternatively, the solid shell can be formed by precipitation of polymer onto the droplets. In that case a mixture is produced with the active ingredients and a polymer in a solvent e.g. the continuous phase. The dispersed phase in then a nonsolvent for that particular polymer. By evaporation of the solvent the microcapsules are formed.[42] Another method, which is physical in nature, is by sintering the stabilizing microparticles at the interface of Pickering emulsion microdroplets.[2,43,44] The encapsulation method that is chosen is logically also dependent on the chemical and physical properties of the capsule material, as well as on the properties/sensitivities of the encapsulant. For instance, if capsules are

produced by sintering of the primary particles at the interface of a Pickering emulsion droplet,[45] then the glass transition temperature or melting point of the polymer should be lower than the boiling points of the liquids in the initial emulsion. Capsule material of polymers produced by polycondensation reactions are typically synthesized by placing the necessary reactants in "opposite phases".[39,40,46–48] In addition, microcapsules with a hybrid organic-inorganic shell are often synthesized by templating Pickering emulsion droplets.[49–54]
As capsule material, silica is very suitable, since it is chemically and biologically inert. Silica is mechanically and thermally stable and non-toxic.[40,55,56] A well-known silica precursor is tetraethyl orthosilicate (TEOS), which reacts with water to silica and can be polymerized by base or acid catalysis at ambient temperatures.[57] Besides that, TEOS has many derivatives (for example 3-(triethoxysilyl)propyl methacrylate), which after polymerization result in materials with different properties.[56] Furthermore, the derivatives can react with various other materials/compounds to yield *e.g.* hybrid materials.[58] For those reasons silica is always part of the capsule material studied in this work.

1.3 Pickering emulsion

As previously described, a Pickering emulsion is a liquid/liquid dispersion which is stabilized by solid particles, see Figure 2.[38,59] Pickering emulsions are characterized by their high stability, which has been discussed in literature extensively.[31,60]

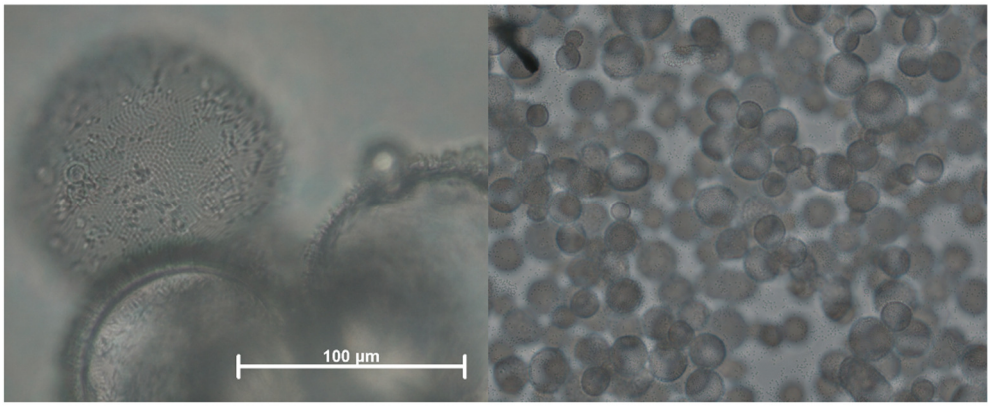
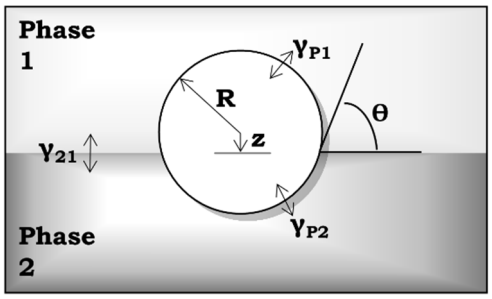


Figure 2. Light microscope images of a Pickering emulsion in which water is the dispersed phase, *n*-heptane is the continuous phase and poly(methyl methacrylate) micron-sized particles are used as stabilizing entities.

This stability is mainly caused by the high energy of detachment (E_B) of the stabilizing particles from the interface, see Equation 1.[61] E_B is in turn related to the three-phase contact angle that the stabilizing particles have with the

interface, in other words the hydrophobicity of the particles, see Figure 3. The value of the three-phase contact angle is governed by the different interfacial energies in the system and can be expressed by Young's equation, see Equation 2.[62,63] Particles are assumed to be effectively irreversibly attached to the interface when the energy of detachment is between 10^2-10^6 k_BT, which is the situation when colloids are used with diameter between 0.01 – 10 μm.[31] In addition the interfacial tension between water/*n*-heptane and between water/toluene is 49 and 35 mN m^{-1} respectively, see Equation 2.[64] Besides the energy of detachment of the particles, coalescence of two Pickering emulsion droplets is also prevented by steric hindrance of the stabilizing particles, but also due to capillary stabilization[65] and drainage.[31,45,66]



$$E_B = \pi R^2 \gamma_{12} (1 - |\cos\theta|)^2 \quad (1)$$

$$\cos\theta = \frac{\gamma_{P2} - \gamma_{P1}}{\gamma_{21}} \quad (2)$$

Figure 3. Schematic representation of a solid particle at a water – oil interface. In Equation 1, θ is the three-phase contact angle, γ represents the interfacial tension between liquid 1 and 2 or with one of the liquids with the solid particle P. In Equation 2, E_B stands for the energy of desorption of a particle from the interface. R is the radius of the particle.

Besides a strong indication for the stability of the Pickering emulsion, the three-phase contact angle is also a measure for what kind of emulsion, can expected to be formed. When particles have a three-phase contact angle with the interface between 94° and 110° they are assumed to produce the most stable water-in-oil Pickering emulsion. If the three-phase contact angle is between 70° and 86° oil-in-water emulsions are assumed to be more stable than water-in-oil emulsions.[64]

1.4 Versatility of Pickering emulsion droplets as templates

As templates for microcapsules, Pickering emulsion droplets are very suitable, see Figure 3. This is in the first place due to their high colloidal stability. The droplets are stable even at elevated temperatures. The system remains segregated even when it is subjected to vigorous stirring. Shell formation at the interface is possible even when the procedure lasts hours. Besides that surfactants or other amphiphiles which influence the interfacial tensions can be added without destabilizing the emulsion.[67,68] Furthermore, the emulsion droplet size can be easily controlled if the size of

the stabilizing particles is known.[31] In that case the, concentration of stabilizing particles per volume element of dispersed phase in a Pickering emulsion to produce droplets with a certain size can be calculated, see Equation 3.

$$r_D = \frac{3V_D}{N_A 2\sqrt{3}R^2} \tag{3}$$

In Equation 3, N_A is the total number of particles attached to the interface of the droplets. R is the radius of the particles. When the particles are hexagonally close-packed, the area that a particle occupies is $2\sqrt{3}R^2$ and V_D is the total volume of the dispersed phase. r_D is the radius of the droplet. Curvature effects are neglected. In addition a feature that makes Pickering emulsion droplets interesting as templates for microcapsules is that there is already a lot of polymer present at the interface. So, besides an interfacial reaction producing another solid at the interface, the microparticles that are already there can be fixed at the interface to produce a shell. The resulting capsules are called colloidosomes, which can be produced with precise control of size, elasticity and permeability.[2,44,54] Therefore, Pickering emulsion droplets have numerous options to be converted into microcapsules, see Figure 4.

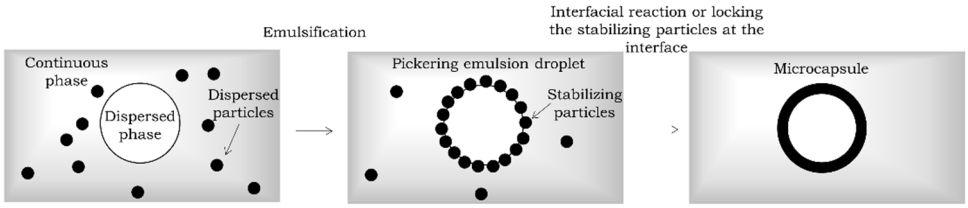


Figure 4. Schematic illustration of the formation of a Pickering emulsion droplet, followed by the formation of a microcapsule.

1.5 Aim

In the field of microencapsulation there is an ambition to precisely control the architecture of microcapsules. This means that the moment of release and the release flux of an active ingredient into its environment need to be exactly controlled. The size of the capsules and the material they are made of are the key factors in this respect. At the same time there is the ambition to preserve the encapsulant in exactly the same state as it was before microencapsulation. This is not trivial for delicate materials such as drugs, enzymes, bacteria and live cells.

There are many methods to produce microcapsules, or even nanocapsules and different materials have been employed to produce these capsules.[69] However, it proved to be difficult to fully understand the

mechanism of formation of the microcapsules.[37,46,70] But it is also a challenge to produce microcapsules at low temperatures and simultaneously to minimize or prevent contamination of the core of the capsules with the shell precursor or the catalyst.

This thesis describes the synthesis of microcapsules based on Pickering emulsion droplets as templates. Two different strategies for the formation of the microcapsules have been used. In the first strategy, the stabilizing microparticles at the interface are interconnected to keep them fixed at the liquid/liquid interface. In the second strategy, microcapsules are produced by an interfacial reaction, which forms a shell separate from the primary stabilizing microparticles. One of the objectives is to produce microcapsules under mild conditions for the encapsulant. These conditions include no or very limited mixing of precursors and catalysts with the core of the microcapsules during and after the synthesis. Furthermore ambient reaction temperatures are applied. Another objective is to fully understand the mechanism of formation of the microcapsules. Different compounds are used to produce the microcapsules, *i.e.* organic and inorganic materials. Finally, bacteria are microencapsulated without losing their viability during and after the encapsulation process.

Outline thesis

Chapter 2 describes different methods to produce microcapsules by templating water-in-oil Pickering emulsion droplets. As a result of comparing the methods, some of the bottlenecks within microencapsulation are revealed. The microcapsules being described are produced by templating Pickering emulsion droplets that are either stabilized by *poly*(methyl methacrylate) (*p*MMA) or silica (SiO_2) microparticles.

Chapter 3 describes details of the formation of *p*MMA-SiO_2 microcapsules that were produced by templating Pickering emulsion droplets. Minimization of mixing of the microcapsule core with catalyst or precursor was achieved by using *n*-hexylamine as catalyst and tetraethyl orthosilicate (TEOS) as silica precursor. Because of the amphiphilic properties of *n*-hexylamine, the emulsion droplets are initially stabilized by the synergistic interactions of *p*MMA microparticles and *n*-hexylamine at the interface. Microcapsules with tunable shell thickness were successfully synthesized.

Chapter 4 outlines the mechanism of silica microcapsule formation. The microcapsules were produced by templating Pickering emulsion droplets that were initially stabilized by *n*-hexylamine and silica microparticles. The shell was produced by the interfacial reaction of TEOS and water, catalyzed by *n*-hexylamine. A comprehensive explanation of the capsule formation, supported by experimental data has been given.

The formation of hybrid *poly*(styrene-*co*-maleic anhydride) (*poly*(St-*co*-MAh)) – silica microcapsules is described in Chapter 5. These microcapsules were produced by locking/fixing the stabilizing microparticles at the

interface of a Pickering emulsion droplet. The emulsion was initially stabilized by amine-functionalized silica microparticles. The amine groups on the microparticles lead to aminolysis of the maleic anhydride residue of *poly*(St-*co*-MAh), which results in microcapsules that can easily be dispersed in water.

Chapter 6 covers the microencapsulation of bacteria of the *Lactobacillus plantarum* strain 423 in *poly*(organosiloxane) microcapsules. The template Pickering emulsion droplets were stabilized by hydrophobized silica microparticles. The shell was produced by the interfacial reaction of alkylchlorosilanes. To protect the bacteria from the pH change caused by the reaction, they were suspended in a growth medium or pH buffer. As a result the *Lactobacillus plantarum* strain 423 stayed viable during and after the encapsulation procedure.

Conclusion of the work are given in Chapter 7, together with an outlook.

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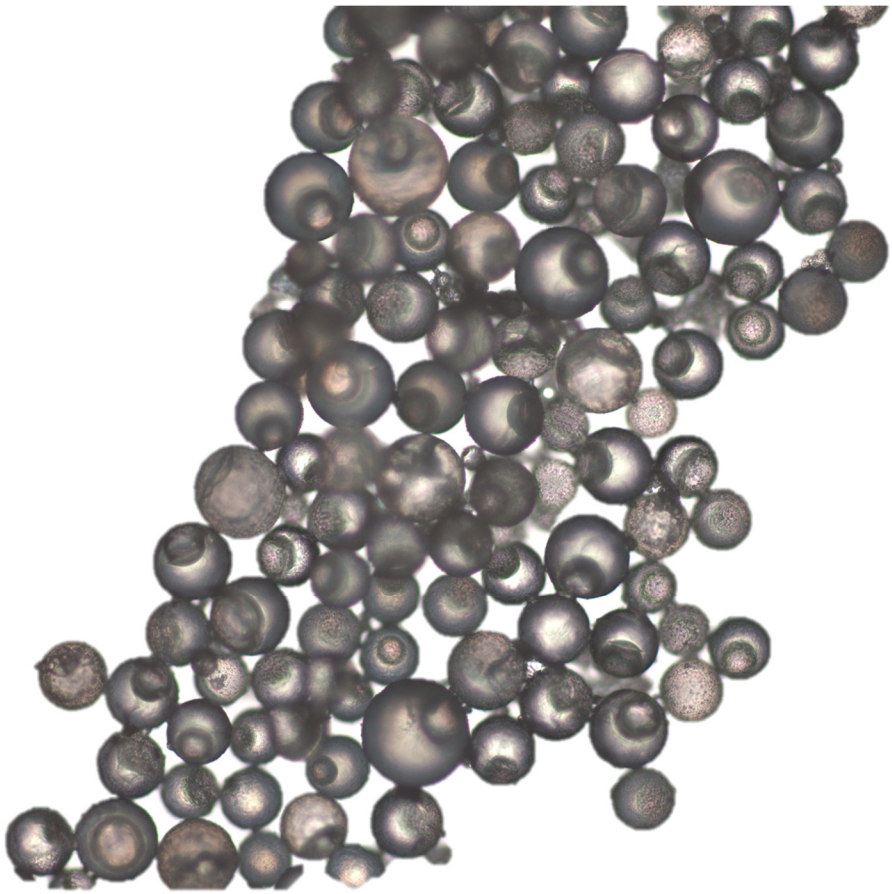
2. Preliminary studies on the formation of microcapsules by templating Pickering emulsions



Abstract

This chapter provides an introduction into the synthesis of microcapsules by templating inverse Pickering emulsions. Section 2.1 reports about water-in-oil Pickering emulsion droplets which are being used as templates for microcapsules. The emulsion droplets are stabilized by either *p*MMA particles or silica particles, which in both occasions are functionalized with reactive groups. Microcapsules are produced by a seeded polymerization of the primary stabilizing particles at the interface of the emulsion droplets. This encapsulation technique turns out to be most successful if the actual polymerization takes place in the oil phase. In a different approach, microcapsules are produced by positioning reactants and catalyst in different phases of the Pickering emulsion. This encapsulation technique is speculated to be successful when the time constant for transport of reactive species from one phase to the other is lower than the time constant of the actual shell forming reaction.
In Section 2.2, we report the synthesis of microcapsules by templating Pickering emulsion droplets with an aqueous core. The Pickering emulsions are initially stabilized by poly(methyl methacrylate) or silica microparticles. Tetraethyl orthosilicate solely or combination with dimethyldimethoxysilane or 1,8-bis(triethoxysilyl)octane are used as shell precursors. In one case, the shell-forming reaction is directed to the interface by functionalizing the stabilizing silica microparticles with catalytically active amine groups. As a result, a very thin silica shell is produced. Most probably, the amine groups on the surface of the primary silica microparticles get engulfed with newly formed silica at some point in time. As a consequence, the reaction basically stops. The shell-forming reaction is also directed to the interface by using amphiphilic catalysts, namely *n*-hexylamine and *n*-hexanoic acid. As a result, when *n*-hexanoic acid was used, the silica shell was very brittle and all the capsules collapse upon drying. If *n*-hexylamine was used to catalyze the reaction and dimethyldimethoxysilane or 1,8-bis(triethoxysilyl)-octane was used as silica precursor in combination with tetraethyl orthosilicate, microcapsules were produced with controllable toughness.

2.1 Microcapsules by growth of the stabilizing particles of a Pickering emulsion

2.1.1 Introduction

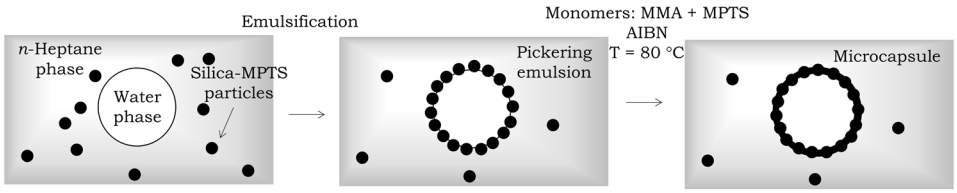
Microencapsulation is the formation of a solid shell around a micron-sized droplet. Microencapsulated active ingredients have a number of potential applications, *e.g.* in the food industry or for the protection of phase change materials.[1–5] In recent years, most interest is focused on the microencapsulation of delicate materials, such as drugs, enzymes or live cells.[6–14] Specific applications ask for properly designed encapsulation techniques and for specific properties of the capsules. For example, if high

temperatures are used during the synthesis of the capsules, this might not be suitable for delicate material as encapsulant. Also, mixing of chemicals with the active ingredients during and after synthesis of the microcapsules might not be suitable when the application is either in the food industry or as phase change materials. For that reason, it can be stated that there is a necessity to control the architecture in combination with the synthesis procedure of microcapsules to be compliant with the different applications.
During the synthesis procedure of microcapsules, microdroplets have to be produced first, which are the templates for the capsules. The microdroplets should be stable, but most of all they should remain stable during the formation of the solid shell. Pickering emulsion droplets are known for their high stability against coalescence and are therefore suitable templates for microcapsules.[15] A Pickering emulsion is an emulsion that is solely stabilized by solid particles. Besides stabilizing entities, the particles at the interface can also play a role in the formation of the shell around the microdroplets. Shell formation proceeds for instance, via sintering and fusing of the particles at the interface, or via a polymerization where the stabilizing particles act as seed particles. This latter method will be referred to as "seeded" polymerization in this chapter.[16–18]
The formation of polymer microcapsules by sintering polymer microparticles at the interface of microdroplets is a known and well-understood method to produce microcapsules.[18,19] During this method, first a Pickering emulsion is produced. A Pickering emulsion is a W/O or O/W emulsion, which is stabilized by solid particles. The solid particles at the interface of the dispersed and continuous phase have a certain three-phase contact angle, which depends on the hydrophobicity of the stabilizing particles and is dependent on the different surface tensions in the system, see Chapter 1 Figure 1.3. When particles have a three-phase contact angle with the interface between 94° and 110° they are assumed to produce the most stable water-in-oil Pickering emulsions.[20] Pickering emulsion droplets are known to be very stable, which is *i.a.* a reason for the droplets to be suitable as templates for microcapsules.
Heating is necessary for sintering and fusing of the stabilizing particles of a Pickering emulsion at the interface, to produce microcapsules. When a stable Pickering emulsion has been produced, the different interfacial tensions in the system change upon heating. For that reason, before sintering, the hydrophobicity of the stabilizing particles needs to be optimized to produce a Pickering emulsion that remains stable also upon heating. Optimization of the hydrophobicity can be achieved by for example tuning the particle stabilizer concentration during the particle synthesis.[21] Because of the high T_g of a number of polymers, sometimes a plasticizer is added to enhance the capsule-forming sintering process.
Another method to produce polymer microcapsules, is by using a seeded polymerization technique. In that case, the stabilizing polymer particles at the interface of the Pickering microdroplets are used as seed and increase in size.[15] This method may be applicable if sintering of the microparticles

does not result in microcapsules. Such a situation may arise if the T_g of the polymer is too high and plasticizers do not have a sufficient effect. Then, swelling the polymer microparticles at the water – oil interface with monomer instead of plasticizer is a logical next step. In this case, first monomer is added to the Pickering emulsion, which is in a reactor while stirred for a certain time period. The Pickering emulsion droplets are *e.g.* stabilized by poly(methyl methacrylate) (*p*MMA) particles. The stabilizing polymer particles are allowed to swell with monomer. Subsequently an oil-soluble azo-initiator or peroxide-initiator, solubilized in additional MMA, is added and by increasing the temperature, the seeded polymerization is started. Because the growing particles are restricted to the interface, at some point they will start to coalesce and overlap and a microcapsule is produced.[17]

2.1.2 Microcapsules based on primary stabilizing silica microparticles

Besides polymer particles, functionalized silica microparticles have also been used as seed particles during seeded polymerizations.[22] This procedure leads to the formation of hybrid polymer – silica microparticles. Such hybrid particles often have superior properties.[23] Similarly, in our work hybrid silica – poly(methyl methacrylate) (*p*MMA) microcapsules were produced by using silica microparticles that are functionalized using 3-(triethoxysilyl) propyl methacrylate (MPTS) as seed particles at the interface of a Pickering emulsion, see Scheme 2.1.



Scheme 2.1 Overview of the synthesis route towards hybrid SiO_2-*p*MMA microcapsules via a "seeded" polymerization technique

Results

For the formation of hybrid microcapsules, monodisperse, micron-sized, silica particles were produced as a first step, see Figure 2.1 A and B. These silica microparticles were produced using a seeded polymerization of tetraethyl orthosilicate (TEOS) as reported by Bogush et al..[24] The seed particles in this case were produced using the well-known Stöber method followed by a seeded polymerization technique since it proved to be challenging to synthesize monodisperse silica microparticles in a reproducible manner by using just the (one-step) Stöber method.[25]

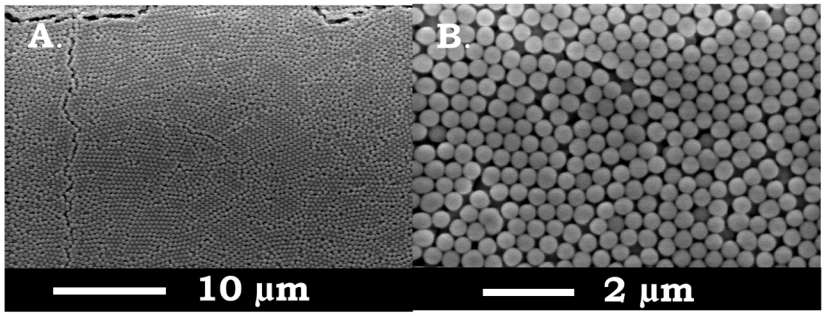


Figure 2.1 Scanning Electron Microscope (SEM) images of silica microparticles; Silica microparticles produced by a seeded polymerization technique, in which the seed particles were produced by the Stöber method.

After the synthesis of the microparticles in the second step, the silica microparticles were modified for two reasons. The first reason was to change their hydrophobicity to make the microparticles suitable for water-in-oil Pickering stabilization, see Chapter 1 Figure 1.3. The second reason was to give them functional reactive groups for the seeded polymerization reaction. Consequently, the silica particles were functionalized using MPTS. This resulted in particles with appropriate hydrophobicity and with reactive methacrylate groups for the radical polymerization reaction. Functionalization of the silica microparticles was done in *n*-heptane and proceeded by the condensation reaction of the triethoxysilyl residue of the monomer with the silanol groups and physically adsorbed water at the surface of the silica microparticles.[26,27] Before the particles could be dispersed in *n*-heptane, they had to be isolated from the ethanol mixture that was used for the synthesis. This could be achieved by settling the microparticles followed by decanting the liquid, after which the particles were dried at ambient conditions. Since silica particles are very hydrophilic, they form large aggregates in *n*-heptane. When the microparticles are hydrophobized they can be well dispersed in *n*-heptane and hence they do not form aggregates anymore. Accordingly, successful functionalization could be visually observed. The next step after the functionalization of the microparticles was the formation of a stable Pickering emulsion. This was accomplished by the addition of water to a predetermined number of particles dispersed in *n*-heptane. The Pickering emulsion was produced by high shear emulsification using a rotor-stator mixer (Ultraturrax©). Because the particles are monodisperse and arrange in a hexagonal close packing at the interface between the water droplets and the continuous phase, the number of particles and the volume of necessary dispersed phase to produce a certain emulsion droplet size can be calculated accurately, as described in by Salari et al..[28] Subsequently, azo-initiator solubilized in monomer was added and the temperature was increased to induce polymerization.

Additional MPTS was added as a comonomer besides methyl methacrylate in order to direct the reaction to the interface. Since the triethoxysilyl moiety can hydrolyze at the interface due to the presence of water, a molecule with amphiphilic properties will be formed. Consequently, the monomer will stay at the interface and thus increases the concentration of reactive groups at the interface.
As a result of the microencapsulation method described *vide supra*, hybrid silica – poly(methyl methacrylate) microcapsules were produced, see Figure 2.2. SEM images of the microcapsules were taken at several moments during the reaction. After 60 min of reaction, an initial solid layer formed under the microparticles on the surface of the microdroplets, see Figure 2.2 A. This is most probably the result of the hydrolysis and *poly*condensation reactions of MPTS. Without base or acid catalysis, this reaction is usually very slow.[29] However, in this case the reaction rate increased significantly because of the elevated temperature. This initial layer is covered with methacrylate groups, so at this time not only the functionalized primary silica particles are seed particles, but the whole capsule acts as a hollow seed particle. After 60 min of reaction, small solid particles were produced that precipitated on the larger stabilizing functionalized silica microparticles, Figure 2.2 A. After 150 min much more newly formed polymer is covering the initial shell and silica particles, Figure 2.2 D. After 205 min of reaction, the primary silica microparticles cannot be observed anymore on the outside of the microcapsules, Figure 2.2 C and B. Finally, after 24 hrs, a thick layer of polymer has been produced around the microdroplets and microparticles, Figure 2.2 E and F. Interestingly, the silica particles seem to be built in the newly formed shell randomly, see Figure 2.3. On the line of fracture of the microcapsules, indentations are visible where the functionalized silica microparticles used to be. Commonly, the indentations are non-spherical, which means that besides the microparticle, also newly formed polymer broke out of the shell, see Figure 2.3. This is a strong indication that the microparticles were covalently bound to the newly formed shell.

It has been shown that sintering and fusing microparticles at the interface of microdroplets can be used to produce microcapsules. Microcapsules are also successfully produced by growing microparticles at the interface of Pickering emulsion droplets via a “seeded” polymerization technique.

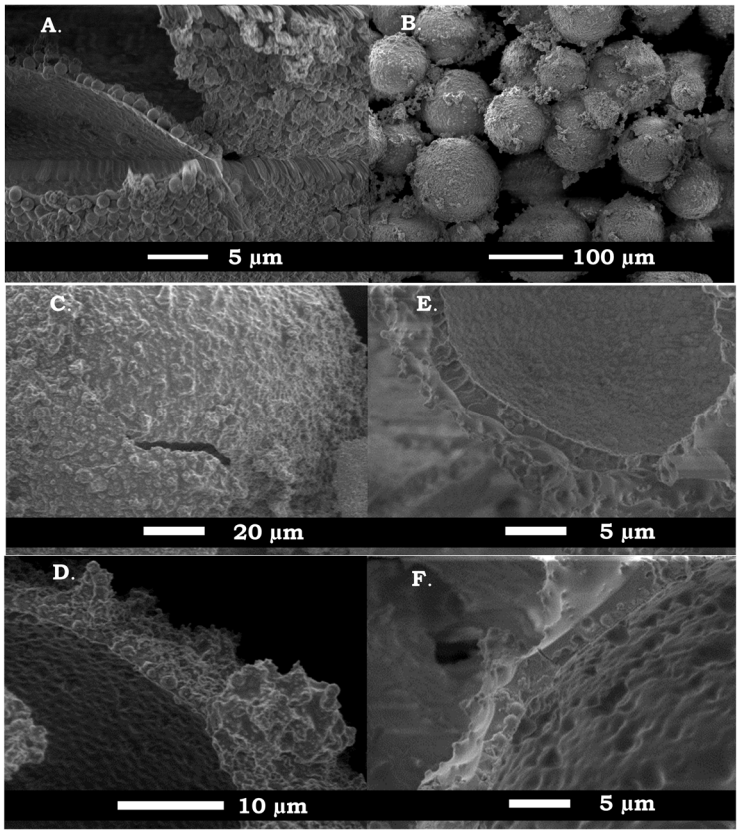


Figure 2.2 SEM images of hybrid microcapsules produced by the interfacial copolymerization of MMA and MPTS by templating Pickering emulsion droplets that were stabilized by silica microparticles. The Morphology as a function of reaction: 60 min (A), 150 min (D), 205 min (B and C) and hrs. (E and F).

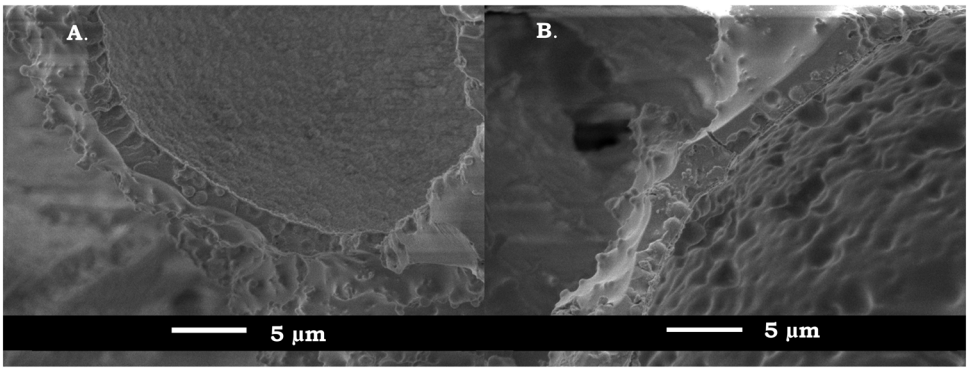
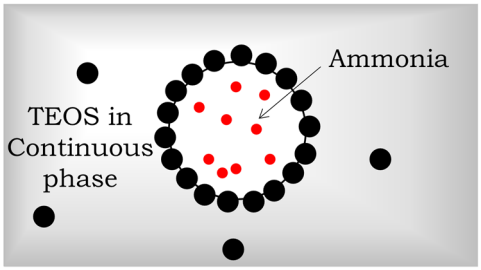


Figure 2.3 SEM images of hybrid microcapsules produced by the interfacial copolymerization of MMA and MPTS by templating Pickering emulsion droplets that were stabilized by silica microparticles.

2.1.3 Microcapsules based on primary stabilizing poly(methyl methacrylate) microparticles or silica nanoparticles

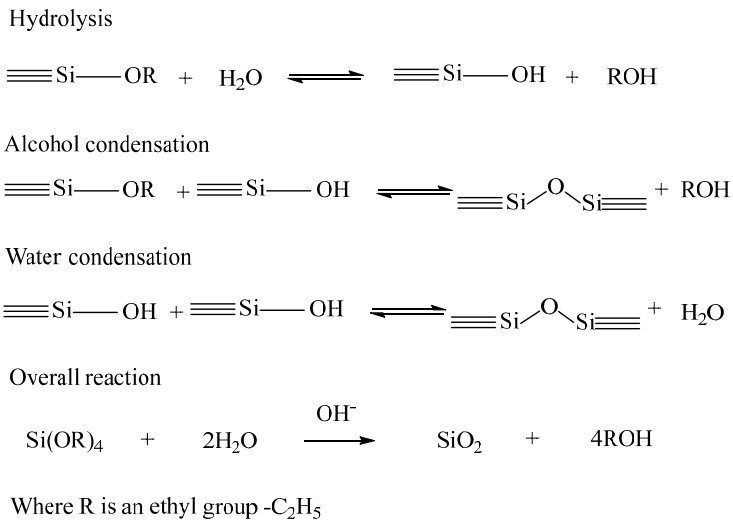
Polymer microparticles are also used as seed for hybrid polymer – silica microparticles.[30] In our work, a Pickering emulsion was produced, stabilized by *p*MMA microparticles that were functionalized with -Si(OEt)$_3$ groups. TEOS was used as monomer to grow the shell and ammonia was present inside the emulsion droplets to catalyze the reaction, see Scheme 2.2.



Scheme 2.2 Starting conditions to produce microcapsules with ammonia in the aqueous core to catalyze the polymerization of TEOS.

The polymerization of TEOS to silica consists of multiple steps. Initially, TEOS is oil-soluble before it hydrolyses. After hydrolysis there are alcohol and water condensation reactions, see Scheme 2.3.
The polymerization of TEOS can be base or acid catalyzed at ambient conditions. When the reaction is base catalyzed, this usually results in a silica network with left-over ethoxy groups. When the reaction is acid catalyzed this results in a network with left-over hydroxy groups that can still condensate at a later stage. This is the result of the relative reaction rates of hydrolysis and condensation. When the polymerization is base

catalyzed, the condensation reactions are faster.[29] In this work, the base catalysis was induced by an ammonia solution and obviously present in the dispersed emulsion droplets. Again, the different concentrations were calculated according to Chapter 1.



Scheme 2.3 Base catalysed polymerization of tetraethyl orthosilicate with water to silica.

Results

Similar to the Pickering emulsion used for the microcapsules from Figure 2.2, the droplet diameter was targeted to be 50 µm and *n*-heptane was used as the continuous phase, Figure 2.4 B and C. The emulsion was produced by the addition of water or/and ammonia to a particle dispersion followed by high shear emulsification. The microparticles that were used as stabilizing entities were produced via the seeded dispersion copolymerization of MMA and 3-(triethoxysilyl) propyl methacrylate (MPTS) in *n*-heptane, with AIBN as initiator and Kraton block copolymer as stabilizer (*poly*(styrene-*block*-(ethylene-*co*-propylene))), see Figure 2.4 A.
Via the adjustment of the Kraton stabilizer concentration during the dispersion polymerization, the hydrophobicity of the particles could be controlled to produce microparticles that sufficiently stabilized the Pickering emulsion.[21] After *p*MMA seed particles were produced, MPTS (20 %(w/w)) was added to produce a layer of reactive triethoxysilyl groups on the surface of the microparticles, for the seeded polymerization of the microparticles at the water – oil interface. After formation of the Pickering emulsion, it was transferred to a reactor and TEOS was added. The amount of TEOS was adjusted to produce a shell thickness of 1 µm.

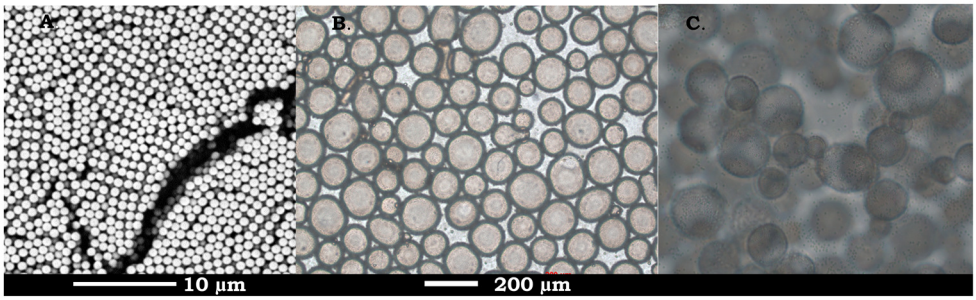


Figure 2.4 SEM images of polymer microparticles and light microscopy (LM) images of a Pickering emulsion. B and C; Pickering emulsion of water in *n*-heptane, stabilized by *p*(MMA-MPTS) microparticles produced by the dispersion polymerization of MMA and MPTS in *n*-heptane (A).

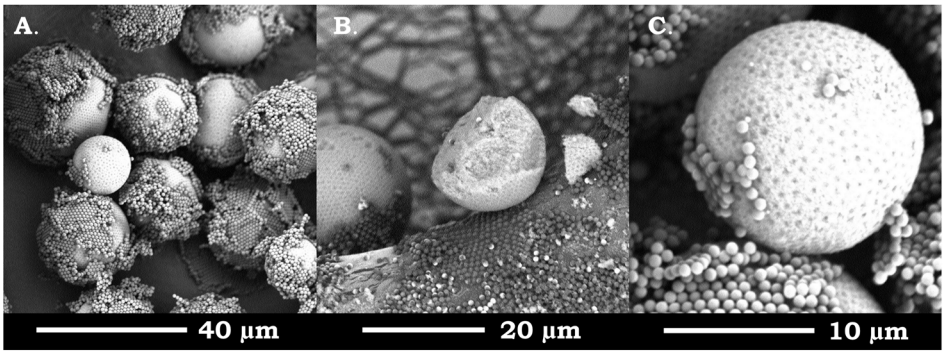


Figure 2.5 SEM images of silica microparticles produced by templating Pickering emulsion droplets when the catalyst (ammonia) was inside the droplets. Initially a Pickering emulsion was produced with *p*(MMA-MPTS) microparticles as stabilizing entities. After the addition of TEOS basically every emulsion droplet was converted into a silica microparticle.

During the seeded polymerization of triethoxysilyl-functionalized *p*MMA particles as stabilizers in a Pickering emulsion, the microparticles did not grow at the interface, see Figure 2.5. Instead every Pickering emulsion droplet turned into a silica particle. So apparently, hydrolyzed TEOS molecules or/and oligomers migrate into the emulsion droplet to further react to a particle according to a nucleation-and-growth mechanism.[31] This result was also found by Sambrook et al.[32] The primary triethoxysilyl-functionalized *p*MMA particles do not seem to be covalently bound to the newly formed silica microparticles. A large fraction of the particles is released from the surface, leaving small indentations on the surface, which give the silica particles a 'golf ball' like appearance, Figure 2.5 C.
The reason for TEOS not to react with the stabilizing silica microparticles is most likely caused by inhibited drainage[17,33] of solvent from the layer of steric stabilizer. This essentially means that the triethoxysilyl-functionalized *p*MMA particles are surrounded with a thin layer of organic solvent even at the interface. The reaction of TEOS continues in water and not in the oil

layer around the microparticles. As such, also indentations are produced, this is where the primary particle surrounded by a very thin oil layer deformed the interface. The reason that microcapsules were not produced is most likely because the catalyst is inside the emulsion droplet. As a result there is no reason for the reaction to preferably take place at the interface. Although there is an exception, specifically when hydrophobized silica *nano*particles were used to stabilize the Pickering emulsion instead of *p*MMA *micro*particles. In this experiment hydrophobized pyrogenic silica nanoparticles (HDK® H30, H30 RM) were used, which are known to stabilize water-in-oil Pickering emulsions and which have an average diameter of 20 nm.[34] The concentrations necessary for a Pickering emulsion with a small droplet size (< 10 µm) were calculated according to Binks *et al.*[34] To produce the Pickering emulsion, a predetermined number of nanoparticles were dispersed in toluene, and ammonia was added. The emulsification continued via high shear emulsification. The volume of dispersed phase, the ammonia concentration and the amount of TEOS were kept the same as the experiment leading to Figure 2.5. Again, the produced Pickering emulsion was transferred to a reactor with overhead stirring and TEOS was added, the shell thickness was targeted to be 85 nm.

