

Synthesis of poly(ethylene glycol) (PEG)-grafted colloidal silica particles with improved stability in aqueous solvents

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Abstract

The known grafting procedures of colloidal silica particles with poly(ethylene glycol) (PEG) lead to grafting layers that detach from the silica surface and dissolve in water within a few days. We present a new grafting procedure of PEG onto silica with a significant improvement of the stability of the grafting layers in aqueous solvents. Moreover, the procedure avoids any dry states or other circumstances leading to strong aggregation of the particles. To achieve the improved water stability, Stöber silica particles are first pre-coated with a silane coupling agent (3-aminopropyl)triethoxysilane (APS) to incorporate active amine groups. The water solubility of the pre-coating layer was minimized using a combination of APS with bis-(trimethoxysilylpropyl)amine (BTMOSPA) or bis-(triethoxysilyl)ethane (BTEOSE). These pre-coated particles were then reacted with *N*-succinimidyl ester of mono-methoxy poly(ethylene glycol) carboxylic acid to form PEG-grafted silica particles. The particles form stable dispersions in aqueous solutions as well as several organic solvents.

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1. Introduction

Composite colloidal particles [1] consist of at least two types of materials, often with one material on the outside and one in the center of the particle. These composite particles combine different material properties such as specific (bio)chemical, optical, electrical, magnetic and mechanical properties. Furthermore, different stabilization layers can also be used to direct the formation of specific mesoscopic structures.

In this paper we present a new synthesis procedure for colloidal dispersions of silica particles terminally grafted with poly(ethylene glycol) (PEG) with a much improved stability in water as compared to known synthesis protocols. Moreover, the procedure avoids any dry states or other circumstances leading to strong aggregation of the particles. For these particles the chemical possibilities for modifications and the biocompat-

ibility of silica are combined with the steric stabilization of the biocompatible PEG. The possibility to vary the molecular weight and the grafting density of the PEG might make the dispersions of interest for fundamental model studies on colloidal dispersions. Furthermore, the particles as well as variations of the synthesis procedure may be of interest for biological and biomedical applications because of the decreased solubility in water of the particle surface layer as compared to the bare silica particle. Indeed, similar coatings have been tested in applications such as drug delivery, biosensors, diagnostics, and medical imaging [2–4]. The grafting procedure presented here, which leads to an improved water stability and renders colloidally stable dispersions, might be of value in such applications.

This paper is organized as follows. In Section 2.1 an overview is given of previous work related to PEG grafting of silica surfaces. These previous synthesis methods have in common that the PEG-grafted layer dissolves in water. Section 2.2 describes relevant work on the water stability of silane coatings. Section 3 introduces the new synthesis method and Section 4

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gives the experimental details of both the pre-coated particles and the subsequent coating with PEG, resulting in grafted silica particles with an improved stability in water. Section 5 presents the characterization and properties of the newly synthesized particles, and Section 6 is a summary of the work presented here.

2. An overview of previous work

2.1. PEG grafting on silica

Already about 25 years ago, Bridger and Vincent [5] reported on the synthesis of poly(ethylene oxide) (PEO) grafting on particulate silica surfaces (whether PEO or PEG depends on how the polymer is synthesized). They first prepared isocyanate-capped PEO, which was reacted with (3-aminopropyl)triethoxysilane to obtain a triethoxysilane-terminated PEO, which was added at the end of a Stöber synthesis [6]. However, after storing the samples for about one month in aqueous solutions particle aggregation was observed and evidence was found for free PEO in solution. They attributed this phenomenon to three possible reasons: hydrolysis of the link between the $(\text{CH}_3\text{CH}_2\text{O})_3\text{Si}-$ moiety and the PEO chain (urethane in their case), oxidative degradation of the PEO in aqueous solution, or slow dissolution of the surface layer of silica (to which the PEO chains are bound). The first reason can be avoided by using a strong linking bond such as a silicon–carbon bond and an amide bond as in our case. The second one can be minimized by adding some isopropanol or ethanol [5,7]. It was suggested that the third reason is the main factor to explain the instability of the grafted layer in water. It may be that the layer formed by the end group $(\text{CH}_3\text{CH}_2\text{O})_3\text{Si}-$ linked to the PEG is destroyed and/or the underlying layer of silica is dissolving, so that the PEG will detach from the surface. The results in our work confirm this suggestion.

Since then, many publications of PEG grafting on silica surfaces have appeared. However, none of them address the stability of the PEG grafting in water, and furthermore most of them deal with the coating of macroscopic surfaces or the coating of dry silica particles. Here we deal with the PEG coating of colloidal dispersions of silica particles avoiding any dry states or other circumstances that lead to irreversible aggregation between the colloidal particles. Especially, the dry state generally leads to strong particle aggregation. As far as we know, four studies avoiding the dry state have been published since the work of Bridger and Vincent [5]. However, none of these studies discusses the problem of the limited water stability.

Xu et al. [3] prepared PEG-coated silica by adding hydroxyl terminated PEG at the start of the preparation procedure of silica particles by a synthesis according to Stöber in a methanol–ammonia mixture (note, that the more common ethanol is not a good solvent for higher molecular weight PEGs). The advantage of the method for biological applications is that one only uses silica and PEG and does not introduce any other chemistry, like linking groups, which might influence the biocompatibility of the particles. The disadvantage is that an unknown amount of the PEG will probably be buried inside the particles making a

quantitative determination of the amount of PEG on the surface almost impossible. Furthermore, for fundamental studies these particles are less well suited because different fractions of the individual PEG chains will stick out of the particles, i.e. this is PEG coating and not PEG grafting.

Oh et al. [8] prepared PEG-grafted silica particles by adding end-functionalized PEG to a preparation of silica in an emulsion. The particles obtained are nicely spherical, but quite poly-disperse. The main limitation of the method is that the synthesis procedure in an emulsion makes it difficult or impossible to coat some specially modified silica particles prepared by another method.

