Nanophase Separated Amphiphilic Microbeads

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Organo-chemical reactions, where the substrates and the catalyst are not soluble or stable in the same solvent, e.g., enzyme-catalyzed reactions in organic solvents and organo-metal-catalyzed reactions in water, are often performed with suspended or immobilized catalysts or as phase transfer reactions. In the first case the catalyst often exhibits lower activity compared to that of its dissolved form. Phase transfer reactions, on the other hand, are usually elaborate because two nonmiscible solvents are used. An alternative that afforded high activity and easy handling was the use of catalysts immobilized on polymeric microbeads.[1,2] Since then, the bead design was mainly focused on large porosity and functional density. Later, the adsorption of molecules was influenced more selectively by molecular imprinting.[3,4] More recently, the benefits of amphiphilic structures to various catalytic applications moved into focus of research.[5] Various efforts to prepare amphiphilic polymer beads have been made in order to achieve high-affinity catalyst supports or high-performance chromatography materials. A common way is the modification of the surface of prestructured inorganic microspheres, e.g., silicate,[6,7] or porous polymeric beads, mostly polystyrene,[8] via physically or covalently grafting polymers or amphiphilic block copolymers. Alternatively, hydrophobic beads made of chemically different but miscible monomers have been prepared and subsequently modified. For example, Yashuda et al. prepared amphiphilic microbeads by amination of beads composed of a mixture containing poly(glycidyl methacrylate) and subsequently statistically modifying them with fatty acids.[9] Further, the cross-linking of amphiphilic block copolymers or dendrimers led to amphiphilic microparticles.[10,11] Mostly, PEG telechelics have been used as cross-linking agent of polystyrene or acrylates to achieve polymer beads swellable in water and organic solvents.[12] Another way to amphiphilic beads was described by Shahidi et al., who prepared microbeads consisting of two interpenetrating networks, being cross-linked rubber and a poly(acrylic acid).[13] Although some of these particles were found to have greater affinity to catalysts or substrates, they all function in the common way; i.e., the catalysts is immobilized on the surface of the mostly porous beads.

In previous work, we could show that nonporous amphiphilic conetworks exhibit a great potential as carriers for catalysts in phase transfer reactions, particularly for biotransformations in organic solvents.[14] The concept for such an application is based on the nanophase separation of two incompatible polymer phases, which can be swollen separately in a suited solvent, being a typical behavior of amphiphilic conetworks.[15] Thereby, one selectively swollen polymer phase is loaded with the catalyst dissolved, e.g., in water. After drying and subsequent swelling of the conetworks in an orthogonal solvent, the substrate, dissolved for instance in *n*-heptane, diffuses into the other polymer phase of the conetwork. The catalyzed reaction takes place at the huge interface between the two polymer phases. Since the demixing of the incompatible macromolecule segments during polymerization is very fast, only thin coatings and membranes that are fully nanostructured could be obtained in water-free systems.[16] Thus, the synthesis of amphiphilic conetwork microbeads could not be realized so far. Here, we describe the first synthesis of such thoroughly nanostructured nonporous amphiphilic polymeric microbeads and their evaluation as catalyst support.

We chose to apply a precursor strategy for amphiphilic conetworks,[17] whereby a hydrophobic polymeric cross-linker (α,ω-methacrylate-terminated poly(dimethylsiloxane), MA-PDMS-MA, degree of functionalization 0.92, MW = 5200 g/mol, PD = 1.3) is copolymerized with trimethylsilylated 2-hydroxyethylacrylate (TMSOEA) to yield an hydrophobic precursor network. Upon subsequent cleavage of the TMS groups, an amphiphilic, nanophase separated conetwork is formed as we could show in previous work.[16] The challenge in transferring this synthesis to a microbead preparation via suspension polymerization was the use of water as polymerization medium because the TMS protecting groups are easily cleaved in an aqueous environment, which immediately leads to a poly(2-hydroxyethyl acrylate) (PHEA) cover on the respective surface.