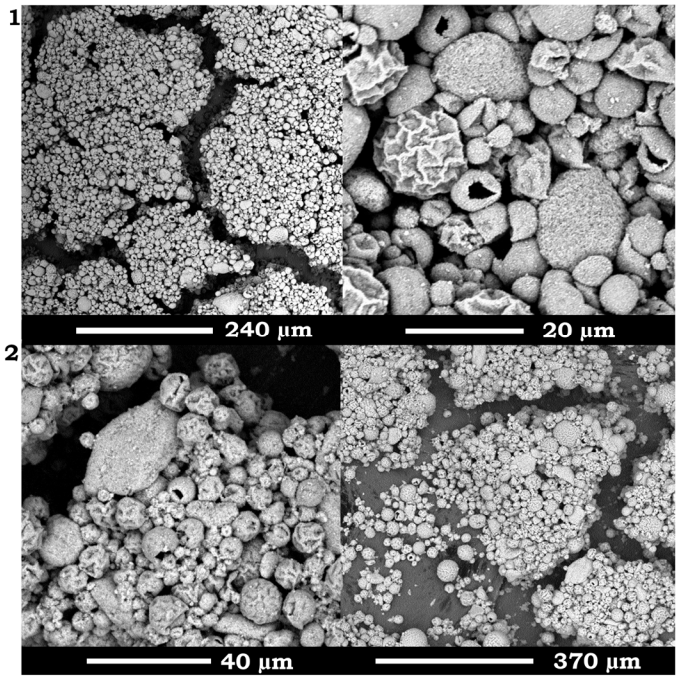


Figure 2.6 SEM images of silica microcapsules produced by templating Pickering emulsion droplets when the catalyst (ammonia) was inside the droplets and TEOS as silica precursor, from two identical experiments (1 and 2). Initially, the Pickering emulsion was produced with hydrophobized pyrogenic silica nanoparticles as stabilizing entities.

Silica microcapsules were produced by templating Pickering emulsion droplets stabilized by silica nanoparticles and utilizing ammonia as catalyst inside the droplets, see Figure 2.6. In this case, the condensation reactions at the interface are speculated to be sufficiently fast to produce a shell before partially hydrolysed TEOS species migrate into the emulsion aqueous droplets to further react to form a microparticle.
To explain the different results from Figures 2.5 and 2.6, the following is suggested. If TEOS loses one ethoxy group because of hydrolysis, two things can happen, *i.e.* it reacts with another TEOS molecule via condensation and stays hydrophobic, or it undergoes hydrolysis where additional ethoxy group(s) is (are) converted and the species formed becomes more hydrophilic. Hydrophobic species prefer the interface and oil phase over the dispersed water phase and hydrophilic species prefer to go to the aqueous phase to respectively hydrolyse completely and precipitate as SiO_2. For that reason, most probably an increase in condensation reactions at the interface will at one point result in capsule formation. During the interfacial reaction of TEOS, a low TEOS concentration is assumedly beneficial for hydrolysis. Then, as a result of the TEOS concentration there is a competition between the hydrolysis reaction and the condensation reactions. It is plausible that when *p*MMA *micro*particles are used to stabilize the Pickering emulsion, the resistance for transport of TEOS to the interface is higher than when hydrophobized silica *nano*particles are used. More transport limitation means a lower concentration of TEOS at the interface, which is beneficial for hydrolysis instead of condensation. The higher resistance for transport could have something to do with the stabilizing particles size, *micro* versus *nano* that might create a thicker or thinner layer, respectively, through which transport has to take place. This is probable since all other conditions are roughly the same.

2.1.4 Conclusion

Microcapsules were produced by templating water-in-oil Pickering emulsion droplets. The emulsion droplets were stabilized by either functionalized *p*MMA microparticles, functionalized silica microparticles or by hydrophobized silica nanoparticles. Microencapsulation was successful via a “seeded” polymerization in the oil phase, when the seed particles stabilize the Pickering emulsion. It was also successful when precursors and catalyst are positioned in the two different liquids phases in the Pickering emulsion. However, it is speculated that transport of reactive species from one phase to the other phase needs to be taken into account. Specifically, the solid forming reaction at the interface needs to have a lower time constant than the time constant for transport of reactive species to the other phase.

2.2 The effect of the synthetic method on shell toughness

2.2.1 Introduction

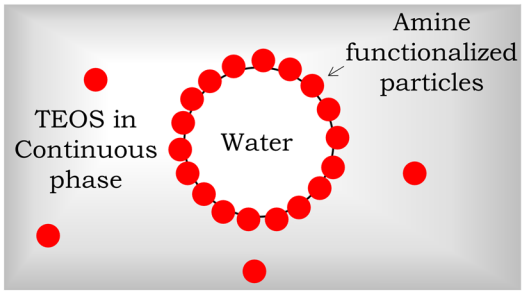
As mentioned (*vide supra*), specific applications ask for specific properties of the capsules. For example, if the application lies in the conversion of liquids into powder form, they should not break upon drying.[35] Besides that, mixing of chemicals with the active ingredients during and after the synthesis, might not be desirable. For that reason, there is a necessity to control the shell toughness in combination with the synthesis procedure of microcapsules to suit the different applications.
In the next sections we report the synthesis of silica microcapsules by templating Pickering emulsion droplets with an aqueous core. The droplets were stabilized with catalytically active amine functionalized silica microparticles, by poly(methyl methacrylate) microparticles or by the synergistic interactions of *n*-hexylamine or *n*-hexanoic acid in combination with silica microparticles. Tetraethyl orthosilicate was used as silica precursor and dimethyldimethoxysilane and 1,8-bis(triethoxysilyl)-octane were used to influence the shell properties. Via Scanning Electron Microscopy, the influence of the different synthesis procedures on the shell toughness was examined.

2.2.2 Catalyst immobilized on the stabilizing microparticles

Considering potential applications, *i.e.* encapsulation of active ingredients, it is convenient to produce capsules with a tunable size and shell thickness, without contamination of the dispersed phase and synthesized at ambient temperatures. In this context TEOS is a suitable precursor, since it can react at ambient conditions and is oil-soluble. To enhance the rate of the shell forming reaction, the catalyst can be situated on the surface of the stabilizing particles of a Pickering emulsion, thus avoiding contamination of the droplets, see Scheme 2.4. Furthermore, reaction of TEOS inside the aqueous droplets is circumvented, since no catalyst is present in the water phase.

Synthesis approach

To accomplish this, in the present work silica microparticles were functionalized with catalytically active amine groups and used to stabilize a water-in-oil Pickering emulsion when the silica precursor TEOS was used, see Scheme 2.4.



Scheme 2.4 Starting conditions to produce microcapsules when the stabilizing particles are functionalized with reactive amine groups and TEOS is used as silica precursor.

Silica microparticles were produced by a seeded polymerization technique based on the Stöber method.[24,25] After the synthesis of the microparticles they were separated from the reaction mixture by leaving them to settle, after which the fluids were decanted, followed by evaporation of the remaining liquids at room temperature. To functionalize the particles, they were first re-dispersed in toluene. Since silica particles are very hydrophilic, they cannot be properly dispersed in toluene, for that reason octadecyltrichlorosilane (OTC, $C_{18}H_{37}Cl_3Si$) was added to hydrophobize the silica particles. Subsequently, (3-aminopropyl)triethoxysilane (APTES, $C_9H_{23}NO_3Si$) was added to the reaction mixture to give the particles functional catalytically active groups. The particle and water concentration in the Pickering emulsion were calculated according to Chapter 1 (Equation 1.3) and the emulsion was produced by high shear emulsification, the droplet size was targeted to be 50 µm. Hence, in the Pickering emulsion, water was the dispersed phase, amine-functionalized hydrophobized silica microparticles were the stabilizing entities and toluene was the continuous phase. Subsequently, TEOS was added under overhead stirring in a reactor.

Results

3.5 Hours after the addition of TEOS to the Pickering emulsion stabilized by amine-functionalized microparticles, a very thin initial shell was produced, Figure 2.7 A – D. However, TEOS was added to produce a shell thickness of a few hundred nanometers. The reaction was allowed to continue in order to achieve a larger shell thickness. Nonetheless, after 24 hrs. the shell had not grown any thicker. Instead, silica structures are covering the surface of the initially stabilizing amine-functionalized silica microparticles, Figure 2.7 E and F. Evidently, when the catalyst is not mobile at the interface, but immobilized on the microparticles, it gets buried and looses its ability to catalyze the hydrolysis of TEOS towards silica. For that reason, engulfment limits the possibility of tuning the thickness of the shell.

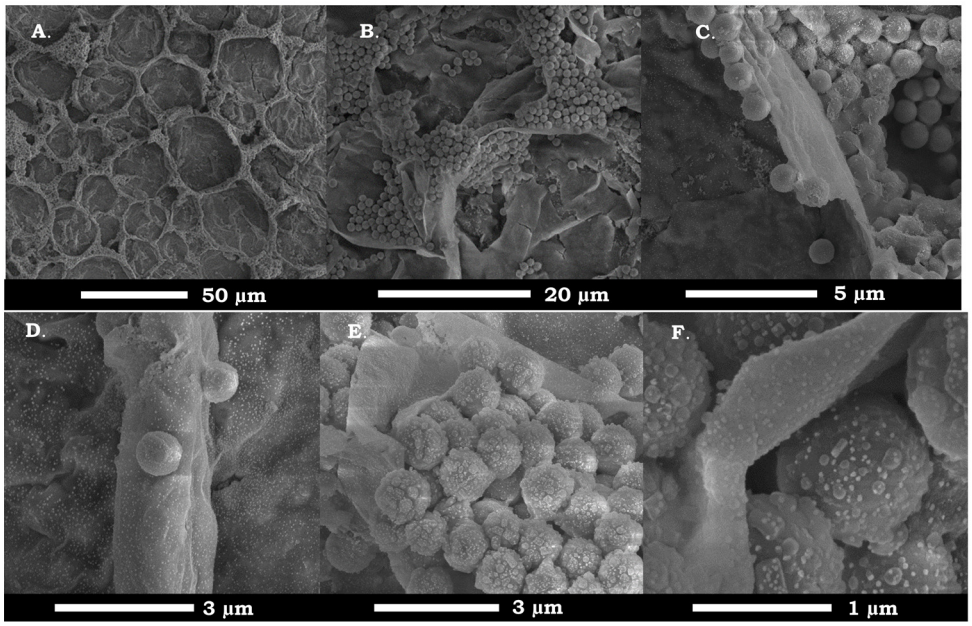


Figure 2.7 SEM images of a very thin silica shell produced around the aqueous Pickering emulsion droplets (A – D) and of silica agglomerates on the primary stabilizing silica microparticles (E and F). The stabilizing hydrophobized silica microparticles were also functionalized with catalytically active amine groups, to catalyze the interfacial reaction of TEOS.

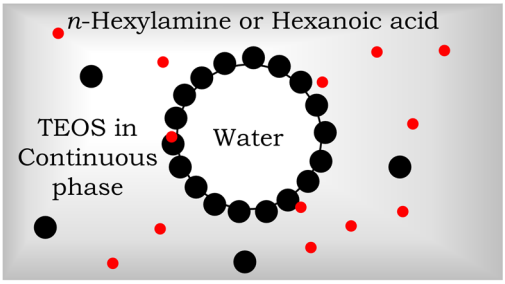
2.2.3 Amphiphilic oil-soluble catalyst

To enable the shell to grow thicker compared to the results reported above, the catalyst should be mobile at the interface, preferably also in the oil phase.[21] Besides that, in the case of TEOS, water should be able to diffuse through the shell to participate in the reaction. This can be achieved by using a catalyst which is oil-soluble and has amphiphilic properties[21] A known catalyst for the polymerization of TEOS that fits this desription is *n*-hexylamine.[36,37] When *n*-hexylamine is used to catalyse the interfacial reaction of TEOS, silica microcapsules with tunable shell thickness are produced, when Pickering emulsion droplets are used as templates.

Synthesis approach

If an amphiphilic catalyst is used, microcapsules can be produced at low temperatures, with a minimum of contamination of the dispersed phase and with tunable size and thickness. In this context, also an acidic amphiphile was used. Specifically, *n*-hexanoic acid was used to catalyze the reaction at the interface, see Scheme 2.5. It was experimentally confirmed that *n*-hexanoic acid catalyzes the polymerization of TEOS satisfactorily. In that

experiment a solution of TEOS and an oil-soluble amphiphilic acid in *n*-heptane, was mixed with water and vigorously shaken. The acids of choice were *n*-hexanoic acid, *n*-propionic acid and *n*-benzoic acid. For comparative reasons, one mixture was based on *n*-hexylamine instead of an acid. After 24 hrs, the silica formation in the different mixtures was determined and the silica formation in the *n*-hexanoic acid mixture was most comparable with the silica formation in the *n*-hexylamine mixture. To synthesize microcapsules, a Pickering emulsion was produced with *p*MMA microparticles as stabilizing entities, produced with Kraton block copolymer as stabilizer at an optimized concentration as investigated earlier in our group.[21] After formation of the Pickering emulsion, *n*-hexanoic acid was added. The concentration of *n*-hexanoic acid that was used is comparable to the concentration of *n*-hexylamine that is used in a typical synthesis of microcapsules.[21]



Scheme 2.5 Starting conditions to produce microcapsules with *n*-hexylamine or *n*-hexanoic acid in the continuous oil phase to catalyze the TEOS reaction.

Results

As a result of using an acid to catalyze the TEOS reaction instead of a base, there are two main differences, namely, the reaction time and the resulting product. Both differences are caused by the relative reaction rates of the hydrolysis and condensation reactions of TEOS with water to silica. When the reaction is acid-catalyzed, the overall reaction rate is lower and a network is produced with more residual hydroxyl groups. This is because the condensation reactions are now slower than the hydrolysis reaction. These left over hydroxyl groups in the silica network can later on still react with each other. Left over ethoxy and hydroxyl groups have a limiting effect on the silica density.[38] For this reason when an acid was used to catalyze the reaction instead of a base, a much more brittle silica product was produced and when the capsules were dried they all broke and collapsed, see Figure 2.8. Nevertheless, others have produced silica capsules by templating microdroplets consisting of an acidic solution. In that case, the silica precursor is added to the continuous oil phase, followed by an interfacial shell forming reaction. However, precautions have to be taken, *e.g.* the precursor is a pre-synthesized polymer before addition, which makes

sure it does not migrate into the droplet and already has the right shell properties.[39,40] Alternatively, the droplets are very small and the produced shell is proportionally thick, resulting in very small microcapsules which are exposed to less mechanical stress.[8,32,41–45]

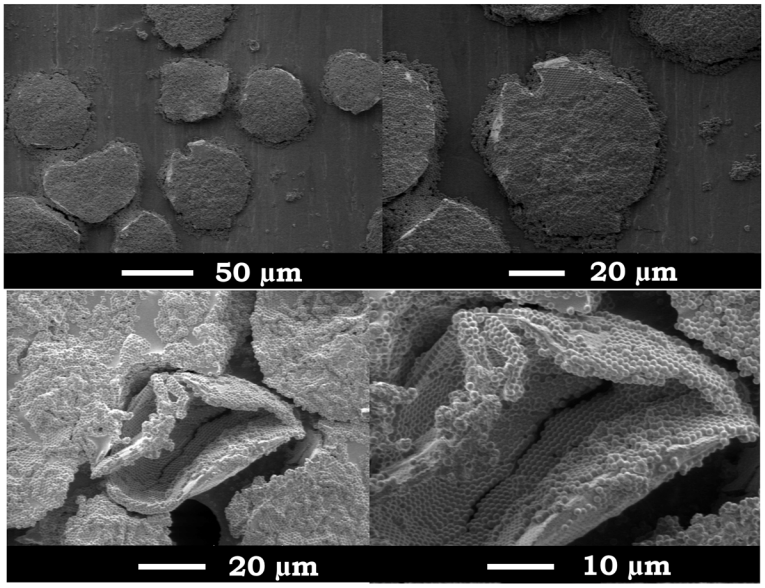


Figure 2.8 SEM images of silica produced at the interface by using an acidic amphiphilic (hexanoic acid) catalyst via templating Pickering emulsion droplets and TEOS as silica precursor.

2.2.4 Choice of monomers: dimethyldimethoxysilane

On the basis of the results described above it can now be concluded that the mechanical properties of the shell are important for a successful synthesis of microcapsules, *i.e.* a successful encapsulation. Mechanical properties comprise among others the toughness, brittleness and density. The choice of the type of silica precursor allows control of the mechanical properties of the shell. For example, a shell can be produced that does not break in solution, but only when liquid on the inside starts to evaporate upon drying. To be precise, when a pressure difference ΔP is generated by evaporation of water through the shell, ΔP is here the Laplace pressure. Usually when TEOS is polymerized via base catalysis, a silica network is produced in which the larger fraction of the silicon atoms possess 4 or 3 siloxane bridges, resulting in a certain silica density.[38,46] When the fraction of siloxane bridges decreases in the silica network, the density decreases as well. The density and the network structure are responsible for the mechanical strength of silica.[47] So, if besides TEOS also dimethyldimethoxysilane (DMDMS) is used to produce the shell of the microcapsules, this would result in less siloxane

bridging, a lower density and for that reason loss of mechanical strength. However, it would not result in brittleness of the microcapsules.

Synthesis and Results

Microcapsules were produced according to the strategy described above, based on a mixture of 50/50 % (w/w) TEOS/DMDMS and catalyzed by *n*-hexylamine.[21] First a Pickering emulsion was produced with pMMA microparticles to which *n*-hexylamine was added to catalyze the reaction. After the emulsion was charged in the reactor the silica precursors were added. The concentrations that were used in the Pickering emulsion were calculated to produce droplets with an average size of 50 μm. The shell thickness was estimated to result in 1 μm.

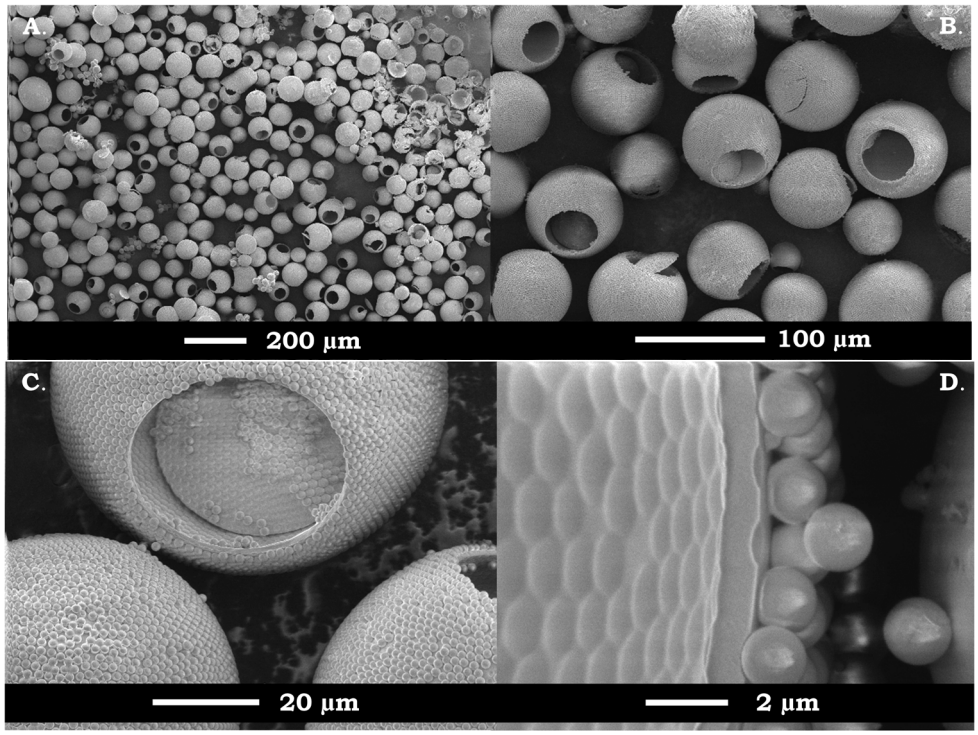


Figure 2.9 SEM images of microcapsules produced by using a mixture of TEOS and DMDMS as shell precursors. The initial Pickering emulsion was stabilized by the synergistic interactions of *p*MMA particles and *n*-hexylamine. 90% of the capsules break upon drying (A and B) with remarkably comparable fractures (C). The shell thickness was targeted to be 1 μm (D).

When Pickering emulsion droplets, were used as templates to produce microcapsules and a mixture of 50/50 % (w/w) of TEOS/DMDMS was used as shell precursor, silica microcapsules were produced, see Figure 2.9 A – D. The microcapsules have a shell thickness of about 1 μm, see Figure 2.9

D. The size distribution of the microcapsules is relatively small, considering that emulsion droplets were used as templates for the capsules. As a result of drying, more than 90 % of the capsules broke. Their fractures are all perfectly spherical, suggesting that the fracture was not caused by impact. Besides that, the fractures also appear to have a narrow size distribution and the fractured part of the shell always ends up inside the microcapsule. These observations basically only leave the option that upon drying the fractures are caused by LaPlace pressure. It is very plausible that the shell breaks at a certain pressure difference, because it has a limited mechanical strength. In addition, when the capsules break in solution, phase separation will take place between the water and oil phase and this was not observed. The percentage of broken microcapsules when they are produced with only TEOS is much lower.[21] This observation points to DMDMS being responsible for less mechanical strength. Hence, microcapsules have been produced that stay intact in solution but break upon drying.

2.2.5 Choice of monomers: 1,8-bis(triethoxysilyl)octane

Another option is the production of microcapsules that do not break upon drying and do not break in solution. This can be achieved by producing microcapsules with a flexible shell. The monomer 1,8-bis(triethoxysilyl)octane (TESO, $C_{20}H_{46}O_6Si_2$), because of its long aliphatic chain will result in a polymer with less network formation and more flexible properties.

Synthesis and Results

For the reason described above, microcapsules were produced similarly to the procedure reported above, but with 25/75 % (w/w) TEOS/TESO.[21] A much larger fraction of TESO was used because the reaction rate of this monomer is lower than the reaction rate of TEOS, and the selected ratio was to make sure that a reasonable fraction of the shell consisted of TESO. Also, microcapsules were produced using only TESO. The Pickering emulsion was stabilized by the synergistic interactions of silica particles and *n*-hexylamine. To the Pickering emulsion the precursors were added after it was charged in the reactor, which reacted with water under base catalysis at the interface to a silica shell.

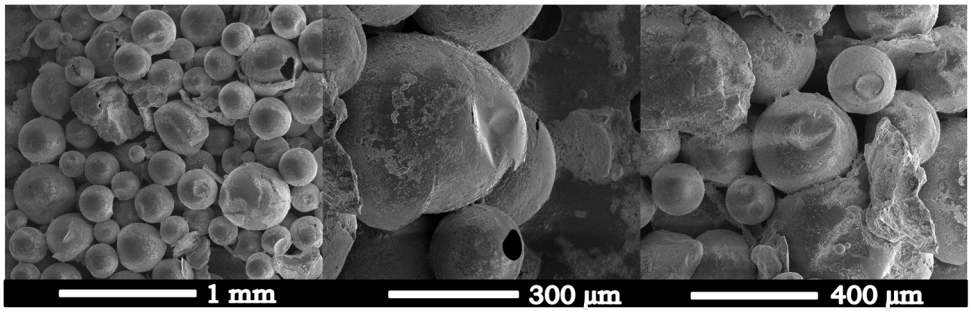


Figure 2.10 SEM images of microcapsules produced by using a mixture of TEOS and 1,8-bis(triethoxysilyl)octane as shell precursors. The initial Pickering emulsion was stabilized by the synergistic interactions of silica particles and *n*-hexylamine.

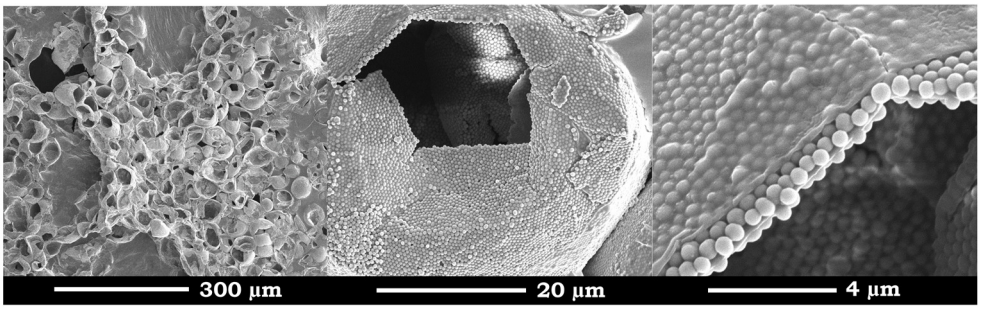


Figure 2.11 SEM images of microcapsules produced with 1,8-bis(triethoxysilyl)octane as shell precursor. The initial Pickering emulsion was stabilized by the synergistic interactions of silica particles and *n*-hexylamine.

The procedure for encapsulation using TEOS and TESO resulted in microcapsules, see Figure 2.10 and 2.11. The difference from the microcapsules shown in Figure 2.9, is that these capsules have indentations instead of fractures and more that 90% of the original microcapsules remain intact. The microcapsules produced with only TESO do not show robustness anymore and upon drying and under the high vacuum conditions that need to be applied for SEM they all collapsed, see Figure 2.11.

2.2.6 Conclusion

Silica microcapsules were produced by templating Pickering emulsion droplets. As stabilizing particles of the Pickering emulsions poly(methyl methacrylate) and silica microparticles were used. If amine groups were immobilized on the surface of the particles to catalyze a reaction of tetraethyl orthosilicate to silica, at one point the shell did not grow thicker. This is most probably caused by the reduced access to the catalyzing amine groups that are engulfed by newly formed silica. In addition, the newly formed silica shell forms a barrier between tetraethyl orthosilicate and water, which are both necessary to produce silica.

If a mobile catalyst (*n*-hexylamine) at the interface is employed and tetraethyl orthosilicate is used as silica precursor, microcapsules can be produced with tunable shell thickness. Although, hexanoic acid as acidic amphiphilic catalyst resulted in microcapsules that are very brittle and collapse upon drying. The flexibility and robustness of the shell can be controlled by the selection of proper shell precursor. When a mixture of 50/50 % (w/w) of tetraethyl orthosilicate/dimethyldimethoxysilane was used as shell precursor, silica microcapsules were produced. As a result of drying, more than 90 % of the capsules broke. When 1,8-bis(triethoxysilyl)octane was used as silica precursor or a mixture of 25/75 % (w/w) tetraethyl orthosilicate/1,8-bis(triethoxysilyl)octane, also silica microcapsules were produced. In that case the capsules have indentations instead of fractures and more that 90% of the original microcapsules remain intact.

2.3 Experimental

Materials were used as received unless indicated otherwise. To remove the inhibitor, prior to use methyl methacrylate (MMA) (99%, Sigma Aldrich) was passed over a column packed with aluminium-oxide (activated basic, Sigma Aldrich) and inhibitor-free MMA was refrigerated for later use. To remove impurities azo-bis-isobutyronitrile (AIBN, initiator) (Sigma Aldrich) was recrystallized twice from methanol. Poly(styrene-blockethylene-co-propylene) (PS-b-EP, Kraton G1702H) , molar mass 155 000 g mol^{-1}. Kraton is a linear block copolymer with low dispersity (Đ = 1.09) and a styrene content of 28 weight percent. *n*-Heptane (>96%, Biosolve), toluene (>96%, Biosolve), ethanol, methanol (99.8%, Biosolve), tetraethyl orthosilicate (TEOS) (Sigma Aldrich), *n*-hexylamine and 32% ammonia (Merck-Chemicals), 3-(trimethoxysilyl) propyl methacrylate (MPTS) (98 % Sigma – Aldrich), octadecyltrichlorosilane (OTC) (>90 % Sigma – Aldrich), (3-Aminopropyl)trimethoxysilane (APTES) (97 % Sigma – Aldrich), acetic acid (>99 % Merck), sodium silicate solution (purum, ≥10% NaOH basis, ≥27% SiO_2 basis, Sigma-Aldrich), dimethyldiethoxysilane (DMDMS) (97 % Fluka) and 1,8-bis(triethoxysilyl)octane (Chemsigma). Pyrogenic silica particles (HDK® H30, H30 RM) were donated by Wacker Silicones.

Monodisperse silica microparticles used in this work were produced by a Stöber seeded polymerization technique.[24,25] First the seed particles were synthesized via the Stöber method. In short, in ethanol (100 mL), tetraethyl orthosilicate (TEOS) (7 g, 33.6 µmol) and water (5 mL, 278 µmol) were solubilized in a three neck round bottom flask (250 mL) at 25 °C. Ammonia (15 mL, 25%) was added after 10 min to catalyze the reaction. After 6 hours of reaction tetraethyl orthosilicate TEOS (2 g, 9.6 µmol) was added, this was repeated with multiple aliquots. To ensure full conversion of the previously added TEOS every new TEOS aliquot was done at least 6 hours after the previous addition. The total concentration of TEOS was adjusted so the

particles will have a diameter between 400 and 600 nm (ρ = 2.15 g cm^{-3}). After completion of the reaction the particles were left to settle, after which the ethanol phase was decanted and the particles were dried at ambient conditions.

Functionalization of the monodisperse silica microparticles was completed by first redispersion of the microparticles (2 g) in *n*-heptane (30 g). This was done in a three neck round bottom flask, stirred with a magnetic stirrer (25 °C). To the particle dispersion 3-(triethoxysilyl) propyl methacrylate (0.2 mL, 0.68 µmol) was added and the reaction was allowed to proceed for 24 hrs. The diameter of the particles was 0.87 µm. Sufficient modification could be visually observed, since hydrophilic silica microparticles form aggregates in *n*-heptane, and after they are hydrophobized they do not.
In the case the microparticles (6.24 g, d_p = 740 nm) were functionalized with reactive amine groups, after redispersion in *n*-heptane (93 mL) first octadecyltrichlorosilane (0.2 mL, 0.5 µmol) was added and the reaction was attained for 24 hrs. Then 3-(amino propyl triethoxysilane) (0.2 mL, 0.95 µmol) was added and the reaction was continued for another 24 hrs.

Poly(methyl methacrylate) (*p*MMA) monodisperse microparticles were synthesized by the dispersion polymerization of MMA in *n*-heptane. The synthesis was performed in a reactor (250 mL) equipped with four baffles. The reaction mixture was stirred with a four-blade pitched impeller and the reactor was provided with condenser which was water-cooled. A thermostatic bath was used to heat the reactor with water that flows through the double-walled cylindrical reactor. First, Kraton (55 mg) and *n*-heptane (110.5 g) were brought into the reactor (stirred at 100 rpm) until the Kraton was completely dissolved. Secondly, AIBN (0.5 g, 3 µmol) was dissolved in MMA (33.2 g, 331 µmol) separately. This solution was added to the *n*-heptane with Kraton mixture in a single aliquot. To remove oxygen the reaction mixture was continuously purged with argon. Initiation took place by heating the mixture (80 °C), the reaction was conducted for 3 hours after which the reaction mixture was cooled to room temperature.

Poly(methyl methacrylate) (*p*MMA - MPTS) triethoxysilyl functionalized monodisperse microparticles were produced by the addition of 3-(triethoxysilyl) propyl methacrylate (6.64 g, 23 µmol) during the dispersion polymerization of MMA (26.56 g, 265 µmol) after 25 min reaction time. The resulting microparticles had a diameter of 0.94 µm.

The exact concentrations that were used for the **Pickering emulsions** produced in this work are given in the experimental description of the microcapsules, *vide infra*. Pickering emulsions were produced by the addition of water to a certain amount of particle dispersion in *n*-heptane or toluene, which was followed by emulsification of the mixture. Before the addition of water, the necessary amount of the particle dispersion was

further diluted with *n*-heptane or toluene if necessary to fill up the reactor in a next step (to about 120 mL). The Pickering emulsion droplets were targeted to be 50 µm unless indicated otherwise. The required concentrations were calculated according to Chapters 1. It was assumed that all particles that cover the water - oil interface are in a close packing and curvature effects are neglected. The Pickering emulsion was formed through the application of shear by stirring with a rotor stator mixer (IKA Ultra Turrax T25 Basic) (16 000 rpm for 2 minutes). A certain shearing time and shear rate is needed to accommodate all the particles at the interface.

The hybrid SiO_2 *p*MMA microcapsules shown in Figure 2.2 and 2.3 were produced as follows. First by the addition of extra *n*-heptane (50 g) to methacrylate-functionalized silica microparticles dispersed in *n*-heptane (15 g) produced as described above. To this particle dispersion water was added (9 mL) and the Pickering emulsion was produced. The Pickering emulsion was placed in the reactor and again extra *n*-heptane was added (20 g) to fill up the reactor to the appropriate volume. The mixture was purged with argon for 30 min at 25 °C. Afterwards, AIBN (0.0815 mg, $4.96 \cdot 10^{-4}$ µmol) which was dissolved in MMA (1.56 g, 15.6 µmol) and MPTS (1.54 g, 5.3 µmol) was added in one aliquot and the reaction was continued for 24 hrs at 70 °C.

The silica microparticles by templating Pickering emulsion droplets shown in Figure 2.5 were produced by first the addition of *n*-heptane (100 g) to the triethoxysilyl functionalized *p*MMA microparticle dispersion (7 g). To this dispersion ammonia (5 mL) and water (5 mL) were added and the Pickering emulsion was produced as described above. To the Pickering emulsion in the reactor TEOS was added (10 g, 48 µmol) and the hydrolysis and condensation reaction was continued for 6 hrs at 25 °C.

The silica microcapsules by templating Pickering emulsion droplets shown in Figure 2.6 were produced by first the dispersion of H30 Fumed hydrophobized silica nanoparticles (3.132 g) in toluene (155 g). To the particle dispersion an ammonia solution was added (31.92 g) and the Pickering emulsion was produced as described above. The size of the Pickering emulsion droplets was aimed to be smaller than 10 µm. After the emulsion was charged into the reactor, TEOS was added (11 g, 53 µmol). The reaction was allowed to proceed for 24 hrs at 25 °C.

The silica microcapsules produced by templating Pickering emulsion droplets from which the stabilizing microparticles were functionalized with catalytic groups, shown in Figure 2.7 were synthesized by the addition of water (25 mL) to a dispersion of amine functionalized silica microparticles (3 g) in *n*-heptane (60 g). Emulsification proceeded as described above. After emulsification, in the reactor TEOS (20 g, 96 µmol) was added and the reaction was attained for 24 hrs. at 25 °C.

The silica microcapsules produced by hexanoic acid, shown in Figure 2.8 were synthesized by the addition of water (23.5 mL) to the *p*MMA particle dispersion in *n*-heptane (7 g) which was produced as described above. Emulsification proceeded as described above. Before emulsification hexanoic acid (6 g, 52 µmol) was added to the mixture. To the Pickering emulsion in the reactor an additional amount of *n*-heptane was added, to fill the reactor to the appropriate volume (150 mL). Subsequently TEOS (10 g, 48 µmol) was added and the reaction was allowed to proceed for 24 hrs. at 25 °C.

The microcapsules produced with dimethyldimethoxysilane shown in Figure 2.9 were synthesized as follows. First *n*-hexylamine (6 g, 59 µmol), was dissolved in *n*-heptane heptane (120 g). To the solution, *p*MMA particles dispersed in *n*-heptane (7 g) was added. Emulsification proceeded as described above, after the addition of water (16.2 mL). The Pickering emulsion was charged into the reactor after which TEOS (5 g, 24 µmol) and dimethyldimethoxysilane (5.2 g, 43 µmol) were added. The reaction was attained for 6 hrs. at 25 °C.

The microcapsules synthesized with a fraction of 1,8-bis(triethoxysilyl)octane shown in Figure 2.10 and 2.11, were synthesized as follows. Silica microparticles (1.64 g, d_p = 420 nm) were first dispersed in a mixture of *n*-hexylamine (7 g, 69 µmol) and toluene (122 g) via ultrasonication. To the particle dispersion water was added (25 mL) and the Pickering emulsion was produced as described above. After the Pickering emulsion was charged in the reactor, TEOS (3 g, 14 µmol) and 1,8-bis(triethoxysilyl)octane (9.5 g, 21.6 µmol) were added or only OTC (9.5 g, 21.6 µmol). The reaction was allowed to proceed for 24 hrs. at 25 °C.

Light microscopy (LM) was performed with a Zeiss Axioplan Universal Microscope. The samples were prepared by placing a droplet of the Pickering emulsion on a microscope slide afterwards it was placed on the specimen table.

Scanning Electron Microscopy (SEM) was performed on FEI Quanta™ 3D FEG low vacuum SEM/Focused Ion Beam (FIB). Samples were prepared by placing a droplet of sample on a sample holder covered by double sided carbon tape. After a sample was dried in air, it was coated with a thin layer of gold, to ensure good conductivity for SEM analysis.

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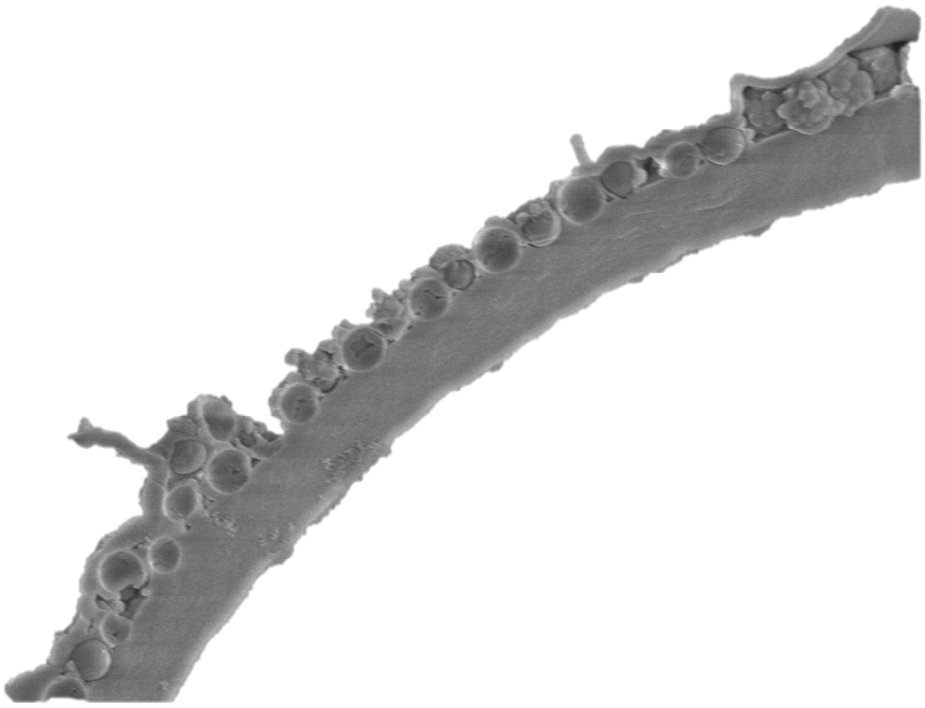
3. Poly(methyl methacrylate)-silica microcapsules



Abstract

In this contribution, we present the silica microencapsulation of hydrophilic compounds by templating Pickering emulsion droplets without contamination of the dispersed phase with either a catalyst or the silica precursor. This is accomplished by the use of an amphiphilic catalyst, which situates around the Pickering emulsion droplets and directs the reaction to the interface. Both silica precursor and the amphiphilic catalyst are soluble in the oil phase and therefore initially do not reside in the hydrophilic microcapsule templates. The thickness of the capsules can be tuned by adjusting the amount of precursor. Thus, the permeability of the capsules can in principle be controlled. The possibility of tuning the permeability holds promise for a variety of applications of the microcapsules. Because of the straightforward synthesis method and by minimizing mixing of the core with contaminants, the technique is potentially suitable for the encapsulation of delicate matter including live organisms, drugs or enzymes.

