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Formation of hybrid Poly(styrene-*co*-maleic anhydride) – Silica microcapsules

Judith van Wijk[1], Nedine van Deventer[2], Elrika Harmzen[2], Jan Meuldijk[1], Bert Klumperman[2,*]

[1]Eindhoven University of Technology, Department of Chemical Engineering and Chemistry, P.O. Box 513, 5600 MB Eindhoven, the Netherlands

[2]Stellenbosch University, Dept Chemistry and Polymer Science, Private Bag X1, Matieland 7602, South Africa

e-mail: bklump@sun.ac.za

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.

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 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 2 and 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.

The main advantages of the synthesis procedure described above to *e.g.* Pickering emulsion polymerization to produce microcapsules[21] is firstly that the core is not contaminated in the process of capsule formation and secondly that the polymer is pre-synthesized before addition, which allows independent control of the polymer properties. 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. In addition, all Pickering emulsions were produced manually to avoid severe conditions, in this case due to high shear emulsification. 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, which is a relatively new concept within the state of the art of microencapsulation.[7,8]

Results and discussion

Figure 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 is 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.[22–24] 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.[22] 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°.[22]

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 1.

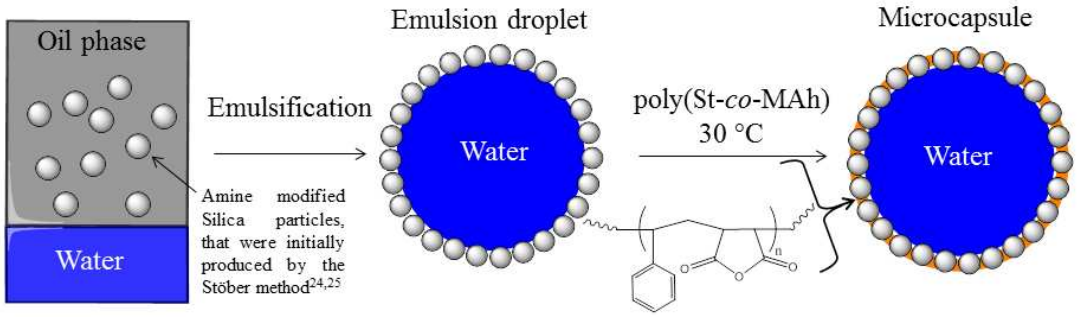

Figure 1. Overview of the synthesis route to produce hybrid poly(St-*co*-MAnh) SiO_2 microcapsules.

The silica particles were synthesized using the well-known Stöber mechanism to produce monodisperse seed silica particles, followed by a seeded polymerization technique.[25,26]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.[25] 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 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.

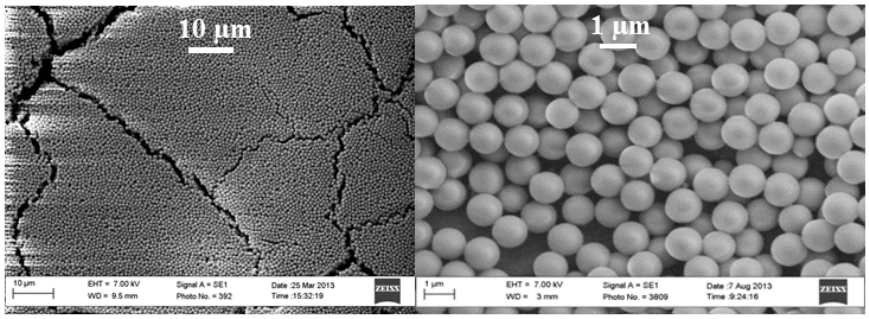

Figure 2. Silica microparticles. Produced by the Stöber mechanism, followed by a seeded polymerization technique. [25,26]

To tune the hydrophobicity of the silica microparticles, octadecyltrichlorosilane (OTC) was used as the first modification agent, see Figure 3A. 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}, Figure 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}, Figure 3A.[27,28] 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.[29] 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, Figure 3A.[30] 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.[31,32] Instead, an OTC-based network structure will be produced and enough hydroxy groups will be left for the next modification step, Figure 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, Figure 3B. After the second modification step, the particles still were suitable for inverse Pickering stabilization. After redispersion of the modified silica particles in ethyl acetate, to get rid of the unreacted agents, water was added and upon emulsification of this mixture, the Pickering emulsion was produced, Figure 4.