Joubert et al. [9] are the only researchers who prepared PEO-grafted silica with the so-called “grafting from” method in which the grafting polymer is grown from the surface of the particles, contrary to the method used by all others (including us) who linked pre-made polymers to the particles, the so called “grafting onto” method. The method yields high grafting densities, but so far the grafted polymers are of low molecular weight and have a broad molecular weight distribution.

Yoon et al. [4] prepared silica-coated magnetic particles and grafted them with a low molecular weight PEG. This was done in a very convenient way by adding a commercially available trimethoxysilane terminated PEG with a polymerization degree of 6 to 9 to the dispersion at the end of the synthesis. The method is essentially similar to the method used by Bridger and Vincent [5], apart from the fact that the latter synthesized the triethoxysilane terminated PEO themselves and also used a molecular weight of 18,000 g/mol. However, after hydrolysis these silane-terminated PEGs are expected to aggregate among each other in solution, which can result in inhomogeneous grafting layers as was shown by Jo and Park for PEG-grafted glass plates [2].

2.2. Relevant work on the water stability of silane coatings

Etienne and Walcarius [10] showed that 67% of the total (3-aminopropyl)triethoxysilane (APS, see Fig. 2) grafted onto silica gel was liberated in solution after about 4 h in contact with pure water. This result seems to be in agreement with the suggestion of Bridger and Vincent [5] that the solubility of the surface layer limits the stability of PEG-grafted silica in aqueous solutions. Our results further support this idea. Much effort has thus been devoted to forming a stable pre-coating layer to which the PEG can be linked. For this purpose, bridged silane coupling agents were introduced in the pre-coating layer. Bridged silane coupling agents, when hydrolyzed, contain two or more $-\text{Si}(\text{OH})_3$ groups. When modifying metal surfaces for corrosion protection and adhesion promotion, a more stable film can be formed with a mixture of APS and 1,2-bis-(triethoxysilyl)ethane (BTEOSE, see Fig. 2) than with pure APS [11,12]. Also bis[3-(trimethoxysilyl)propyl]amine (BT-MOSPA, see Fig. 2) emerged as an outstanding example in corrosion protection of metals in recent years [13,14]. The improved stability of the metal coatings may be attributed to the 6-fold hydrolyzable groups which are expected to have enhanced cross-linking ability [15] and maybe the hydrophobic

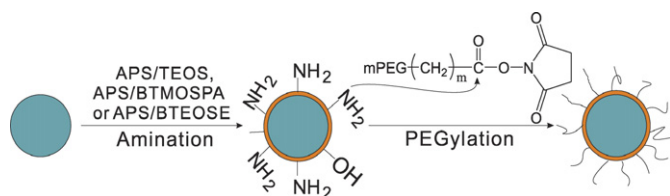
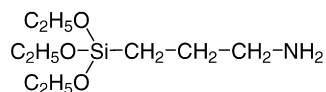
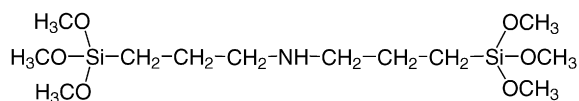


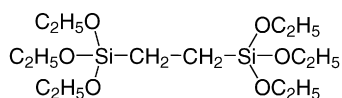
Fig. 1. Synthesis of PEG-g-silica particles.



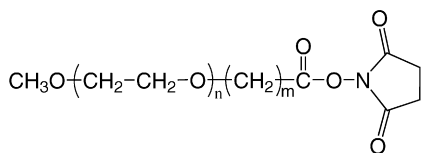
APS



BTMOSPA

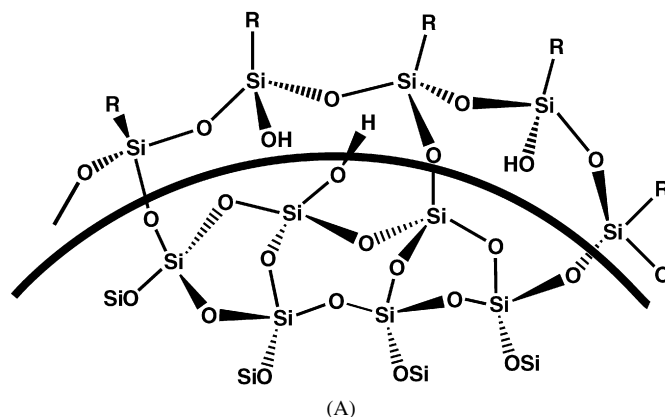


BTEOSE

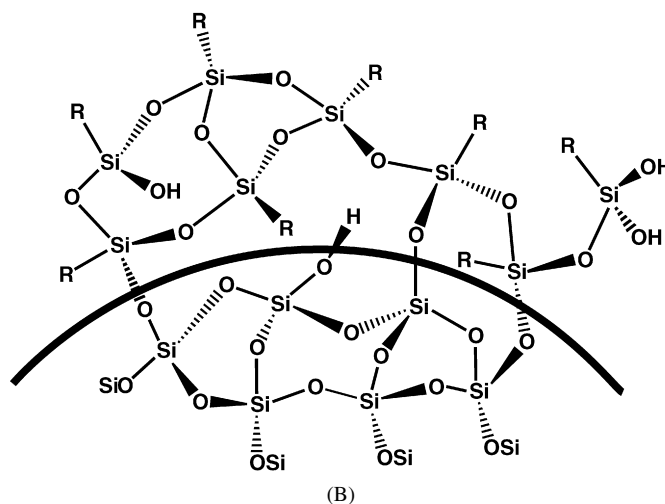


N-succinimidyl ester of methoxy PEG carboxylic acid

Fig. 2. Structure of agents used in this work.



(A)



(B)

Fig. 3. Structure of the layer formed from coupling agents or silanated PEG on the surface of silica particles (redrawn from Ref. [26]): (A) monolayer, (B) multilayer.

alkyl moieties which limit the contact with water [10]. These results promoted the use of BTEOSE and BTMOSPA in this work. In the present paper we will demonstrate by solid-state ^{29}Si NMR that these bridged silanes indeed form an enhanced degree of cross-linking.