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3.1 Introduction

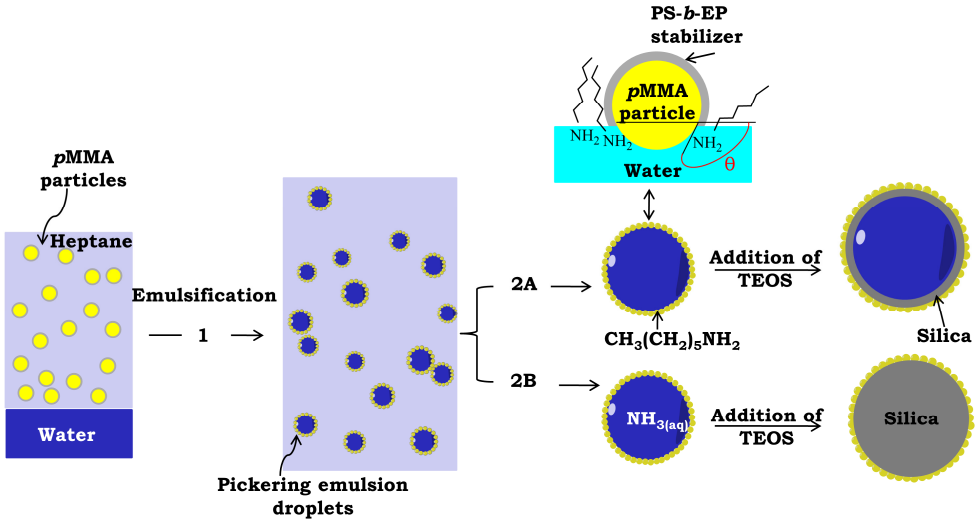
Microencapsulation is the process in which small (µm-range) particles or droplets are surrounded with a thin solid layer to form so-called microcapsules. During the past decade there has been a growing interest in the microencapsulation of active ingredients. The growing interest is, among others things, caused by the diversity of potential applications such as in drug delivery, the food industry, in phase transfer catalysis and for phase change materials.[1-4] The material inside the microcapsule is generally referred to as the 'core' and the layer that surrounds the core is called the 'shell'. Besides core-shell encapsulation, molecules can also be entrapped in a solid matrix.[5] Each type of morphology has different properties and therefore suits different applications. For example, solid polymer particles have recently been investigated for the encapsulation and controlled release of drugs,[6] while core-shell particles are suited for the permanent immobilization and protection of phase change materials.[3] In general it can be stated that there is a need to control the architecture of the microcapsule to suit different applications.
Microencapsulation can be performed, among other techniques, by templating emulsion droplets.[7-14] There are many variations on this method, but the general procedure is as follows. First, the substance of interest, or a solution of this substance, is emulsified in another (immiscible) fluid to obtain small droplets dispersed in a continuous phase. Second, a shell-forming reaction is performed in the presence of the emulsion droplets to achieve the actual microencapsulation.

Silica as a shell material provides chemical and physical stabilization of the encapsulated active ingredient. Furthermore, silica has useful physical properties, for instance a low permeability and a high thermal resistance.[15] Droplets stabilized by solid particles, i.e. Pickering emulsion droplets, are effective templates for microencapsulation, because of their extreme stability against coalescence.[16,17] During the encapsulation of hydrophilic compounds, water droplets are stabilized by solid particles. Silica (SiO_2) is formed by the hydrolysis and polycondensation of tetraethyl orthosilicate (TEOS). This reaction is enhanced by a catalyst, which is usually an amine.[18]
One of the main drawbacks of silica as a capsule material is that contamination, by surfactant or silica precursor or catalyst, of the hydrophilic core has been unavoidable until now.[19-25] D.E. Morse *et al.* demonstrated that short chain aliphatic amines are efficient catalysts for the hydrolysis and polycondensation reactions of silicon alkoxide precursors.[26] Ikeda *et al.* performed investigations on the effects of amines during the *in situ* silica generation in an hydrophobic environment and reported that *n*-hexylamine was the most preferable catalyst for effective *in situ* silica production.[27]
In this contribution we report the synthesis of silica microcapsules by using an amphiphilic catalyst to encapsulate aqueous (Pickering) emulsion droplets stabilized by poly(methyl methacrylate) (*p*MMA) particles. The catalyst, *n*-hexylamine, is highly soluble in the oil phase, but is also present at the interface of the emulsion droplets because of its amphiphilic characteristics. The effect of an amphiphilic catalyst is clearly shown in a comparative experiment where ammonia is used as a water-soluble catalyst.

3.2 Synthesis approach

Scheme 3.1 outlines the approach for the silica microencapsulation of hydrophilic molecules by templating Pickering emulsion droplets without mixing of the dispersed phase with a catalyst or a silica precursor. Initially, a Pickering emulsion was prepared with poly(methyl methacrylate) (*p*MMA) particles as stabilizing entities. The *p*MMA particles were produced by dispersion polymerization in *n*-heptane, using poly(styrene-*block*-(ethylene-*co*-propylene)) (PS-*b*-EP) as stabilizer. PS-*b*-EP not only provides colloidal stability of the particles in dispersion but also prevents coalescence of the Pickering emulsion droplets.[28] Consequently, in the Pickering emulsion, *n*-heptane was the continuous phase and double de-ionized water was used as the dispersed phase. Prior to high shear emulsification, *n*-hexylamine was added to the *n*-heptane phase. Since *n*-hexylamine has amphiphilic characteristics, this results in synergism between the two emulsifiers.[29] We speculate that *n*-hexylamine will have its hydrophilic NH_2 head-group in the aqueous phase after formation of the Pickering emulsion, which increases the

particles three-phase contact angle (Scheme 3.1). To stimulate a fast reaction, the *n*-hexylamine concentration was maximized in combination with the critical concentration of PS-*b*-EP for colloidal stability, still resulting in a stable water-in-oil Pickering emulsion. Tetraethyl orthosilicate (TEOS) was added to the Pickering emulsion, the amount of TEOS used was estimated from the aimed shell thickness when 100% conversion of TEOS is reached. A reference experiment was completed in which all conditions were the same except for the type and location of the catalyst. Instead of *n*-hexylamine, an equimolar amount of ammonia was used to catalyze the reaction, which is present inside the emulsion droplet. Scheme 3.1 shows the two synthesis routes.



Scheme 3.1 Schematic overview of the synthesis routes leading to silica microcapsules and silica microparticles. Formation of a Pickering emulsion by emulsification (1), formation of a silica capsule when *n*-hexylamine as catalyst was added *prior* to emulsification (2A) and formation of a silica particle when an ammonia solution is used as catalyst and forms the dispersed phase (2B).

The produced Pickering emulsions were analyzed by light microscopy (LM) on stability and droplet size. The shell thickness and structures of the resulting capsules were analyzed by Scanning Electron Microscopy (SEM). Gas Chromatography (GC) measurements were performed to monitor the conversion of TEOS. Thermogravimetric analysis was used to examine the permeability.

3.2.1 Pickering emulsion

Figure 3.1 shows a LM image of a typical stable Pickering emulsion that was used to produce silica capsules. The emulsion droplets consist of double de-ionized water and are stabilized by *p*MMA particles and *n*-hexylamine.

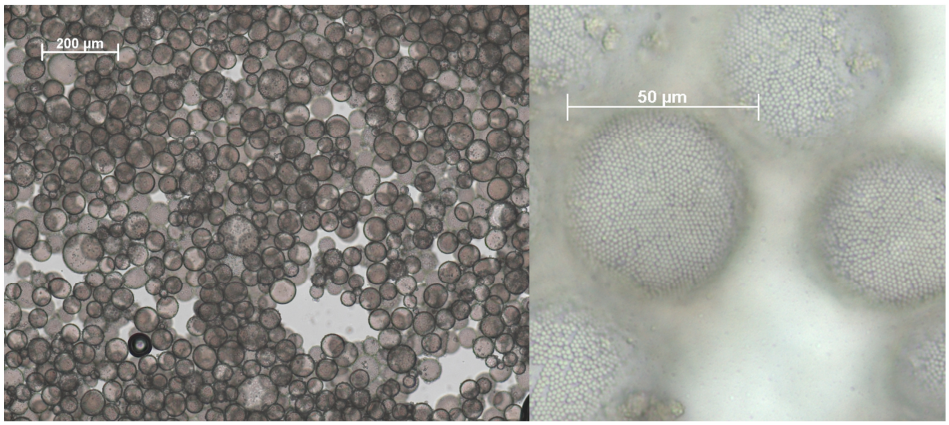


Figure 3.1 Light microscopy images of a Pickering emulsion. The emulsion is stabilized by *p*MMA particles produced with a PS-*b*-EP concentration of $1.5 \cdot 10^{-3}$ [$g_{PS\text{-}b\text{-}PE}/g_{MMA}$] and 3 g *n*-hexylamine. The left image gives an overview of the emulsion droplets and when focusing on one emulsion droplet and zooming in, close hexagonal packing of the particles at the interface can be observed.

The three-phase contact angle (θ) of the stabilizing particles at the water oil interface is by far the most important parameter that determines the stability of a Pickering emulsion, see Figure 3.2 θ can be described mathematically with Young's equation and is a function of the different interfacial tensions in the emulsion (Equations 1 and 2).[30]

$$\cos\theta = \frac{\gamma_{po} - \gamma_{pw}}{\gamma_{ow}} \quad (1)$$

$$\sin\theta = \frac{w}{2R} \quad (2)$$

In equations 1 and 2, γ represents the interfacial tension between oil (o) and water (w) and particle (p), w and R are the relevant distances as shown in Figure 3.2. An inverse Pickering emulsion is most stable when the particles have a contact angle between 94° and 110° [31-34]. This means that a change of the different interfacial tensions indicates a change in θ and consequently changes the stability of the emulsion. The influence of *n*-hexylamine in heptane on the interfacial tension of a water-heptane-system is represented in Figure 3.3. The lower heptane-water interfacial tension, at higher *n*-hexylamine concentrations, will increase θ and negatively affect the stability of the Pickering emulsion.

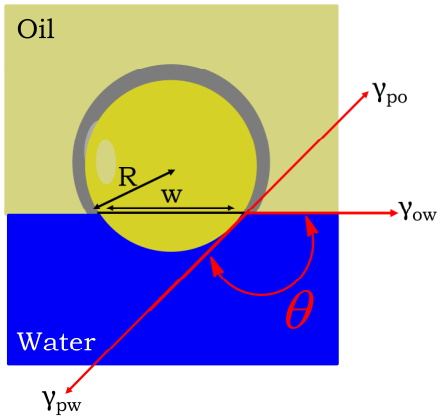


Figure 3.2 Schematic representation of a *p*MMA particle at the water-oil interface.

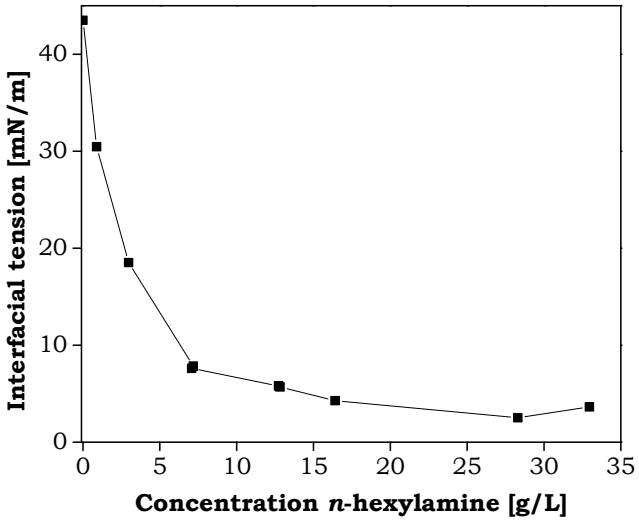


Figure 3.3 Interfacial tension between water and *n*-heptane as a function of concentration of *n*-hexylamine in heptane.

3.2.2 The stabilizing particles and amphiphile

Initially, *p*MMA particles synthesized with a stabilizer concentration of $4.2 \cdot 10^{-3}$ [$g_{PS\text{-}b\text{-}PE}/g_{MMA}$] were used to stabilize the Pickering emulsions, which proved to result in highly stable emulsions.[35] However, when a certain amount of *n*-hexylamine was added to the Pickering emulsion this resulted in phase separation and it was visually observed that the *p*MMA particles, after phase separation, resided in the *n*-heptane phase. Thus, there is a maximum concentration of *n*-hexylamine in *n*-heptane, which can be added to a Pickering emulsion stabilized by particles produced

with a stabilizer concentration of $4.2 \cdot 10^{-3}$ [$g_{PS\text{-}b\text{-}PE}/g_{MMA}$] while maintaining a stable Pickering emulsion.[36] This indicates that a fraction of the added *n*-hexylamine is present at the interface of the emulsion droplets (also shown in Figure 3.3) and that both emulsifiers are responsible for the final stability of the emulsion. This leads us to conclude that with increasing concentration of *n*-hexylamine, the three-phase contact angle of the *p*MMA particles at the water/oil interface of the emulsion, also increases.

The colloidal stability of dispersion particles is generally enhanced, when in the dispersion polymerization the concentration of the steric stabilizer PS-*b*-PE is increased. When the particles in *n*-heptane are colloidally stable, they are not suitable for Pickering stabilization, because in that case they prefer to stay in the n-heptane phase instead of at the water-oil interface. With an increase of PS-*b*-PE at the particle interface they become more hydrophobic, which leads to an increased three-phase contact angle if the *p*MMA particles are used in a Pickering emulsion. The stabilizer concentration affects the three phase contact angle and therefore also the emulsion stability.[36] To investigate the effect of the amine concentration on the formation rate of silica, it was necessary to optimize the emulsion stability. So, the three-phase contact angle of the *p*MMA particles with the water/oil interface needs to be decreased, in order to find the maximum allowed concentration of *n*-hexylamine. The critical stabilizer concentration for colloidal stability of *p*MMA particles in the dispersion polymerization was experimentally determined to be $0.8 \cdot 10^{-3}$ [$g_{PS\text{-}b\text{-}PE}/g_{MMA}$], resulting in a surface concentration of PS-*b*-PE on the *p*MMA particles of $5 \cdot 10^{-3}$ [$g_{PS\text{-}b\text{-}PE}/m^2$], as deduced from size exclusion chromatography concentration measurements in the continuous *n*-heptane phase. The experiments were done by decreasing the stabilizer concentration in several dispersion polymerizations until the lower limit for colloidal stability was reached, see Figure 3.4. An *n*-hexylamine concentration in *n*-heptane of $5.2 \cdot 10^{-2}$ [g/mL] is the highest concentration that was used in combination with *p*MMA particles synthesized with the lowest stabilizer concentration necessary for colloidal stability of the *p*MMA particles.

3.3 Results

SEM images of silica microcapsules produced by templating Pickering emulsion droplets and using *n*-hexylamine as the catalyst, are represented in Figure 3.5. The silica shells have an estimated thickness of about 300 nm and impressions of the spherical *p*MMA particles can be observed on the inside of the silica microcapsules (Figure 3.5 C). In other words, on the inside of the silica shell the spherical shape of where the *p*MMA particles used to be at the water-oil interface is visible. This

observation suggests that the hydrolysis and polycondensation reactions take place in the aqueous phase.

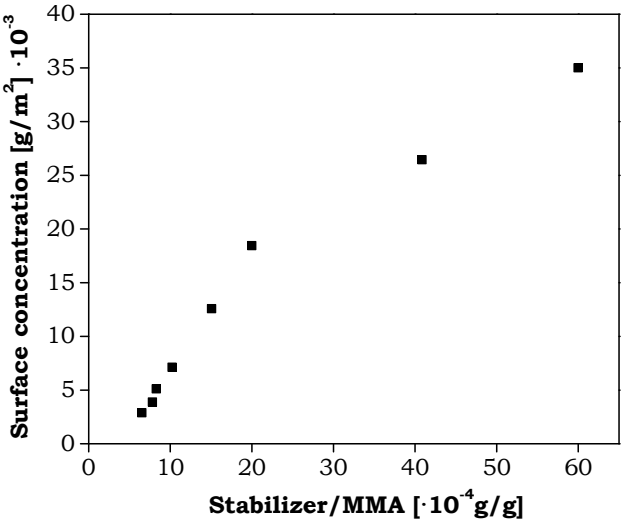


Figure 3.4 The surface concentration of PS-*b*-PE on *p*MMA particles as a function of the PS-*b*-PE to MMA ratio during the dispersion polymerization of MMA, measured using SEC.

Because of the hydrophilic character of silica, a fraction of the more hydrophobic *p*MMA particles is released from the microcapsule surface into the *n*-heptane phase. The PS-*b*-EP stabilizer anchored on the surface of polymer particles, besides providing colloidal stability of the particles, also prevents coalescence of the pMMA particles at the water-oil interface of the emulsion and in a later stage prevents aggregation of the growing microcapsules.[28] Although a fraction of the stabilizing polymer particles is released during and after the growth of the silica microcapsules, there are no aggregates observed. The fractures of the microcapsules are remarkably spherical (Figures 3.5 B and 3.5 C). The fractures are most likely caused by a pressure difference that is generated during the drying process, when water evaporates through the silica shell. Nevertheless most capsules are still intact after drying and under the high vacuum conditions needed for SEM analysis, which is an indication that they are robust. The close packing of polymer particles at the w/o interface of the original Pickering emulsion droplets is similar to the packing of the particles on the silica microcapsules (Figure 3.5 D).

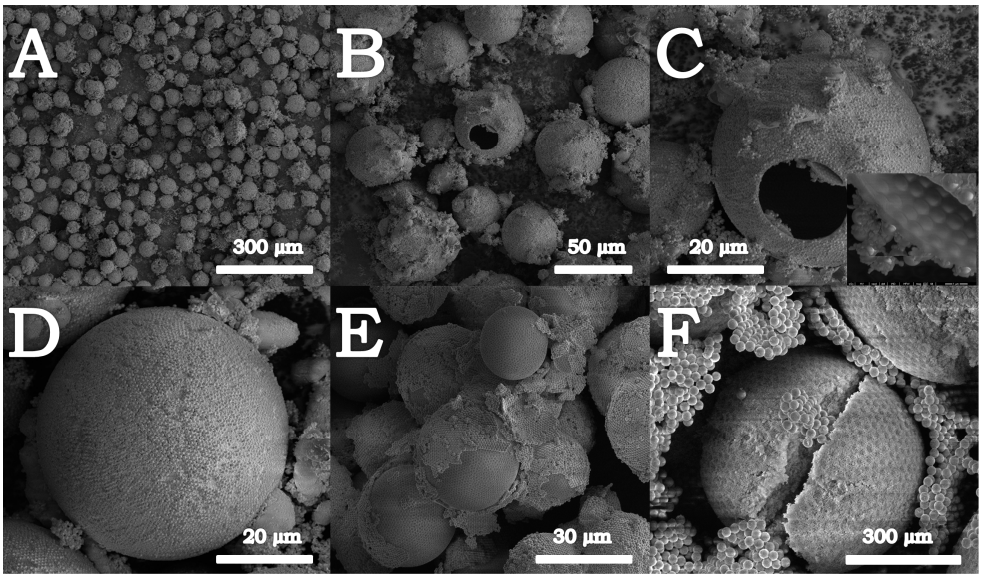


Figure 3.5 Scanning electron microscope images of hollow silica microcapsules and solid silica particles. The capsules were synthesized using *n*-hexylamine to catalyze the hydrolysis and polycondensation reactions of TEOS (A-D) and the particles synthesized using ammonia to catalyze the hydrolysis and polycondensation reactions of TEOS (E-F), respectively.

When ammonia was used to catalyze the reaction, solid silica spheres were produced, see Figures 3.5 E and F. Due to its water-solubility, ammonia is present in the dispersed aqueous phase, every single Pickering emulsion droplet is converted into a silica sphere.

On the basis of the results described above it can be proposed that at the interface of the dispersed water droplets there is *n*-hexylamine is present. Also water is present and TEOS is transferred from the continuous phase to the locus of reaction, *i.e.* the water-*p*MMA interface. Since all three are necessary for the reaction to silica and they only meet at the interface this is reason to assume that an interfacial reaction will take place.
In the case of ammonia dissolved in water, apparently the condensation reactions at the interface are not fast enough to consume (partially) hydrolyzed TEOS available at the interface. Diffusion of these (partially) hydrolyzed TEOS products into the core of the water droplet allows condensation everywhere in the droplets, which leads to a silica particle. This allows the conclusion that to achieve a core-shell silica microencapsulation, the catalyst should be present at the interface in combination with the silica precursors water and TEOS in the dispersed and continuous phase, respectively.

3.3.1 Kinetics

The kinetics of shell formation is investigated via the conversion of TEOS as a function of time (Figure 3.6), measured by using GC analysis during a typical synthesis of the *p*MMA/silica-microcapsules. Also, the thickness of the shell over time was monitored by using SEM images. The diameter of the emulsion droplets was calculated to be 50 µm and the amount of TEOS in this experiment was specified to produce a shell thickness of 500 nm, using Equations 3 and 4.[35]

$$r_D = \frac{3V_D}{N_A 2\sqrt{3}R^2} \tag{3}$$

$$m_{TEOS} = \frac{3\delta_{shell}\rho_{SiO_2}V_D}{0.3r_D} \tag{4}$$

In Equations 3 and 4, N_A is the number of particles attached to the interface of the droplets and R is the radius of the particles, when the particles are hexagonally close-packed, the area that a particle occupies is $A_p = 2\sqrt{3}R^2$ and V_D is the total volume of the dispersed phase. r_D is the radius of the droplet. In the recipe $0.3 \cdot m_{TEOS}$ is the mass fraction of TEOS that is converted to silica, δ_{shell} is the thickness of the shell that is aimed for and ρ_{silica} is the theoretical density of silica (≈ 2.15 g/mL).[37] The concentration of *n*-hexylamine was $2 \cdot 10^{-2}$ [$g/mL_{heptane}$]. The amount of the continuous phase in every experiment was 0.174 L ($\rho_{n\text{-}heptane}$ = 0.689 g/mL) to which TEOS was added after formation of the Pickering emulsion. After 33 hours, 99% conversion of TEOS was reached and consequently the maximum shell thickness. Considering the long reaction time of 33 hours, this emphasizes that the stability of the Pickering emulsion is prerequisite for successful encapsulation.[28]

For different concentrations of *n*-hexylamine, the conversion of TEOS in the *n*-heptane phase was also determined as a function of time, using GC analysis. Besides the concentration of TEOS, also the concentrations of ethanol and *n*-hexylamine were measured in the *n*-heptane phase. The concentration of *n*-hexylamine in the continuous *n*-heptane phase turns out to be virtually constant throughout the experiment. In every experiment, the same number of *p*MMA particles was used, produced with a stabilizer concentration of $1.5 \cdot 10^{-3}$ [$g_{PS\text{-}b\text{-}EP}/g_{MMA}$]. The droplet diameter in every experiment was estimated to be 50 µm (Equation 3).

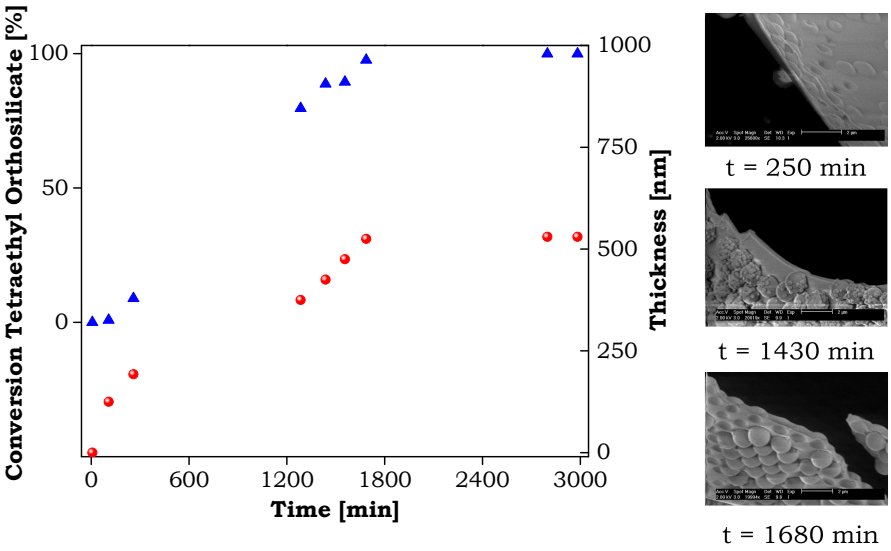


Figure 3.6 Conversion of TEOS and shell thickness as a function of reaction time. The conversion of TEOS (▲) was determined by GC measurements. The capsule thickness (●) was measured from SEM images. The concentration of *n*-hexylamine in *n*-heptane was $2 \cdot 10^{-2}$ [g/mL].

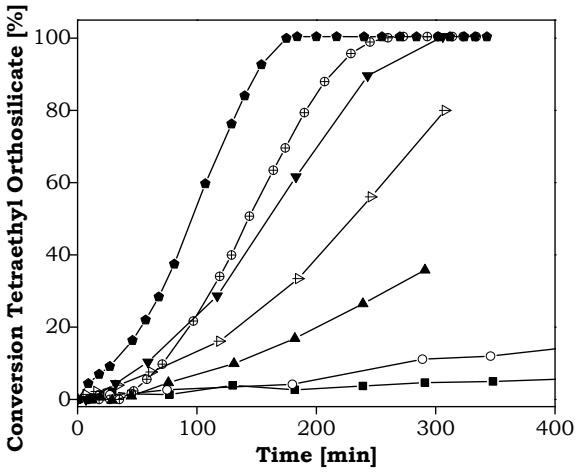


Figure 3.7 The conversion of TEOS, as a function of reaction time, for different initial concentrations of catalyst. The initial *n*-hexylamine concentrations were 0.5 (■)/1.4 (o)/2.8 (▲)/3.8 (▷)/4.2 (▼)/4.4 (⊗)/5.2 (⬟) [$\cdot 10^1$ $g_{n\text{-hexylamine}}/L_{heptane}$]. The initial TEOS concentrations were 5.9 (■)/6.2 (o)/5.0 (▲)/6.5 (▷)/5.7 (▼)/4.0 (⊗)/5.8 (⬟) [$\cdot 10^1$ $g_{TEOS}/L_{heptane}$].

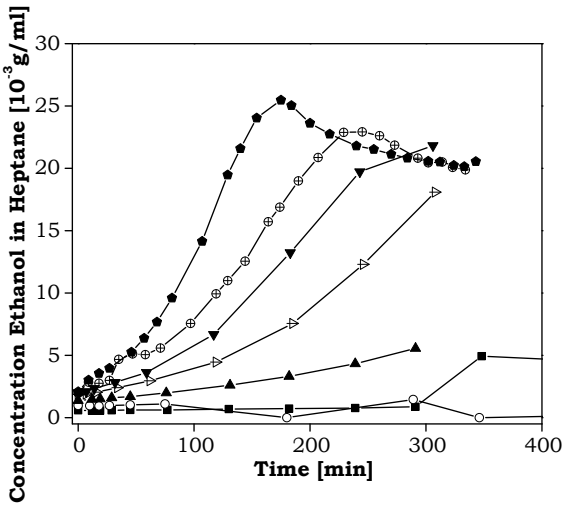


Figure 3.8 The concentration of ethanol in heptane, as a function of time, for different initial concentrations of catalyst. The initial *n*-hexylamine concentrations were 0.5 (■)/1.4 (o)/2.8 (▲)/3.8 (▷)/4.2 (▼)/4.4 (⊗)/5.2 (⬟) [·10^1 $g_{n\text{-hexylamine}}/L_{heptane}$]. The initial TEOS concentrations were 5.9 (■)/6.2 (o)/5.0 (▲)/6.5 (▷)/5.7 (▼)/4.0 (⊗)/5.8 (⬟) [·10^1 $g_{TEOS}/L_{heptane}$].

The results clearly demonstrate that the reaction rate of TEOS increases with increasing *n*-hexylamine concentration. As a result of the increasing conversion rate of TEOS also the rate of ethanol formation increases. For the two highest amine concentrations full conversion of TEOS is reached within the duration of the experiment, see Figure 3.7. In case of the two highest amine concentrations, the ethanol concentration only reaches 80% of the calculated value for full TEOS conversion into ethanol and silica, see Figure 3.8. After the maximum concentration of ethanol is reached, which coincides with the time at which TEOS is just completely converted, a decrease of the concentration of ethanol in the heptane phase is observed (Figure 3.8). This decay is attributed to partitioning of ethanol between the water and *n*-heptane phase, which involves transport through the capsule wall into the water phase. It can now be concluded that the time constant for the reaction of TEOS to form silica and ethanol is faster than the diffusion partitioning process of ethanol to the water phase, otherwise no build-up of ethanol in the heptane phase should be observed (Figure 3.8). In other words, the decay in ethanol concentration after the maximum concentration in ethanol is reached, can be attributed to a diffusion process and not to a reaction process. Besides that, for the experiments with the highest amine concentration (4.4 and 5.2 [·10^1 $g_{n\text{-hexylamine}}/L_{n\text{-heptane}}$]), see Figure 3.9, also the highest ethanol concentration during the reaction in heptane is observed. Eventually, the ethanol concentrations seem to level off to the same

value. For the highest amount of amine, the time constant for reaction in comparison to the time constant for diffusion was higher. So more ethanol was present in the *n*-heptane phase after all the TEOS had reacted. Based on this observation, we preliminarily conclude that silica growth takes place on the outside of the capsule. Namely, if silica would form on the inside of the capsule, an excess concentration of ethanol would occur in the dispersed aqueous phase rather than in the continuous *n*-heptane phase. This is another reason to assume that *n*-hexylamine is present at the interface of the emulsion droplets in the *n*-heptane phase and at a later stage on the outer surface of the growing capsules.

3.3.2 Shell thickness and morphology

Another set of experiments is performed in which the shell thickness is tuned by the successive addition of TEOS. Figure 3.9 shows scanning electron microscopy images of microcapsules, with an aimed shell thickness of respectively 300 nm, 900 nm and 1.4 μm. From the images it can be concluded that after addition of a certain amount of TEOS, the shell does not grow uniformly anymore. A very porous layer of silica is formed as a precipitate on the *p*MMA particles or as poorly defined particle agglomerates in the heptane phase, see Figure 3.10 B – F. The microcapsules were produced in one batch and have an average diameter of 65 μm with a dispersity of 1.19, determined using SEM images in combination with the software imageJ.[38] The dispersity is defined as the ratio of volume-average particle diameter and the number-average particle diameter.
Figure 3.10 shows SEM images of 65 μm microcapsules with a wall thickness that was aimed to be 1 μm that have been prepared in an almost identical way to those shown in Figure 3.9. However, before every new addition of TEOS, the continuous heptane phase was replaced by new heptane and *n*-hexylamine. Compared to the microcapsules in Figure 3.9 there are no poorly defined particle agglomerates observed, nor is there a porous layer of silica observed on the surface of the capsules. Also, the thickness of the shell in Figure 3.9 resulted in 1.25 μm, instead of 1.4 μm that was aimed for, probably as a result of TEOS that converted to agglomerates instead of producing a uniform shell.

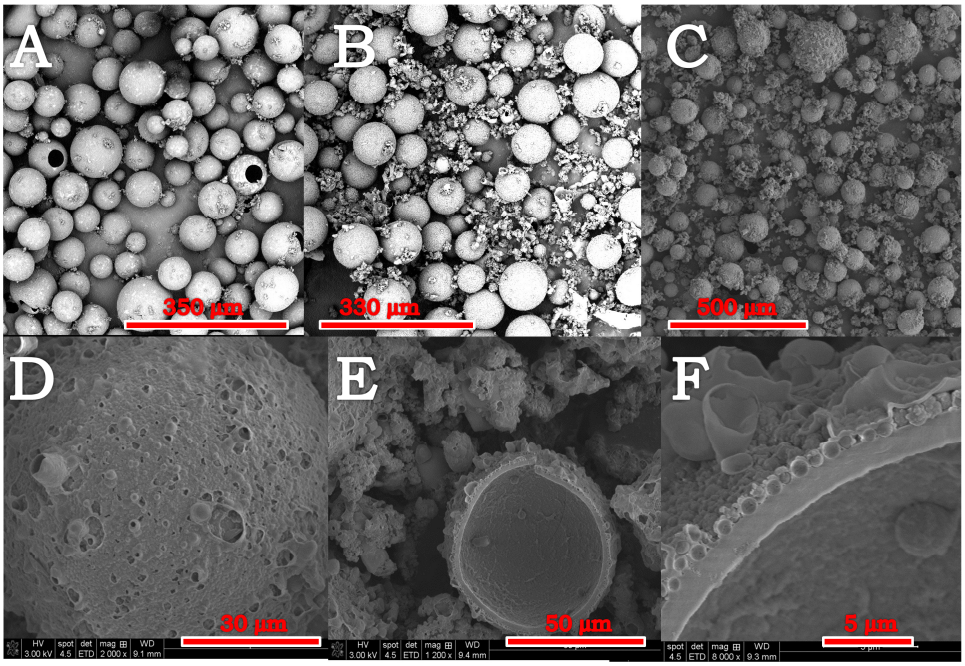


Figure 3.9 SEM images of *p*MMA/SiO_2 microcapsules. The shell thickness derived from the SEM images is 300 nm for A and after multiple additions of TEOS the shell thickness in B is 900 nm and for C-F is 1.25 µm. The larger magnification images are shown in pictures D-F.

Most probably, ethanol that is produced during the capsule synthesis works as a co-solvent, similar to its role in the Stöber mechanism.[18] Throughout the reaction ethanol is constantly produced, diffusing through the shell and present in the *n*-heptane phase and around the shell where it can co-solubilize water and (partially) hydrolyzed TEOS as well as non-hydrolyzed TEOS. When the concentration of ethanol in the heptane phase is low, so is the co-solubilizing effect. The water concentration on the outside of the shell will be lower and the reaction of TEOS with water to form poorly defined particle agglomerates and a porous layer of silica that precipitates on the capsules will not occur (Figures 3.9 and 3.10). Nevertheless the shell-forming reaction will not stop, because of the hydrophilic nature of silica, water from inside the capsules will be able to diffuse through the shell to react with TEOS. Sol-gel silica is known for its ability to physically adsorb water.[39] In addition, because of the hydrophilic nature of silica and because of the aqueous core, the more hydrophobic *n*-hexylamine does not prefer to diffuse to the inside of the capsules.

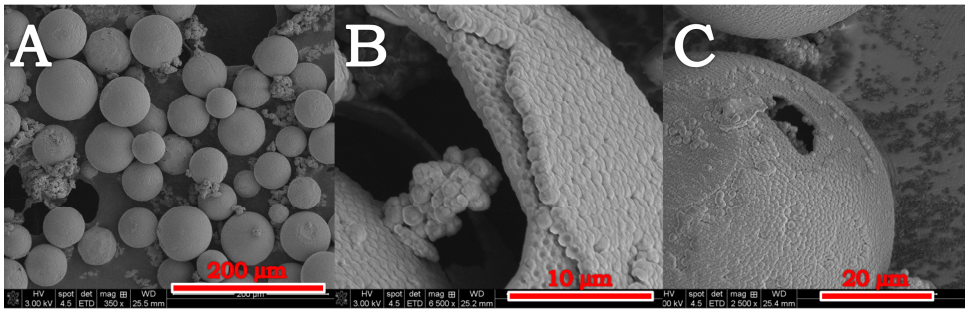
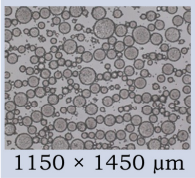
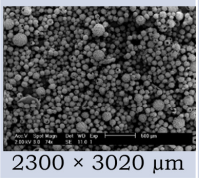
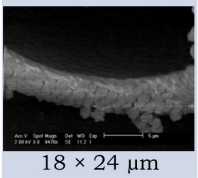
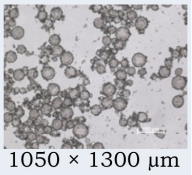
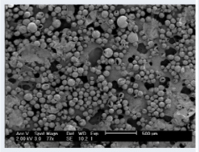
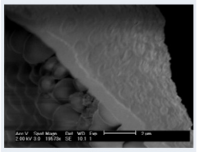
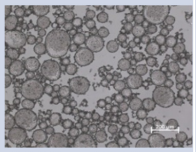
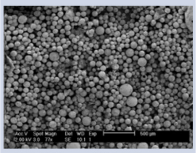
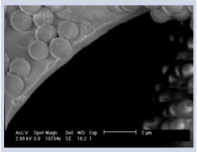
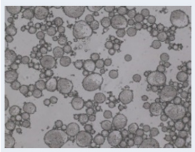
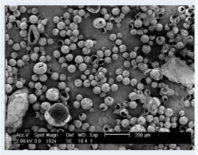
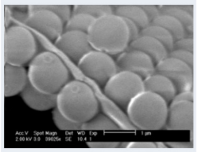


Figure 3.10 SEM images of *p*MMA/SiO_2 microcapsules. Produced by multiple additions of TEOS. Before every TEOS addition the heptane phase was replaced by fresh heptane and *n*-hexylamine. The shell thickness derived from the SEM images is 1 µm.

An increase of the shell thickness leads to a decrease of the diffusion time of ethanol and water through the shell. This in turn leads to a non-uniform shell production, because of the co-solvent effect of ethanol. Reduction of the concentration of ethanol in *n*-heptane decreases the concentration of water in *n*-heptane and as a result a uniform shell is produced.
For protection and controlled release applications, control over the capsule shell thickness is important. The most convenient and accurate method to gain control over the capsule shell thickness is by adjusting the concentration of the silica precursor. Table 1 shows the calculated as well as the experimentally determined shell thickness, when the average Pickering emulsion droplet diameter was 50 µm and the concentration of *n*-hexylamine was $5.2 \cdot 10^{-2}$ g /mL. Notable is the difference between the calculated and the experimentally determined thickness, this is probably due to a difference in the silica density used in the calculations and the actual silica density. Nevertheless the results indicate a clear increase in shell thickness and therefore also indicate controllability over the capsule shell thickness. In view of the mechanical properties of the capsules, logically, the fraction of broken capsules increases when the capsule shell thickness decreases. When comparing the Pickering emulsion droplet size with the *p*MMA/silica capsules size, in Table 3.1, they closely resemble each other, underlining the template function of the Pickering emulsion droplets.

Table 3.1 Control over the capsule thickness via adjustment of the concentration of silica precursor.

Calculated thickness (nm)*	Resulted thickness range (nm)	LM image of the Pickering emulsion	SEM image of the capsules	SEM image of the silica shell
500	600-800			
Dimension image	length×broadness	1150 × 1450 µm	2300 × 3020 µm	18 × 24 µm
300	600-700			
Dimension image	length×broadness	1050 × 1300 µm	2300 × 3020 µm	9 × 12 µm
200	400-450			
Dimension image	length×broadness	1100 × 1350 µm	2300 × 3020 µm	9 × 12 µm
100	150-200			
Dimension image	length×broadness	1150 × 1450 µm	1100 × 1450 µm	4.5 × 6 µm

*The capsule thickness was calculated using Equations 3 and 4, $V_D = 16 \cdot 10^{-3} dm^3$ is the total volume of the dispersed phase. $r_D = 25 \mu m$ is the radius of the droplet. ρ_{silica} is the theoretical density of silica (≈ 2.15 g/mL).[37] The concentration of *n*-hexylamine was $5.2 \cdot 10^{-2}$ [$g/mL_{heptane}$].