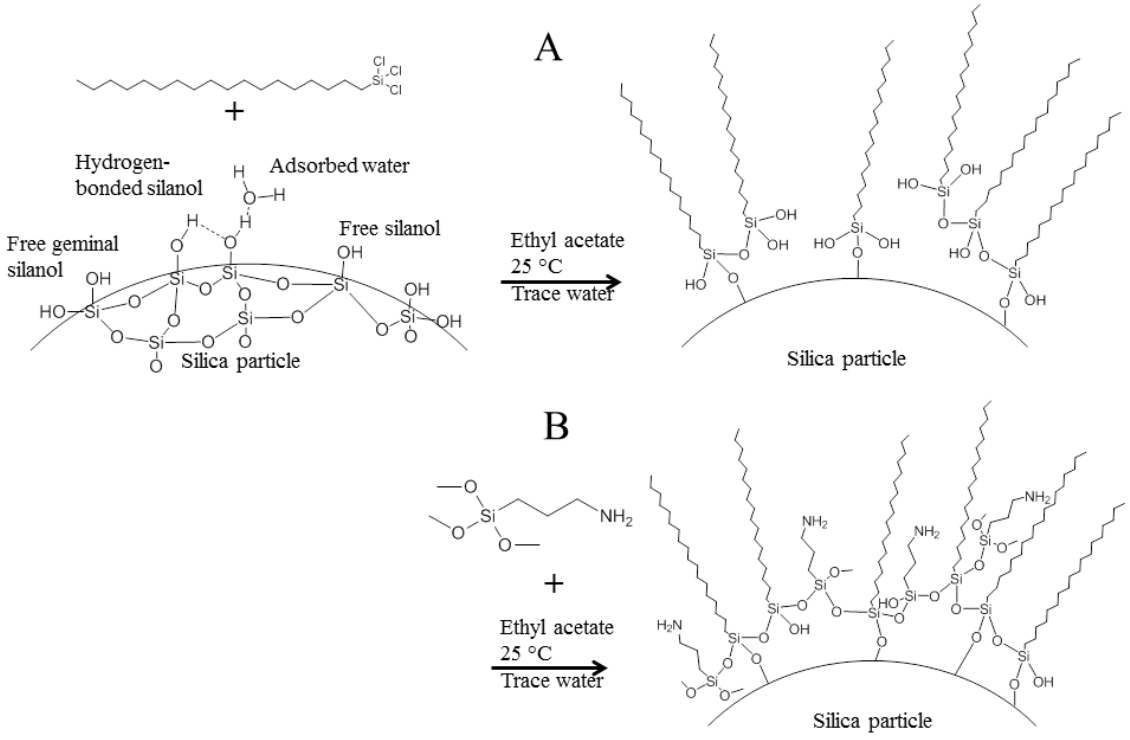

Figure 3. Modification steps of the silica microparticle surface. The reaction of octadecyltrichlorosilane (OTC) with the reactive silanol groups on the silica surface, with the adsorbed water and with each other (A) to make the particles more hydrophobic. The reaction of residual

hydroxy groups on the hydrophobized silica microparticles with aminopropyl trimethoxysilane (APTMS) (B).

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.[33]

$$D_D = \frac{6V_D}{N_A 2\sqrt{3}R^2} \qquad 1.$$

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.