3. The new grafting procedure

Here we present a new method to prepare PEG-grafted colloidal silica particles, that addresses the problem of the water stability of the grafting by combining the ideas from Section 2.1 with the ideas from Section 2.2. So far, the low stability in water of PEG-grafted silica colloids only has been mentioned by Bridger and Vincent [5] and no synthesis routes have been described in literature to produce PEG-grafted silica colloids with an improved water stability. Moreover, the procedure avoids any dry states or other circumstances leading to strong aggregation of the particles.

As shown in Fig. 1, our method can be described by two steps: amination and PEGylation. In amination, the hydrolysis and condensation reaction of the silane coupling agent APS (Fig. 2) is used to introduce amine groups to the surface of the silica particles. A schematic representation of the proposed structure of the surface layer is shown in Fig. 3. For a low degree of coating a monolayer might be obtained as

shown in Fig. 3A, but here the multilayer structure in Fig. 3B is probably more close to reality. The water solubility of the pre-coating layer is reduced using a combination of APS with BTMOSPA or BTEOSE. In PEGylation, *N*-succinimidyl esters of mono-methoxy poly(ethylene glycol) carboxylic acids (Fig. 2) are used to graft PEG to silica through amide bond formation by the reaction between the amine group and the *N*-succinimidyl group. The active *N*-succinimidyl group can react fast with a primary amine group at room temperature. PEGylation is also widely used to graft PEG onto proteins [16]. Several kinds of *N*-succinimidyl-activated PEGs with different molecular weight and narrow polydispersity are commercially available. The inert methyl end group ensures that PEG can be grafted with only one end, and both of the two steps take place in the dispersed state. To our best knowledge, there is no literature that focuses on applying this chemistry to dispersed colloidal silica particles.

4. Experimental

4.1. Materials

All agents were purchased from Sigma–Aldrich except where noted otherwise. *N*-succinimidyl ester of mono-methoxy

Table 1
Results of DLS, elemental analysis and stability test

Sample ^a	Agents ^b	R_{DLS}/PDI (nm)/–	Elemental analysis ^c			Stability test ^d
			C%	H%	N%	
B		126/0.007	1.65/0.18	1.64/1.46	0.04/0.02	–
PreB1	APS/TEOS (2:1)		2.50/0.93	1.86/1.53	0.16/0.09	–/–
PEG-PreB1	PreB1 + mPEG-SCM	133/0.067	–/0.96	–/1.67	–/0.14	+/–
PreB2	APS/BTEOSE (1:1)	117/0.006	3.61/0.95	2.03/1.66	0.23/0.17	–/–
PEG-PreB2	PreB2 + mPEG-SCM		7.13/2.30	2.52/1.74	0.24/0.22	+/+
PreB3	APS/BTEOSE (2:1)	141/0.075				–/–
PEG-PreB3	PreB3 + mPEG-SCM	254/0.227	4.92/1.64	2.12/1.66	0.19/0.15	+/+
PreB4	BTMOSPA	146/0.037				–/–
PEG-PreB4	PreB4 + mPEG-SCM					–/–
PreB5	APS/BTMOSPA (1:2)	136/0.019				–/–
PEG-PreB5	PreB5 + mPEG-SCM	198/0.069				+/+
S		34.1/0.141				–
PreS1	APS/BTMOSPA (1:2)	38.4/0.170	9.06/8.32	2.71/2.59	1.86/1.76	–/–
PEG-PreS1	PreS1 + mPEG-SCM	44.4/0.111	11.96/9.44	2.91/2.76	1.81/1.74	+/+

^a B, S refer to bare silica particles with different size (big and small); prefix “Pre” means particles pre-coated with coupling agents and “PEG” means particles grafted with PEG. For example, “PEG-PreB2” refers to the pre-coated particles PreB2 grafted with PEG.

^b For 100 mL B sample, the total volume of silanes is 0.18 mL, for 25 mL S sample, the total volume of silanes is 0.18 mL. The value in parentheses is the volume ratio.

^c Elemental analysis is the average out of five runs. Before slash: samples washed with methanol buffer; after slash: samples washed with methanol buffer then water buffer.

^d Representative of the results of both 5% NaCl aqueous solution and *o*-chlorophenol. Stability criteria are explained in the text. +: stable, –: unstable. Before slash: samples washed with methanol buffer; after slash: samples washed with methanol then water buffer.

poly(ethylene glycol) acetic acid (average molecular weight 5000 g/mol, hereafter referred to as mPEG-SCM, see Fig. 2) was purchased from Fluka. *N*-succinimidyl ester of monomethoxy poly(ethylene glycol) propionic acid (hereafter referred to as mPEG-SPA, average molecular weight 20,000 g/mol) was obtained from Shearwater. Tetraethoxysilane (TEOS) (99%, Fluka) was freshly distilled. (3-Aminopropyl)triethoxysilane (APS) (98%), bis-(trimethoxysilylpropyl)amine (BTMOSPA) (90%), bis-(triethoxysilyl)ethane (BTEOSE) (96%), ammonium hydroxide (25%, Merck, hereafter referred to as ammonia), *o*-chlorophenol (99%), methanol (99.9%), absolute ethanol (99.9%, KMF Laborchemie Handels GmbH) and Lipophilic Sephadex LH-20 Gel (bead size: 25–100 μ m) were used as received.

4.2. Silica particles

Two sizes of silica particles were synthesized according to the Stöber method [6]. For the S samples (Table 1), 9.0 mL TEOS was added into a mixture of 250 mL methanol and 17.5 mL ammonia under strong stirring. The radius of the resulting particles is 30 nm by dynamic light scattering (DLS). For the B samples (Table 1), 12.5 mL TEOS was added into a mixture of 300 mL ethanol and 25 mL ammonia under strong stirring. The radius of the resulting particles is 126 nm by DLS. All of the above syntheses were performed at room temperature.