3.3.3 Permeability

After the synthesis, the microcapsules can be easily isolated from the *n*-heptane phase because they quickly sediment. The clear *n*-heptane phase can then be decanted and the remaining heptane can be evaporated. Once the *p*MMA/silica microcapsules are isolated the water starts to evaporate as the shell is porous, see Figure 3.11. *p*MMA/silica microcapsules from which water is evaporating can be observed with light microscopy by the darker sections that indicate air inside the capsules, see Figure 3.11 B. The capsules were synthesized by multiple

additions of TEOS and before a new addition, the *n*-heptane phase was replaced with new *n*-heptane and *n*-hexylamine

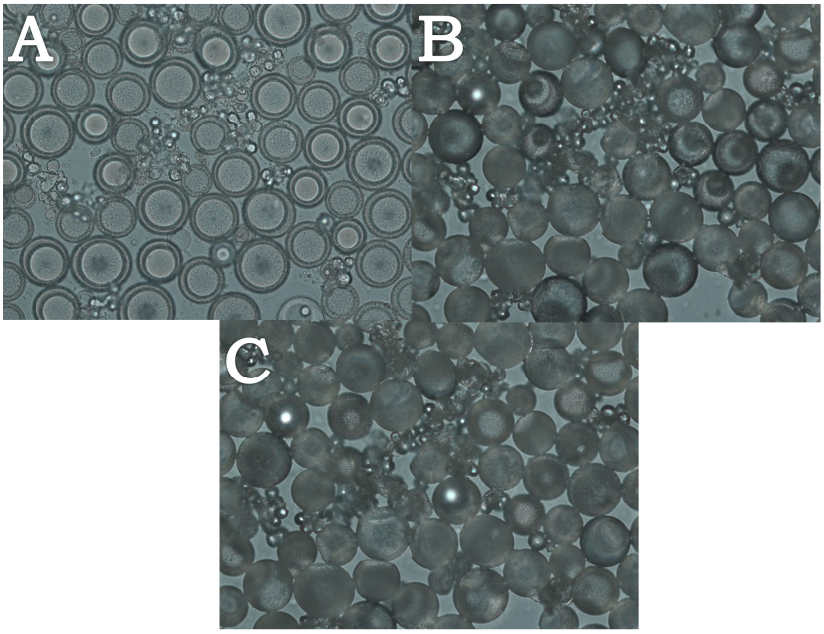


Figure 3.11 Light microscope images of *p*MMA/silica microcapsules in *n*-heptane containing water (A), *p*MMA/silica microcapsules without *n*-heptane (B), dry *p*MMA/silica microcapsules (C). The microcapsules that were used had an average diameter of 50 µm and a shell thickness of 340 nm. The pMMA particles used were produced have a PS-*b*-EP concentration of $1.5 \cdot 10^{-3}$ [$g_{PS\text{-}b\text{-}EP}/g_{MMA}$].

Thermogravimetric analysis allows monitoring the drying process of the *p*MMA/silica microcapsules at constant temperatures of 30 °C, 50 °C and 70 °C, see Figure 3.12. The microcapsules that were used had an average diameter of 50 µm, a shell thickness of 340 nm and the *p*MMA particles used were produced with a PS-*b*-EP concentration of $1.5 \cdot 10^{-3}$ [$g_{PS\text{-}b\text{-}EP}/g_{MMA}$]. The capsules were synthesized by multiple additions of TEOS and before a new addition the *n*-heptane phase was replaced with new *n*-heptane and *n*-hexylamine, see Figure 3.12. The difference between first the evaporation of *n*-heptane (A) followed by the evaporation of water (B) can be distinguished. For every measurement a different weight of sample was used which resulted in an evaporation rate of water of 0.4 mg/min at 30 °C, 0.9 mg/min at 50 °C and 2.3 mg/min at 70 °C for 30 mg, 53 mg and 46 mg of sample, respectively.
For small molecules like water, the capsules have a high permeability and after separation from the continuous phase, the water directly starts to evaporate from the capsules. In case of potential applications this should be taken into account.

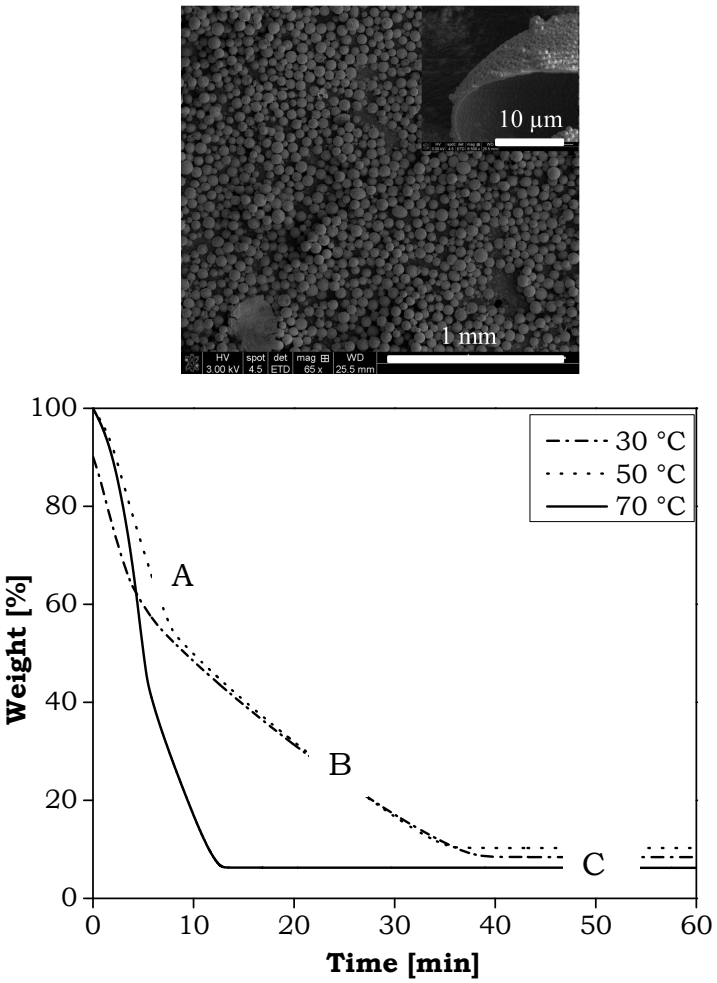


Figure 3.12 TGA curves of the evaporation of heptane (A), and water (B) from the *p*MMA/SiO_2 capsules in the right SEM image. Dried capsules are depicted by the horizontal section (C). Isothermal measurements at 30 °C, 50 °C and 70 °C. The microcapsules that were used had an average diameter of 50 µm and a shell thickness of 340 nm. The pMMA particles used were produced have a PS-*b*-EP concentration of $1.5 \cdot 10^{-3}$ [$g_{PS\text{-}b\text{-}EP}/g_{MMA}$].

We found that re-dispersion of the capsules in water is possible without prior isolation from the *n*-heptane phase. Since the microcapsules readily sediment, most of the *n*-heptane can be decanted. The microcapsules with some residual heptane can then be directly mixed with water. The *n*-heptane phase is on top of the water phase and due to gravity and the hydrophilic nature of silica the *p*MMA/silica microcapsules subside in the water phase. Afterwards the *n*-heptane phase can be removed. When bigger molecules are encapsulated the permeability will be lower and direct evaporation after isolation from the continuous phase might not

occur. Besides that, a thicker shell will also decrease the evaporation rate.

3.5 Conclusion

In this contribution we have presented *p*MMA/silica microcapsules with a tunable shell thickness, synthesized by templating Pickering emulsion droplets. An amphiphilic catalyst is used that situates around the emulsion droplets, which prevents contamination of the dispersed phase by catalyst or silica precursor. Tetraethyl orthosilicate has been used as the precursor which reacts with water to silica and ethanol. It was found that ethanol, after the interfacial reaction was completed, partitions between the continuous and dispersed phase. To avoid ethanol from diffusing to the water droplets, replacement of the continuous phase by fresh one after or during the interfacial reaction is necessary. In addition, substitution of the *n*-heptane phase during the reaction also leads to uniform shell formation. To explain the formation of the capsules, a mechanism has been proposed based on the sol-gel process with appropriate positioning of reactants, catalyst and side-product during the course of the reaction. Sol-gel silica is known for its complex interior structure. Therefore, larger molecules or even macromolecules, will probably diffuse trough the silica shell at a different rate than smaller molecules. In addition, most likely there is a maximum molecular weight that can diffuse through the shell. Because the permeability of the capsules can in principle be controlled by the thickness of the silica layer, this method is suitable for protection and controlled release of active ingredients. For example, the controlled release of drugs or selective reactants. Since the capsules are produced without contamination of the dispersed phase with catalyst or precursor, this opens the possibility of encapsulation of *e.g.* enzymes.

3.6 Experimental

Material, methyl methacrylate (MMA) (99%, Sigma Aldrich) was passed over a column packed with aluminium-oxide (activated basic, Sigma Aldrich) prior to use to remove the inhibitor. The inhibitor-free MMA was refrigerated for later use. *n*-Heptane (> 96%, Biosolve) was used as received. Azo-bis-isobutyronitrile (AIBN, initiator) (Sigma Aldrich) was recrystallized twice from methanol to ensure impurities were removed. Methanol (99.8%, Biosolve) was used as received. Poly(styrene-*block*-ethylene-*co*-propylene) (PS-*b*-EP, Kraton G1702H) was used as received. The molar mass of Kraton is 155,000 g/mol. Kraton is a linear block copolymer with low dispersity ($Đ$ = 1.09); the styrene content is 28 weight percent. Tetraethyl orthosilicate (TEOS) (Sigma Aldrich) was used as received. *n*-Hexylamine and ammonia 32% (Merck-Chemicals) were used as received.

Synthesis of *p*MMA particles, *p*MMA particles were synthesized by dispersion polymerization of MMA in *n*-heptane. The synthesis was performed in a 250 mL double-walled cylindrical reactor equipped with four baffles. A four-blade pitched impeller was used to stir the reaction mixture. The reactor was provided with a water-cooled condenser. A thermostatic bath was used to heat the reactor with water that flows through the reactor wall. The bath was heated to 80 °C. First, 50 mg Kraton and 110.5 g *n*-heptane were brought into the reactor and stirred at 100 rotations per minute (rpm) until the Kraton was completely dissolved.
Second, 0.5 g AIBN was dissolved in 33.2 g MMA separately and added to the heptane/Kraton mixture by a single addition. The reactor was constantly purged with argon to remove oxygen from the mixture. Initiation took place by heating the mixture to 80 °C, the reaction mixture was stirred for at least 3 hours at this temperature and finally cooled to room temperature. Different amounts of Kraton were used in the dispersion polymerizations, to obtain *p*MMA particles with different surface concentrations of Kraton (Table 3.2).

Table 3.2 Amounts of Kraton used in the different dispersion polymerizations of MMA in *n*-heptane and the corresponding surface concentration of Kraton on the particles at full conversion of MMA.

Amount of Kraton G1702H [mg]	199	139	66	50	34	28	26	22
Overall concentration Kraton [10^{-3} $g_{PS\text{-}b\text{-}EP}/g_{MMA}$]	6	4	2	1.5	1	0.83	0.78	0.65
Surface concentration Kraton* [10^{-3} g/m^2]	35	26.5	18.5	12.6	7.1	5.1	3.8	3.0

* The adsorption of Kraton on the *p*MMA particles was determined by measuring the concentration of unadsorbed Kraton in heptane with SEC, by using the method as described in ref. 28.

Preparation of Pickering emulsions. Pickering emulsions were produced by the addition of 16 g of double de-ionized water to 7 g of the *p*MMA particle dispersion in *n*-heptane followed by emulsification of the mixture. The *p*MMA particles were produced with a stabilizer concentration of $1.5 \cdot 10^{-3}$ [$g_{PS\text{-}b\text{-}EP}/g_{MMA}$], the particle diameter was 0.95 µm and the latex solid content was 23% g/g. Before addition of the dispersed phase, the necessary amount of the particle dispersion was further diluted with *n*-heptane, until the total weight of *n*-heptane was 120 g. The Pickering emulsion droplets were targeted to be 50 µm under the assumption that all particles are covering the w/o interface in a close-packing and that curvature effects can be neglected.[35] The Pickering emulsion was formed through the application of shear by stirring at 15.000 rpm for 2 minutes, with a rotor stator mixer (IKA Ultra Turrax T25 Basic). A certain shear rate is necessary to create enough energy to accommodate a particle at the interface and a certain shearing

time to accommodate all the particles at the interface. The Pickering emulsion was imaged by Light Microscopy (LM).

Synthesis of *p*MMA/Silica capsules and Silica particles. *p*MMA/silica microcapsules and silica particles were synthesized by the polymerization of TEOS, forming either a silica capsule around the emulsion droplets or a silica particle inside the emulsion droplets. The Pickering emulsion, as described above, was charged into a 250 mL double-walled reactor. Then 9.2 g of *n*-hexylamine was added, after which 10 g of TEOS was added. The emulsion was sufficiently stirred, to avoid settling of the emulsion droplets on the bottom but nevertheless did not coalescence. Stirring was adjusted so, that agglomeration did not occur. The thickness of the capsules was adjusted by variation of the amount of TEOS that was added, all other experimental conditions were kept the same. In the case of the synthesis of the silica particles, an equimolar amount of ammonia in water, with respect to *n*-hexylamine, was added as the dispersed phase. The capsules were imaged with Scanning Electron Microscopy (SEM). The capsule size and capsule size distribution were determined from SEM images with the freeware program ImageJ.[38] The concentrations of TEOS, *n*-hexylamine and ethanol in the heptane phase, as a function of time, were determined by Gas Chromatography (GC) measurements of the continuous *n*-heptane phase using hexadecane as internal standard. Since the microcapsules readily sediment a sample could be taken from the clean *n*-heptane phase.
During the *p*MMA/silica microcapsule synthesis, the *n*-hexylamine concentration was varied to determine the effect on the TEOS conversion as a function of time, everything else but the *n*-hexylamine concentration was kept the same as described above. Amounts of *n*-hexylamine used in different pMMA/silica microcapsule syntheses were 0.94/2.53/5.1/6.6/7.3/7.6/9 [g].

In one experiment, TEOS was added in multiple additions instead of a single addition. Before every new addition, a sample was taken for SEM analysis, to follow the shell morphology as a function of the shell thickness, see Table 3.3. New TEOS was added after at least 6 hours of reaction time, to ensure full conversion of the previous TEOS addition. Everything else was kept the same as described above.

Table 3.3 Amounts of TEOS added in multiple additions to follow the shell morphology as a function of shell thickness.

Percentage droplets left after sampling [%] [a]	100	90	80	70	60
Added TEOS [g] [b]	4	4	2.8	3	2
Calculated thickness [nm] [c]	290	670	900	1200	1400

[a] For analysis, before a new addition of TEOS, samples were taken from the reaction mixture after full conversion of the previous TEOS addition was reached, the percentage of droplets left in the reaction mixture is presented here.

[b] To follow the morphology as a function of the shell thickness, TEOS was added in multiple additions, the amount of TEOS that was added after the previously added TEOS reached full conversion and after sampling is presented here.
[c] The capsule thickness was calculated using Equations 3 and 4, $V_D = 16 \cdot 10^{-3} dm^3$ is the total volume of the dispersed phase. $r_D = 25 \mu m$ is the radius of the droplet. ρ_{silica} is the theoretical density of silica (≈ 2.15 g/mL)

To examine the role of ethanol on the microcapsule morphology, an experiment was done in which TEOS was added in multiple additions. Before every new addition, the continuous *n*-heptane phase was replaced by new *n*-heptane with the same concentration of *n*-hexylamine, and a sample was taken for SEM analyses, see Table 3.4. Since the microcapsules readily sediment, most of the heptane can be decanted and replaced with new heptane. Every new addition of TEOS was done after at least 6 hours of reaction time, to ensure full conversion of the previous TEOS addition. Everything else was kept the same as described above.

Table 3.4 Amounts of TEOS added in multiple additions to follow the shell morphology as a function of shell thickness and ethanol concentration. Before every TEOS addition the *n*-heptane phase was replaced by new *n*-heptane and *n*-hexylamine.

Percentage droplets left after sampling [%] [a]	100	90	80	70	60
Added TEOS [g] [b]	2.5	2	2	2	1.2
Calculated thickness [nm] [c]	180	340	520	720	900

[a] For analysis, before a new addition of TEOS, samples were taken from the reaction mixture after full conversion of the previous TEOS addition was reached, the percentage of droplets left in the reaction mixture is presented here.
[b] To follow the morphology as a function of the shell thickness, TEOS was added in multiple additions, the amount of TEOS that was added after the previously added TEOS reached full conversion and after sampling is presented here.
[c] The capsule thickness was calculated using Equations 3 and 4, $V_D = 16 \cdot 10^{-3} dm^3$ is the total volume of the dispersed phase. $r_D = 25 \mu m$ is the radius of the droplet. ρ_{silica} is the theoretical density of silica (≈ 2.15 g/mL)

In four comparable experiments, the TEOS concentration was varied to produce capsules with different shell thickness, see Table 3.5. Everything else was kept the same as described above.

Table 3.5 The thickness of the shell of *p*MMA/silica microcapsules was varied by using different amounts of TEOS in four experiments, while everything else but the TEOS concentration was kept the same.

Added TEOS [g]	6.88	4.13	2.75	1.38
Thickness [nm] [a]	500	300	200	100

[a] The capsule thickness was calculated using Equations 3 and 4, $V_D = 16 \cdot 10^{-3} dm^3$ is the total volume of the dispersed phase. $r_D = 25 \mu m$ is the radius of the droplet. ρ_{silica} is the theoretical density of silica (≈ 2.15 g/mL).

Light microscopy was performed on a Zeiss Axioplan Universal Microscope. The samples were prepared by placing a droplet of the Pickering emulsion on a microscope slide before placing it on the specimen stage.

Scanning Electron Microscopy (SEM) was performed on FEI Quanta™ 3D FEG low vacuum SEM/Focused Ion Beam (FIB). Samples were prepared by placing a droplet of sample on a sample holder covered by double sided carbon tape. After a sample was dried in air, it was coated with a thin layer of gold, to ensure good conductivity for SEM analysis.

Gas Chromatography was performed on a GC Flame Ionization Detector (FID) set-up consisting of a Shimazu GC-2010, with an apolar column (Varian Chrompack Capillary GC Column CP-SIL 5CB 25X0.25X0.25 μm) with a packing/coating that consists of 100% polydimethylsiloxane. The injection volume was 1 μL and the oven temperature was 300 °C. The used temperature program started at 50 °C, and retained this temperature for 1 min, then heated with a rate of 20 °C/min until 300 °C was reached and retained 300 °C for 1 min, the total program took 14.5 min. The split ratio was set at 100 and the column flow rate was 1.52 mL/min. Data processing and acquisition was done with the software program 'ThermoFisher Scientific GC solution software'.

Thermogravimetric analysis (TGA) was performed on a TA Instruments high resolution TGA Q500 V6.7 apparatus. Using a constant temperature or a heating rate of 10 °C /min under a nitrogen flow of 50 mL/min.

Size Exclusion Chromatography (SEC) was performed on a Waters Alliance system equipped with a Waters 2695 two-column separation module, with a Waters 2414 refractive index detector and a Waters 2487 UV detector. As eluent tetrahydrofuran with 1% (v/v) acetic acid was used at a flow rate of 1.0 mL/min. The injection volume that was used is 50 μL.

Interfacial tension between water and *n*-heptane as a function of the *n*-hexylamine concentration in *n*-heptane was measured using a Dataphysics DCAT 11 Dynamic Contact Angle Meter and Tensiometer apparatus. For every measurement a new solution with a certain concentration was prepared and the Wilhelmy Plate Method was used to determine the surface tension. The width of the Wilhelmy plate used was 19.9 mm and it is made of Platinum-Iridium. The temperature was 20 °C and before use the plate was heated by a flame until it turned red.

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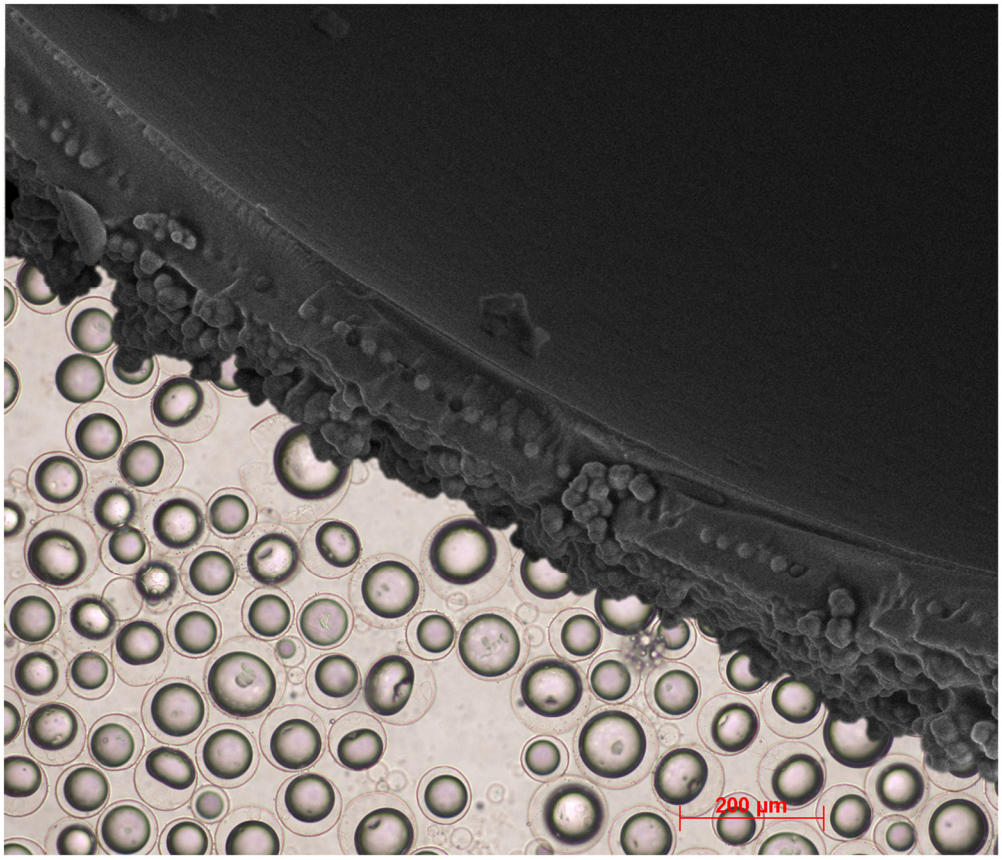
4. Silica Microcapsules by Templating Pickering Emulsion Droplets: Mechanism of Formation



Abstract

Several descriptions about the mechanism of formation of tetraethyl orthosilicate to silica particles and microcapsules in water or in alcohol with water mixtures have been put forward.[1] Nevertheless, little is known about the mechanism of formation of silica in organic solvents and about the growth direction of silica. On the basis of various analytical techniques, in this work a detailed explanation is given about the mechanism of formation of aqueous-core silica microcapsules, produced by templating Pickering emulsion droplets stabilized by silica microparticles. By the consecutive addition of two fluorescently labeled silica precursors and Confocal Fluorescence Microscopy, a novel procedure was used to determine that the shell is growing from the inside to the outside. Therefore water has to diffuse through the shell to participate in the reaction in the continuous oil phase.

4.1 Introduction

The synthesis and characterization of aqueous-core silica microcapsules has received significant attention in recent years, because of the myriad of applications that can be envisioned. Examples are the use of microcapsules for the stabilization of delicate matter, such as cells[2], bacteria[3] and enzymes[4,5], and the delivery and controlled release of active ingredients (food and drugs).[6–8] Various hypotheses have been put forward regarding the mechanism of formation of such microcapsules.[9–11] Most of these hypotheses are based on the notion that the hydrophilicity of the silica precursor changes, as a result of the hydrolysis reaction, during the course of the interfacial reaction. As a consequence, the silica precursor can either react at the interface to form a microcapsule or dissolve into the aqueous phase. However, experimental proof regarding the precise mechanism of formation is lacking. A clear understanding of the mechanism of formation is crucial for the careful tuning of material properties, in particular for the encapsulation of delicate matter, which are often sensitive towards (chemical) changes in their surroundings. A more comprehensive understanding of the location of the silica precursors, catalysts and other reactants during the interfacial reaction will make it possible to precisely choose reaction conditions that are non-destructive for the encapsulant and at the same time improves the control over the capsule formation. In this contribution, we present a novel method to determine the direction of silica growth, using confocal microscopic imaging. A fluorescein-labeled and a rhodamine-labeled silica precursor are added consecutively during the capsule synthesis and the direction of growth is determined by observation of the relative location of the dyes in the shell, using confocal fluorescence microscopy. Knowledge of the growth direction leads to a solid conclusion about the locus of reaction and provides insight into transport of reactants and products through the shell during the silica microcapsule formation. In combination with analytical results obtained from Scanning Electron

Microscopy, Light Microscopy, Gas Chromatography, ^{29}Si NMR and Karl Fisher titration, a comprehensive explanation of the mechanism of formation of the silica microcapsules is presented in this contribution.

4.2 Synthesis approach

The synthesis method for silica microcapsules in this work was developed from the basic hypothesis that the components necessary for the silica reaction only meet at the oil-water droplet interface. The interface is therefore expected to be the only place where the reaction takes place.

Silica microcapsules, with an aqueous core, were synthesized by polymerization of tetraethoxy orthosilicate (TEOS) in the presence of Pickering emulsion droplets as a template and by using *n*-hexylamine as an amphiphilic catalyst, (Fig. 4.1).[12] During this encapsulation method, silica particles are initially ultrasonically dispersed in a mixture of toluene and *n*-hexylamine. Water is emulsified in the silica particle dispersion by the use of a rotor-stator (Ultra Turrax®) high-shear mixing device to yield a water-in-oil emulsion. Such a so-called inverse Pickering emulsion is most stable when the particles have a three-phase contact angle between 94° and 110°.[13–16] However, *n*-hexylamine has amphiphilic properties (Supplementary information Fig. S1) and when an amphiphile is added to a Pickering emulsion, the stability will be determined by synergistic interactions of the amphiphilic molecules with the stabilizing particles.[17] As a result of the synergistic interactions between the silica particles and *n*-hexylamine, it proved to be possible to use hydrophilic silica particles, with a contact angle < 10°,[18,19] to stabilize an inverse Pickering emulsion (Supplementary information Fig. S2 and Table S1). The Pickering emulsion was loaded in a reactor with overhead stirring and TEOS was added subsequently.
Since water, as a reactant, is present in the emulsion droplet and TEOS is in the continuous organic phase, the precursor and water in theory should only meet at the interface. However, it has been shown in previous work that the precursor can diffuse into the water droplet upon hydrolysis and the reaction no longer takes place at the interface.[12] Moreover, it has been shown that the use of *n*-hexylamine strictly directs the reaction to the interface and not to the inside of the emulsion droplets.[12]

4.3 Results

The silica particles that were used for the Pickering emulsions were synthesized using a seeded Stöber reaction.[20,21] This resulted in silica microparticles with a narrow size distribution $D_Z/D_V \approx 1.01$, (Fig. 4.2 A). The particle concentration in the Pickering emulsion to produce a certain droplet size (assuming close-packing on the interface), and the concentration of TEOS to produce a certain shell thickness were calculated as previously reported.[12]

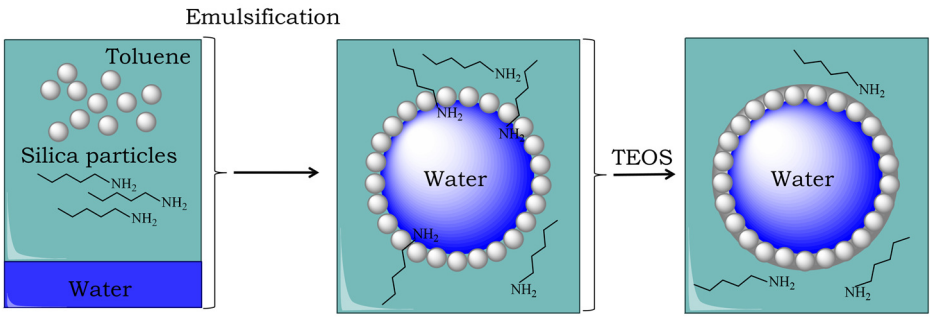


Figure 4.1 Schematic overview of the synthesis of silica microcapsules. Left) all components of the Pickering emulsion are added together. Middle) Application of shear results in the formation silica-particle-stabilized water droplets. Right) capsule formation occurs after the addition of TEOS.

Accurate tuning of the shell thickness of the microcapsules demands for a sufficiently narrow emulsion droplet size distribution. To produce a Pickering emulsion with a narrow droplet size distribution, the particles should be well dispersed in the oil phase before emulsification, to be able to form a close packing at the oil-water interface. Understandably, hydrophilic silica particles cannot be sufficiently dispersed in toluene. However, when *n*-hexylamine is added, the particles can be properly dispersed in toluene and therefore arrange in a hexagonal close packing at the interface. This resulted in an emulsion with a droplet size distribution characterized by $D_z/D_v \approx 1.1$ (Fig. 4.2 B). After the addition of TEOS, a solid amorphous silica shell was produced around the emulsion droplets. The size distribution of the capsules was identical to that of the original Pickering emulsion droplet templates (Fig. 4.2 B and C and Supplementary information Figs. S2 and S3). The SEM images show that the majority of the silica microcapsules are intact, even under the high vacuum conditions required for SEM, which means they are robust. The capsules that are broken have remarkably spherical fractures. We speculate that the formation of these spherical fractures is caused by a pressure difference that is generated upon the evaporation of water outwards and slow diffusion of air inwards. This causes a pressure difference resulting in the spherical fractures.

Interestingly, the silica particles that initially stabilize the Pickering emulsion droplets are incorporated in the shell, but can still be distinguished from the newly formed silica (Fig. 4.2 C and Supplementary information Fig. S3). It is also visible that some of the silica particles detach from the fracture surface and leave indentations. The silica particles do not seem to be covalently bound to the newly formed silica shell, since the indentations are nicely intact and no broken particles are observed at the fracture surface. From the SEM images in Figure 4.2 C it can also be concluded that the outside of the silica based microcapsules has an

irregular surface in contrast to the smooth surface of the primary silica particles and of the inside of the capsules.

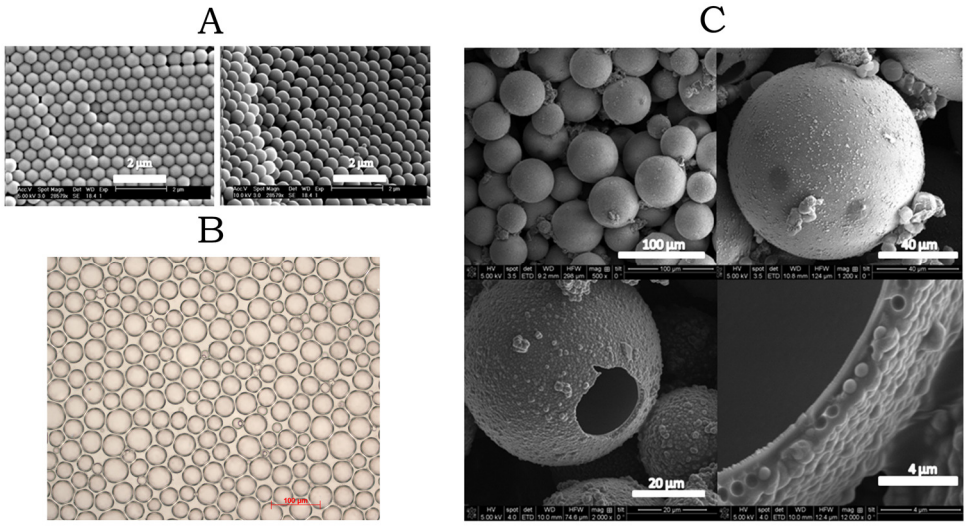


Figure 4.2 Images of various stages during the silica microcapsule synthesis. SEM images of primary silica microparticles (A), light microscopy image of the Pickering emulsion droplets (B) and SEM images of silica microcapsules (C).

The apparent lack of covalent binding between the primary particles and the shell was not anticipated, for the reason that the silanol groups on the surface of the stabilizing silica particles are reactive towards the hydrolyzed TEOS at the interface. The particles were expected to grow and be covalently bound to the newly formed silica shell, similar to the seeded Stöber polymerization technique described above.[21] Furthermore, the shell was expected to be uniform. It was anticipated that at a certain moment, the shell would form a barrier between the two phases and would cause the reaction to stop or at least to slow down significantly.

Part of the observations described above can be explained if the shell is growing from the inside to the outside. In that situation, water has to diffuse through the already formed shell to participate in the reaction. This means that the time constant for diffusion of water to the locus of reaction increases upon increasing shell thickness. As a consequence, the shell would not grow significantly beyond a certain thickness. This hypothesis could also explain the differences in silica morphology, since the reactant concentrations at the locus of reaction would change with the shell thickness. Gas chromatography (GC) was used to gain insight into the concentration of TEOS, *n*-hexylamine and ethanol in the continuous organic phase. ^{29}Si one-pulse solid state NMR was applied to detect differences in the silica structure as a function of the shell thickness. Confocal Fluorescence Microscopy was used to determine the growth direction of the shell.

4.3.1 Kinetics

During five different reactions to produce silica microcapsules, the concentration of TEOS and the concentration of ethanol in toluene were determined during the course of the reaction by using GC analysis (Fig. 4.3 and Supplementary information Figs. S4 and S5). The concentration of TEOS was varied among the five experiments, while the other conditions were kept constant. The initial concentrations of TEOS in toluene were 23, 16, 12, 8, and 7 [g mL^{-1}]. A steep initial drop in the concentration of TEOS within the first 2 minutes of the reaction was found, after which the TEOS conversion follows a sigmoidal trend (Fig. 4.3 A). Consistent with that, the ethanol concentration in toluene shows a steep initial increase, pointing to a fast initial reaction (Fig. 4.3 B). Within the first two minutes of the reaction, a silica shell cannot yet be observed with SEM or light microscopy. Besides that, using gas chromatography–mass spectrometry (GC-MS) analysis, as intermediate products the dimer and trimer of TEOS were identified in toluene, *i.e.* respectively, hexaethoxy disiloxane and octaethoxy trisiloxane, (Supplementary information Fig. S9). The relative concentration of hexaethoxy disiloxane in toluene was plotted as a function of time (Fig. 4.3 C). Additionally, a decay in the ethanol concentration in toluene could be observed after TEOS was completely converted. When TEOS has fully reacted, the ethanol concentration in the organic phase reaches its maximum. The subsequent decrease in ethanol concentration is ascribed to the partitioning of ethanol over the toluene and the water phase by transport, through the shell. This phenomenon provides indirect evidence that ethanol is produced during the interfacial reaction in the toluene phase and that the time constant of the condensation reaction is lower than the time constant for diffusion of ethanol through the shell.

^{29}Si solid state NMR was used to determine the ratio of siloxane bridges (Q4) to isolated silanols and/or ethoxy groups (Q3) in the stabilizing silica particles and the silica microcapsules with increasing shell thickness (Fig. 4.3 D and Supplementary information Figs. S6, S7 and S8). An increase of Q3 was observed when the shell became thicker upon measuring the shell thickness. After the shell reached a thickness of about 400 nm, the ratio leveled off. So, the molar concentration of isolated silanols and/or ethoxy groups in the primary silica particles is lower than in the newly formed shell. It appears that a shell is being produced with a higher ratio of Q3 to Q4 and so with more isolated silanols and/or ethoxy groups than in the primary silica. The molar ratio was measured as an average over the entire shell including the primary particles. The leveling off can be an asymptotic increase to the ratio of the shell material that differs from the ratio in the primary particles. More Q3 than Q4 in the silica shell may be due to incomplete hydrolysis, caused by a deficiency of water at the locus of reaction.

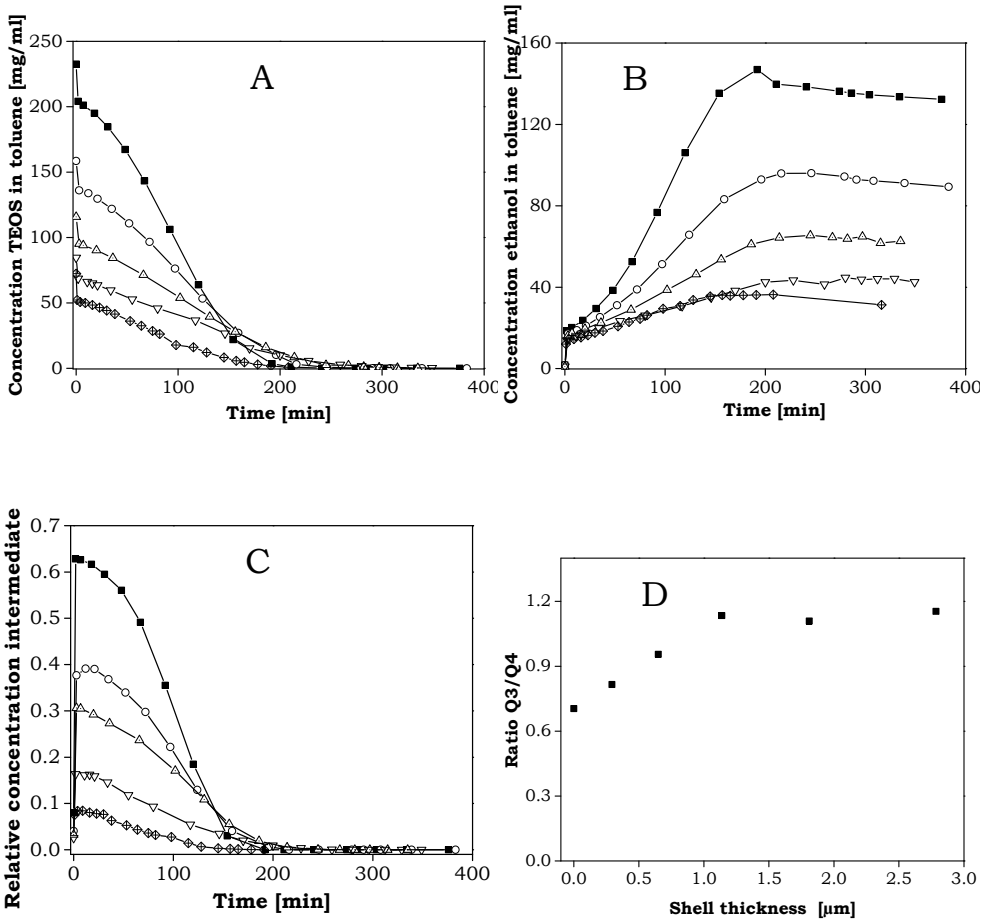


Figure 4.3 Concentration versus time profiles in toluene for various relevant species during microcapsule synthesis. The concentration of TEOS (A), of ethanol (B) and the relative concentration of hexaethoxy disiloxane (C) as a function of time, for different initial concentrations of TEOS. (D) The ratio of isolated silanols or/and ethoxy groups (Q3) to siloxane bridges (Q4) as a function of shell thickness, as determined by ^{29}Si solid-state NMR spectroscopy.