The synthesis was performed with a mixture of MA-PDMS-MA, TMSOEA, and the photoinitiator Irgacure 651 (3 wt % regarding to monomer mixture) emulsified in a surfactant solution in 100 mM phosphate buffer, pH 7.0. The resulting emulsion (10 wt % monomer mixture) was UV-radiated at 340 nm for 3 min. The monomer mixture was adjusted to 50 vol % PDMS, and four different surfactants were used. While the negatively charged surfactants SDS and Disponil FES77 effectively stabilized the emulsion of the PDMS/TMSOEA mixture in phosphate buffer at concentrations as low as 0.2 wt %, the neutral surfactants Lutensol AT25 and Pluronic F68 needed to be used at 2 wt % to avoid aggregation during polymerization. In all cases, a dispersion of spherical polymer particles was obtained. The beads were filtered off, were washed with the phosphate buffer to remove the respective surfactant, and were then immersed into a water/methanol mixture (1:1, v/v) to cleave the TMS groups. The obtained polymer beads of the amphiphilic conetworks are perfectly round shaped and do not show any porosity as seen in the ESEM (environmental scanning electron microscope) image displayed in Figure 1a on the example of beads prepared with the aid of Disponil. The diameter of the particles was measured to be in a range of 30−80 μm. Additional measurements with light scattering confirmed the particle's diameter with a maximum of 53 μm and a standard deviation of 27 μm, which also shows the relatively broad distribution of the polymer bead size. Similar results regarding particle

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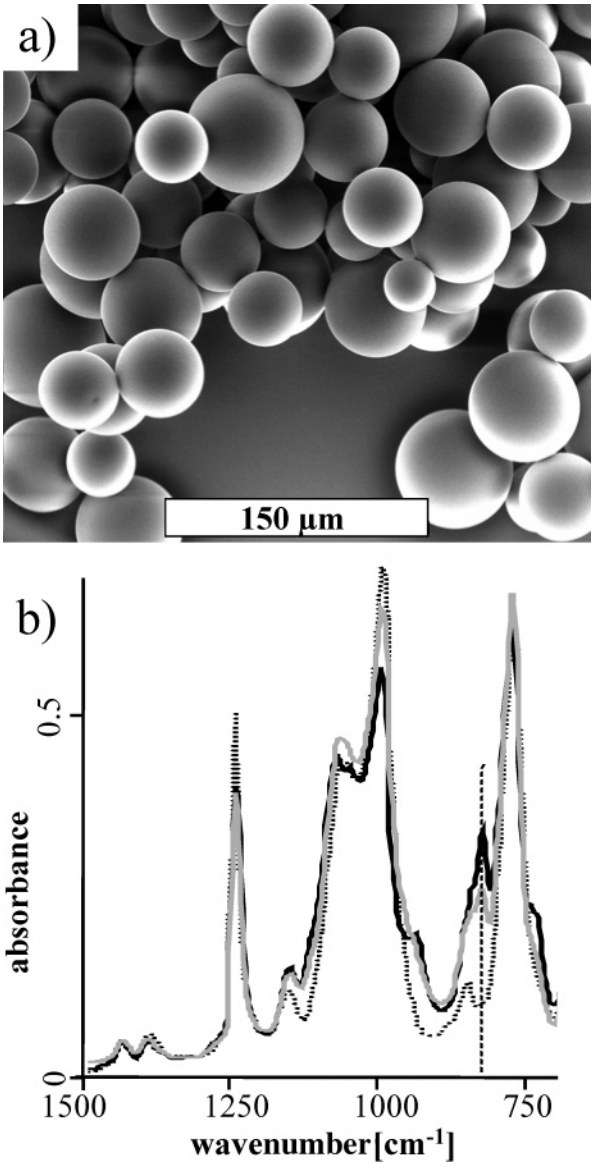


Figure 1. (a) ESEM image of PHEA-*l*-PDMS microparticles prepared with Disponil and subsequent immersion in water/methanol for 24 h. (b) ATR-FTIR spectra of microparticles synthesized from MA-PDMS-MA (50 vol %) and TMSOEA prepared with Disponil (black —), with SDS (gray —), and with Disponil and subsequent immersion in water/methanol for 24 h (- - -).

shape and size were found for polymer beads prepared with SDS, Lutensol, and Pluronic.

ATR-FTIR was used to investigate the chemical composition of the particles near their surface. A signal at 836 cm^{-1} corresponds to the O−Si bond of the TMS-protected PHEA chain segments and disappears upon cleavage of the TMS groups. As seen in Figure 1b, when preparing the microbeads with Disponil, the spectrum is that of a water-free fully protected PTMSOEA-*l*-PDMS conetwork.[18] Samples prepared with Pluronic and Lutensol (spectra not shown) look similar to that displayed in Figure 1b, i.e., no significant cleavage of the TMS group occurred. However, when using SDS as surfactant, the signal of the TMS-group is significantly lower compared to that of the pure PTMSOEA-*l*-PDMS network (cf. Figure 1b). Altogether, according to the IR data, only polymeric surfactants regardless their charge are suitable to protect the TMS-modified HEA during polymerization, while the low molecular weight compound SDS cannot provide this. However, subsequent transformation of the obtained precursor networks into amphiphilic ones by cleavage of the TMS groups in methanol/water is fully achieved. All ATR-FTIR spectra reveal the complete disappearance of the TMS-typical signal at 836 cm^{-1} as shown in the example of the PHEA-*l*-PDMS microbeads prepared with Disponil (Figure 1b).