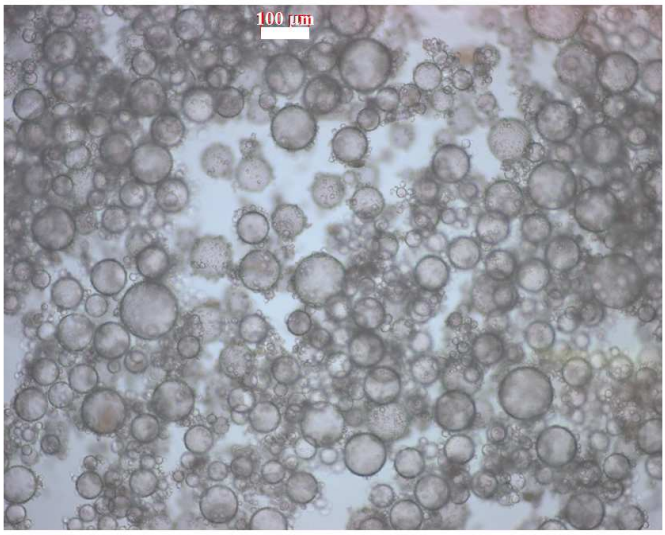

Figure 4. Light microscopy image of an inverse Pickering emulsion. The Pickering emulsion was produced by the addition of 3 mL distilled water to 0.35 g silica particles (d_p = 740 nm) dispersed in 40 mL ethyl acetate. The particles were functionalized with 18 µmol m^{-2} OTC and 90 µmol m^{-2} APTMS.

Since the Pickering emulsions were produced manually, exact control of the droplet size and the droplet size dispersity proved to be difficult, compared to emulsification using high shear, see Figure 4.[33] It takes a certain force to position a particle at the interface and a certain time to position them all

at the interface. Manual emulsification was selected to avoid severe conditions, since high shear is not desirable in the case of encapsulation of delicate materials, *e.g.* live bacteria.

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.[34] 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 5B. 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, Figure 5A.

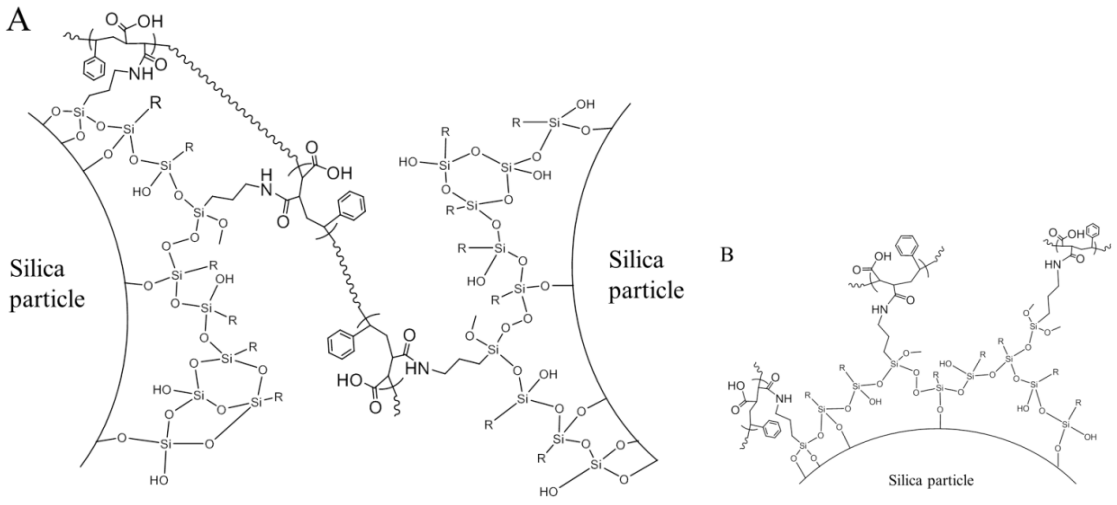

Figure 5. Schematic illustration of the crosslinking of amine and octadecyl (-R) functionalized silica microparticles with poly(St-*co*-MAh). Successful crosslinking will be the result if one polymer chain reacts with more than one amine group, on different particles (A), otherwise crosslinking will not take place (B)

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. 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 6A 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[35], 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 in water of the capsules was favored by their hydrophilic properties. Although the microcapsules can be re-dispersed in pure water, the process can be (and was in the current study) further enhanced by the addition of some acetone 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*, and micro*capsules* would not be observed with LM. Figure 6B, 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, Figure 6B. The debris observed in Figure 6B is most probably due to particle aggregates. This can be caused by *e.g.* insufficient re-dispersion of the microparticles in ethyl acetate before modification. Furthermore, since emulsification took place manually, the size distribution in the

primary Pickering emulsion was broad in comparison to the scenario where high shear would have been used. Therefore, residual microparticles were present in the continuous phase and aggregated with the microparticles at the interface of the microdroplets.