4.3.2 Confocal Fluorescence Microscopy

A fluorescein-labeled and a rhodamine-labeled triethoxysilicate precursor were added consecutively during the capsule synthesis and the direction of growth was determined by observation of the relative location of the dyes in the shell, using confocal fluorescence microscopy. Throughout the synthesis of the labeled microcapsules, TEOS was added in 5 aliquots and to the second and fourth aliquot, one of the silica precursor dyes was added. Every new addition was done after at least 3 hours of reaction time, to ensure sufficient conversion of the previously added TEOS. The labeled silica precursors were synthesized according to a literature procedure by the

addition reaction between fluorescein isothiocyanate or rhodamine B isothiocyanate and aminopropyltriethoxysilane in ethanol.[22] The amount of labeled silica precursors added was in the order of milligrams, relative to the total newly formed silica, which was in the order of grams. Both dyes were dissolved in ethanol upon addition, because they were taken directly from the crude reaction mixture. The extra ethanol that was added with the dyes did not influence the interfacial reaction, since ethanol is produced in much larger quantities during the course of the interfacial reaction. Also, when not reacted with aminopropyltriethoxysilane, the dyes do not chemically or physically adsorb to the newly formed silica shell.[22] Before the rhodamine-fluorescein-labeled silica microcapsules were imaged by confocal fluorescence microscopy, they were washed with ethanol twice to get rid of un-reacted dyes. As a result, when the fluorescein-labeled silica precursor was added before the rhodamine-labeled precursor, the confocal fluorescence microscope images clearly show that the inside of the silica microcapsules is green fluorescein-labeled and the outside is red rhodamine-labeled and *vice versa*, (Fig. 4.4 and Supplementary information Fig. S10). This provides ultimate proof that the shell grows from the inside to the outside. Besides that, the silica microparticles that initially stabilized the Pickering emulsion can be distinguished from the newly formed fluorescein-labeled silica shell. This is in agreement with the SEM images, (Figs. 4.2 and 4.4). At a later stage, the newly formed silica engulfs the particles and silica formation takes place on the whole surface of the shell. This can be observed via the dye that was added last and that as a result covers the entire surface. Also visible, is the difference in shell morphology between the inner and outer side of the microcapsules, the outer side appears to have a more irregular morphology. In addition, as the shell grows from the inside to the outside and residual ethoxy or hydroxy groups have a limiting effect on the density of silica particles[23], this means that according to the ^{29}Si-NMR results the density of the is different from the primary stabilizing silica microparticles.

Given that the shell grows from the inside to the outside, meaning that the reaction takes place on the “outside” of the microcapsules. A consequence of this is that water has to diffuse through the growing shell to participate in the reaction. Also, during the reaction on the “outside” of the capsules, the concentrations of TEOS and ethanol continuously change. This explains the changes in the morphology as well as the changes in the silica structure (Q3 to Q4 ratio).[24] It also clarifies the transport due to partitioning of ethanol from the organic to the water phase at a later stage, because the ethanol is produced in the organic phase faster than it can be transported to the water phase through the shell.

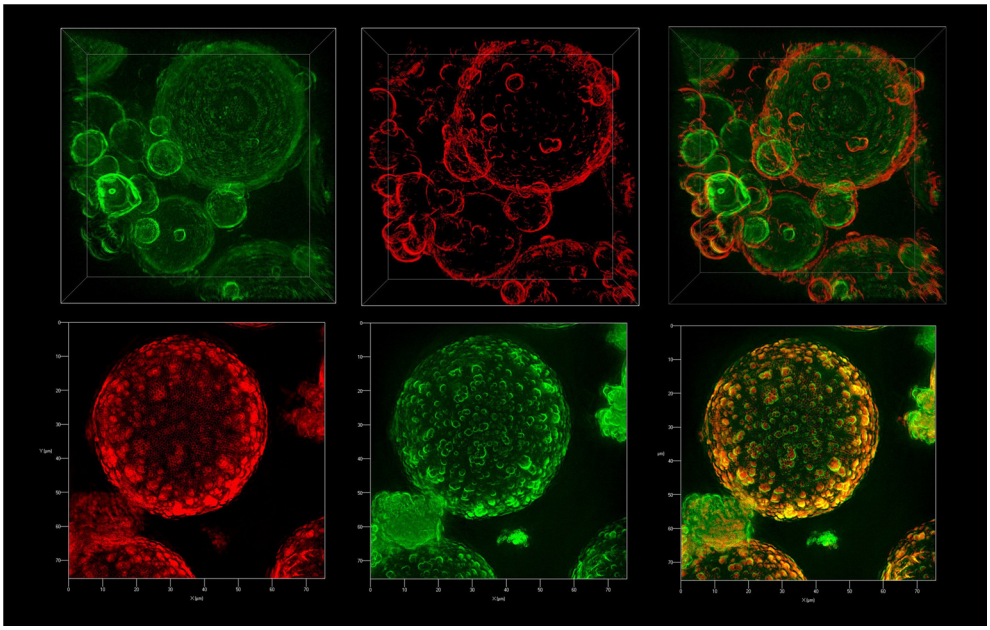


Figure 4.4 Fluorescence confocal microscopy images of Rhodamine- Fluorescein-labeled silica microcapsules (top images) and Fluorescein-Rhodamine- labeled silica microcapsules (bottom images). The two dye-labeled triethoxy silicate precursors have been added in aliquots two and four out of five total aliquots of TEOS addition.

The explanation regarding the growth direction of silica does not account for the initial drop in the TEOS concentration and the initial increase in the ethanol concentration. Also, is does not explain why the silica particles do not seem to be covalently bound to the newly formed silica shell. However, the initial reaction could be due to the presence of water in the organic phase. It was shown that the presence of *n*-hexylamine increases the concentration of water in the toluene phase, *vide supra*. In this water-saturated organic phase, the reaction (rapidly) proceeds. This would mean that the mechanism is slightly different from that previously described, *vide supra*. Namely, initially a reaction of TEOS with water takes place in the toluene phase, producing ethanol and TEOS-oligomers. When the water in the organic phase is mostly depleted because it has been converted, it starts to diffuse from the inverse Pickering emulsion droplets to the toluene phase, because of partitioning to reach the equilibrium concentration again. Upon diffusion to toluene, water rapidly reacts with TEOS to form the shell from the inside to the outside, as established from the confocal fluorescence microscope images (Fig. 4.4). Therefore, now the reaction rate is limited by the diffusion of water from the droplets to the toluene phase, through the newly formed silica shell to the outer surface.

4.3.3 The effect of ethanol

To verify this assumption, an additional experiment was conducted in which the *n*-hexylamine concentration in toluene was varied. Water was added to this mixture after which it was vigorously shaken. After phase separation, which occurred within a few minutes, part of the now water-saturated organic phase was separated from the mixture and TEOS was added. Directly after the TEOS addition, GC analysis was used to determine the conversion of TEOS, the concentration of ethanol and the relative concentration of hexaethoxy disiloxane (Fig. 4.5 B - D). Besides that, the water concentration in the organic phase was determined as a function of the *n*-hexylamine concentration using Karl Fischer titration on separate fractions of the water-saturated organic phase (Fig. 4.5 A). Except for the *n*-hexylamine concentration, all other parameters were kept constant and reaction conditions were chosen that were close to those used for the microcapsule synthesis. Moreover, TEOS conversion, the ethanol concentration and the relative concentration of hexaethoxy disiloxane were determined of the same samples but now after 48 hours and 72 hours (Fig. 4.5 B, 4.5 C and D, respectively). From reference experiments it was concluded that when dry toluene was used, the reaction did not take place, regardless of the *n*-hexylamine concentration.

From Figure 4.5 it can be observed that an increasing *n*-hexylamine concentration, leads to higher saturation concentrations of water in toluene. As a consequence, more TEOS is able to react. After a certain time the reaction essentially stops in toluene, since all water has been depleted by the hydrolysis of TEOS. During the microcapsule synthesis, the reaction does not stop but continues at the interface. At the interface, the reaction rate is limited by diffusion of water through the growing silica shell.

The *n*-hexylamine concentration *i.a.* governs the equilibrium concentration of water in the organic phase (Fig. 4.5). During one experiment, in the course of the microcapsule forming reaction, extra *n*-hexylamine was added. Every time *n*-hexylamine was added, the TEOS concentration showed another drop and the ethanol concentration increased again in an almost stepwise fashion. This is similar to the respective *initial* decrease and increase of TEOS and ethanol (Supplementary information Fig. S11). Besides that, the TEOS concentration and ethanol concentration decreased and increased faster than before the extra addition. When additional ethanol was added during the microcapsule synthesis procedure, this also increased the solubility of water in the organic phase. As a result, the total reaction time of TEOS and ethanol decreased from about 200 [min] to 120 [min] (Supplementary information Figs. S11 and S14). The increase of the reaction rate due to a higher solubility of water in the organic phase is a strong indication of faster transport of water to the organic phase, *i.e.* the reaction is diffusion limited. This observation is ascribed to the new

equilibrium conditions, *i.e.* a new equilibrium water concentration in the organic phase adjusts instantaneously.

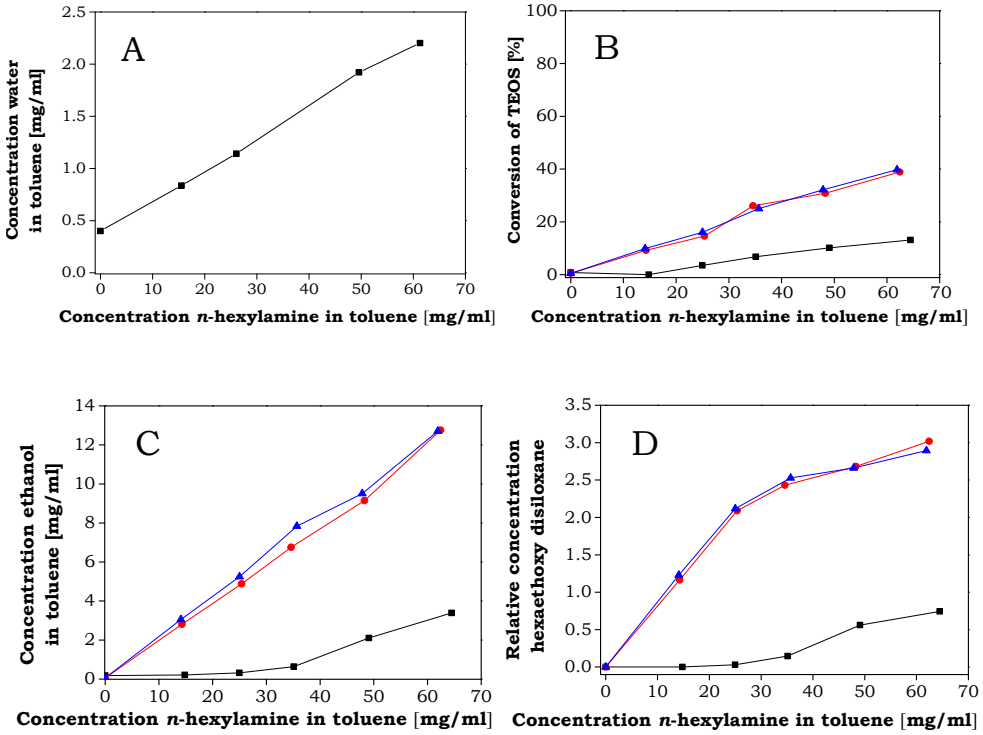


Figure 4.5 The reaction of TEOS in water-saturated toluene. The water concentration in the organic phase prior to the reaction (A), the conversion of TEOS (B), the concentration of ethanol (C) and the relative concentration of hexaethoxy disiloxane (D) as a function of the *n*-hexylamine concentration in a water-saturated organic phase. Measured directly (●), after 48 hours (▲) and after 72 hours (■).

From visual inspection of the fractured surface of the capsules it seems that the particles that initially stabilize the Pickering emulsion have very limited bonding to the newly formed silica shell. This judgment is based on the complete detachment of the particles from the fracture surface (Fig. 4.6 A plus insert and Supplementary Figs. S12 A-C). With an initial amount of ethanol in the continuous toluene phase, however, the visual difference between the newly formed silica shell and the initially stabilizing silica microparticles becomes less apparent. Detachment of the particles from the fracture surface is no longer observed (Fig. 4.6 B and Supplementary information Figs. S12 D-F). An additional observation during the reaction with increased ethanol concentration in toluene is the formation of silica gel in the continuous organic phase. When the silica microcapsules were used as seed during a seeded Stöber polymerization, the primary silica particles

appear to be completely integrated in the newly formed silica shell, (Figs. 4.6 C, 4.6 D and Supplementary information Figs. S12 G-I).

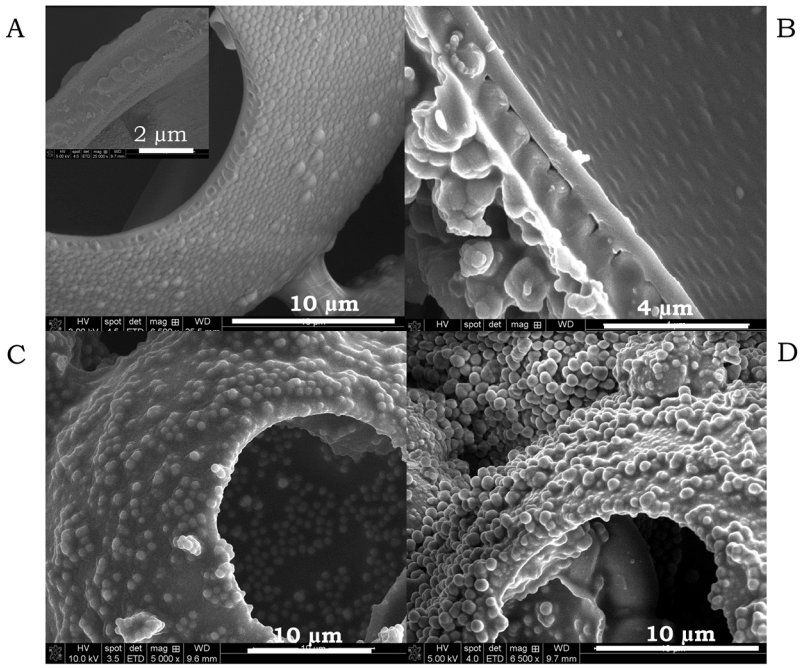


Figure 4.6 Silica microcapsules produced with different ethanol concentrations. A) By frequent replacement of a large fraction of the organic phase, the ethanol concentration was kept low; B) by addition of extra ethanol when a few nanometers of the shell was produced, the ethanol concentration was kept high from the start; C) and D) Silica capsules are used as seed during a seeded Stöber polymerization.

The main difference between the microcapsule synthesis procedure and the Stöber process is that during the Stöber process, the concentrations of the relevant species, *i.e.* reaction products, reactants and catalyst, are uniform throughout the mixture, while during the capsule synthesis this is clearly not the case. When the concentration of ethanol during the microcapsule synthesis is increased, the conditions come closer to those of the Stöber process.

4.4 Mechanism

Based on the results described above, the microencapsulation procedure can be subdivided into 3 stages. In the first stage, the water that is initially solubilized in the organic phase reacts with TEOS, followed by condensation to produce oligomers. This stage results in the initial steep drop in the TEOS concentration (Fig. 4.3).

The second stage starts when all water dissolved in the continuous phase has reacted with TEOS and all species necessary for the reaction are in direct contact only at the oil/water interface of the Pickering emulsion droplets, which results in the formation of a very thin silica shell around the emulsion droplets.

The third stage comprises the growth of the shell, *i.e.* thickening of the shell, from the inside to the outside (Supplementary information Fig. S8). In order for the reaction to take place, water has to diffuse through the shell. Two important observations can be made in relation to the third stage. The first observation relates to the conversion *versus* time histories that show a sigmoidal behavior, *i.e.* the reaction initially speeds up and later on slows down again. Our tentative explanation for this behavior is that the increased rate is caused by the gradual increase in ethanol concentration. It is well known that ethanol is a powerful dehydrating agent.[25] As such its increasing concentration in the continuous phase will lead to an enhancement in the driving force for water to diffuse through the silica shell. Therefore, the presence of ethanol in toluene from the start of the reaction, avoids a slow start up of the hydrolysis of TEOS as a consequence and the sigmoidal conversion – time history is no longer observed (Supplementary information Fig. S14). The later decrease of the rate is likely due to either the increased diffusion barrier (thickness of the silica shell), or the decrease in silica precursor concentration in the continuous phase. The second observation is the engulfment of the primary (initially stabilizing) silica particles. This engulfment takes place somewhere during the third stage, but certainly not directly from its onset. In other words, the primary particles seem to move with the outer surface of the silica layer up to a certain point, after which they are being engulfed by the newly formed silica layer. This can also be seen from the changing silica structure as a function of the shell thickness (Fig. 4.3 and Supplementary information Figs. S6, S7 and S8). We hypothesize on two relevant mechanisms for this sequence of events. As reported in earlier work, it takes time for drainage of the interfacial layer to take place.[26] As a consequence, when the primary particles absorb at the interface, a thin layer of the complete organic phase is in between the particle and the water droplet. Polymerization of TEOS can take place in this layer to form silica in between the water droplet and the particle, thus pushing the particle away from the water droplet to some extent. Simultaneously, silica oligomers are formed in the organic phase. After reaching a certain size, these oligomers will become insoluble in the continuous phase and precipitate on the growing capsules. This happens on the primary particles as well as in the interstitial areas. Further condensation and growth of these oligomers will eventually lead to engulfment of the particles. Dependent on the relative rate of the two processes described above, engulfment will take place earlier or later in the polymerization, thus leading to a thinner or thicker inner layer that separates the primary particles from the water droplet. Because of the

physical nature of the engulfment process, also non-silica microparticles are being engulfed during the encapsulation procedure, *e.g.* poly(methyl methacrylate) particles (Supplementary information Fig. S13).[12]

4.5 Conclusion

We managed to produce silica microcapsules by templating inverse Pickering emulsion droplets and by using tetraethyl orthosilicate (TEOS) as silica precursor. The aqueous Pickering emulsion droplets were stabilized by the synergistic interactions of amphiphilic *n*-hexylamine and silica microparticles. Since *n*-hexylamine acts as a catalyst for the synthesis of silica, it will perform this function in the continuous organic phase as well as on the outer surface of the inverse Pickering emulsion droplets.

The microcapsule formation mechanism was found to comprise three stages. The process starts by the addition of TEOS to a stable inverse Pickering emulsion. The first stage entails the hydrolysis of TEOS in the continuous organic phase by water that is initially solubilized in this continuous organic phase. The amount of water in the organic toluene phase is approximately linearly dependent of to the amount of dissolved *n*-hexylamine. During the first stage, oil-soluble TEOS oligomers are formed, and the first stage ends when the dissolved water is depleted due to the hydrolysis of water. In the second stage an initial thin silica shell is formed around the emulsion droplets. This takes place when most of the water in the continuous organic phase is converted. Now the reaction continues at the oil/water interface of the emulsion droplets. During the third stage, the thickness of the shell is increased from the inside to the outside. Therefore, water has to diffuse through the shell, to react on the outside of the microcapsules with TEOS. It was also found that the ethanol that is produced during the reaction has a profound effect on the morphology of the microcapsules. The formation of ethanol increases the driving force for water to diffuse through the silica shell.

Finally, the direction of silica growth was unequivocally confirmed by the addition of fluorescently labeled silica precursors that were added consecutively during the interfacial reaction and by determining the relative position of the dyes in the shell by using fluorescence confocal microscopy. This procedure is possibly useful for other studies on growth direction and morphology in the micrometer and nanometer size-range.

4.6 Experimental

Monodisperse silica microparticles. The monodisperse silica microparticles were prepared by a seeded Stöber polymerization technique.[21] First the seed particles were synthesized via the Stöber method.[20] In short, in 100 mL ethanol, 7 g TEOS (33.6 μmol) and 5 mL of water (278 μmol) were

solubilized in a three neck round bottom flask of 250 mL at 25 °C stired by a magnetic stirrer. After 10 min, 15 mL of ammonia 25% was added to base-catalyze the reaction. After at least 6 hours of reaction time 2 g of tetraethyl orthosilicate (TEOS) (9.6 μmol) was added, this was repeated multiple times. Every new 2 g TEOS (9.6 μmol) addition was done at least 6 hours after the previous addition, to ensure full conversion of the previously added TEOS. The total amount of TEOS was adjusted so the monodisperse particles would have a diameter between 500 and 1000 nm (if ρ = 2.15 g cm^{-3}). Once the reaction was completed, the particles were left to settle, after which the liquid phase was decanted and the particles were air-dried.

Pickering emulsion. The inverse Pickering emulsion was prepared by first the redispersion of the prepared and dried silica microparticles in a solution of toluene and *n*-hexylamine, using ultrasonication, until a translucent dispersion was produced. The amounts of toluene, the silica microparticles and water were calculated according to Salari et al.[27] For all prepared Pickering emulsions, formulations were chosen so that the average droplet diameter would be 50 μm. A number of concentrations of *n*-hexylamine in toluene was used. *n*-Hexyalmine in toluene concentration of 51 and 50 mg mL^{-1} resulted in a stable Pickering emulsion, see Supplementary Fig. S2 and Table S1. The inverse Pickering emulsion was produced by applying high shear via a rotor stator device (Ultra Turrax ® T18 Basic). Operating at 20,000 rpm. The time needed to obtain a proper emulsion was 2 min. The exact concentrations used in the Pickering emulsions produced in this work are described below.

Synthesis of silica microcapsules. The silica microcapsules were produced by the addition of TEOS to the inverse Pickering emulsion. The reaction proceeded under continuous overhead stirring at 25 °C. The amount of TEOS was adjusted to produce microcapsules with a certain shell thickness, calculated according to previous work.[12]

The silica microcapsules shown in Fig. 4.2 were produced with 2 g of silica microparticles (diameter 850 nm), 15.1 mL water and 115 mL toluene with 69 mg mL^{-1} *n*-hexylamine (0.67 μmol mL^{-1}). After the Pickering emulsion was produced, 17.35 g of TEOS (83 μmol) was added. The shell thickness for this formulation was estimated to be 1.33 μm.

The rhodamine-fluorescein-labeled silica microcapsules shown in Fig. 4.4 were produced with 2 g of silica microparticles (diameter 830 nm), 16 mL water and 150 mL of toluene with 52 mg mL^{-1} *n*-hexylamine (0.51 μmol mL^{-1}). After the Pickering emulsion was produced, TEOS was added in 5 batches of 4, 4, 3.7, 4.1 and 2 g (19, 19, 18, 20 and 9.6 μmol). Every new batch was added after at least 6 hours of reaction time, to ensure complete conversion of the previously added TEOS. Together with the second TEOS batch 0.5 mL fluorescein-labeled silica precursor in ethanol was added, with a

concentration of 18 mg mL^{-1} (7.8 µmol mL^{-1}). Together with the fourth TEOS batch 0.05 mL rhodamine-labeled silica precursor in ethanol was added, with a concentration of was 20 mg mL^{-1} (10.6 µmol mL^{-1}).

The fluorescein-rhodamine-labeled silica microcapsules shown in Fig. 4.4 were produced with 1.5 g of silica microparticles (diameter 750 nm), 12.8 mL water and 150 mL toluene with 47 mg mL^{-1} *n*-hexylamine (0.47 µmol mL^{-1}). After the Pickering emulsion was produced 2.5, 3, 2.4, 2.6 and 2 g batches of TEOS (12, 14, 12, 13 and 9.6 µmol) were added, after at least 6 hours of reaction time, to ensure complete conversion of the previously added TEOS. Together with the second TEOS batch, 0.05 mL rhodamine-labeled silica precursor solution in ethanol (20 mg mL^{-1}, 10.6 µmol mL^{-1}) was added. Together with the second TEOS batch, 0.7 mL fluorescein-labeled silica precursor solution in ethanol (18 mg mL^{-1}, 7.8 µmol mL^{-1}) was added.

The fluorescein-labeled and rhodamine-labeled silica precursors were synthesized by the addition reaction of (3-aminopropyl)triethoxysilane with rhodamine B isothiocyanate or with fluorescein isothiocyanate, according to van Blaaderen et al..[22,28] In short, in 5 mL anhydrous ethanol, 0.2 mmol of (3-aminopropyl)triethoxysilane (44 mg) was reacted with 0.1 mmol of the dye (53 mg rhodamine B isothiocyanate and 39 mg fluorescein isothiocyanate). The reaction proceeded for 17 hours in a nitrogen atmosphere at 25 °C.

The silica microcapsules shown in Fig. 4.6 A were produced with 2 g of silica microparticles (diameter 500 nm), 24 mL water and 175 mL of toluene with 62 mg mL^{-1} *n*-hexylamine (0.6 µmol mL^{-1}). After the Pickering emulsion was produced, 2 g of TEOS (9.6 µmol) was added. After at least 6 hours, the microcapsules were allowed to settle and 70% of the organic phase was replaced by fresh toluene (175 mL), and *n*-hexylamine (62 mg mL^{-1}, 0.6 µmol mL^{-1}). The organic phase after the reaction consisted of toluene (175 mL), *n*-hexylamine (62 mg mL^{-1}, 0.6 µmol mL^{-1}) and ethanol (1.77 g, 38 µmol). To this new mixture again 2 g of TEOS (9.6 µmol) was added. This procedure was repeated 4 times.

The silica microcapsules shown in Fig. 4.6 B were produced with 2 g of silica microparticles (diameter 700 nm), 18 mL water and 120 mL toluene with 57 mg mL^{-1} *n*-hexylamine (0.56 µmol mL^{-1}). After the Pickering emulsion was produced, 1 g of TEOS (4.8 µmol) was added, to form a very thin initial shell. Note that adding extra ethanol will destabilize the Pickering emulsion. After 3 hours reaction time, 8 g ethanol (174 µmol) and 20 g TEOS (96 µmol) were added. The shell thickness was estimated to be 1.3 µm.

The silica microcapsules from Fig. 4.6 C-D were synthesized by using the silica microcapsules (1.9 g) from Fig. 4.2, produced with 16 g mL^{-1} TEOS (77 µmol mL^{-1}), as seed in the Stöber process. In 127 g ethanol (2.8 mol), 5 mL water (0.28 mol), 15 mL ammonia (32%) and 6 g TEOS (29 µmol) an amount

of 1.9 g of the silica microcapsules were dispersed. During the reaction also new silica particles were produced which partially fused with the seed microcapsules, besides the reaction of TEOS with the seed microcapsules.

The reaction of TEOS in a water saturated organic phase. First, different mixtures were prepared of 7.8 mL toluene with varying concentrations of *n*-hexylamine and 1 mL water was used to saturate the toluene/*n*-hexylamine mixture with water. The mixture was vigorously shaken. After which the mixture was allowed to phase separate. Then, 4 g of the water saturated organic phase was separated from the mixture and 0.2 g TEOS (0.96 μmol) was added to react with water in the organic phase. The *n*-hexylamine concentrations in toluene were: 0, 15, 27, 38, 53, and 68 mg mL^{-1}, respectively, 0, 0.15, 27, 38, 52 and 67 μmol mL^{-1}.

Kinetic studies. Gas chromatography was used to determine the concentrations of TEOS, ethanol, *n*-hexylamine and the relative concentration of the intermediate product, hexaethoxy disiloxane, as a function of time (Fig. 4.3 and Supplementary Figs. S4 and S5). The relative concentration of hexaethoxy disiloxane is the ratio of the peak area of hexaethoxy disiloxane and the peak area of the internal standard hexadecane. This was done during the silica microcapsule synthesis procedure as described above, for different initial TEOS concentrations. The inverse Pickering emulsion droplets and at a later stage the growing silica microcapsules, readily sediment as soon as stirring is stopped. Samples for GC analysis were taken from the clear organic phase on the top of the reaction mixture while the stirring was shortly stopped. This interruption of stirring did not influence the course of the reaction. Note that time during which the stirring is stopped is much smaller than the total reaction time (seconds versus hours). As an internal standard, hexadecane was used, which was added simultaneously with TEOS. See also Supplementary Information for the details of the preparation and more details about the characterization of the microcapsules for the kinetic studies done with GC analysis.
^{29}Si solid state NMR was used to determine the ratio of siloxane bridges to isolated silanols and/or ethoxy groups, Q4 to Q3 (See Supplementary Information S7), in the stabilizing silica particles and silica microcapsules with increasing shell thickness (Fig. 4.3 D and Supplementary information Figs. S6, S7 and S8). In which Q is a silicon-oxygen tetrahedron and the number indicates to how many other tetrahedrons it is attached.[24] Before analysis, the silica microparticles and microcapsules were dried under vacuum at 25 °C. See also Supplementary Information for the details of the preparation and more details about the characterization of the microcapsules for the kinetic studies done with ^{29}Si solid state NMR.

A complete overview of the used materials and more detailed information concerning the used analysis techniques is presented in the Supplementary Information.

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Supplementary information

Materials All chemicals were used as received, unless indicated otherwise. Tetraethyl orthosilicate (TEOS), (3-aminopropyl)triethoxysilane (99%), Rhodamine B isothiocyanate ($C_{29}H_{30}ClN_3O_3S$, λ_{ex} 543 nm, λ_{em} 580 nm in methanol) and hexadecane (minimum 99%) were purchased from Sigma Aldrich. *n*-Hexylamine, ethanol anhydrous (99.8%) and ammonia 32% were purchased from Merck-Chemicals. Toluene (AR) and Ethanol absolute (dehydrated AR) were purchased from Biosolve. Fluorescein 5(6)-isothiocyanate ($C_{21}H_{11}NO_5S$, λ_{ex} = 495 nm, λ_{em} = 525 nm) (90% HPLC) was purchased from Fluka. The water used was double de-ionized water produced by an Elix Millipore purification system.

Preparation of microcapsules for the kinetic studies The silica microcapsules used to generate the results shown in Fig. 4.3 A-B, from which the organic phase was used for Gas Chromatography analysis, were produced in 5 different experiments, respectively. With silica microparticles (experiment 1 – 4; 2 g and experiment 5; 2 g and the silica particles had a diameter of experiment 1 – 4; 400 nm and experiment 5; 490 nm), water (experiment 1 – 4; 23 mL and experiment 5; 25 mL), and toluene (experiment 1 – 4; 150 mL and experiment 5; 140 mL), with a concentration *n*-hexylamine (experiment 1 – 4; 60 (mg mL^{-1}), 59 (μmol mL^{-1}) and experiment 5; 70 (mg mL^{-1}), 69 (μmol mL^{-1})). And after the Pickering emulsion was produced TEOS was added, so the shell thickness was targeted to be 1.8, 1.3, 0.8, 0.6, 0.46 μm, respectively, see table 1.

Table 1. To examine the reaction kinetics as a function of time microcapsules were produced with different initial TEOS concentrations.

Experiment	1	2	3	4	5
Concentration TEOS (g mL^{-1})	23	16	11	8	7
Concentration TEOS (μmol mL^{-1})	111	76	53	38	34
Targeted shell thickness (μm)	1.8	1.3	0.8	0.6	0.46

The silica microcapsules used to generate the results in Fig. 4.3 D, S6, S7 and S8, that were used for ^{29}Si solid state NMR, were produced with 2 g of silica microparticles (diameter 400 nm), 15.9 mL water and 170 mL toluene with a *n*-hexylamine concentration of 70 mg mL^{-1} (0.69 μmol mL^{-1}) After the Pickering emulsion was formed, 5 batches of 4 g TEOS (19 μmol) were added, each batch after at least 6 hours of reaction time to ensure complete conversion of the previous TEOS addition. Before every new addition, a sample was taken for analysis. Based on the volumes of the samples, after the first sample, 91% of the microcapsules were left in the reaction mixture. Before the third, fourth and fifth addition 79%, 65% and 53% of the original amount of microcapsules was left, respectively. As a result of the various concentrations, the shell thicknesses in the samples were estimated to be 0.29, 0.65, 1.1, 1.8 and 2.8 μm, respectively.

The silica microcapsules shown in Fig. S11 are from two separate experiments, in which the organic phase was used for gas chromatographic analysis. For both experiments, 2 g (diameter = 950 nm) of silica microparticles were used in conjunction with 13.6 mL water. The average droplet size of the inverse Pickering emulsion was estimated to be 50 µm. In one experiment three times 3 g TEOS (14 µmol) was added during the course of the reaction (at t = 23, 70 and 134 min). In this experiment, 156 mL toluene with 62 mg mL^{-1} *n*-hexylamine (0.61 µmol mL^{-1}) and initially 10.7 g TEOS (51 µmol mL^{-1}) were used. In the other experiment, extra *n*-hexylamine was added three times during the reaction. In that experiment 160 mL toluene with initially 46 mg ml^{-1} *n*-hexylamine (0.46 µmol mL^{-1}) and 15.15 g TEOS (73 µmol) were used. Subsequently, 4, 1.88 and 2.1 g additional *n*-hexylamine (40, 19 and 21 µmol) were added, respectively (at t = 21, 40 and 100 min).

Imaging studies (Light microscopy (LM), Scanning Electron Microscopy (SEM) and Fluorescence Confocal Microscopy (FCM)) Optical microscopy was used to image the produced Pickering emulsions. The microscopy was performed on a Zeiss Axioplan Universal Microscope. Samples were prepared by placing a droplet of the Pickering emulsion on a glass microscope slide, before placing it on the specimen stage.
Scanning electron microscopy (SEM) was used to examine the silica microcapsules on, amongst other things, morphology and shell thickness. SEM was performed on a FEI Quanta™ 3D FEG low vacuum SEM/Focused Ion Beam (FIB) instrument. The samples were prepared by placing a droplet of the sample on a sample holder covered by double-sided carbon tape. After the sample was dried in air, it was sputter-coated with a thin layer of gold, for good conductivity during SEM analysis.
The Rhodamine B and Fluorescein labelled silica microcapsules were analysed with a fluorescence confocal microscope (FCM). The microscope used for FCM is a Carl Zeiss LSM 780 with Elyra S.1 superresolution platform. The images were made with a 561 nm (100 mW) laser (red) and with a 488 nm (100 mW) laser (green).

Preparation of Pickering emulsions. The concentrations of the Pickering emulsions from S2 were calculated according to Salari et al..[1] The average emulsion droplet diameter was estimated to be 50 µm. For stabilization 1 g of silica microparticles was used, with an average particle diameter of 340 nm. The total volume of the dispersed droplets was 18 mL. For that reason, in every sample 18 mL toluene was mixed with 18 mL water, so water or toluene could be the dispersed or continuous phase. To the different samples 0.12 (S2-A), 0.36 (S2-B), 0.72 (S2-C), 1.2 (S2-D) and 1.35 (S2-E) mL *n*-hexylamine was added. Experiments S2-D and S2-E resulted in stable inverse Pickering emulsions.

The poly(methyl methacrylate)-silica microcapsules shown in Figure S13 were produced according to previous work[2]. In short, 1.6 g of *p*MMA particles stabilized by poly(styrene-*block*-(ethylene-*co*-propylene)) (diameter = 785 nm) dispersed in a 156 mL solution of *n*-hexylamine in *n*-heptane(32 mg mL^{-1}, 0.32 μmol mL^{-1}) and 23.5 mL water were used. The average droplet diameter of the inverse Pickering emulsion was estimated to be 50 μm. Enough TEOS (24 g, 117 μmol) was added to the Pickering emulsion to produce a silica shell with a thickness of 2.4 μm.

GC methods The concentrations of various compounds in the organic phase during the microcapsule synthesis were determined with gas chromatography (GC), with hexadecane as internal standard. The GC analysis was performed on a GC Flame Ionization Detector (FID) with a Shimazu GC-2010, equipped with an a-polar column (Varian Chrompack Capillary GC Column CP-SIL 5CB 25X0.25X0.25 μm, with a packing/coating that consists of 100% poly(dimethyl siloxane)). The injection volume was 1 μL and the oven temperature was 300 °C. The oven temperature program started at 50 °C, retained this temperature for 1 min. The oven was then heated with a rate of 20 °C/min until a temperature of 300 °C was reached and retained 300 °C for 1 min. The total program took 14.5 min and the split ratio was set at 100. The column flow rate was 1.52 mL/min. The software program 'ThermoFisher Scientific GC solution software' was used for data processing and acquisition.

NMR methods. To determine the ratio of Q3 and Q4 (see Figure 3) in silica microparticles and in silica microcapsules as a function of their shell thickness, solid state ^{29}Si one pulse MAS NMR was used. Q is a silicon-oxygen tetrahedron and the number indicates to how many other tetrahedrons it is attached.[3] The instrument is an SS NMR Varian spectrometer (VNMRS WB 500 solids) with a static magnetic field strength of 11.7 T (corresponding to a proton larmor frequency of 500 MHz) and a probe of 6 mm MAS. For the used parameters, the MAS frequency was 4000 Hz, the pulse length used was 4.75 μs (90° ^{29}Si), the ^{1}H decoupling field was 55 kHz, the relaxation delay was 60 s (no complete relaxation) and for processing the line broadening was 100 Hz.

Interfacial tension. The interfacial tension between water and toluene, as a function of the *n*-hexylamine concentration in toluene was measured using a Dataphysics DCAT 11 Dynamic Contact Angle Meter and Tensiometer apparatus, see Fig. S1. A new solution with a predefined concentration was prepared for every measurement. The Wilhelmy Plate Method was used to determine the surface tension. The Wilhelmy plate was made of Platinum-Iridium and the width of the plate was 19.9 mm. Before use the plate was heated by a flame until it turned red and every measurement was performed at a temperature of 20 °C.

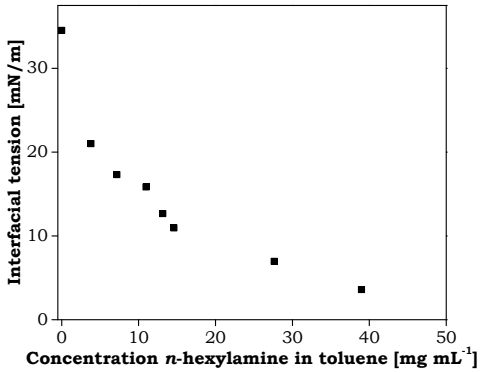

Figure S1. Interfacial tension between water and toluene as a function of the *n*-hexylamine concentration in toluene. Before the addition of water a solution of n-hexylamine in toluene was made (0, 3.8, 7.2, 11, 13, 15, 28 and 39 mg mL^{-1}). After the addition of water the measurement was done.