4.3. Pre-coating and purification

The pre-coating can be carried out in situ at the end of the Stöber synthesis. APS or a mixture of APS with TEOS, BTEOSE or BTMOSPA was diluted with methanol and then

added to the silica dispersions under vigorous stirring. The amount of coupling agents used was calculated as follows. As the density of a layer obtained with BTEOSE, BTMOSPA and APS is not known, the same volume of reagent was used as the amount of TEOS needed for a silica layer of about 2 nm. We estimate that this corresponds to a pre-coating layer thickness between 2 and 8 nm. For instance, 0.06 mL APS and 0.12 mL BTMOSPA (volume ratio 1:2) were mixed and diluted with 0.36 mL methanol. The resulting mixture was ultrasonicated for a very short time to form a homogeneous solution. The solution was added into 25 mL dispersion of S sample under vigorous stirring. Then the sample was stirred slowly for at least 12 h.

After pre-coating the sample was purified to remove ammonia and excess silanes (either in monomeric or oligomeric form). Especially removing the ammonia is important, because free ammonia is more active than the primary amino groups on the surface of the pre-coated particles and would consume most of the active PEG in the following PEGylation. Centrifugation and redispersion in methanol buffer (0.1 mM NaHCO₃ in methanol) was successfully used for the B samples. For the S samples aggregation was observed after five centrifugation/redispersion cycles, even at very slow centrifugation speeds that take a long time.

In order to circumvent this problem, gel permeation chromatography was used. A 350-mm-long glass column (25 mm internal diameter) packed with Sephadex LH₂₀ Gel (bed volume 170 mL) was used to remove ammonium and residual silane oligomers. Methanol buffer (0.1 mM NaHCO₃ in methanol) was used as mobile phase. In each round 17 mL dispersion can be purified with this column. Two rounds are enough to purify pre-coated particles from the above-mentioned materials. Finally, pre-coated silica particles in methanol buffer were obtained ready for the PEGylation.

4.4. PEGylation and purification

mPEG-SCM with a molecular weight of 5000 g/mol was used in all but one experiment to PEGylate the purified pre-coated particles with primary amine groups on the surface. All PEGylation was conducted in methanol (buffer) at room temperature. In methanol, the hydrolysis of the succinimidyl ester, which is the pronounced side reaction in aqueous solution, can be effectively avoided. The efficiency of this step depends on the extent of ammonia-removing in the purification step.

In a typical experiment, 1 mL the pure pre-coated dispersion was mixed with 10 mg mPEG-SCM. The resulting mixture was put in an ultrasonic bath to accelerate dissolution of mPEG-SCM. At the same time, ultrasonication keeps the mixture homogeneous, which is especially important for the big particles (B samples). The reaction was allowed to continue for 3 h. The resulting particles were separated from unreacted mPEG-SCM by three cycles of centrifugation and redispersion in methanol buffer (0.1 mM NaHCO₃). Finally, PEG-g-silica particles were transferred to the solvent of choice in the same way.

4.5. Elemental analysis

Samples of pre-coated particles as well as samples of PEG-coated particles were both purified by washing with methanol buffer as described above. Then a part of the particles was washed with water buffer (1 mM NaHCO₃, pH 9.0) three times. The results from elemental analysis (C, H and N) of the samples washed with water buffer are compared to the samples only washed with methanol buffer. In this way the stability in water of the PEG grafting and the pre-coating was assessed. Before analysis all samples were dried for 72 h at 50 °C under a nitrogen flow. A low drying temperature and the nitrogen atmosphere were used to avoid decomposition of the PEG-grafted on the silica. Considering the accuracy of the elemental analysis, the discussion will be focused on the carbon content.

4.6. Colloidal stability in a 5% salt solution and *o*-chlorophenol

Two solvents were used to demonstrate the steric stabilizing effect of PEG chains on the particles: 5% NaCl aqueous solution and *o*-chlorophenol. Both solvents have a high refractive index difference of 1.45–1.33 and 1.56–1.45 to the silica particles, so that flocculation and the light scattering of the dispersed particles are easily observed by the eye. Bare silica particles and all pre-coated particles flocculate in both solvents. Therefore, the colloidal stability in these solvents is used as proof of a successful PEG grafting.

Several drops of the pre-coated or PEG-grafted dispersion were added into 1 mL of the above solvent and mixed to form an (initial) homogeneous dispersion. If the dispersion did not flocculate immediately, it was left for 5 days. Then the following criterion was used to assess the colloidal stability of these dispersions: a dispersion was called stable if it was still a homogeneous sol or could be redispersed to a homogeneous sol

after sedimentation to the bottom of the container. Sedimentation is considered here in connection to the B samples, because these big particles will sediment under gravity.

4.7. Dynamic light scattering (DLS)

Dynamic light scattering was used to monitor every step for the change in hydrodynamic radius and check for aggregates. All measurements were carried out at 22.0 °C on highly dilute, dust-free dispersions. Data analysis was performed with the second cumulant fit according to Frisken [17] using intensity autocorrelation functions collected from ten scattering angles between 30° and 120°. The hydrodynamic radius (R_{DLS}) was calculated from the average diffusion coefficient using the Stokes–Einstein relation. The average of the second cumulant μ_2 , which is equal to the variance of the decay rate Γ , was normalized as $\mu_2/\langle\Gamma\rangle^2$, and will be referred to as the polydispersity index (PDI).

4.8. Transmission electron microscopy (TEM)

TEM was performed with a Philips CM200 transmission electron microscope. Samples for TEM were prepared by dropping and evaporating the particle suspension onto a carbon-coated copper grid.