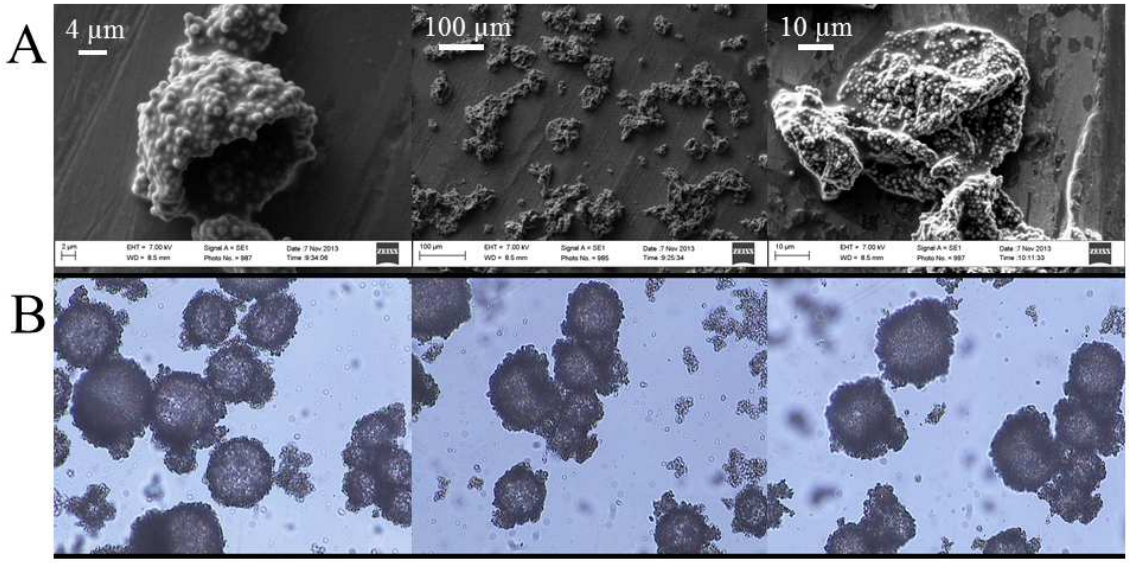

Figure 6. Scanning Electron Microscope (A) and Light Microscope (B) images of hybrid poly(St-*co*-MAh) – SiO_2 microcapsules, produced by poly(St-*co*-MAh)-induced crosslinking of the stabilizing silica microparticles of an inverse Pickering emulsion at the interface.

Although it can now be concluded that poly(St-*co*-MAh) – SiO_2 microcapsules have been produced (Figure 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. 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 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 trimethoxysilane, see Figure 7.[36] The green fluorescent poly(St-*co*-MAh) was produced by the partial aminolysis of MAh with 5-aminofluorescein, see Figure 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.