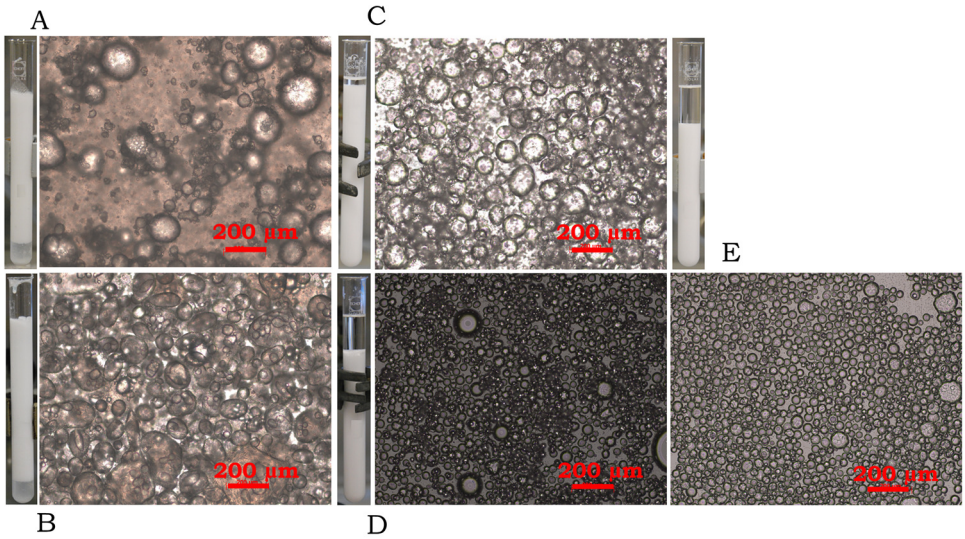

Figure S2. Phase inversion[4] as a result of *n*-hexylamine concentration. Light microscopy images, of Pickering emulsions (A and B) and inverse Pickering emulsions (C to E) stabilized with silica particles. The concentrations of *n*-hexylamine in the toluene phase were 5, 15, 31, 51 and 57 mg mL^{-1}, the other concentrations were kept the same for all emulsions.

In the case that inverse Pickering emulsions are stabilized by silica microparticles and *n*-hexylamine, the stability will be determined by synergistic interactions of the amphiphile with the stabilizing particles.[4–6] Table S1. presents a summary of experimentally determined data of recipes

for *stable* water in toluene Pickering emulsion droplets. All the concentrations, except the *n*-hexylamine concentration, were calculated according to Salari et al. with an average droplet diameter of 50 µm.[1]

Table S1. Summary of experimentally determined data of recipes for *stable* water in toluene Pickering emulsion droplets.

Volume dispersed phase [mL]	Diameter silica particles [µm]	Concentration *n*-hexylamine in toluene [mg mL^{-1}]	Total surface of the emulsion droplets [m^2]	Total mass of the used silica particles [g]
2	0.4	39	0.2	0.1
3	0.4	48	0.4	0.2
4	0.5	53	0.5	0.3
4	0.4	52	0.5	0.2
4	0.8	32	0.1	0.5
10	1.0	63	1.2	1.5
12	1.0	67	1.4	1.9
13	0.8	47	1.5	1.5
14	1.0	62	1.6	2.0
14	1.0	49	1.6	2.0
14	1.0	60	1.6	2.1
15	0.9	69	1.8	2.0
15	0.8	53	0.5	1.8
15	0.8	54	1.9	2.0
18	0.5	59	2.1	1.4
18	0.5	54	2.2	1.5
23	0.4	45	2.8	1.5
25	0.5	65	3.0	2.1
30	0.3	62	3.6	1.6
32	0.4	62	3.8	2.0
32	0.3	46	3.9	1.7

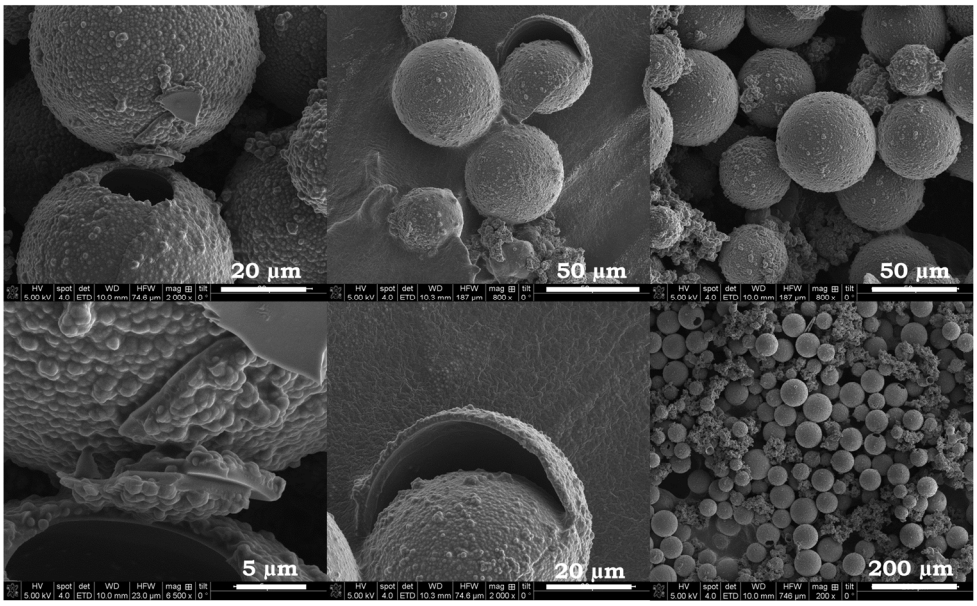

Figure S3. SEM images of silica microcapsules produced by templating inverse Pickering emulsion droplets. The silica microcapsules are the same as in Fig.2 and were produced with 2 g of silica microparticles (diameter 850 nm), 15.1 mL water and 115 mL toluene with 69 mg mL^{-1} *n*-hexylamine. After the Pickering emulsion was produced, 17.35 g of TEOS was added. The shell thickness was estimated to be 1.33 µm.

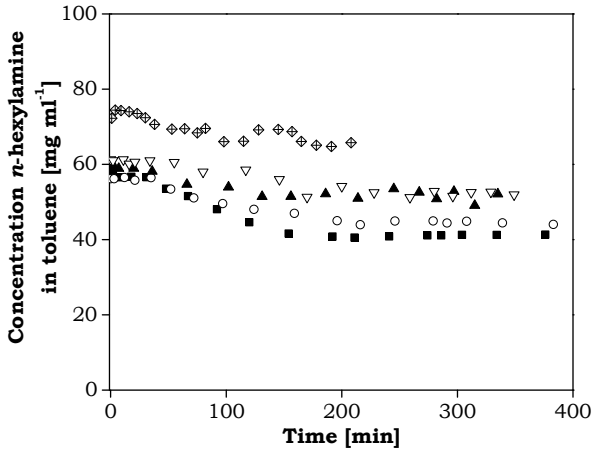

Figure S4. Concentrations of *n*-hexylamine in toluene as function of time during the interfacial silica microcapsule forming reaction, for different initial concentrations of TEOS determined by GC analysis. The decay in the concentration of *n*-hexylamine is attributed to adsorption on the silica shell and onto the pores of the growing shell. The total area of the shell, onto which the amine can adsorb, is increasing as a function of time. *n*-Hexylamine is known to adsorb onto silica gel[7]. The starting concentrations of TEOS (23 (■), 16 (⬤), 11 (▲), 8 (◣) and 7 (●)(g mL^{-1}) which is 111, 76, 53, 38 and 34 (µmol mL^{-1}), respectively) were different, all other conditions were kept the same.

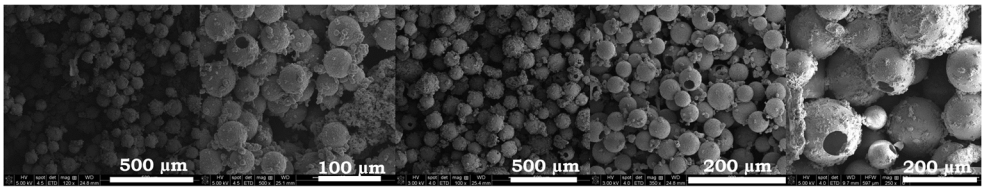

Figure S5. Silica microcapsules produced in the kinetic studies, see Figure 3. The starting concentrations of TEOS (23, 16, 11, 8 and 7 (g mL^{-1}) which is 111, 76, 53, 38 and 34 (µmol mL^{-1}), respectively) were different, all other conditions were kept the same.

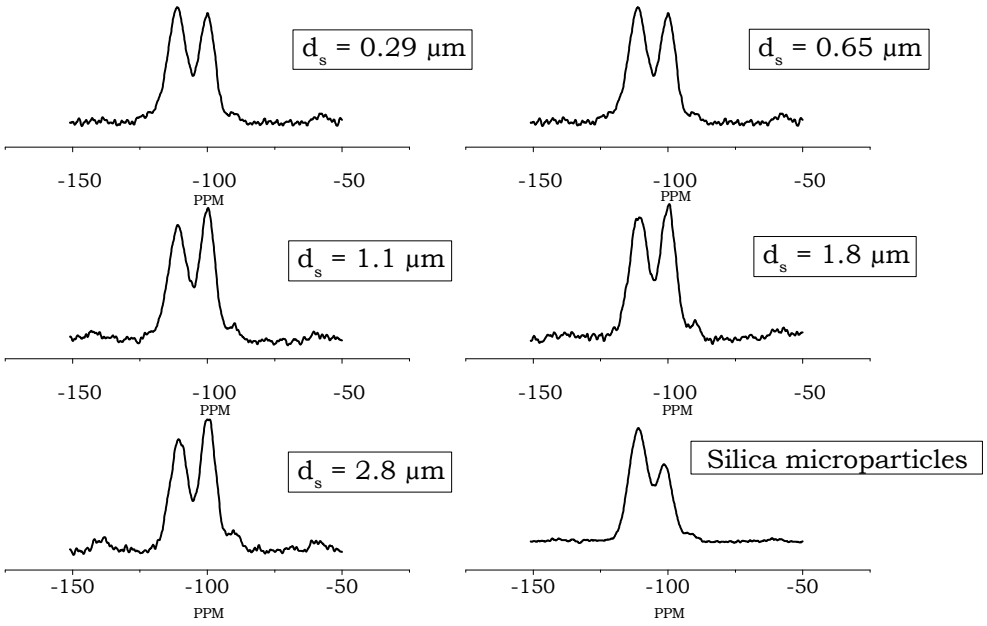

Figure S6. ^{29}Si one-pulse MAS NMR spectroscopy results, of silica microparticles produced by the Stöber method[8] and of silica microcapsules produced by templating Pickering emulsion droplets. The shell thickness (d_s) was estimated to be 0.29, 0.65, 1.1, 1.8 and 2.8 µm.

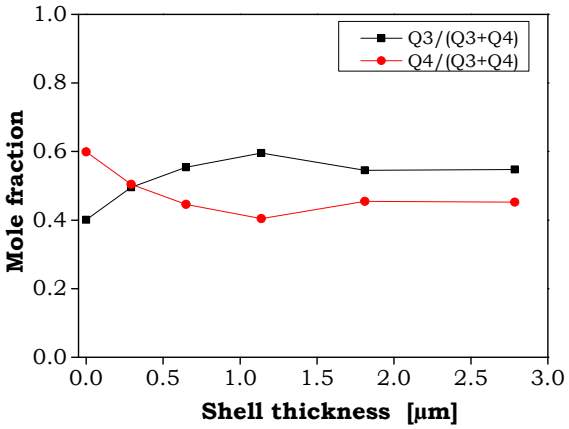

Figure S7. Mole fractions Q3 and Q4 in the silica microparticles produced by the Stöber method[8] (shell thickness = 0 µm) and silica microcapsules, calculated from the ^{29}Si NMR results. The shell thickness (d_s) was estimated to be 0.29, 0.65, 1.1, 1.8 and 2.8 µm. In the calculations Q2 was ignored.

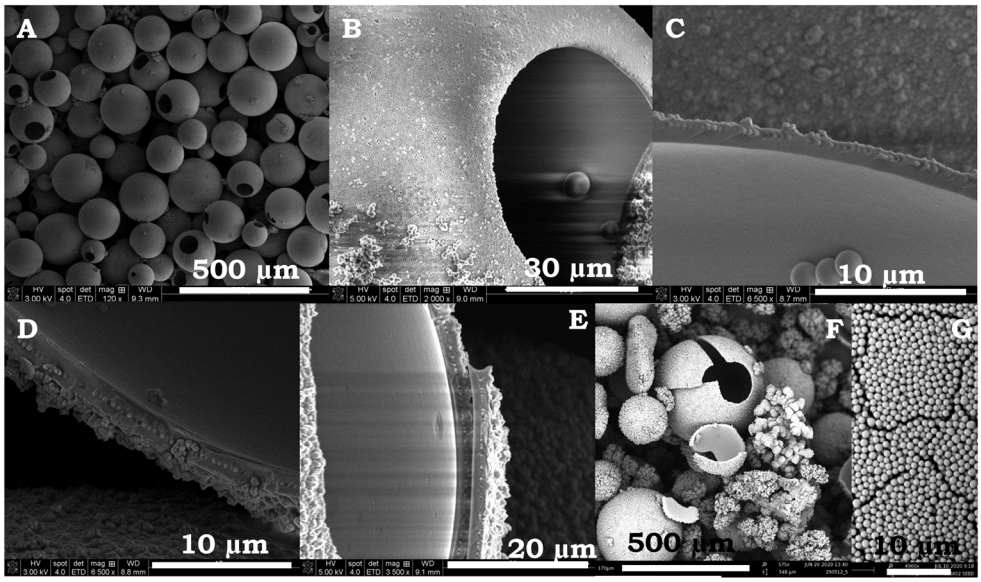

Figure S8. SEM images of the silica microcapsules that were used for ^{29}Si NMR analysis. (A): overview of the synthesized microcapsules. The shell thickness was estimated to be 0.29 µm for (B), 0.65 µm for (C), 1.1 µm for (D), 1.8 µm for (E) and 2.8 µm for (F). The primary silica microparticles used for the microcapsule synthesis are presented in image (G).

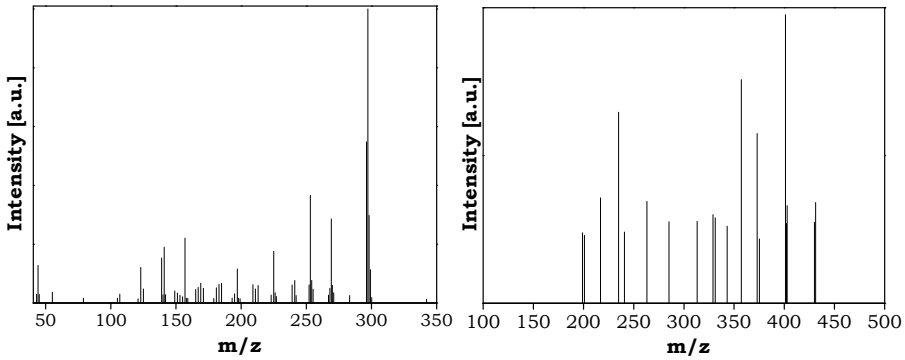

Figure S9. GC-MS spectrum of hexaethoxy disiloxane (left) and octaethoxy trisiloxane (right).

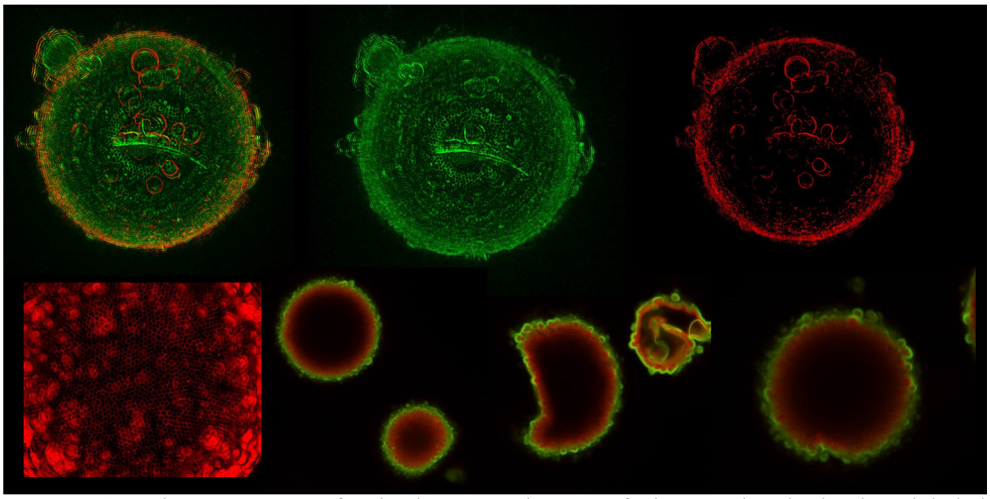

Figure S10. Fluorescence confocal microscopy images of Fluorescein-Rhodamine- labeled silica microcapsules (top images) and Rhodamine- Fluorescein-labeled silica microcapsules (bottom images). Determination of the growth direction of the shell, from the inside to the outside, from observation of the order of two different dyes in the silica shell. The two dye-labeled triethoxy silicate precursors have been added in aliquots, two and four out of five total aliquots of TEOS addition.

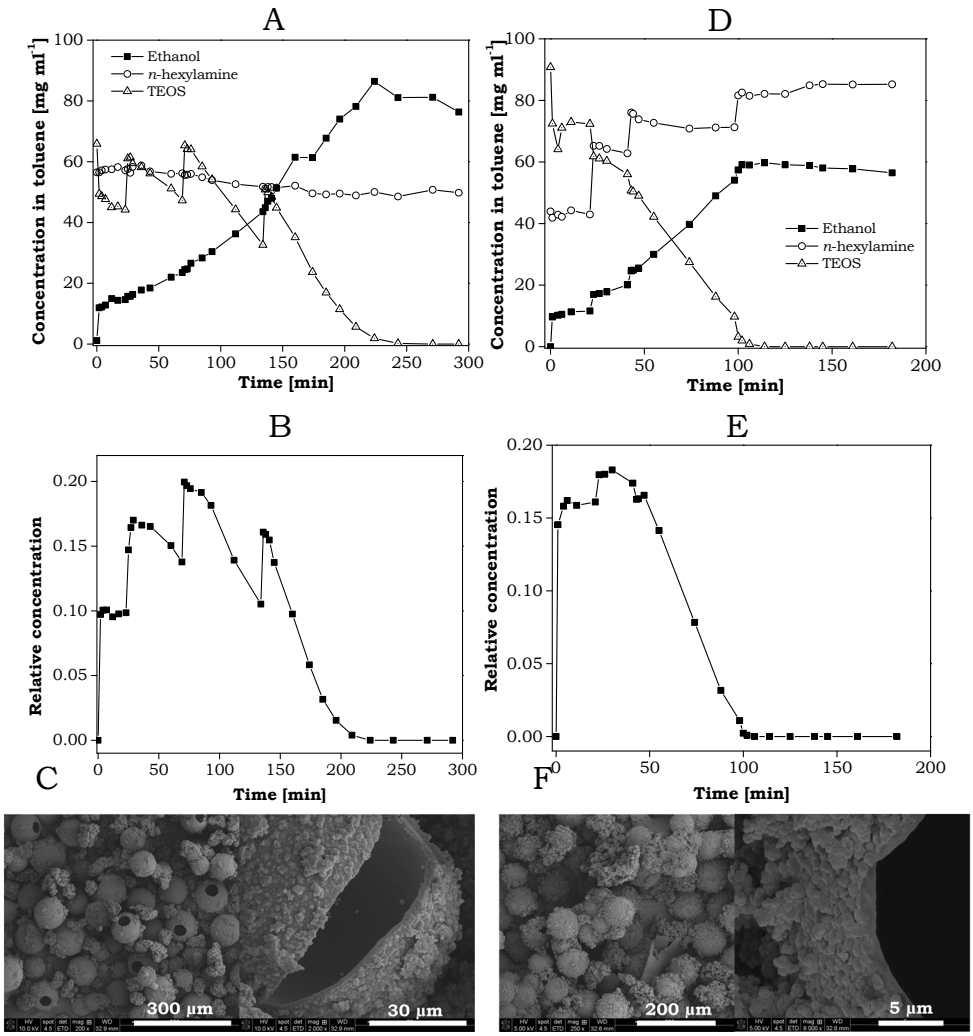

Figure S11. Concentrations of TEOS, ethanol and *n*-hexylamine in toluene and the relative concentration of hexaethoxy disiloxane as function of time determined with GC analysis and SEM images of the produced silica microcapsules. During one experiment extra TEOS was added 3 times during the course of the reaction, (A) presents the concentration in toluene of ethanol (●), *n*-hexylamine (▲) and TEOS (■) as a function of time, (B) presents the relative concentration of hexaethoxy disiloxane in toluene as a function of time. The relative concentration is the ratio of the peak area of the intermediate and the peak area of hexadecane which was used as internal standard. Finally (C) shows SEM images of the produced silica microcapsules.
During one experiment extra *n*-hexylamine was added 3 times during the course of the reaction, (D) presents the concentration in toluene of ethanol (■), *n*-hexylamine (●) and TEOS (▲) as a function of time, (E) presents the relative concentration of hexaethoxy disiloxane in toluene as a function of time. The relative concentration is the ratio of the peak area of the intermediate and the peak area of hexadecane which was used as internal standard. Finally (C) shows SEM images of the produced silica microcapsules.

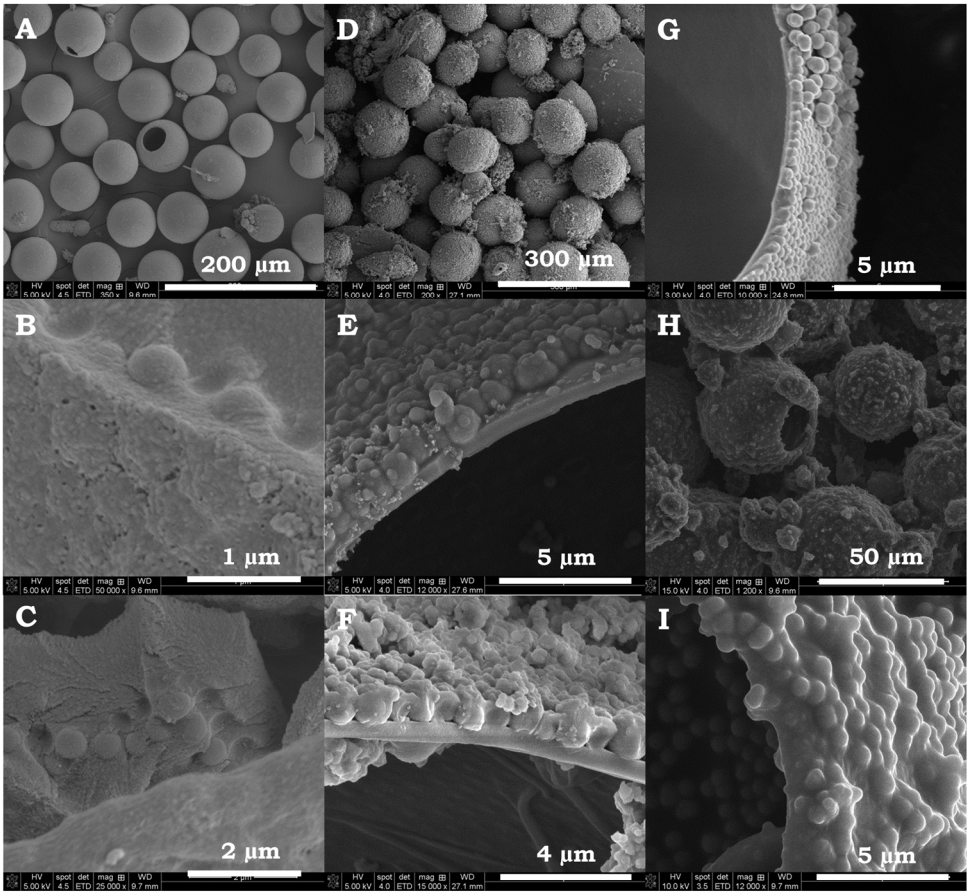

Figure S12. SEM images of the shell morphology for different ethanol concentrations during the reaction. A-C: Frequent replacement of a large fraction of the organic phase kept the ethanol concentration low; D-E: by addition of extra ethanol after a few nanometers of shell was produced kept the the ethanol concentration high from the start; G: The silica capsules are used as seed during a seeded Stöber polymerization; H-I: Silica microcapsules after the seeded Stöber polymerization technique.

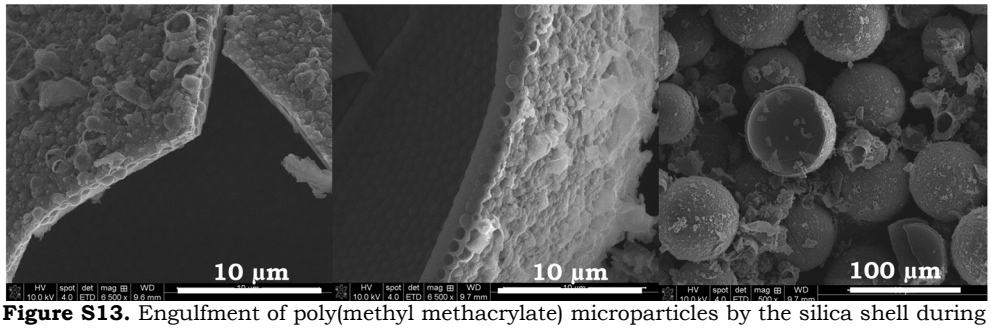

Figure S13. Engulfment of poly(methyl methacrylate) microparticles by the silica shell during a microcapsules synthesis according to chapter 3. The average droplet diameter of the inverse Pickering emulsion was estimated to be 50 µm. Enough TEOS was added to the Pickering emulsion to produce a silica shell with a thickness of 2.4 µm.

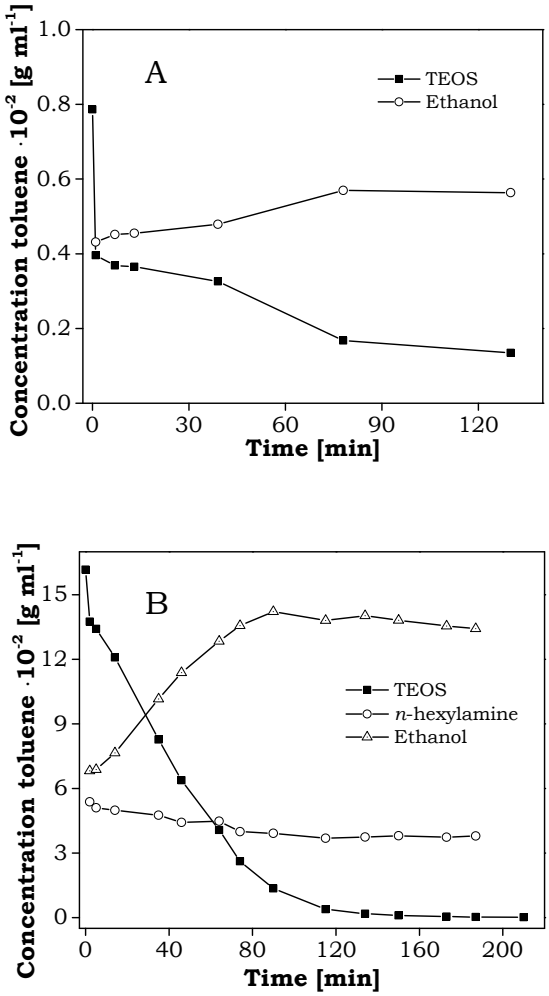

Figure S14. Concentration profiles of TEOS, ethanol and *n*-hexylamine in toluene during a microcapsule synthesis procedure. Additional ethanol was added to keep the ethanol concentration in the organic phase high early in the reaction. During the synthesis procedure, before addition of extra ethanol an initial amount of TEOS was used to produce an initial shell. After 3 hours reaction, more ethanol and TEOS were added. For the reason that, ethanol influences the varioust interfacial Gibbs energies in the inverse Pickering emulsion, addition of ethanol will result in destabilization and phase separation of the emulsion. (A): Concentrations of TEOS and ethanol in toluene as function of time during the interfacial reaction, concentrations were determined with GC, for the formation of the very thin initial shell (90 nm). The initial *n*-hexylamine concentration is 57 mg mL^{-1} (0.56 µmol mL^{-1}) (B): Concentrations of TEOS, ethanol and *n*-hexylamine in toluene as function of time during the further growth of the silica shell, after a batch wise addition of ethanol and TEOS to the reaction mixture.

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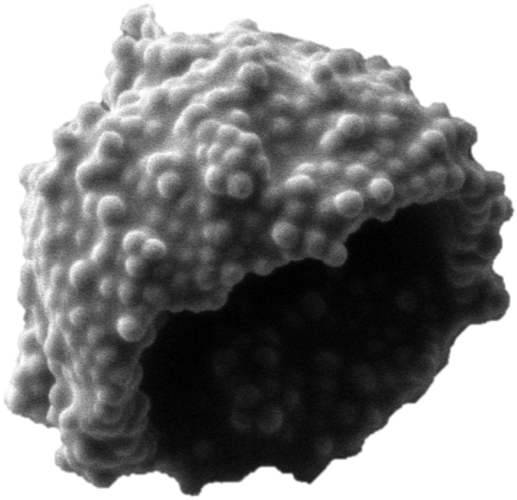
5. Hybrid Poly(styrene-*co*-maleic anhydride) – Silica microcapsules



Abstract

In this contribution we report the synthesis of hybrid poly(styrene-*co*-maleic anhydride) – SiO_2 microcapsules by crosslinking of the stabilizing particles of an inverse Pickering emulsion droplet at the interface. This was achieved by the ring-opening aminolysis reaction of the maleic anhydride residue of poly(St-*co*-MAh), with amine-functionalized silica particles, that stabilized the Pickering emulsion. The crosslinking reaction is clearly shown by labeling the polymer with a green dye and the silica particles with a red dye, followed by confocal fluorescence microscopy analysis. Because poly(St-*co*-MAh) is a versatile polymer that can react with different other polymers, this opens the possibility of producing microcapsules with versatile properties. Encapsulation of delicate matter, *e.g.* live cells or enzymes might be suitable as a results of the straightforward synthesis method.

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5.1 Introduction

In view of different controlled release applications for microcapsules, the synthesis of microcapsules has gained increased attention during the past decade, *e.g.* in the food industry or in the drug industry.[1–4] In the process of microencapsulation, typically a solid shell is formed around a micrometer-sized droplet. For controlled release applications, the shell material should be semi-permeable and should possess a certain strength and toughness, so that it does not easily break. This strength is among others necessary upon exposure to external forces, *e.g.* during re-dispersion of the capsules in a different medium.
Pickering emulsion droplets are suitable templates for microencapsulation, caused by their extreme stability against coalescence.[5,6] A Pickering emulsion is an emulsion solely stabilized by solid particles. The crosslinking of inorganic particles at the interface of a Pickering emulsion droplet proved to be a powerful method to produce microcapsules.[7–10] In that situation, the interstitial space among the stabilizing particles of a Pickering emulsion droplet can be filled with polymer. In order to synthesize microcapsules by templating Pickering emulsion droplets, a polymer is necessary that can interconnect the particles at the interface, also referred to as crosslinking of the microparticles at the interface. A very useful feature of the particles that stabilize the Pickering emulsion is that they should be easy to chemically modify. This will make it straightforward to tune the hydrophobicity and to make the particles suitable for stabilization of the emulsion.[11–13] In addition, the same feature will allow the introduction of reactive groups for the crosslinking (interconnecting) reaction. Alternatively, the crosslinking polymer may also be able to react with a different polymer that can add a responsive property, so the capsules are responsively permeable.

Poly(styrene-*co*-maleic anhydride) is a versatile polymer that can react with different other polymers, which results in tunable properties of the polymer material formed.[14–16] Silica particles are known to be suitable for Pickering stabilization.[11,17,18] This is largely caused by their tunable hydrophobicity upon modification.[19,20] Modification of silica particles is possible because of the large number of reactive silanol groups at the surface. These silanol groups are also suitable for the introduction of reactive groups for the crosslinking reaction on the particle surface.
In this contribution, we report the facile synthesis of hybrid poly(St-*co*-MAh) – SiO_2 microcapsules by crosslinking of the stabilizing particles of an inverse Pickering emulsion droplet, see Figure 5.1. This was achieved by the ring-opening aminolysis reaction of the maleic anhydride residue of poly(St-*co*-MAh), with amine-functionalized stabilizing silica particles (Figures 5.2 and 5.3), which produces an amide and a carboxylate group. The location of the components is clearly shown by labeling the polymer with a green dye and the silica particles with a red dye, followed by confocal fluorescence microscopy analysis. The crosslinking reaction is shown via a redispersion experiment, in aqueous media, followed by observation via light microscopy analysis. After redispersion in water, the capsules stay intact. If cross-linking of the particles that initially stabilized the interface had not happened, redispersion would result in a dispersion of silica micro*particles* and microcapsules would not be observed.
Because the synthesis method is straightforward without severe condition changes in temperature or chemicals inside the microdroplets and microcapsules, this opens the possibility to encapsulate delicate material. Besides that, there is an option to use versatile polymers to crosslink the particles together at the oil – water interface, which opens the possibility to produce microcapsules that are responsive to external stimuli.

5.2 Synthesis Approach

Figure 5.1 schematically shows the approach for the formation of poly(St-*co*-MAh) – SiO_2 microcapsules. Initially, an inverse Pickering emulsion is produced, which is a water-in-oil emulsion that is stabilized by solid particles.[5] The continuous phase of the inverse Pickering emulsion was chosen to be ethyl acetate and the stabilizing particles are modified silica particles. A strong indicator towards the stability of a Pickering emulsion, is the three-phase contact angle that the particles have with the oil – water interface.[21–23] The closer this contact angle is to ninety degrees, the higher the energy of detachment of the particles from the interface, which results in an emulsion with a high stability.[21] More than half of the volume of a particle is in the oil phase when its contact angle at the water-oil interface is larger than 90°. In that case, predominantly for geometric reasons, an inverse emulsion (w/o) is most stable. The particles are then more hydrophobic in nature and from literature it is known that an inverse Pickering emulsion is most stable when the stabilizing particles have a three-phase contact angle with the interface between 94° and 110°.[21]

In this work, the silica particles that stabilize the inverse Pickering emulsion had to be modified for two reasons. Firstly, silica particles are very hydrophilic and as a consequence, their three phase contact angle is smaller than 10°.[20] In the present study, the particles were hydrophobized to make them suitable for the stabilization of an inverse Pickering emulsion. Secondly, the silica particles were modified to provide them with primary amine containing groups for the reaction with MAh residues of poly(St-*co*-MAh).
The basic concept is that the poly(St-*co*-MAh) before ring-opening has a more hydrophobic nature and will therefore be oil-soluble. After ring-opening, the generated acid groups, make the polymer much more hydrophilic. However, since ring-opening takes place during the reaction that links the polymer to the primary particles, the polymer does not have the opportunity to migrate into the aqueous phase. Reaction of the polymer with the primary amine functional groups on the SiO_2 particles results in microcapsules with hydrophilic properties, which makes the capsules easy to re-disperse in water or other hydrophilic fluids at a later stage. In practice, first, water is added to a dispersion of hydrophobized and amine-functionalized silica microparticles in ethyl acetate. After emulsification, a stable inverse Pickering emulsion is produced. To the Pickering emulsion, poly(St-*co*-MAh) is added, which reacts with the amine functional groups on the particle surfaces and this process links the particles together, see Figure 5.1.

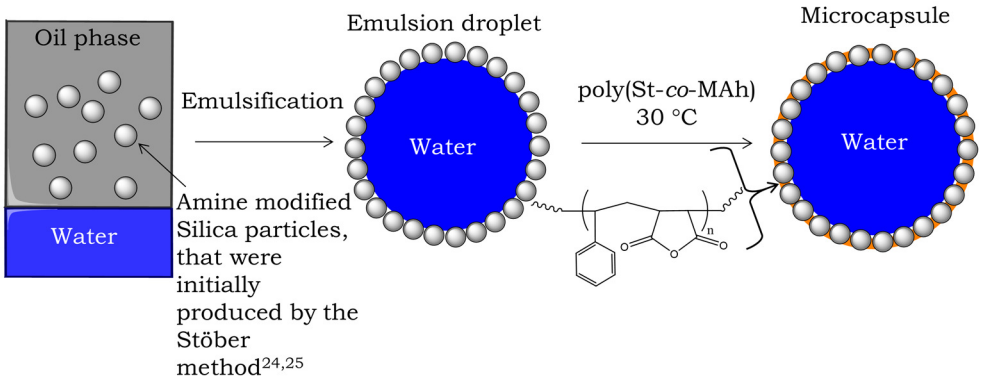

Figure 5.1 Overview of the synthesis route to produce hybrid poly(St-*co*-MAnh) SiO_2 microcapsules.

5.2.1 Functionalized silica microparticles

The silica particles were synthesized using the well-known Stöber mechanism to produce monodisperse seed silica particles, followed by a seeded polymerization technique.[24,25]A seeded technique was used since it proved to be difficult to produce monodisperse silica particles in the micron-

size range via a one-step Stöber process.[24] The silica particles were then dried, re-dispersed in ethyl acetate and modified by two different modification agents. After the modification steps, the particles were re-dispersed in ethyl acetate twice, to get rid of residual modification agents. Figure 5.2 shows typical SEM images of the produced monodisperse silica particles. A narrow particle size distribution is a prerequisite, since the surface of the silica particles is the basis for calculation of the amount of modification agents in the formulation. The diameter of the silica particles is also necessary to calculate the recipe of the inverse Pickering emulsion.

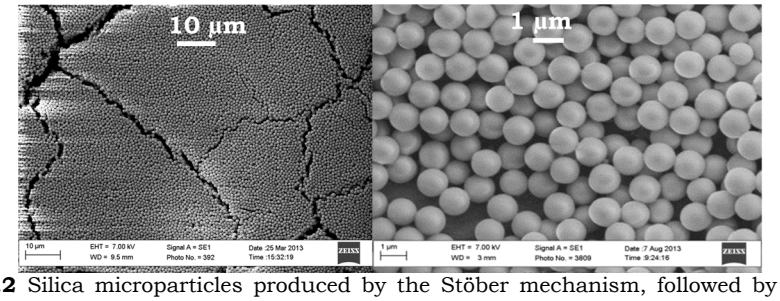

Figure 5.2 Silica microparticles produced by the Stöber mechanism, followed by a seeded polymerization technique. [24,25]

To tune the hydrophobicity of the silica microparticles, octadecyltrichlorosilane (OTC) was used as the first modification agent, see Figure 5.3 A. OTC is known to be a suitable modifier.[19,20] To determine the surface concentration of OTC that results in the most stable Pickering emulsion, the silica particles were reacted with different concentrations of OTC. Subsequently, the silica particles with varying hydrophobicity were used to produce an inverse Pickering emulsion. The particle surface concentration of OTC that resulted in the most stable inverse Pickering emulsions was determined to be 18 µmol m^{-2}, see Figure 5.4. For this system, phase separation did not take place within days after emulsification. The average concentration of reactive silanol groups on the surface of silica particles that are produced by the Stöber method is 8 µmol m^{-2}, see Figure 5.3 A.[26,27] This means that the introduction of OTC on the surface of the particles proceeds via polymerization of the trihydroxy silicate moiety. A hydroxy group is produced by the reaction of a chloride moiety of OTC with water.[28] There is plenty of water present in the particle dispersion mixture, since the silica particles were not dried under vacuum or/and high temperature and water physically adsorbs onto amorphous silica, see Figure 5.3 A.[29] Besides that, the ethyl acetate that was used was not dried before use. These two drying steps are unnecessary, since the particles will subsequently be used in a water-oil emulsion. In addition, the three-phase contact angle of the particles could be tuned satisfactorily although the reaction was not performed under dry conditions. As a result of the reaction of OTC, not only with the particle surface, but also with water and with each other, a monolayer of OTC will not be formed on the surface.[30,31] Instead, an

OTC-based network structure will be produced and enough hydroxy groups will be left for the next modification step, see Figure 5.3.
To give the particles reactive functional groups for the crosslinking of with poly(St-*co*-MAh), aminopropyl trimethoxysilane (APTMS) was added. An excess of APTMS was used (90 µmol m^{-2}), to ensure that enough amine groups were present at the surface of the silica microparticles for the cross-linking reaction, see Figure 5.3 B. After the second modification step, the particles still were suitable for inverse Pickering stabilization. After redispersion of the silica particles in ethyl acetate, water was added and upon emulsification of this mixture, the Pickering emulsion was produced, see Figure 5.4.