4.9. Solid-state ²⁹Si NMR

The chemical structure of the silanes was determined by solid-state ²⁹Si NMR spectroscopy. A Varian INOVA™ unity equipped with a 6 mm narrow bore probe and operating at 119.192 MHz for ²⁹Si was used to acquire all the spectra. The temperature was set at 25 ± 0.1 °C and magic angle spinning (MAS) was carried out at a spinning rate of 6000 ± 1 Hz. The experiments were collected by using 1 k data points over an acquisition time of 20 ms. A first qualitative characterization was obtained quickly through measurements using cross-polarization (CP) of the ²⁹Si nuclei through magnetization transfer from ¹H nuclei (see supplementary material), but to obtain quantitative results for each type of silicon environment classical direct polarization (Bloch decay) experiments (DP) were carried out. The DP/MAS experiments were recorded with a 90° pulse length of 5 μs. The necessary recycle delay of 900 s was determined in a separate experiment (see supplementary material). If not indicated otherwise, in each DP experiment 256 free induction decays (FID) were accumulated. Chemical shift values are given in parts per million relative to tetramethylsilane. Signal attribution was conducted as described in the literature [18,19]. Spectra elaboration was conducted with MestRe-C version 4.9.9.9 software. All the FIDs were transformed by applying, first a 2 k zero filling, and then, an exponential filter function with a line broadening (LB) of 30 Hz. A second-order polynomial with Bernstein algorithm was used for baseline correction of the NMR spectra. The DP spectra were then deconvoluted by fitting Gaussian lineshapes adjusting their intensity, width and frequency shift. The integrated

areas of the curves were then used to calculate the silicon percentages.

5. Results and discussion

5.1. Pre-coated particles

Results of different pre-coated silica particles are summarized in Table 1. Because the silanes contain carbon, distinct carbon content differences between bare particles and pre-coated particles demonstrate successful pre-coating. After washing with water the carbon content of the pre-coated particles decreases again, showing a limited stability in water for the pre-coating layer, which will be discussed extensively later. For the B samples, most radii of the pre-coated particles from DLS are usually a little bit higher than the calculated value using an estimated 2–8 nm pre-coating thickness (depending on the silane used and the density of the pre-coating layer). This might be because of a minor aggregation (the PDI values are small) or due to some fractionation in the centrifugation procedure used to purify the dispersions. For the smaller particles (S samples), primary experiments in ethanol–ammonia indicated that non-aggregated pre-coated particles can be obtained only when pre-coated with pure APS. With the mixture of APS/BTEOSE or APS/BTMOSPA, flocs were formed within several hours and sometimes soon after adding the reagents (depending on the concentration of particles and ammonia). After using methanol instead of ethanol, non-aggregated small particles were obtained if pre-coated with the mixture of APS and BTMOSPA, but pre-coating with APS/BTEOSE still ended up with aggregation. The increase in DLS radius after pre-coating with APS/BTMOSPA is given in Table 1 and is in agreement with the estimated pre-coating thickness range. After the pre-coating, we proceeded with the purification as soon as possible, because otherwise some film-like flocs will appear on the top of the bare silica dispersion or the pre-coated dispersion in methanol several days after the synthesis. The modified procedure in methanol–ammonia used to coat the small particles, was also tested on a B sample (PreB5) with a positive result. The pre-coated sample could also be purified by gel permeation chromatography instead of centrifugation. So the pre-coating procedure with APS/BTMOSPA in methanol/ammonia seems to be the more universal method.

5.2. PEG-grafted particles

Table 1 shows the results from elemental analysis, DLS and stability tests of the grafted silica particles. Elemental analysis after PEGylation and washing of the particles shows an increase in carbon content, which is a strong indication of a PEG-coating of the particles. Furthermore, several dispersions are stable in a 5% salt solution, which in combination with the results from elemental analysis demonstrates PEG grafting. The results in Table 1 also show that for some particles the stability depends on whether the particles are washed in water or not, which is in agreement with the result from Bridger and Vincent [5]. The stability of the coating in aqueous solvents will be discussed

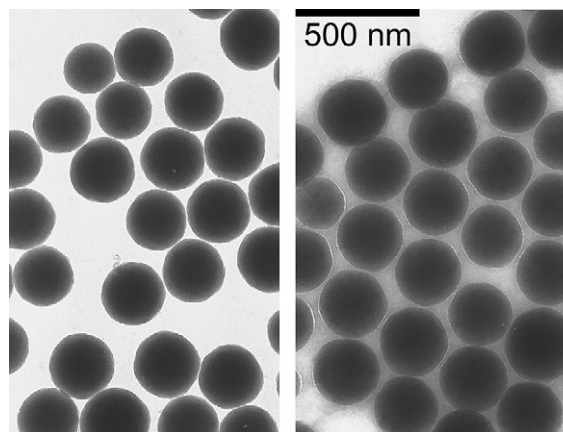


Fig. 4. TEM images of PreB5 (left) and PEG-PreB5 (right).

more extensively later. The results from dynamic light scattering show that the S samples could be coated without significant aggregation. For the B samples, however, DLS indicates some aggregation.

One of the pre-coated particles, PreB2, was also grafted with mPEG-SPA of molecular weight 20,000 g/mol following the above method. The resulting PEG_{20,000}-PreB2 was washed with methanol and water buffer and was stable in a 5% NaCl solution, demonstrating that the synthesis is not restricted to the PEG with a molecular weight of 5000 g/mol as used throughout this paper.

PEGylation of pre-coated particles was also tried in water (with 1 mM NaHCO₃) instead of the methanol buffer, but otherwise the procedure was the same. Also the resulting particles were washed with methanol as usual. When dispersed in 5% NaCl solution in water, these particles aggregated quickly. Probably, the mPEG-SCM did react with water and lost its activity. The hydrolysis half-life of mPEG-SCM in water is only about 0.75 min. Although PEGylation of proteins is often applied in water it seems that hydrolysis is dominant in the present form of PEGylation. Maybe, because of the large particle size in our samples, the concentration of –NH₂ groups is lower than in the usual protein PEGylation.