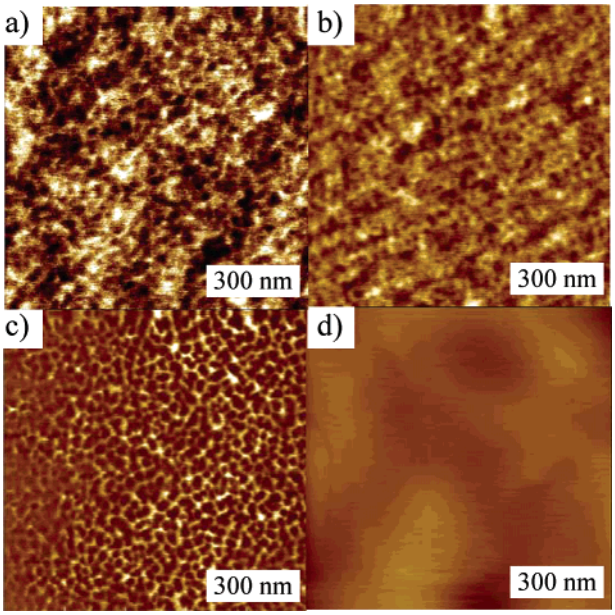


Figure 2. AFM phase mode images of the surfaces of amphiphilic PHEA-*l*-PDMS (60 wt % PDMS) microbeads prepared with (a) Disponil, (b) Lutensol, (c) Pluronic, and (d) SDS as emulsifier.

It was now investigated with atomic force microscopy (AFM) if both polymer phases are present on the surface of the particles, which is crucial for the application of the microbeads in the phase transfer catalysis. Figure 2 displays the AFM images in phase mode (discriminates soft (PDMS, dark) and hard (PHEA, bright) material) of the surfaces of PHEA-*l*-PDMS microbeads. While the particles prepared with polymeric surfactants (Figure 2a−c) show indeed both polymer segments on their surface, the SDS-prepared microbeads do not. The latter is most likely due to a surface demixing of the hydrophilic PHEA segments during the polymerization, which occurs according to the IR data shown in Figure 1b. This apparently leads to a surface covered with PHEA. However, it seems that every surfactant affords a specific surface morphoplogy. Thereby, Pluronic results in beads with the most distinguishable morphology.

To investigate the phase separated bulk morphology of the PHEA-*l*-PDMS microspheres, samples prepared with Pluronic as emulsifier were embedded in a silicone matrix and cut under liquid nitrogen, and the cross sections were again explored with AFM. As seen in Figure 3a, the amphiphilic polymer microbead shows a continuous nanostructure from the inside to the surface. Thereby, the PHEA segments form a spongelike continuous phase with domains sizes of about 3−6 nm and the PDMS domains (18−30 nm) are embedded into the PHEA structure. Even the surface morphology, which looks similar, is evident in this picture (cf. Figure 2c). Images with comparable morphologies were observed for all preparations with polymeric emulsifiers. Comparing surface (Figure 2) with bulk morphology (Figure

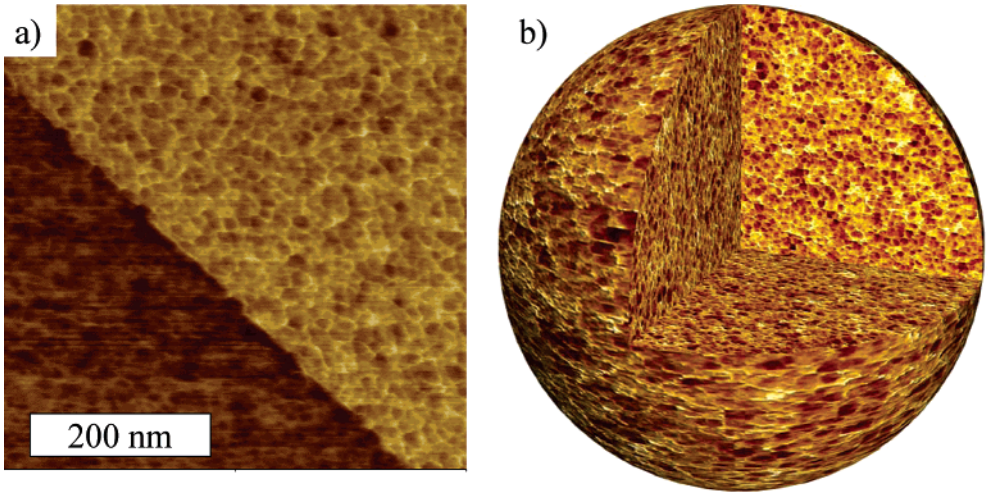
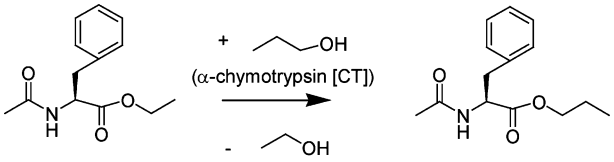