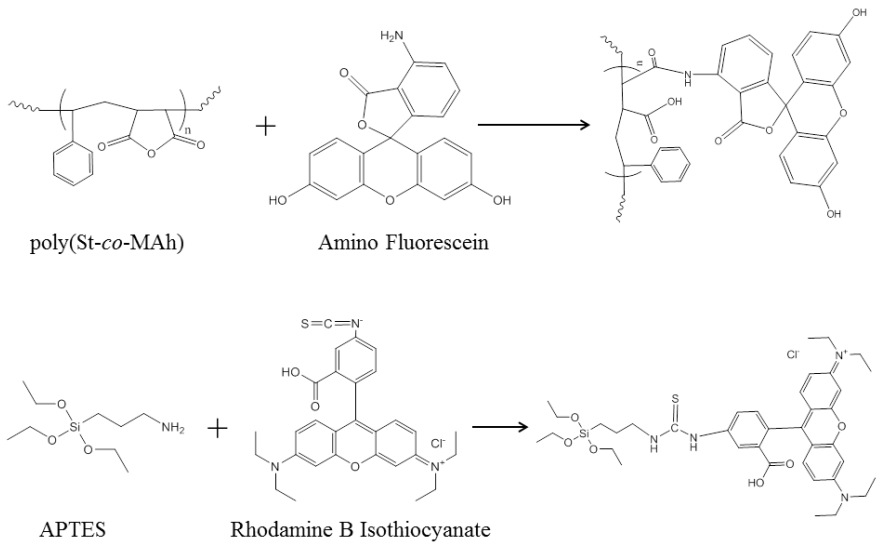

Figure 7. Synthetic scheme for the labeling of poly(St-*co*-MAh) with 5-aminofluorescein and of APTMS with rhodamine B isothiocyanate.

As a result, after the microcapsule synthesis with the labeled precursors, the silica particles that initially stabilized the emulsion could be observed, see Figure 8B. Also, the labeled polymer could be observed, Figure 8A. When the two individual images are overlaid, it can be concluded that the majority of the polymer in in contact with the amine-functionalized particles, see Figure 8C. 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.

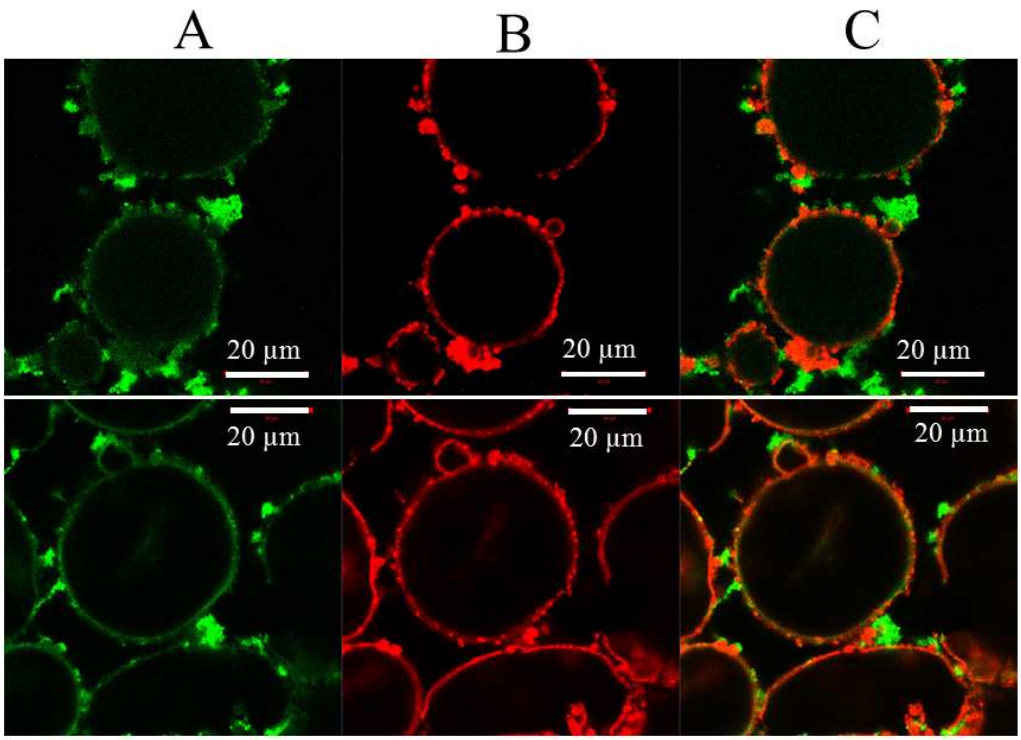

Figure 8. Confocal Fluorescence microscope images of hybrid poly(St-*co*-MAh) – SiO_2 microcapsules (C). Produced with green fluorescein-labeled poly(St-*co*-MAh) (A) and red rhodamine B-labeled silica microparticles (B).