Fig. 4 shows TEM images of a PEG-grafted sample and the corresponding pre-coated particle. The TEM image of the PEG-grafted particles shows gray zones of low contrast that are not shown on the image of the pre-coated particles. The same difference before and after PEG grafting was also found by Joubert et al. [9], who attributed the gray zones to the PEG grafting. The agreement between their results and our result is an indication that we can see the grafted PEG with TEM. Still, to really prove it is indeed PEG here, more systematic studies would have to be performed, as microscopy images can show misleading results.

The carbon content from elemental analysis is used to estimate the grafting density Γ assuming a smooth spherical geometry by $\Gamma = (\text{mPEG}/\text{msilica})\rho R/3$. Here, mPEG and msilica are the mass of PEG and silica respectively, ρ is the density of silica for which we used 2.0 g/cm³, and R is the radius of the silica spheres as obtained from DLS. Assuming that the carbon content difference between pre-coated and PEGylated particles is attributed to PEG chains, we can estimate mPEG/msilica,

which results in a grafting density of $\sim 1.3 \text{ mg/m}^2$ for PEG-PreS1 and $\sim 5.4 \text{ mg/m}^2$ for PEG-PreB2. In addition we tried to estimate the grafting density directly from the amount of residual mPEG-SCM after grafting, but the accuracy of this determination was found to be too low (more than 90% of the PEG is residual). Nevertheless, the approximate amount of residual PEG is in agreement with the grafting densities obtained from elemental analysis. The approximate grafting densities we obtain are similar to grafting densities reported by Bridger and Vincent [5] of 0.9 mg/m^2 when using silanated PEG (molecular weight 18,000 g/mol) with Stöber silica particles and Joubert et al. [9] who obtained 3.6 and 6.5 mg/m^2 for their grafted Stöber silica samples using polymerization from the particle surface (grafting from).

Another experiment was performed to demonstrate that the PEG is indeed chemically bound to the APS. Therefore, silica particles were pre-coated only with BTMOSPA, without any APS. The resulting pre-coated particles, PreB4, were mixed with mPEG-SCM under the identical condition of PEGylation of other particles. The final particles, PEG-PreB4 were washed with methanol as usual and then dispersed in 5% NaCl aqueous solution. Aggregates appeared soon in this dispersion on the same time scale as with the corresponding pre-coated particles, PreB4. PreB4 should have surface properties similar to the ones pre-coated with APS or APS/BTMOSPA because APS and BTMOSPA have similar chemical structure (see Fig. 2). The only difference is that the former one only has secondary amine groups while the latter two have primary amine groups on the surface. But under the conditions applied, mPEG-SCM does not react enough with the secondary amine groups to result in sterically stabilized colloidal dispersions. In other words PEG will not graft enough on to particles pre-coated with BTMOSPA due to the absence of $-\text{NH}_2$ groups on the surface. This qualitative result suggests that the PEG is chemically bound through the APS to the silica.

5.3. Stability in water

Table 1 summarizes the effect of TEOS, BTEOSE and BTMOSPA on the stability of the pre-coating layer as well as the final PEG grafting.

5.3.1. TEOS

After washing with water buffer three times, the carbon content of PreB1 decreased from 2.5 to 0.93%, i.e. about 63% of the carbon was lost (Table 1). If attributed all of the lost carbon to APS moieties, 63% APS moieties in the pre-coating layer are lost. This estimate is quite comparable to published results [10] where 67% aminopropylsilane on silica gel was found free in solution when dispersed in pure water for 4 h. But it should be noted that there are also unhydrolyzed ethoxy groups in the pre-coating layer and silica core if dried from a dispersion in alcohol. Hydrolysis of these unhydrolyzed ethoxy groups during washing may also contribute to the above decrease of the carbon content. For instance, 1.65% carbon exists in bare silica particles B dried from the ethanol/ammonia mixture as used in the synthesis of the particles, as compared to a carbon con-

tent of 0.18% after washing with water (Table 1). Significant carbon contents for silica samples dried from dispersions in alcohols were also observed by others [19,20].

After grafting PreB1 in methanol with mPEG-SCM, the resulting PEG-PreB1 can keep stable in 5% NaCl solution due to the steric stabilization by the grafted PEG. But the stability is lost after washing with water buffer (Table 1), which is consistent with the low carbon content of washed PEG-PreB1. As seen from Table 1, only 0.96% carbon exists in washed PEG-PreB1, the same as the corresponding pre-coated particles, PreB1. This indicates that almost all of the PEG is lost after washing the particles with water.

5.3.2. BTEOSE

The effect of BTEOSE (see Fig. 2) on the stability of the pre-coating layer is subtle. BTEOSE has been expected to be a promising candidate for stabilizing the pre-coating layer as discussed in the introduction. But the carbon content of PreB2, which was pre-coated with an APS/BTEOSE (1:1) mixture, decreases from 3.61 to 0.95% after washing with water buffer. Compared with APS/TEOS, BTEOSE seems to have no effect on the stability of the pre-coating layer, given almost the same carbon content is found for washed PreB1 and PreB2. However, the performance of PEG-PreB1 and PEG-PreB2 is different. In contrast to PEG-PreB1, PEG-PreB2 can keep stable in 5% NaCl aqueous solution even after washing with water buffer. The carbon content of washed PEG-PreB2 decreases to 2.30%, but is much higher than washed PEG-PreB1 (0.95%). The stability in 5% NaCl can only be explained by the steric effect of PEG chains considering the instability of corresponding washed PreB2 in the same solvent. In the case of PreB3 and PEG-PreB3, where a different ratio of APS and BTEOS was used, a similar result as for PreB2 and PEG-PreB2 is found. These results show that at least a part of the PEG still binds on the surface of PEG-PreB2 or PEG-PreB3 after washing with water.