Figure 3. (a) AFM phase mode image of a cross section of a silicone embedded PHEA-*l*-PDMS microbead (60 wt % PDMS, 500 × 500 nm^2). (b) Proposed structure according to the AFM images measured on and within the PHEA-*l*-PDMS microbeads.

Table 1. Initial Activities of Free and PHEA-*l*-PDMS Embedded α-Chymotrypsin (CT) in the Transesterification of APEE with Propanol in Dry *n*-Octane (Water Content < 0.02 wt % According to Karl Fischer Titration) at 30 °C, 30 mM APEE, and 1 M Propanol



APEE APPE

PDMS content of carrier	CT loading[a] (wt %)	activity of CT		activity enhancement[d]
		TOF[b] (min^{-1})	U/mg[c]	
free enzyme		0.08	2×10^{-6}	
40 wt %	2.4	2.0	50×10^{-6}	25
60 wt %	1.5	13.4	355×10^{-6}	165

[a] mg of CT/mg of carrier. [b] TOF = turnover frequency [mol of APPE/(mol of CT min)]. [c] μmol of APPE/(min mg of CT). [d] v(embedded enzyme)/v(free enzyme).

3a) leads to the conclusion that the prepared PHEA-*l*-PDMS are truly thoroughly nanophase separated microbeads, as illustrated in Figure 3b.

The suitability of the amphiphilic PHEA-*l*-PDMS microbeads as phase transfer matrix for catalysis was explored in the example of the α-chymotrypsin(CT)-catalyzed transesterification of *N*-acetyl-L-phenylalanine ethyl ester (APEE) with propanol in *n*-octane to *N*-acetyl-L-phenylalanine propyl ester (APPE), a common reaction to explore the performance of CT in organic solvents.[19] To this end, PHEA-*l*-PDMS microbeads of two different compositions (40 and 60 wt % PDMS) prepared with Pluronic as surfactant were loaded with the enzyme by immersing them into a buffered aqueous solution of the enzyme (10 mg CT/mL 10 mM phosphate buffer, pH 7.8) overnight. The loaded amount was determined by measuring the decrease in absorbance of the enzyme at 280 nm in the aqueous solution. As expected, greater PHEA content resulted in increased CT loading (see Table 1). However, taking into account the volume fraction of PHEA, the enzyme loading into the PHEA phase is nearly constant. Further, the enzyme is greatly concentrated (5−10-fold) into the network compared to the surrounding enzyme solution. The particles were then simply air-dried overnight, which lowered the water content below the detection limit of some 0.1 wt %. The initial rates of the transesterification reaction shown in Table 1 reveal the great activation potential of the amphiphilic network beads with the best activity being 165 times greater than that of the merely suspended native enzyme. The enzyme does not leach in the medium during the reaction as proven by a complete halt of the product formation after removing the particles.

The activation of the immobilized CT increases with greater PDMS content. This is probably due to smaller hydrophilic phases at greater PDMS content, affording the loaded enzyme molecules to closer proximity to the interface between the hydrophilic and the hydrophobic domains of the network. Exploring this phenomenon will be part of future investigations, applying networks loaded with labeled enzymes to high-resolution microscopy such as AFM and TEM. Comparing the results to literature experiments, where CT was activated upon entrapment, shows the good performance of the amphiphilic microbeads. The best results so far were obtained by Novick and Dordick,[20] who covalently immobilized CT on a polysaccharide matrix and afforded a 34-fold activation of the enzyme, and by van Unen et al.,[21] who entrapped the enzyme into a siloxane matrix via a sol−gel technique, which led to a 45-fold activation of CT.

In closing, we could demonstrate the first successful synthesis of nonporous nanophase separated amphiphilic conetwork microbeads. The beads show the phase separation in bulk as well as on the surface and could be shown to be effective as solid-phase transfer medium on the example of a α-chymotrypsin-catalyzed biotransformation in octane.

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