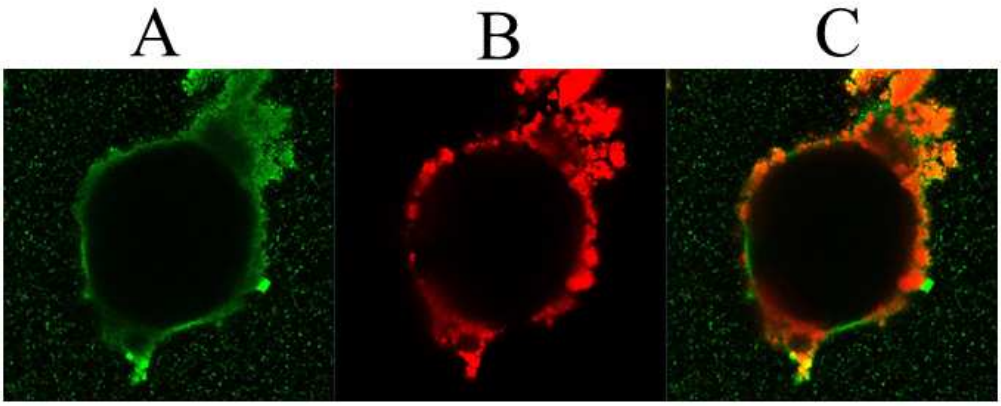

Figure 9. Confocal Fluorescence microscope images of hybrid poly(St-*co*-MAh) SiO_2 microcapsules (C). Produced with excess green fluorescein-labeled poly(St-*co*-MAh) (A) and red rhodamine B-labeled silica microparticles (B).

When excess polymer was added, this could be observed by the green dye in the continuous ethyl acetate phase, Figure 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, Figure 9 A and C.

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. Future work will also combine the present work with the use of microfluidics to allow the formation of uniform capsule sizes under low-shear conditions. [37,38]

Materials

All chemicals were used as received, unless indicated otherwise. Tetraethyl orthosilicate (TEOS), (3-aminopropyl)trimethoxysilane (99%) (APTMS), 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.

Methods

The silica microparticles were produced by an initial synthesis of monodisperse seed silica microparticles (+/- 500 nm) using the well-known Stöber technique.[26] 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.[25] 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 trimethoxysilane, according to a procedure described by van Blaaderen *et al*.[36,39] Briefly, in 5 mL anhydrous ethanol, aminopropyl trimethoxysilane (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.[36,39]

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.[40] 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, APTMS (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.[41]

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.

Analysis

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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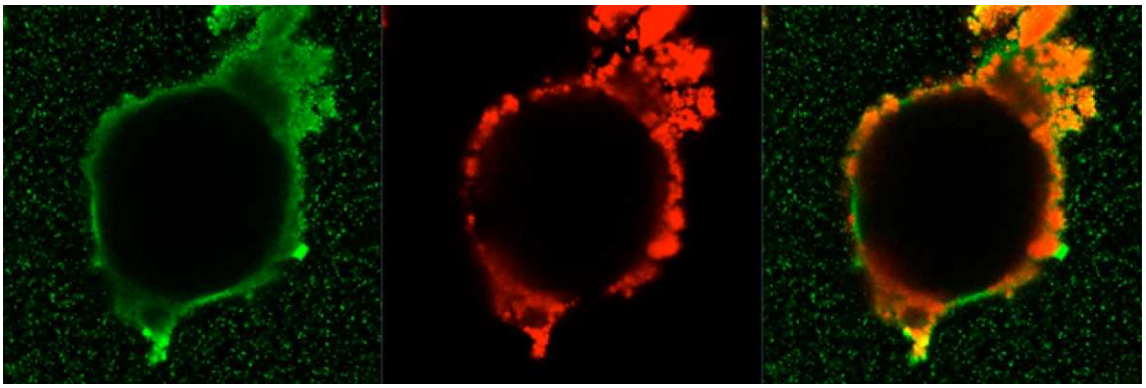

A technique for the micro-encapsulation of a contamination-free aqueous droplet is presented.