On the basis of the above results, another idea to stabilize the coating was tested. Silica particles were pre-coated with pure APS then grafted with mPEG-SCM. The resulting PEG-grafted silica was further coated with BTEOSE in ammonia/methanol. However, the resulting particle aggregates in 5% NaCl solution even without washing with water. It is possible that PEG chains are buried under the layer formed by BTEOSE.

BTEOSE works well with big silica particles (radius 126 nm). For the small particles (in this work, particles with radii 50 and 30 nm were tested), the pre-coating with a mixture of APS and BTEOSE always ended up with aggregates. The loss of stability of these particles during pre-coating may be due to the weaker charge stabilization of small particles as compared to larger particles in combination with the increased hydrophobicity after introduction of BTEOSE.

5.3.3. BTMOSPA

When BTMOSPA (see Fig. 2) was used with APS instead of BTEOSE, pre-coated particles, PreB4, PreB5 and PreS1 without significant aggregation can be obtained for both big particles and small particles. Most markedly, the loss rate of carbon

content of PreS1 is only 8% (from 9.06 to 8.32%, Table 1) after washing with water buffer. Compared with about 70% loss rate of PreB1 and PreB2, the pre-coating layer of PreS1 is almost intact after washing. Furthermore, the corresponding PEG-grafted particles, PEG-PreS1 are stable in 5% NaCl solution before and after washing with water. Still the loss rate of carbon content of PEG-PreS1 is 20% (from 11.96 to 9.44%). This higher loss rate than that of 8% for PreS1 shows that also here a part of the PEG is lost during washing. Nevertheless, the particles are more stable towards aqueous solutions than particles pre-coated with APS/TEOS. Why a pre-coating of small particles with BTMOSPA results in a colloidal stable dispersion, whereas the same reaction with BTEOSE results in aggregation, is not clear.

5.3.4. Discussion of the stability in water

The use of BTMOSPA/APS and BTEOSE/APS instead of TEOS/APS results in coatings with increased stability towards washing with water. This result is in agreement with results on the formulation of corrosion protective coatings where improved performance was obtained by using BTMOSPA [13,14] and BTEOSE [11,12]. Bridger and Vincent [5] suggested a slow dissolution of the surface layer of silica as the most probable reason for the limited stability of their PEG-grafted silica particles. Indeed, also pure amorphous silica will dissolve in aqueous media to about 115 ppm [21], clearly demonstrating the equilibrium nature of the formation and breaking of Si–O–Si bonds in water. Because BTMOSPA and BTEOSE can form six siloxane bonds compared to four for TEOS, they are able to give a stronger cross-linking in the surface coating, which probably results in a lower water solubility. Because the PEG itself is only bound to the surface by the 3 siloxane bonds from the APS, the increased stability in water probably has to be attributed to a partial burying of these 3 siloxane bonds in the layer with a lower water solubility. This also explains why a part of the PEG can still be washed away, since this part does not have buried linking bonds, whereas the other part has an improved water stability. Our findings therefore strongly indicate that the limited stability of the Si–O–Si bond against hydrolysis is the main reason for the limited stability in water. Furthermore, the hydrophobic alkyl groups in BTMOSPA and BTEOSE may lead to some additional decrease of the water solubility.

5.3.5. Degree of cross-linking by ^{29}Si NMR

The degree of cross-linking of three pre-coated samples was analyzed by solid-state ^{29}Si NMR. To get a good NMR signal for the silicons in the pre-coating layer, only small core particles (S) were used. In our samples six different types of silicon environments can be distinguished in the NMR spectra. Signal attribution was conducted as described in the literature [18,19]. The silicon atoms found in the silica core of the particles are able to form 4 Si–O–Si bonds and are denoted by a Q with a superscript 4, 3 or 2 giving the actual number of Si–O–Si bonds. All silicon atoms in the pre-coating layer have one Si–C bond, therefore are only able to form 3 Si–O–Si bonds and are denoted by a T and the superscript 3, 2 or 1 gives the actual number of Si–O–Si bonds. Fig. 5 displays an example of a quantitative

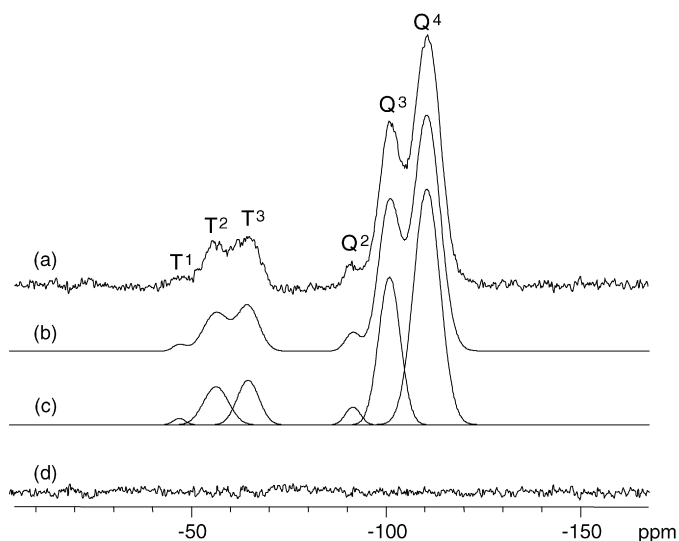


Fig. 5. Quantitative DP/MAS ^{29}Si spectrum of PreS2 and deconvoluted spectrum: (a) original spectrum of PreS2 (delay 900 s, 256 scans), (b) sum of deconvoluted Gaussian curves, (c) deconvoluted Gaussian curves, (d) residual spectrum after deconvolution.