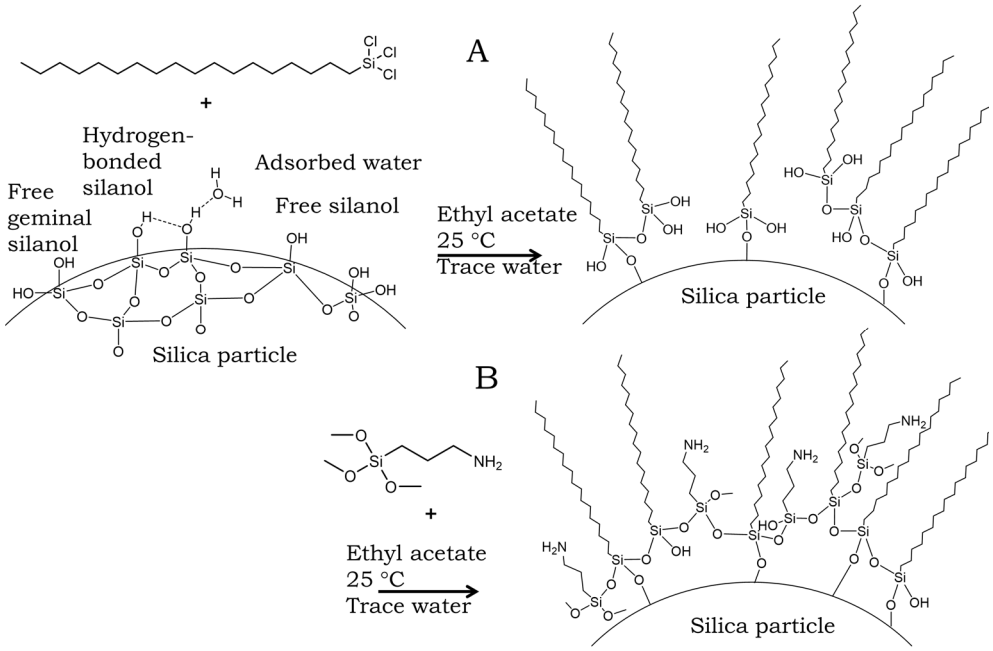

Figure 5.3 Modification steps of the silica microparticle surface. The reaction of octadecyltrichlorosilane (OTC) with the reactive silanol groups on the silica surface, with the adsorbed water and with each other (A) to make the particles more hydrophobic. The reaction of residual hydroxy groups on the hydrophobized silica microparticles with aminopropyl trimethoxysilane (APTMS) (B).

5.2.2 Pickering emulsion and microcapsule formation

The different concentrations in the inverse Pickering emulsion were calculated according to Equation 1, in which the droplet diameter is always set at 50 µm, unless indicated otherwise.[32]

$$D_D = \frac{6V_D}{N_A 2\sqrt{3}R^2} \quad (1)$$

In Equation 1, N_A is the total number of particles attached to the interface of the total amount of droplets and when the particles are hexagonally close-packed. R is the radius of the modified silica particles, the area that a particle occupies is $A_p = 2\sqrt{3}R^2$. V_D is the total volume of the dispersed phase and D is the diameter of the emulsion droplets. After the addition of water, emulsification was achieved by manually shaking the mixture for 15 to 30 seconds.

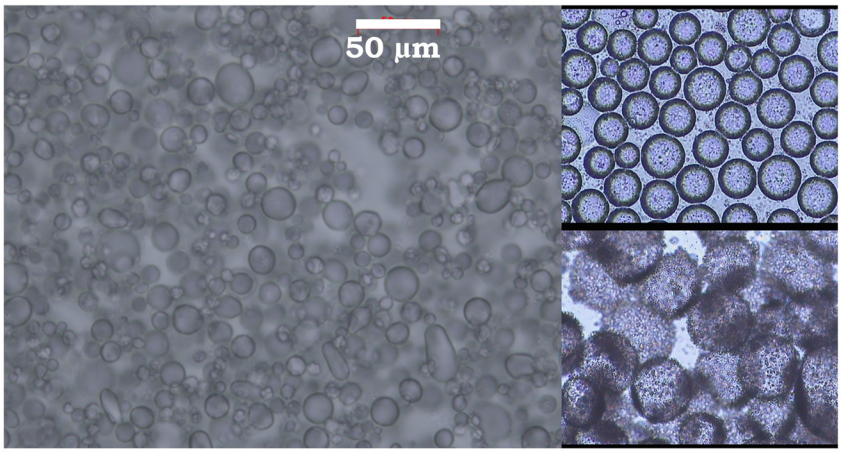

Figure 5.4 Light microscopy images of an inverse Pickering emulsion. The Pickering emulsion was produced by the addition of 3 mL distilled water to 0.5 g silica particles (d_p = 1 µm) dispersed in 40 mL ethyl acetate. The particles were functionalized with 18 µmol m^{-2} OTC and 90 µmol m^{-2} APTMS.

After a stable inverse Pickering emulsion was produced, it was charged into a three-neck round bottom flask with overhead stirring. To the stable inverse Pickering emulsion a certain amount of a poly(St-*co*-MAh) solution in ethyl acetate was added and crosslinking took place within several minutes.[33] This could be observed, since some flocculation of the microcapsules in the continuous oil phase occurs. The hydrophobized silica particles that initially stabilize the inverse Pickering emulsion are now covered by polymer that has a more hydrophilic character, which is deemed responsible for the flocculation. In order for the crosslinking reaction to be successful, the concentration of poly(St-*co*-MAh) had to be optimized. If an insufficient amount of polymer is added, not all silica microparticles at the interface of the emulsion droplets will be linked together. However, if an excess of polymer is used, every amine group will just react with one polymer chain, which would also lead to unsuccessful crosslinking of the particles, Figure

5 B. The alternating poly(St-*co*-MAh) that was used had a number average molecular weight (M_n) of $48.4 \cdot 10^3$ g mol^{-1}, the average number of MAh residues in one chain therefore is 239 (M_{STY} = 104 and M_{MAh} = 98 g mol^{-1}). To every inverse Pickering emulsion, 0.14 µmol poly(St-*co*-MAh) chains per m^2 of particle interface was added. An amount of 90 µmol aminopropyl trimethoxysilane per m^2 of particle interface was used to give the silica microparticles reactive groups. Consequently, it can be assumed that one polymer chain will react with more than one amine group, and bridge between particles, which results in crosslinking of the particles, see Figure 5.5 A.

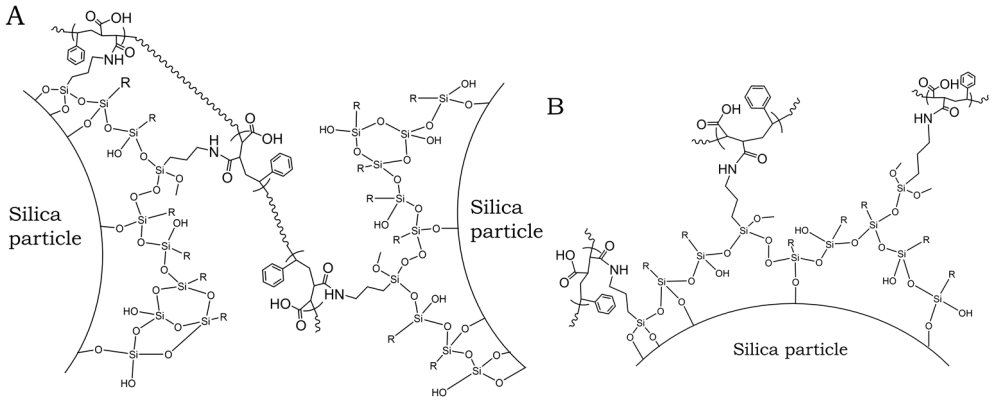

Figure 5.5 Schematic illustration of the crosslinking of amine and octadecyl (-R) functionalized silica microparticles with poly(St-*co*-MAh). Successful crosslinking will be the result if one polymer chain reacts with more than one amine group, on different particles (A), otherwise crosslinking will not take place (B)

5.3 Results

After addition of poly(St-*co*-MAh) to an inverse Pickering emulsion stabilized by OTC and APTMS-modified silica microparticles, hybrid poly(St-*co*-MAh) – SiO_2 microcapsules were produced, see Figure 5.6. When the stabilizing silica particles were not amine-functionalized, the capsule forming reaction did not take place. This is evidenced by an additional experiment in which non-amine functionalized microparticles were used to stabilize the Pickering emulsion and all other conditions were kept the same. In the case of amine-functionalized particles, after the aminolysis reaction of poly(St-*co*-MAh), the produced carboxylic acid residues give the polymer more hydrophilic properties. These carboxylic acid groups consequently result in the aggregation of the produced hybrid microcapsules. Aggregation can be visually observed by a clean transparent continuous phase. When the primary stabilizing particles were not amine-functionalized, this was not observed. Also, when light microscopy was used as analysis method, no

microcapsules were detected when using SiO_2 particles that are not amine-functionalized.

Figure 5.6 A shows Scanning Electron Microscope (SEM) images of the produced microcapsules. The SEM images are unable to reveal the presence of poly(St-*co*-MAh) on the surface of the microcapsules, apart from a few exceptions. In contrast to the formation of a robust shell with a thickness of (at least) a few nanometers[34], in this case only new polymer is covering the silica microparticles and bonding them together, which is virtually impossible to detect with SEM. The most important observation from the SEM images is that the microcapsules stay intact, even though they usually collapse under the high vacuum conditions. In the absence of crosslinking, the microcapsules do not form and SEM images only show individual SiO_2 particles, or clusters, but no microcapsules. In order to collect additional evidence for the successful crosslinking of the particles, light microscopy (LM) analysis was used. Before LM analysis, the microcapsules were superficially dried and redispersed in water with a trace of acetone (1 wt%). Isolation of the capsules was relatively easy, since they settle readily due to gravity (water droplets of 50 μm in diameter, dispersed in a lower density organic solvent), after which the continuous phase was decanted. Redispersion of the capsules was favored by their hydrophilic properties. To enhance the process even more, some acetone was used to increase the solubility of the residual ethyl acetate. Due to the permeability of the capsules, the fluid is the same inside and outside the capsules, after redispersion. As a consequence, they settle much slower after redispersion, namely hours relative to seconds. However, the most important observation from this redispersion experiment is that the capsules stay intact. If crosslinking of the particles that initially stabilized the interface had not happened, redispersion in aqueous media would result in a dispersion of micro*particles*. Figure 5.6 B, are LM images of poly(St-*co*-MAh) – SiO_2 microcapsules, that were redispersed in water with a trace of acetone. Evidently, the crosslinking reaction took place as intact microcapsules are observed, see Figure 5.6 B.

Although it can now be concluded that poly(St-*co*-MAh) – SiO_2 microcapsules have been produced (Figure 5.6), it is not exactly clear which fraction of the polymer reacted with the amine-functionalized particles. It is possible that only part of the polymer reacted, which would lead to residual polymer in the oil phase, or as a result of ring-opening, polymer may have diffused into the dispersed water phase.

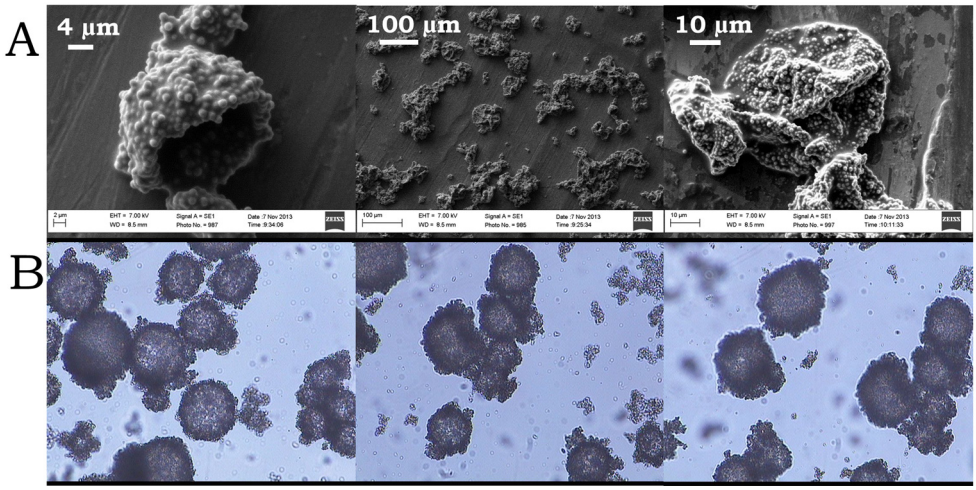

Figure 6. Scanning Electron Microscope (A) and Light Microscope (B) images of hybrid poly(St-*co*-MAh) – SiO_2 microcapsules, produced by poly(St-*co*-MAh)-induced crosslinking of the stabilizing silica microparticles of an inverse Pickering emulsion at the interface.

5.4 Confocal Fluorescence Microscopy Analysis

In an additional experiment, silica microparticles were labeled with a red fluorescent dye and were crosslinked with poly(St-*co*-MAh) that was labeled with a green fluorescent dye in order to synthesize microcapsules. Confocal Fluorescence Microscopy (CFM) allowed the determination of the individual locations of the microparticles and the polymer, see Figure 5.8. The red fluorescently labeled silica microparticles were synthesized by the addition of a very small amount of labeled silica precursor during the synthesis procedure. The labeled silica precursor in turn was synthesized by the addition reaction of Rhodamine B isothiocyanate with aminopropyl triethoxysilane, see Figure 5.7.[35] The green fluorescent poly(St-*co*-MAh) was produced by the partial aminolysis of MAh with 5-aminofluorescein, see Figure 5.7. An amount of 1 mol% of 5-aminofluorescein was used relative to the MAh groups in the polymer. Hence, sufficient MAh groups were left for the crosslinking reaction.
As a result, after the microcapsule synthesis with the labeled precursors, the silica particles that initially stabilized the emulsion could be observed, see Figure 5.8 B. Also, the labeled polymer could be observed, see Figure 5.8 A. When the two individual images are overlaid, it can be concluded that the majority of the polymer is in contact with the amine-functionalized particles, see Figure 5.8 C. In addition, hardly any green poly(St-*co*-MAh) was observed in the oil or water phase. This means that almost all the polymer is in close proximity of the silica shell around the water droplets.

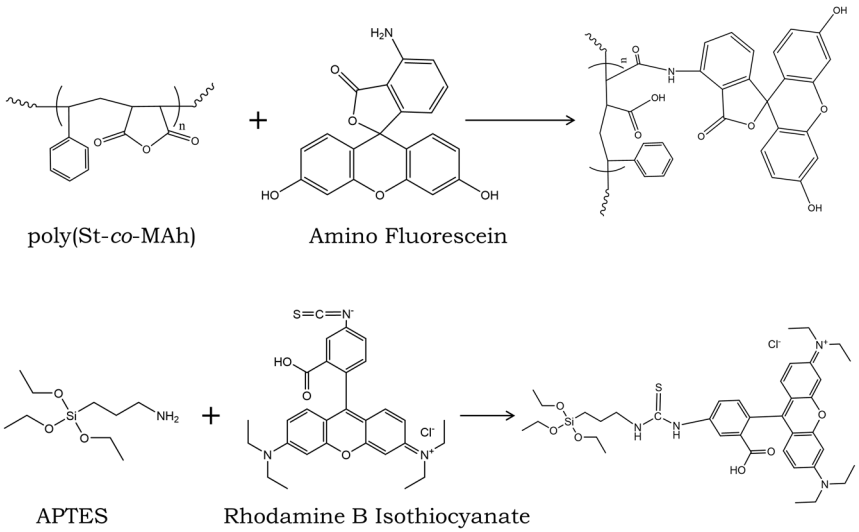

Figure 5.7 Synthetic scheme for the labeling of poly(St-*co*-MAh) with 5-aminofluorescein and of APTES with rhodamine B isothiocyanate.

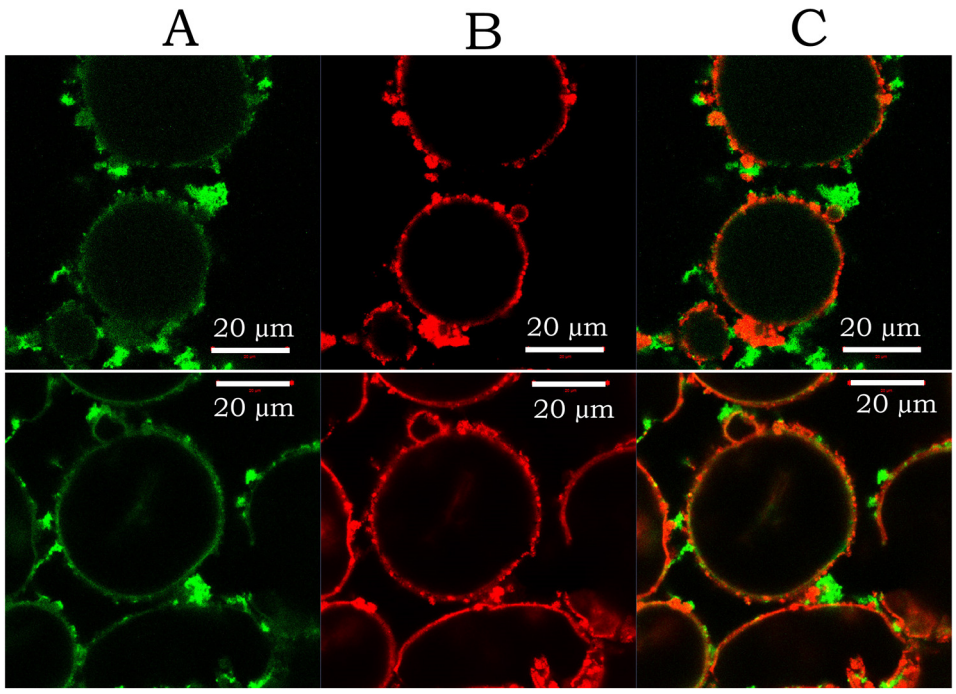

Figure 5.8 Confocal Fluorescence microscope images of hybrid poly(St-*co*-MAh) – SiO_2 microcapsules (C). Produced with green fluorescein-labeled poly(St-*co*-MAh) (A) and red rhodamine B-labeled silica microparticles (B).

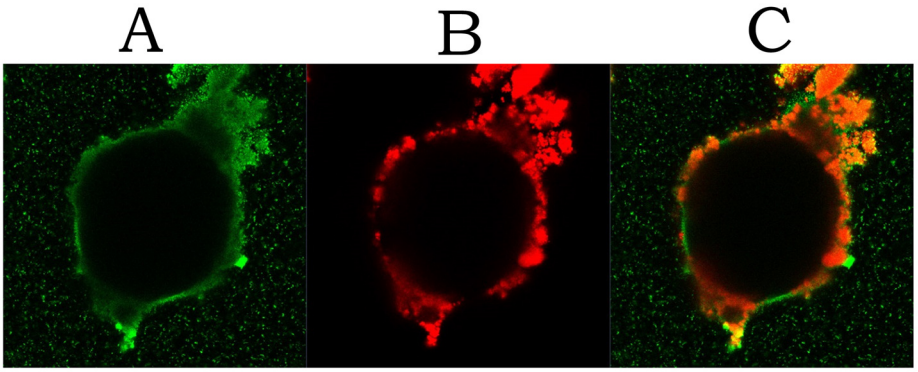

Figure 5.9 Confocal Fluorescence microscope images of hybrid poly(St-*co*-MAh) SiO_2 microcapsules (C). Produced with excess green fluorescein-labeled poly(St-*co*-MAh) (A) and red rhodamine B-labeled silica microparticles (B).

When excess polymer was added, this could be observed by the green dye in the continuous ethyl acetate phase, see Figure 5.9. In that case most probably all the amine groups on the modified primary stabilizing silica particles had reacted. It is noticeable that in this specific experiment, the poly(St-*co*-MAh) does not seem to react with water. Such hydrolysis reaction would increase the hydrophilicity of the polymer, which would then be expected to migrate into the water phase. Instead it resides in the continuous oil phase, see Figure 5.9 A and C.

5.5 Conclusion

Hybrid poly(styrene-*co*-maleic anhydride) – silica microcapsules were successfully synthesized by templating inverse Pickering emulsion droplets. The inverse Pickering emulsion droplets were stabilized by surface-modified silica microparticles. The surface modification was carried out in a two-step process. Initially, the surface was hydrophobized using octadecyl trichlorosilane. Subsequently, remaining hydroxy groups were reacted with aminopropyl trimethoxysilane, which resulted in reactive amine groups on the silica surface. After addition of poly(styrene-*co*-maleic anhydride) to an inverse Pickering emulsion stabilized with amine-functionalized silica microparticles, crosslinking of the microparticles at the interface took place and microcapsules were produced.
Since poly(styrene-*co*-maleic anhydride) is a versatile polymer that can be reacted with different other polymers this opens the possibility of producing microcapsules with versatile properties. Furthermore, because of the straightforward synthesis method the procedure might be applicable for the encapsulation of delicate material, for example live cells or enzymes.

5.6 Experimental

Materials. All chemicals were used as received, unless indicated otherwise. Tetraethyl orthosilicate (TEOS), (3-aminopropyl)triethoxysilane (99%) (APTES), octadecyl trichlorosilane (>90%), 6-aminofluorescein ($C_{20}H_{13}NO_5$, λ_{ex} 590 nm, λ_{em} 520 nm in 0.1 Tris pH 9) and Rhodamine B isothiocyanate ($C_{29}H_{30}ClN_3O_3S$, λ_{ex} 543 nm, λ_{em} 580 nm in methanol) were purchased from Sigma-Aldrich. Ethanol anhydrous (99.8%) and ammonia 32% were purchased from Merck-Chemicals. Ethanol absolute (dehydrated AR) was purchased from Biosolve. The water used was double de-ionized water from an Elix Millipore purification system. Styrene monomer was purchased from Fluka chemika (99.5%). Maleic anhydride (99%), methyl ethyl ketone (≥99.7%), tetrahydrofuran (CHROMASOLV® Plus, for HPLC, ≥99.9%) and deuterated acetone (99.9 atom %, Acetone-d_6) were purchased from Sigma-Aldrich. 2, 2'-Azo-bis (isobutyronitrile) (AIBN) was purchased from Riedel de Haen, recrystallized twice using methanol and dried under vacuum before use.

The silica microparticles were produced by an initial synthesis of monodisperse seed silica microparticles (+/- 500 nm) using the well-known Stöber technique.[25] A three-neck round bottom flask was charged with ethanol (100 g), TEOS (7 g) and water (5 mL). The mixture was left to stir for 10 minutes with a magnetic stirrer at 30 °C. Subsequently, an ammonia solution (15 mL, 25%) was added and the reaction was conducted for 6 hours. The particles were grown through a seeded polymerization technique to the required diameter, while retaining a narrow size distribution.[24] The seeded polymerization entailed the addition of five aliquots of TEOS (2 g), which were added with 6 hour intervals.

The precursor for the dye-labeled silica particles was synthesized by the addition reaction of rhodamine B isothiocyanate with aminopropyl triethoxysilane, according to a procedure described by van Blaaderen *et al.*[35,36] Briefly, in 5 mL anhydrous ethanol, aminopropyl triethoxysilane (0.2 mmol, 44 mg) was reacted with rhodamine B isothiocyanate (0.1 mmol, 53 mg). The reaction was allowed to proceed for 17 hours in a nitrogen atmosphere.

The labeled silica microparticles were synthesized by the addition of 0.05 mL of the above-described mixture (0.1 mmol, 97 mg, labeled silica precursor in 5 mL EtOH) during the synthesis of the seed silica microparticles.[35,36]

Modification of the surface of the microparticles proceeded in a three-neck round bottom flask under continuous magnetic stirring at 30 °C. Before modification, centrifugation was used to separate the microparticles from the ethanol solution, after which the ethanol solution was decanted

and the particles were air-dried. In a typical modification procedure, silica microparticles (0.5 g) with an average diameter of 1 µm were dispersed in ethyl acetate (10 g). If a density of 2.15 [g cm^{-3}] is used, the total surface area of the particles in the reaction mixture is 2.79 m^2.[37] To the microparticles, OTC (18 µmol m^{-2}, *i.e.* 50 µmol, 19 mg) was added. The reaction was allowed to proceed for 24 hours. Subsequently, APTES (90 µmol m^{-2}, *i.e.* 251 µmol, 55 mg) was added to the mixture, and the reaction continued for 24 hours. After modification, again centrifugation was used to isolate the particles and the ethyl acetate phase was decanted. The particles were air-dried, after which they were redispersed in ethyl acetate, to get rid of the unreacted modification agents.

A typical Pickering emulsion was produced by the addition of water (1 mL), to modified silica microparticles ($8 \cdot 10^{-2}$ g) dispersed in ethyl acetate (15 g). The droplets were calculated to have a diameter of 50 µm, see Equation 1. The Pickering emulsion was produced manually by vigorous shaking of the mixture for about 30 sec.

The Poly(styrene-*co*-maleic anhydride) – Silica microcapsules were synthesized by the addition of poly(St-*co*-MAh) (0.14 mol per m^{-2} of silica particles), to a stable Pickering emulsion. So, if the above described Pickering emulsion was used, the total surface area of the modified silica microparticles ($8 \cdot 10^{-2}$ g), with a diameter of 1 µm, is equal to 0.45 m^2. Therefore, poly(St-*co*-MAh) ($6.3 \cdot 10^{-2}$ µmol, 3 mg) was added to the emulsion. The reaction was continued for 1 hour under continuous overhead stirring at 30 °C. The poly(St-*co*-MAh) was solubilized in ethyl acetate (10 mL) before addition.

Alternating poly(styrene-*co*-maleic anhydride) was synthesized by conventional radical copolymerization of styrene and maleic anhydride monomers in a 1:1 molar ratio styrene: maleic anhydride.[38]
In methyl ethyl ketone (250 mL) were dissolved maleic anhydride (192 mmol, 18.82 g), styrene (192 mmol, 19.99 g) and 2, 2' azobis(isobutyronitrile) (3.96 mmol, 0.65 g). The reaction mixture was purged with argon (45 min) at room temperature and emerged into a preheated oil bath (60 °C). Subsequently, the argon needle was taken out of the solution, but kept in the round bottom flask for another 50 min. The reaction was conducted for an additional 15 hours. The polymer solution was allowed to cool to room temperature, after which the polymer was precipitated in diethyl ether. The polymer was dried under vacuum at 80 °C for 1.5 hours and left under vacuum for 16 hours to remove any unreacted monomer and residual solvent. Analyses were done using SEC, which resulted in $M_n = 48.4 \cdot 10^3$ [g mol^{-1}], $Đ = 4.09$ and a MAh content of 50 mol%. ^{1}H-NMR analysis was used to calculate the respective maleic anhydride contents, this was done by integrating the ratio of the aromatic protons of the styrene residue, to the methine protons of the maleic

anhydride residue. It was determined that the poly(St-*co*-MAnh) was alternating, *i.e.* a 1:1 ratio of maleic anhydride to styrene (50%) was found.

Scanning Electron Microscopy (SEM) was used for imaging of the microparticles and microcapsules. SEM was performed on a Zeiss Evo MA15VP scanning electron microscope. Samples were prepared by placing a droplet of the sample on a sample holder, which was covered by double sided carbon tape. After the sample had dried it was coated with a thin layer of gold. The gold was to ensure good conductivity during the measurements.

Light microscopy (LM) was used for the imaging of the inverse Pickering emulsion and of the microcapsules. An Olympus CX31 Light Microscope was used to perform the light microscopy. The samples were prepared by placing a droplet of sample on a glass slide after which it was placed on the specimen stage.

The Confocal Fluorescence Microscope (CFM) images of the labelled microcapsules were visualized with a Carl Zeiss LSM 780 with Elyra S.1 superresolution platform. The images were taken with a 561 nm (100 mW) laser (red) and a 488 nm (100 mW) laser (green). Sample preparation was done by placing a droplet of sample in a glass microscope dish, for the sample to be measured in solution.

Size Exclusion Chromatography (SEC) was used to determine the molar mass and dispersity of poly(styrene-*co*-maleic anhydride). Two PLgel (Polymer Laboratories) 5 µm Mixed-C (300 x 7.5 mm) columns and a pre-column (PLgel 5 µm Guard, 50x7.5 mm) were used. The SEC instrument consists of a Waters 1515 isocratic HPLC pump, a Waters 717plus auto-sampler, Waters 600E system controller (run by Breeze Version 3.30 SPA) and a Waters in-line Degasser AF. A Waters 2414 differential refractometer was used at 30 °C in series with a Waters 2487 dual wavelength absorbance UV/Vis detector operating at variable wavelengths. Tetrahydrofuran (THF, HPLC grade, stabilized with 0.125% BHT) was used as eluent at flow rates of 1 mL min^{-1}. The column oven was kept at 30 °C and the injection volume was 100 µL. Calibration was done using narrow polystyrene standards ranging from 580 to $2x10^6$ g mol^{-1}. All molecular weights were reported as polystyrene equivalents. Sample preparation was done dissolving sample in BHT stabilized THF (2 mg mL^{-1}). Sample solutions were filtered via syringe through 0.45 µm nylon filters before subjected to analysis.

Nuclear Magnetic Resonance spectroscopy (^{1}H-NMR) was used to verify if the synthesis of poly(styrene-*co*-maleic anhydride) resulted in an alternating copolymer. Therefore the spectra were obtained on a Varian Unity INOVA 400 MHz spectrometer, with a pulse width of 3 µs (45°) and a 2 second acquisition time. The samples were prepared using deuterated acetone.

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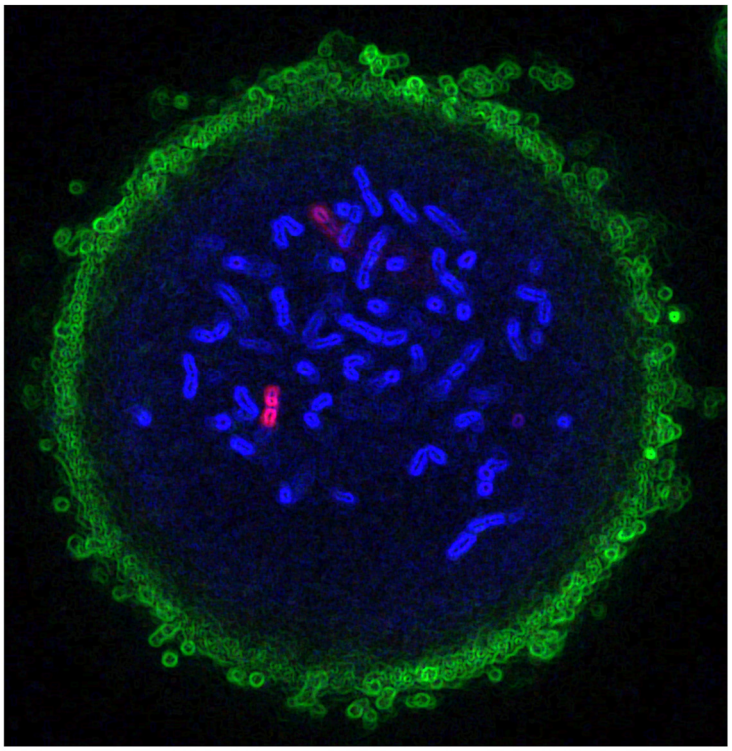
6. Compartmentalization of Bacteria in Microcapsules



Abstract

Lactobacillus plantarum strain 423 was encapsulated in hollow *poly*(organosiloxane) microcapsules by templating water-in-oil Pickering emulsion droplets via the interfacial reaction of alkylchlorosilanes. The bacteria were suspended in growth medium or buffer to protect the cells against pH changes during the interfacial reactions with alkylchlorosilanes. Confocal fluorescence microscopy has shown that strain 423 remained viable in the Pickering emulsion droplets, during as well as after the synthesis of the silica microcapsules. The used fluorescent tracers were 4',6-diamidino-2-phenylindole (DAPI) and bis-benzimide trihydrochloride (H 33342), and also LIVE/DEAD® BacLight™. The results of this work open up novel avenues for the encapsulation of microbial cells.

6.1 Introduction

Encapsulation of viable microbial cells has several novel applications in pharmaceutical, food and agricultural industries. Microencapsulation of bacterial cells has been successfully used to protect probiotic strains from gastric juices and low pH in the gastro-intestinal tract (GIT).[1,2] Microencapsulation has, however, also been used to protect therapeutic molecules and enzymes, with applications in areas of *e.g.* biocontrol, biosensing and bioremediation.[3] One of the advantages of microencapsulation is that metabolic products, such as antibiotics, can be easily be separated from cells.[1,2] If the capsules are permeable for therapeutic molecules or enzymes, produced by the encapsulated bacteria, those products can more easily be separated from the fermentation broth than if the bacteria are in the same suspension.[1,2] Encapsulated antibiotic(s)-producing bacteria may be incorporated into nanofibers to develop wound dressings, or may be used in tissue engineering.[4] Encapsulated microorganisms are enzyme-producing microreactors and may be used as catalysts in chemical reactions.[5] Encapsulated microbial cells may also be used in cell signaling studies.[6] Critical for these potential applications is that the microcapsules are permeable for small molecules and even macromolecules, but impermeable for the encapsulated microbial cells. It is also a prerequisite that the encapsulated cells remain viable during and after encapsulation. This is challenging, as most microorganisms are sensitive to changes in environmental conditions such as temperature, pH and the presence of cytotoxic chemicals.

Viable cells are usually being encapsulated in gels, often in the form of microbeads. These include hydrogels[7,8], calcium alginate[9] and sol-gel products[10–12]. Hollow calcium carbonate capsules produced by a layer-by-layer technique have been used to encapsulate individual *E. coli* cells[13]. Beneficial characteristics of sol-gel silica as encapsulation material are *e.g.* the controllable porosity and mechanical properties. These characteristics can be controlled by the selection of precursors, modification agents and synthesis conditions.[14] Pickering emulsion droplets are suitable templates

for microcapsules because of their high colloidal stability and their controllable droplet size.[15–17]

This chapter reports on the encapsulation of *L. plantarum* in hollow poly(organosiloxane) microcapsules. These microcapsules are synthesized from water-in oil emulsions stabilized by silica particles by an interfacial reaction of alkylchlorosilanes, see Figure 6.1 A. The alkylchlorosilanes have hydrophobic properties, *i.e.* they are not miscible in water before and after hydrolysis. Alkylchlorosilanes are very reactive towards water and contribute in stabilization of the water droplets by the microparticles. Hence, the monomer is oil soluble and when in contact with the stabilizing particles or the water phase at the interface of the water droplets, it rapidly polymerizes. The viability of the bacteria was determined in the Pickering emulsion droplets as well as in the microcapsules by using confocal fluorescence microscopy.

6.1 Synthesis approach

Microencapsulation entails the formation of a solid shell around a microdroplet. In this study Pickering emulsion water droplets were used as templates for the microcapsules. The methods used to prepare microcapsules is schematically presented in Figure 6.1. These droplets are very suitable as templates since they have a high stability against coalescence.[18] The objective of this work was to encapsulate viable bacteria and as a consequence, a water-in-oil Pickering emulsion was required, also called an inverse Pickering emulsion. A water-in-oil Pickering emulsion was required as bacteria generally do not survive in organic media, with only very few exceptions.[19] The three-phase contact angle (θ) that the particles have at the oil-water interface, when used in a Pickering emulsion, is a very strong indication for the stability of the emulsion. The three-phase contact angle is related to the interfacial tensions between the particles and oil (γ_{po}), between the particles and water (γ_{pw}) and between oil and water (γ_{ow}) by Young's equation, equation 1.[20,21]

$$\cos\theta = \frac{\gamma_{po} - \gamma_{pw}}{\gamma_{ow}} \qquad (1)$$

The most stable inverse Pickering emulsion is produced when the stabilizing particles have a three-phase contact angle between 94° and 110°.[22,23] Silica microparticles that are modified by alkylsilanes are known to have a three-phase contact angle suitable for inverse Pickering stabilization.[24] Water was added to the dispersion of modified particles in *n*-heptane, for the production of the inverse Pickering emulsion, see Figure 6.1. The water, oil and particle concentration in the emulsion were calculated according to Equation 2 in which R_D is the droplet radius, which was always set at 25 μm, unless indicated otherwise.[17]

$$R_D = \frac{3V_D}{N_A 2\sqrt{3}R^2} \quad (2)$$

In Equation 2, V_D is the total volume of the dispersed phase, N_A is the number of particles that are attached to the droplet interface and $2\sqrt{3}R^2$ is the droplet area that a particle occupies when in a hexagonally closed packing at the interface, neglecting the curvature effects. R stands for the radius of the stabilizing particles. N_A/ V_D is the number of particle per unit of water. The Pickering emulsion was produced manually, by shaking the mixture of water, *n*-heptane and the hydrophobic SiO_2 particles for 30 seconds. The reason for choosing this methodology is that bacteria are not able to survive high shear or ultra-sonication, which is usually applied to effect emulsification. Alkylchlorosilanes were added to the inverse Pickering emulsion that reacted with water at the interface, see Figure 6.1 B. The reaction of these alkylchlorosilanes proceeds first by a rapid hydrolysis of the monomer with water, producing a surfactant-resembling monomer (Eq. 3).[25] The hydrolysis is followed by the condensation reactions (Eq. 4), producing the shell around the microemulsion droplets, as depicted in Figure 6.1 B.

$RSiCl_3$ (liq) + H_2O → $RSi(OH)_3$ (interface) + HCl (aq)
Hydrolysis reaction (3)

2 $RSi(OH)_3$ (interface) → $R(OH)_2SiOSi(OH)_2R$ (interface) + H_2O
Condensation reaction (4)

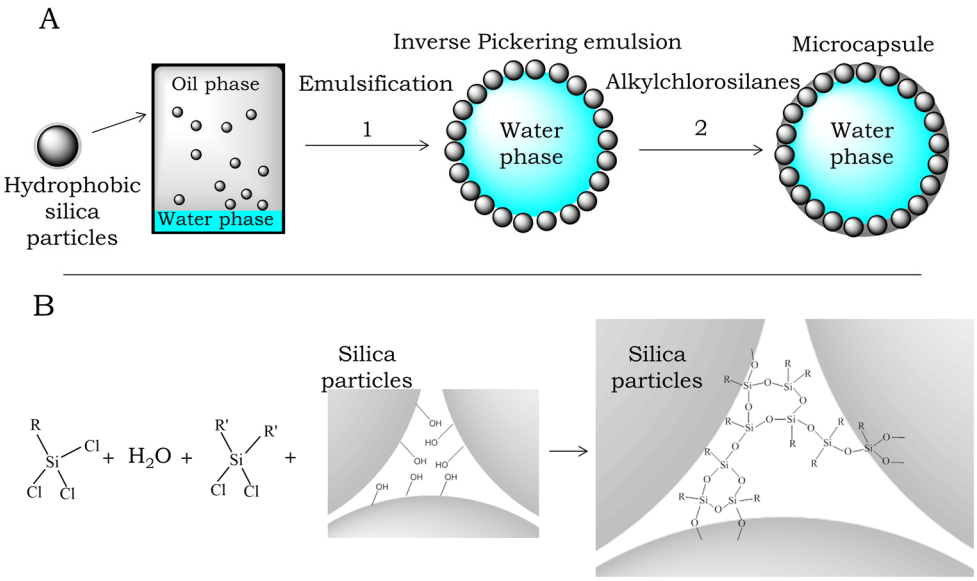


Figure 6.1 Methodology for the synthesis of microcapsules. (A) An inverse Pickering emulsion is produced by the emulsification of water in a dispersion of hydrophobic silica particles, in *n*-heptane (1). The addition of alkylchlorosilanes to the inverse Pickering emulsion results in the reaction of these alkylchlorosilanes at the interface with water to produce hydrochloric acid and polymer, thus forming microcapsules (2). (B) The reactive silanol groups on the primary stabilizing silica particles will also react with the poly(alkyl siloxane).