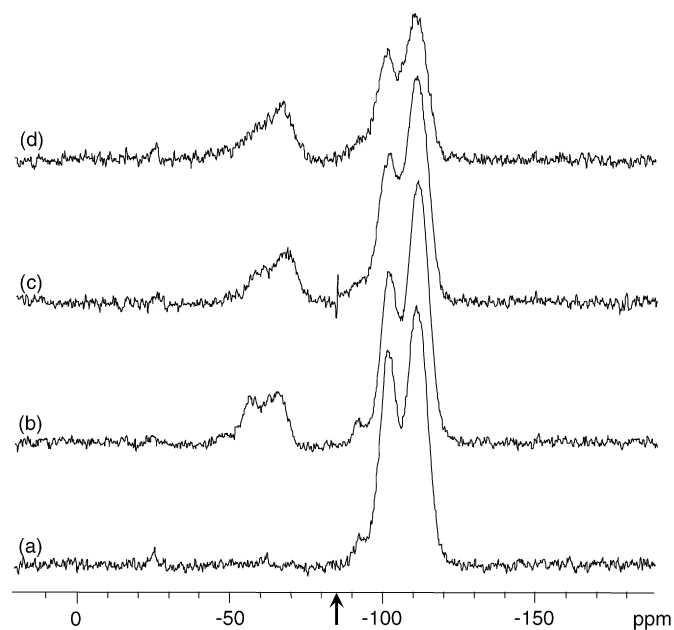


Fig. 6. Quantitative DP/MAS ^{29}Si spectra: (a) S, (b) PreS2, (c) PreS3, (d) PreS4 (arrow—central emitter frequency).

^{29}Si NMR-spectrum obtained through direct polarization of the ^{29}Si and shows how the spectra are deconvoluted to obtain the integrated intensities of each of the six possible silicon environments. Fig. 6 gives an overview of the spectra of all samples and Table 2 presents the results of the deconvoluted and integrated Gaussian curves for all of the samples.

N_Q and N_T give the average number of Si–O–Si bonds per Q or T silicon, respectively. The degree of cross-linking in terms of the average number of Si–O–Si bonds per bridged silane molecule (which contains two silicon atoms) is given by $2N_T$, whereas N_Q immediately gives the degree of cross-linking for the silica in the core. On average for all samples in Table 2 for

Table 2
Pre-coated samples for the ^{29}Si NMR measurements and the percentages^a of the different types of silicon environments

Sample ^b	Agent	Q ⁴	Q ³	Q ²	T ³	T ²	T ¹	N _Q	N _T
S		58	38	3.9				3.54	
PreS2	BTEOSE	52	28	2.7	8.6	8.0	0.7	3.60	2.46
PreS3	APS/BTMOSPA (1:2)	52	29	2.7	10.3	6.2	0	3.59	2.62
PreS4	BTMOSPA	41	28	4.2	13.5	9.9	3.6	3.51	2.37

^a The estimated error is about 2–4% for percentages above 25% and about 1–2% for percentages below 15%.

^b Sample preparation as described for the samples in Table 1, apart from the washing and drying procedure. The samples were only washed twice with methanol, then re-dispersed in water and dried by freeze drying.

the silica in the core a degree of cross-linking of 3.56 is obtained, in good agreement with published results [18,19]. For the pre-coating layer of the pure bridged silanes (PreS2 and PreS4) however, an average degree of cross-linking of 4.83 is obtained. These results demonstrate that the bridged silanes in the pre-coating layer have an increased cross-linking density as compared to silica.

5.3.6. Long time stability test

After finishing all other experiments, we performed a 3 months stability test. Six freshly prepared dispersions (with a BTMOSPA/APS pre-coating) were stored for 3 months in: water with 15% isopropanol, 15% ethanol, 0.1% sodium azide, 1 mM NaHCO₃, pure water and pure methanol. Elemental analysis of these samples before and after the 3 months (and again washed with their respective solvents) is sometimes lower, sometimes higher than the original carbon contents, but on average no significant decrease in carbon content was found. This indicates that no significant amount of PEG is lost during the three months storage. The stability test, however, gives a strange result. Before storage, a small part of all samples was used in our 5 days stability test in aqueous 5% NaCl solution and as expected from the preceding results all dispersions passed the test. After 3 months storage, all dispersions were still stable in each of the solvents used. However, in 5% NaCl all dispersions (including the dispersion stored in methanol, where no hydrolysis and no loss of PEG is expected!) were only stable for 1–2 days. We have no proof about the origin of these results that do not seem to fit in our preceding results. It seems that a new effect with PEG-grafted silica shows up. In this context we note that in many light scattering studies on pure PEG or PEO solutions aggregates have been found [22–24], which probably reflects the marginal solubility of PEG in water. Maybe this also plays a role with our PEG-grafted silica dispersions and although grafting layers with an improved stability in water have been obtained, the marginal solubility of PEG in water limits the steric stabilizing effect against particle aggregation.

6. Summary

A new two-step method is described to graft colloidal silica particles with PEG, avoiding any dry states or other circumstances leading to strong aggregation of the particles, using commercially available succinimidyl activated poly(ethylene

glycols) (PEG). First, the silica particle surface is activated by pre-coating with the silane coupling agent (3-aminopropyl)-triethoxysilane (APS) to introduce amine groups (amination). Second, the activated pre-coated particles react with the *N*-succinimidyl ester of a mono-methyl poly(ethylene glycol) carboxylic acid to form PEG-grafted silica particles (PEGylation). The particles obtained can be dispersed in water and several organic solvents that are good solvents for PEG.

In addition, our results confirm the suggestion by Bridger and Vincent [5] that the limited stability of PEG-grafted silica in water is probably caused by the hydrolysis of Si–O–Si bonds in the surface layer where the PEG is linked to the particle. The water stability of our PEG-grafted silica particles could be improved by using a mixed pre-coating of APS and bis[3-(trimethoxysilyl)propyl]amine or 1,2-bis-(triethoxysilyl)ethane. This pre-coating is possibly also of interest to improve the water stability in other applications where some molecules are linked to a silica surface by silane coupling agents. These applications might range from silica particles that have been specially coated to pass the cell membrane in bio imaging applications [4,25] to the preparation of solid extractants for heavy metal ions [10].

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Supplementary material

The online version of this article contains additional supplementary material.

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