6.2.1 Functionalized silica microparticles

The particles that stabilize the inverse Pickering emulsion were produced by the Stöber method.[26] These silica particles were used in a seeded silica polymerization to produce monodisperse particles in the micron-size range.[27] The particles were separated from the reaction mixture by centrifugation, after which the liquid phase was decanted. The particles were air-dried and re-dispersed in *n*-heptane. Subsequently, the SiO_2 particles obtained were modified in *n*-heptane to make them hydrophobic character.[24] 3-(trimethoxysilyl)-propyl methacrylate (MPTS) was found to be a suitable hydrophobizing agent[24] and the amount of MPTS agent necessary, to provide the proper hydrophobicity to produce a stable inverse Pickering emulsion, was determined to be $1 \cdot 10^{-2}$ $mL \cdot m^{-2}_{particle}$, which corresponds to 42 $\mu mol \cdot m^{-2}_{particle}$, see Equation 5.

$$H_2C=C(CH_3)CO_2(CH_2)_3Si(OCH_3)_3 + HO\text{-(Silica particle)} \rightarrow H_2C=C(CH_3)CO_2(CH_2)_3(OCH_3)_2Si\text{-O-(Silica particle)} + CH_4O \quad (5)$$

The average concentration of reactive silanol groups on silica particles that are produced by the Stöber method is equal to 8 $\mu mol \cdot m^{-2}_{particle}$.[28] Besides reactive silanol groups, physically adsorbed water is also present at the

surface of the silica microparticles. Amorphous silica is well known to physically adsorb water and the adsorbed water will also react with the modification agent.[29] Hence, it is assumed that after the modification step, the surface of the silica microparticles is covered with a layer of MPTS. This again resulted in residual reactive hydroxy groups from the MPTS. Figure 6.2 A is a Scanning Electron Microscope (SEM) image of silica microparticles that were produced by the Stöber method, followed by a seeded polymerization technique.

6.2.2 Pickering emulsion

Before modification, the surface area of the microparticles was calculated by using the freeware package ImageJ.[30] Water was added to the particle dispersion after modification, followed by manual vigorous shaking of the mixture to produce a stable inverse stable Pickering emulsion, see Figure 6.2 B and C.

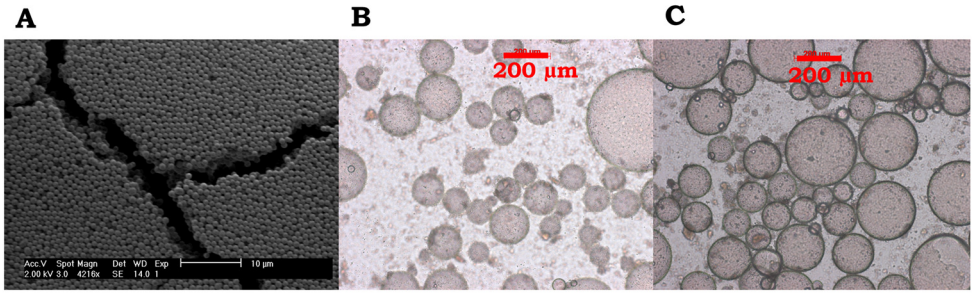


Figure 6.2 SEM image of silica microparticles (A) and light microscopy images of inverse Pickering emulsion droplets (B and C). The silica microparticles were produced first by the synthesis of seed particles using the Stöber method, followed by a seeded polymerization technique (A). After hydrophobization of the silica particles in *n*-heptane, water was added to the particle dispersion and subsequent emulsification resulted in an inverse Pickering emulsion (B and C)

When the microparticles used to stabilize the Pickering emulsion are monodisperse and have the proper three phase contact angle, they arrange in a hexagonal close packing on the water-oil interface. For this reason Pickering emulsions with a narrow size distribution can be produced with a distribution of $D_z/D_v \approx 1.1$.[31] However, it takes a certain amount of energy to transfer a particle to the interface and a specific time to bring them all at the interface. When emulsification takes place manually, the amount of energy and the dispersion time are not sufficient to create a Pickering emulsion with narrow size distribution, which is clear from the polydispersity index $D_z/D_v \approx 5.3$, see Figure 6.2 B and C. Despite the broad droplet size distribution, the produced emulsions were very stable and macroscopic phase separation was not observed over the course of days.

6.3 Results

Alkylchlorosilanes are frequently used for the modification of silica surfaces to improve dispersion properties, or to effect crosslinking for the immobilization of catalysts or biomolecules.[32] When alkylchlorosilanes are used to modify surfaces in the presence of water, they are known to yield a variety of nanostructures, besides a smooth polymer layer on the relevant surface.[33–35] The underlying reason for the formation of the nanostructures is the hydrolysis and self-condensation of the alkylchlorosilanes in the presence of water that occurs parallel to reactions with the surface-bound silanol groups.[33] The formation of the nanostructures is further dependent on the alkyl chain length of the alkylchlorosilane and the nanostructure size, shape and concentration is as well dependent on the chain length.[33]
Reaction of the alkylchlorosilanes with the silanol groups on the particles and the network formation between the silica particles should be sufficient to produce a stable microcapsule when a solid shell around an inverse Pickering emulsion is produced. Hydrolysis of a chlorosilane leads to an hydrophilic intermediate due to the produced hydroxy groups, see Equation 3 and 4. It is important that the intermediates do not migrate into the emulsion droplets but stay at the interface. To restrict the reaction to the interface and avoid migration into the emulsion droplet, octadecyltrichlorosilane (OTC) was used. To stimulate complete network formation, dimethyldichlorosilane (DMDCS) was used, which is more reactive but also partially hydrophobic after hydrolysis. So, after a stable Pickering emulsion was produced, a solution of OTC and DMDCS in *n*-heptane was added, see Figure 6.1. Silica based condensation products that have not reacted with silica particles or with the newly formed silica shell are hydrophobic and will therefore stay in the continuous oil phase.
Figure 6.3 shows light microscopy (LM) and SEM images of microcapsules produced after the addition of OTC and DMDCS to a stable Pickering emulsion. A polyalkylsiloxane shell had formed around the microdroplets, as a result of the interfacial reaction. The reaction of the monomers at the interface with each other, and with the silanol groups at the surface of the particles, also resulted in particle detachment from the interface because of their increased hydrophobicity, see Figure 6.3 A. Nevertheless, this particle detachment did not destabilize the emulsion to such an extent that macroscopic phase separation took place, before encapsulation. The newly produced polymer surrounding the initially stabilizing silica microparticles can be clearly distinguished when focusing in on the shell, see Figure 6.3 F. In contrast to the inverse Pickering emulsion droplets, the microcapsules do not appear to be perfectly spherical, see Figure 6.3. Deformation is due to the low glass transition temperature (T_g) of the polymer of -95 °C as determined by Differential Scanning Calorimetry (DSC) analysis, see Figure 6.5. The capsules need to be dried before SEM analysis, and the analysis itself takes place under high vacuum. Consequently, broken capsules and

collapsed capsules were expected as a result of evaporation of water from the capsules, see Figure 6.3 B - F.

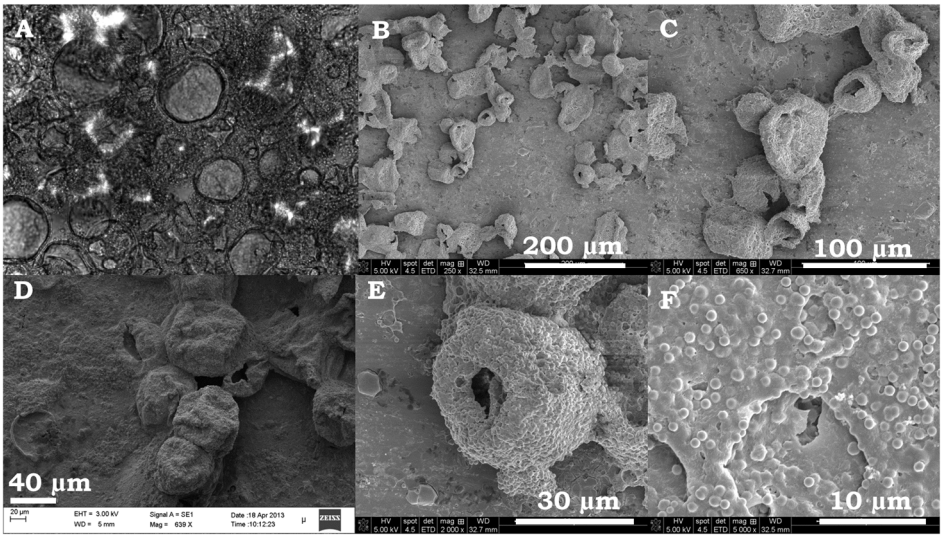


Figure 6.3 Light microscopy (A) and SEM (B – F) images of the produced microcapsules. Microcapsules were synthesized by the interfacial reaction of octadecyltrichlorosilane and dimethyldichlorosilane at the interface of inverse Pickering emulsion droplets, stabilized by hydrophobized silica microparticles. When zooming in on the shell, the newly formed polymer can be distinguished from the primary silica particles (F).

6.3.1 Thermal analysis

Figure 6.4 presents the weight loss of the polyalkoxysiloxane capsules from Figure 6.3 B, C, E and F as a function of temperature, determined with Thermogravimetric Analysis (TGA).
In Figure 6.4, the first weight loss region up to about 200 °C is attributed to physically adsorbed water. The weight loss between 200 and 250 °C is attributed to dehydroxylation, and the third region up to 400 °C is assigned to the degradation of the alkyl moieties. The last step up to 650 °C is assigned to the loss of polymer/copolymer backbone and the residual weight is silica. The glass transition temperature and melting temperature of the poly(organosiloxane) microcapsules were determined with Differential Scanning Calorimetry (DSC), see Figure 6.5. The T_g was -95 °C and the melting temperature was determined to be 62 °C. A T_g of -95 °C is in line with the T_g of DMDCS, which is -122 °C and the melting temperature was expected, since the melting temperature of polyoctadecylsiloxane is 63 °C, probably caused by the octadecyl side chains.[25,36,37] The melting peak around -30 °C could be of physically adsorbed water, which is known to decrease when adsorbed on silica gel.[38]

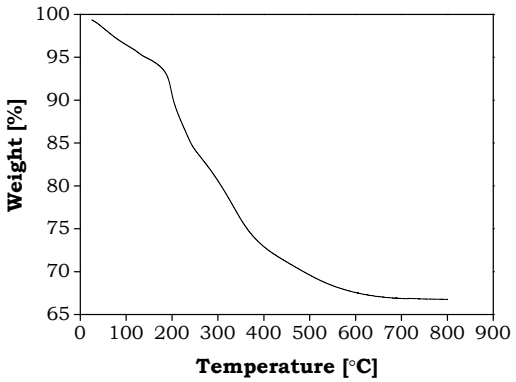


Figure 6.4 Thermogravimetric Analysis (TGA) curve of microcapsules. Microcapsules were synthesized by the interfacial reaction of octadecyltrichlorosilane and dimethyldichlorosilane at the interface of inverse Pickering emulsion droplets, stabilized by hydrophobized silica microparticles.

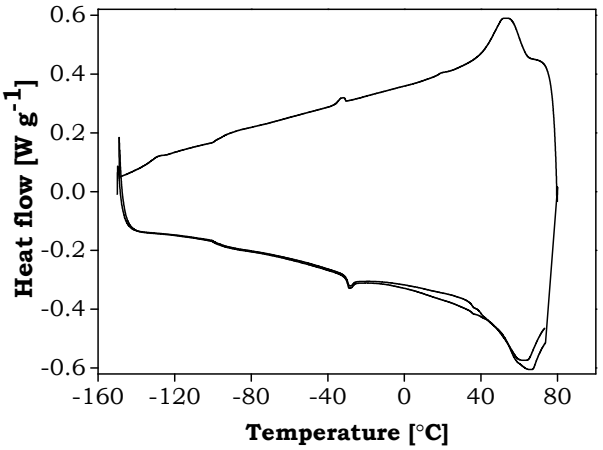


Figure 6.5 Differential Scanning Calorimetry result of microcapsules synthesized by the interfacial reaction of octadecyltrichlorosilane and dimethyldichlorosilane at the interface of inverse Pickering emulsion droplets, stabilized by hydrophobized silica microparticles. T_g of the polyalkoxysiloxane capsules was determined to be -95 °C.

6.4 Compartimentalization of *L. plantarum* 423 cells

Microcapsules and the procedure employed for the encapsulation of bacteria, need to have certain characteristics. For example, the capsules should provide a favorable environment for the bacteria to ensure survival and, in view of future applications, the capsules should also be permeable to allow transport of molecules into the external environment. The focus of the current study is on the development of a method to encapsulate viable

L. plantarum 423 cells[39] and to ensure viability throughout and after the encapsulation. An inverse Pickering emulsion was produced by using a *L. plantarum* 423 suspension in De Man, Rogosa, and Sharpe (MRS) broth as the dispersed phase. The growth media for the bacteria, besides other ingredients, also contain salts and the surfactant Tween 80 that could have an influence on the different interfacial energies in the system. However, the presence of these ingredients did not influence the three phase contact angle to such an extent that macroscopic phase separation took place. Classical microbiological techniques, such as plating out to determine colony-forming units (CFUs), could not be used to determine viability, as the bacteria will be exposed to *n*-heptane after destabilization of droplets. We therefore utilized a staining technique, using fluorescent dyes, to evaluate viability of *L. plantarum* 423 upon dispersion in the droplets of a Pickering emulsion. 4',6-diamidino-2-phenylindole (DAPI, blue) and bis-benzimide trihydrochloride (SYTO9, green) were added, which should stain the bacteria, although, interestingly, SYTO9 only stained the silica particles, see Figure 6.6. In addition, propidium iodide (red) was used, which can only penetrate a cell when the cell membrane is compromised, which is indicative of non-viable bacteria, see Figure 6.6.

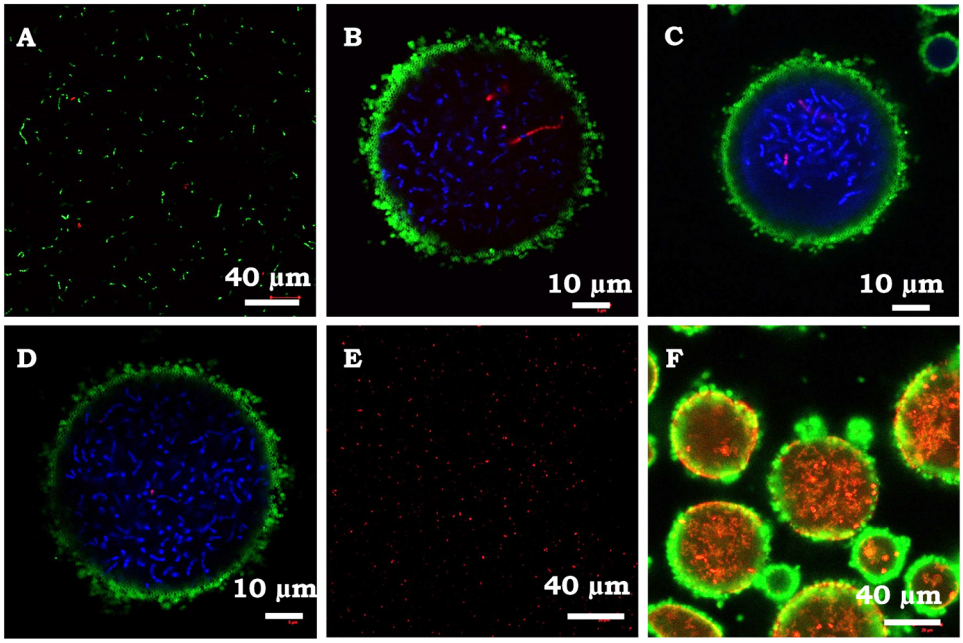


Figure 6.6 Inverse Pickering emulsion with *L. plantarum* 423 imaged by confocal fluorescence microscopy (CFM). A: positive control, B-D: encapsulated *L. planatarum* 423 in an inverse Pickering emulsion. E: negative control, F: negative control for an inverse Pickering emulsion. Blue: DAPI, stains the nucleus of all cells; Red: propidium iodide, stains cells with a permeable membrane (non-viable); Green: SYTO9, stains the stabilizing silica particles.

To investigate the viability of *L. plantarum* 423 in the emulsion droplets, also a positive and a negative control were imaged, see Figure 6.6 A and E. In these control experiments, the bacteria culture was dispersed in a growth media with SYTO9 (green) and propidium (red dye). Confocal fluorescence microscopy (CFM) was used to image the live bacteria (Figure 6 A) and after some ethanol was added CFM was used to determine the fraction of dead bacteria (Figure 6 E). Besides that, by the addition of some *n*-hexylamine to an inverse Pickering emulsion, all the bacteria inside the microdroplets died, so this image is a negative control for bacteria in emulsion droplets (Figure 6.6 F). When comparing *L. plantarum* 423, dispersed in MRS broth and emulsified in the Pickering emulsion droplets, with the different control samples it became apparent that a very large fraction of the bacteria stayed viable in the emulsion droplets after the emulsification procedure, see Figure 6.6 B-D. The experiment was repeated 3 times and it could be concluded that at least 90% of the *L. plantarum* 423 cells inside the droplets remained viable during the Pickering emulsification.

Finally, after a stable inverse Pickering emulsion containing viable *L. plantarum* 423 was produced, alkylchlorosilanes were added to produce a shell around the droplets for final encapsulation. A pH buffer was added to the water phase to counteract the pH change that would otherwise be caused due to the hydrochloric acid production from the polymerization of alkylchlorosilanes. The fluorescent dyes were added before emulsification and the interfacial reaction, see Figure 6.7.
The encapsulation procedure resulted in viable *L. plantarum* 423 cells inside the microcapsules (Figure 6.7). The staining technique used is similar to the one used for the Pickering emulsion, using fluorescent dyes, to evaluate viability of *L. plantarum* 423. Again DAPI (blue) and SYTO9 (green) were added, which should stain the bacteria. In addition, propidium iodide (red) was used, which can only penetrate a cell when the cell membrane is compromised, see Figure 6.6 E-F. Figures 6.7 A and B are independent bacterial cultures from 6.7 C, but all 3 experiments showed a similar trend. After the interfacial reaction of alkylchlorosilanes, the produced capsules were not stained by SYTO9 as in Figure 6.6 B-C, D and F. For the reason that the formed polysiloxanes possess very different characteristics from amorphous silica. Therefore, the green dye was not added in all the experiments, only during the experiment of Figure 6.7 C. Again the viable bacteria could be recognized by the blue color caused by DAPI and the non-viable bacteria by their red color via the addition of propidium iodide. From the CFM images it could be concluded that after the interfacial reaction a large fraction of the bacteria stayed viable, see Figure 6.7. Figures 6.7 A is a series of 2D focus stacking images of an individual capsule and this also accounts for Figure 6.7 B and C. This means that images were made by focusing up and down through the sample/microcapsule.[40] In Figure 6.7 A the deformation of the capsules can be recognized, since the water phase is

also mildly stained blue. The images also indicated that the bacteria mostly reside on the bottom of the capsules, most likely caused by gravity.

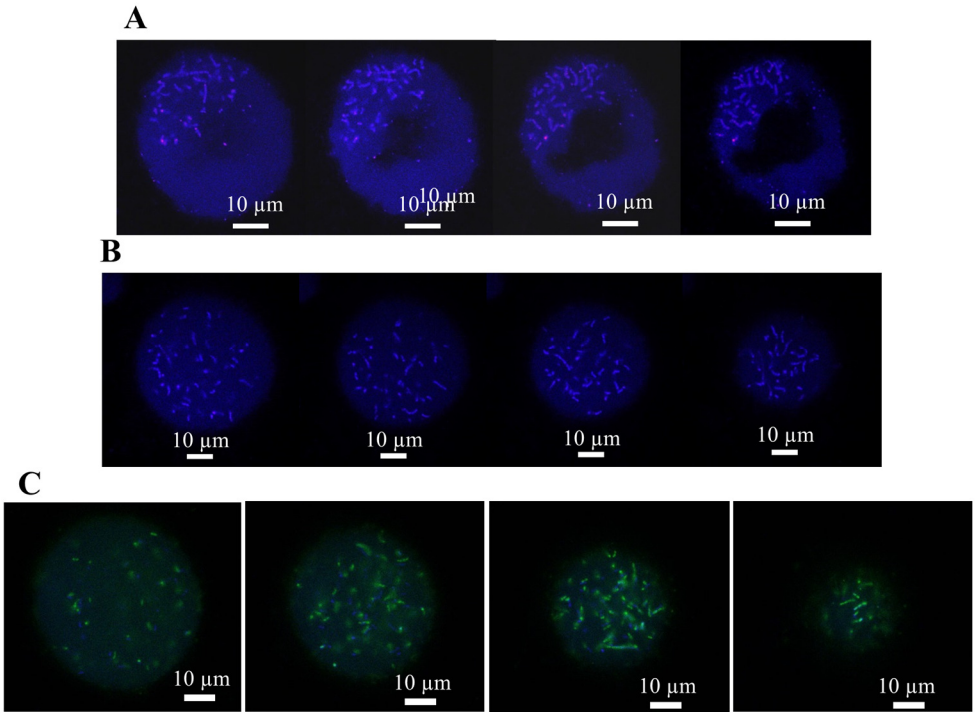


Figure 6. Confocal Fluorescence Microscopy images of isolated *L. plantarum* 423 bacteria in microcapsules. Microcapsules were synthesized by the interfacial reaction of octadecyltrichlorosilane and dimethyldichlorosilane at the interface of inverse Pickering emulsion droplets, stabilized by hydrophobized silica microparticles. Before the interfacial reaction the emulsion droplets consisted of a growth media and a buffer solution in which the bacteria were dispersed.

6.5 Conclusion

In this contribution we reported the microencapsulation of *L. plantarum* 423 and showed that the majority of the cells remained viable during and after encapsulation. Encapsulation of *L. plantarum* 423 was accomplished by formation of a Pickering emulsion of an aqueous suspension of bacteria in *n*-heptane stabilized by hydrophobized silica particles followed by the interfacial reaction of alkylchlorosilanes at the interface of inverse the Pickering emulsion droplets that were stabilized by hydrophobized silica microparticles. The bacteria remained viable during and after microcapsule synthesis. The results of this work open up novel avenues for the encapsulation of e.g. bacteria, enzymes and live cells.

6.6 Experimental

Materials. All chemicals were used as received, unless indicated otherwise. Tetraethyl orthosilicate (TEOS), octadecyltrichlorosilane (OTC) (≥ 90%), dimethyldichlorosilane (DMDCS) (≥ 99%), Trizma®base and trimethoxysilyl propyl methyl methacrylate (MPTS) (≥ 98%) were from Sigma-Aldrich (PO Box 10434, Aston Manor 1630, South Africa). Ammonia (32%), *Tris* hydrochloride (molecular biology grade) and *n*-heptane (≥ 99%) were from Merck-Chemicals (1, Friesland Drive, Longmeadow Business Estate, Modderfontein, 1645, South Africa). Ethanol (dehydrated AR) was from Biosolve (P.O. Box 53402, Kenilworth, Cape Town, and 7745 South Africa). Double de-ionized water from an Elix Millipore purification system was used. *Lactobacillus plantarum* 423 was cultured in De Man, Rogosa, and Sharpe (MRS) broth (Biolab Diagnostics, Midrand, South Africa).

The membrane-less permeable DNA fluorescent stain 4',6-diamidino-2-phenylindole (DAPI), the LIVE/DEAD® BacLight™ Bacterial Viability Kit and DNA probes were from Life Technologies (Kwartsweg 2, 2665 NN Bleiswijk, Netherlands).

The silica microparticles were synthesized in a two-step process. In the first step the silica microparticles were synthesized using the Stöber technique.[26,27] In brief, ethanol (100 g), water (5 mL) and TEOS (7 g) were stirred with a magnetic stirrer bar at 30 °C in a three-neck round bottom flask. After 15 min, ammonia (15 mL, 25% (v/v)) was added and the reaction allowed to proceed for 6 h. In the second step 5 aliquots of TEOS (2 g) were added to the microparticles with time intervals of at least 6 h. So a particle diameter of 800 nm was achieved assuming a density of 2.15 g mL^{-1}.[41] This method allows for the synthesis of silica particles in the micron-size range while keeping them monodisperse.[28]

The surface of the silica microparticles was modified to make them hydrophobic. The reaction with MPTS proceeded in a three-neck round bottom flask under continuous magnetic stirring at 25 °C. The silica microparticles (0.5 g) were suspended in *n*-heptane (10 g). Microparticles were separated from the ethanol solution by centrifugation (15 min, 1500 RPM) and dried at room temperature (25 °C). To this suspension 21 mg (73 µmol) MPTS was added (equivalent to 42 µmol $m^{-2}_{particle}$) and the reaction was allowed to proceed for 24 h. As the surface of the microparticles changed from hydrophilic to hydrophobic, the microparticles dispersed into the *n*-heptane phase.

The inverse Pickering emulsions were produced by adding 2 mL water to 0.25 g modified microparticles dispersed in 15 g *n*-heptane. The droplets were calculated to have a diameter of 50 µm (equation 2). The Pickering emulsion was produced by vigorous shaking the mixture for approximately 30 seconds.

Lactobacillus plantarum 423 was cultured, without aeration, in an MRS broth at 37 °C for 24 h. Cells were harvested by centrifugation (8 000 x g, 10 min, 25 °C) and re-suspended in an MRS broth or a Tris-HCl buffer (pH = 8.0) to an Optical Density$_{600nm}$ of 0.01(equivalent to $1 \cdot 10^6$ Colony Forming Unit ml^{-1}). 2 mL bacterial suspension was added to the dispersed phase of the inverse Pickering emulsion, prepared as described in the previous paragraph.

Microcapsules were produced by the addition of the alkylchlorosilanes to the Pickering emulsion. First, OTC (0.4 mL, 1 µmol) was added to DMDCS (0.22 mL, 1.6 µmol) dissolved in *n*-heptane (4.4 mL). Two thirds of the obtained solution was added to the inverse Pickering emulsion to ensure proper mixing of the monomers before the start of the interfacial reaction. No microcapsules were formed when only OTC or DMDCS was added. The interfacial reaction proceeded for 1 h under continuous overhead stirring at 37 °C in a three-neck roundbottom flask of 50 mL.

Scanning Electron Microscopy (SEM) was used for imaging the microcapsules and the microparticles. SEM was performed either on a Zeiss Evo MA15VP scanning electron microscope or on a FEI Quanta™ 3D FEG low vacuum SEM/Focused Ion Beam (FIB) instrument. Samples were prepared by placing a droplet of the sample on a sample holder, covered by a double-sided carbon tape, and then dried at room temperature (25 °C). The samples were sputter-coated with a thin layer of gold for electrical conductivity.

Light microscopy (LM) was used for the imaging of the inverse Pickering emulsion and the microcapsules. An Olympus CX31 Light Microscope and a Zeiss Axioplan Universal Microscope were used. Samples were prepared by placing a droplet of the inverse Pickering emulsion on a glass slide.

Confocal Fluorescence Microscope (CFM) imaging was conducted on a Carl Zeiss LSM 780 with an Elyra S.1 superresolution platform. The images were acquired with a 561 nm (100 mW) laser (red) and a 488 nm (100 mW) laser (green). Samples were prepared by placing a droplet of the inverse Pickering emulsion on a glass slide.

Thermogravimetric analysis (TGA) was performed on a TA Instruments high resolution TGA Q500 V6.7 apparatus. The measurements were either conducted using a constant temperature, or a heating rate of 10 °C min^{-1} from 25 °C to 800 °C, in a nitrogen flow of 50 mL/min.

Differential Scanning Calorimetry (DSC) was performed on a DSC Q100 from TA Instruments. Measurements were carried out from -150 to 80 °C, with heating and cooling rates of 20 °C min^{-1} under a nitrogen flow of 50 mL

min^{-1}. Glass transition temperature (T_g) readings were obtained from the inflection point of the curve recorded during the first heating run.

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7. Conclusions and Outlook

In the course of the last four years of research, summarizing and reporting results, I never lost my interest in the topic of microencapsulation. This is mainly due to the versatility of the topic. For example, from the point of view of chemistry, different chemical approaches can be used to produce different shell materials. Alternatively, the research can be focused on the physical conditions that are necessary for successful encapsulation, *e.g.* template droplet characteristics or conditions to favour interfacial reactions. Research can also be focused on applications and then focuses on a more technical direction, such as microcapsules filled with a key component in a reaction mixture or filled with phase change materials.

To contribute to the broad field of microencapsulation, we tried to design a capsule synthesis method that would leave the core unaffected. This should be a synthesis method that can be employed for a very wide variety of active ingredients. Since this project followed on the project of Joris W.O. Salari, it started with optimized water-in-oil Pickering emulsion droplets as templates for the microcapsules, stabilized by *p*MMA particles, described extensively in his work.[1] After a literature review, silica turned out to be a suitable capsule material as described in Chapter 1. A number of encapsulation methods are described in Chapter 2. All reported procedures intend to leave the core uninfluenced. Much of the work was focused on the oil-soluble TEOS as a silica precursor, in combination with an amphiphilic oil-soluble catalyst *n*-hexylamine that is known to efficiently catalyze the polymerization of TEOS.[2] This method resulted in well-defined *p*MMA – silica microcapsules with a tunable shell thickness. This has been described in Chapter 3. Although the capsules were produced at ambient temperatures, it turned out that a comprehensive investigation of the mechanism of formation is necessary to decide whether or not contamination of the core can be expected. A comprehensive explanation about the influence of shell-forming chemistry on the core has been reported in Chapter 4. This explanation is based on the experimental data of Chapters 3 and 4. The prospects towards contamination-free hydrophilic capsule cores were promising, because it turned out that the reaction takes place on the outside of the capsules in the oil phase and the shell therefore grows from the inside to the outside.

Based on the encouraging results of Chapters 3 and 4, we embarked to encapsulate bacteria and keep them viable during and after the synthesis. The segregation of viable bacteria in Pickering emulsion droplets was successful. Unfortunately, bacteria are extremely sensitive towards *n*-hexylamine and its surface activity at the water-oil interface is enough to leave no viable bacteria within seconds after addition. However, with the knowledge we acquired about directing a reaction to the interface, viable bacteria were successfully encapsulated in polyalkylsiloxane microcapsules as described in Chapter 6. Another promising method to encapsulate

delicate material such as bacteria and live cells is by interconnecting the stabilizing particles of a Pickering emulsion droplet with a pre-synthesized polymer. A successful step in that direction was reported in Chapter 5, where stabilizing amine-functionalized silica microparticles are connected by the crosslinking aminolysis reaction of the maleic anhydride residue of *poly*(styrene-*co*-maleic anhydride).

The silica microcapsules that were produced using an amphiphilic catalyst (*n*-hexylamine) as reported in Chapters 3 and 4 are permeable. Within a few minutes after the start of drying, the hydrophilic core starts to evaporate. Nevertheless we strongly believe there is a cut-off molecular weight and that this feature can be used for different applications. A very promising application for these microcapsules is the encapsulation of catalytically active enzymes.[3] The enzyme-containing microcapsules are then suspended in a reaction mixture and substrate molecules diffuse through the shell into the 'microreactor' interior. Inside the microcapsules, the substrate is converted to product. This product can diffuse through the shell to the continuous phase. Through this procedure, reaction rates and products can be controlled to a larger extent than in a homogeneous reaction mixture. Product inhibition can be avoided or at least suppressed. Encapsulation of enzymes or *e.g.* large volume transition metal-based catalysts may be promising candidates for this technology. Applications of catalyst-containing capsules in (milli-sized) packed reactors leads to high volumetric reaction rates. This application is a nice example of process intensification. The biological model for this application is the selective permeability through membranes in biological cells.
Concerning the encapsulation of delicate materials, we also believe that encapsulation of bacteria that produce antimicrobial compounds is especially interesting. Since, it can find an application in much more specialized treatments against infections and infectious diseases. For example, the bacteria-containing microcapsules could be processed in a wound dressing. This is relevant because antimicrobial resistance is an increasingly public health problem.[4] Moreover, it can also be useful in research towards finding better antimicrobial treatments via cell signaling studies. Note that when the capsules are permeable for therapeutic molecules or enzymes, those products can more easily be separated from the fermentation mixture and analysed compared to the situation where the bacteria are in the same suspension. However, to realize these applications more control over the architecture of the microcapsules is necessary.

A novel concept is the design of stimuli-responsive microcapsules.[5] The microencapsulation method described in Chapter 5 is a suitable starting point for the encapsulation of bacteria in stimuli-responsive microcapsules. In that case, instead of *poly*(styrene-*co*-maleic anhydride), a polymer could be synthesized with responsive properties. Maleic anhydride groups are then used for the reaction with the amine-functionalized stabilizing

microparticles. If it turns out that the amine groups on the stabilizing particles in the Pickering emulsion leave no viable bacteria, then the process can be inverted. Or in other words, the stabilizing particles should contain the maleic anhydride groups and the pre-synthesized stimuli-responsive polymer should contain the amine groups for the aminolysis reaction.
More than Pickering emulsion droplets, the droplets that are produced via microfluidics offer large control over the architecture of microcapsules.[6] We think the synthesis method for compartmentalization of bacteria as described in Chapter 6 might be improved when instead of Pickering emulsion droplets, microfluidic droplets are used as templates for the microcapsules. The mixing of chemicals and the flow around the droplets seems to be better controllable with microfluidics. This is important when a very fast interfacial reaction takes place in order to ensure uniformity of solid formation around the droplets. A typical example of a fast reaction is the case of the chlorosilane reaction with water at the interface of the droplets in emulsion. Besides that, other chlorosilanes might yield favorable shell properties, which may induce permeability of the shell. If the capsules with the bacteria have a certain permeability, the two main criteria for encapsulation of bacteria are met. Namely, to preserve viability during the capsule synthesis procedure and thereafter, but also due to permeability, nutrients can diffuse to the inner part of the capsule and compounds produced by the bacteria such as antimicrobial polypeptides, can diffuse to the continuous phase, see Figure 7.1.

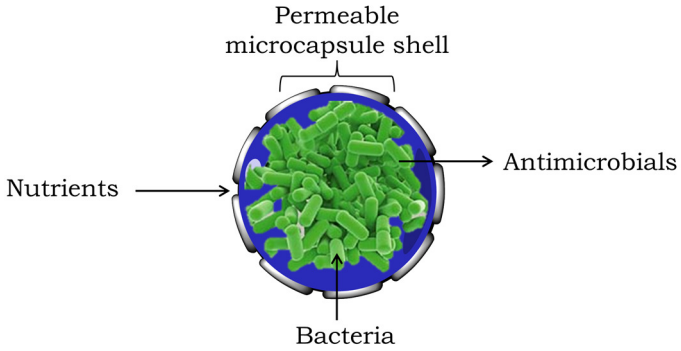

Figure 7.1 Compartmentalized antimicrobial peptide-producing bacteria within a permeable capsule.

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Judith van Wijk, Nedine van Deventer, Elrika Harmzen, Jan Meuldijk, Bert Klumperman, Formation of hybrid Poly(styrene-*co*-maleic anhydride) – Silica microcapsules, *J. Mater. Chem. B*, 2014, 4826-4835

Judith van Wijk, Joris W.O. Salari, Neomy Zaquen, Jan Meuldijk, Bert Klumperman, Poly(methyl methacrylate)-silica microcapsules by templating Pickering emulsion droplets, *J. Mater. Chem. B*, 2013, 1, 2394-2406

Judith van Wijk, Tiaan Heunis, Leon M.T. Dicks, Jan Meuldijk, Bert Klumperman, Compartmentalization of bacteria in microcapsules, (Submitted to *ChemComm*)

Judith van Wijk, Joris W.O. Salari, Jan Meuldijk, Bert Klumperman, Silica microcapsules by templating Pickering emulsion droplets: Mechanism of formation (In preparation)

Judith van Wijk, Bas van Ravensteijn, Joris W.O. Salari, Jan Meuldijk, Bert Klumperman, Some conditions for successful microcapsule synthesis by templating water-in-oil Pickering emulsion droplets (In preparation)

Presentations

J. van Wijk, B. Klumperman, J. Meuldijk, J.W.O. Salari, Silica Microcapsules by Templating Pickering Emulsion Droplets: Mechanism of Formation, *Oral presentation at the Dutch Polymer Days 2014, March, Lunteren, The Netherlands.*

J. van Wijk, B. Klumperman, J. Meuldijk, J.W.O. Salari, poly(methyl methacrylate)/Silica Microcapsules by Templating Pickering Emulsion Droplets, *Oral presentation during the Graduate Research Seminar, June, 2013 Fudan University, Shanghai, PR China*

J. van Wijk, B. Klumperman, J. Meuldijk, J.W.O. Salari, poly(methyl methacrylate)/Silica Microcapsules by Templating Pickering Emulsion Droplets, *Poster presentation at the International Polymer Colloids Group Conference, June 2013 Fudan University, Shanghai, PR China*

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J. van Wijk, B. Klumperman, J. Meuldijk, J.W.O. Salari, A Phase Change Material Slurry to Store and Transport Thermal Energy, *Poster presentation at* the Nederlands Process Technology Symposium, October, 2010, *Veldhoven, The Netherlands*

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Curriculum Vitae

Judith van Wijk was born on 30-11-1983 in Gorinchem, The Netherlands. After finishing the pre-university secondary education (HAVO) in 2003 at Nassau Scholengemeenschap in Breda, she studied Chemical Engineering at Eindhoven University of Technology in Eindhoven. In 2010 she graduated within the Development group on Reduction of residual monomer from polymer latex with super critical CO_2, supervised by prof.dr. J. Meuldijk. Before that, she obtained her BSc degree in Chemical Engineering at the University of professional education, Avans Hogeschool in Breda. In 2007 she graduated on process improvement of chemical cleansing of varnished metal. From 2010 she started a PhD project at Eindhoven University of Technology in Eindhoven of which the results are presented in this dissertation, supervised by prof.dr. J. Meuldijk and prof.dr.ir. L. Klumperman. This project was a collaboration between the Materials Chemistry group and the Polymer Reactor Engineering group of the Department of Chemical Engineering and Chemistry in Eindhoven, and the Advanced Macromolecular Architectures group of the Department of Chemistry and Polymer Science Stellenbosch University (South Africa). As a part of this collaboration, she spent 6 months at Stellenbosch University.