

Amine Grafting on Silica Nanoparticles in Water: A Systematic Study

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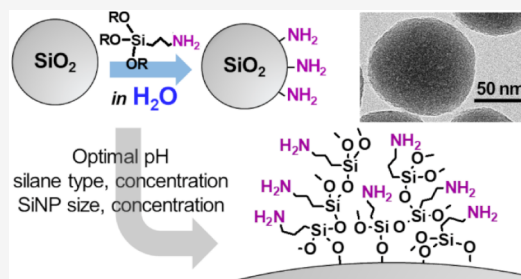


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ABSTRACT: The amine functionalization of silica nanoparticles (SiNPs) using aminotrialkoxysilanes is typically conducted in organic solvents and is widely applied across diverse fields. Recent studies have demonstrated that this modification can also be achieved in aqueous media at low temperatures; however, the impact of water as the primary solvent on the formation and morphology of alkoxyisilane grafting remains unexplored. To address this gap, we systematically investigated the influence of key experimental parameters (including particle size and concentration, the type and concentration of aminoalkoxyisilanes, and pH) on grafting efficiency. Our results reveal that the highest amine grafting density in water is achieved using a large excess of (3-aminopropyl)trimethoxysilane (APTMS) in dilute SiNP solutions with relatively large particle diameters ($D_h \sim 150$ nm) under basic conditions. The so-modified SiNPs exhibit a smooth surface with a reproducible surface amine density of ~ 12 NH_2 nm^{-2} (among the highest recorded in the literature). Such density is attributed to the grafting of short, polymerized aminotrialkoxyisilane chains on the silica surface. In addition, while this dense APTMS graft is sensitive to hydrolytic degradation, storage at low temperatures preserves a high grafting density (~ 10 NH_2 nm^{-2}) over several weeks. This study provides a comprehensive understanding of the experimental conditions governing amine grafting on SiNPs in aqueous media and offers practical guidelines for the preparation of SiNPs with dense amine functionalization.



INTRODUCTION

The surface modification of silica nanoparticles (SiNPs) with amine groups is used in a wide range of applications including drug and gene delivery,^{1–3} bioprinting,^{4,5} dye removal,⁶ and antifouling.⁷ Additionally, it serves as a crucial intermediate step in the functionalization of SiNPs with various compounds, such as fluorescent dyes,⁸ contrast agents,⁹ bioactive molecules (e.g., sugars,¹⁰ phenylboronic acid,^{11–13} polyethylenimine¹⁴) and other functional groups like carboxylic acid,^{15–17} poly(methacrylic acid),^{16,18} and azide.^{11,13}

Amine grafting onto the surface of SiNPs is commonly achieved through the hydrolysis and condensation of an aminotrialkoxyisilane on the silanol groups present on the silica surface (a process often referred to as “silanization”, Figure 1-A). In a vast majority of cases, this modification is performed in organic solvents (ethanol,^{15,19–21} methanol,²² toluene,^{14,17,18,23–25} acetonitrile²⁶) at elevated temperature (typically ≥ 70 $^{\circ}\text{C}$).^{14,17–19,23–25} In recent years, following the work of Kneuer and colleagues,¹ numerous studies have reported that the amine modification of SiNPs could also be performed in aqueous solutions.^{1,2,4,5,7,27–32} This method, though based on the same chemical principles, effectively minimizes the use of hazardous solvents. Moreover, it can be carried out at low temperatures (≤ 40 $^{\circ}\text{C}$),^{2,4,5} which also reduces energy consumption in accordance with the principles of green chemistry.³³

Despite these benefits and to the best of our knowledge, no studies have yet systematically examined the impact of using water as the primary solvent on the grafting of alkoxyisilanes on SiNPs. This is particularly important because water plays a key role in the silanization process. Previous research on modifying flat silica surfaces in organic solvents has demonstrated that silanization occurs only when trace amounts of water are present on the silica surface (see Figure 1-A (*)).^{34,35} This water enables localized hydrolysis, which in turn promotes condensation on the silica surface (as shown in Figure 1-A) rather than the self-condensation of alkoxyisilanes (as shown in Figure 1-B).^{35–37} However, the surface water content must be carefully controlled, as any excess (up to 1 vol %) accelerates hydrolysis, leading to silane polymerization^{35,38} and resulting in uneven grafting layers that increase surface roughness.³⁵ Based on these findings for flat silica substrates, it could be expected that modifying SiNPs in water would presumably result in disordered and polymerized grafting. Still, such observation has yet to be made.

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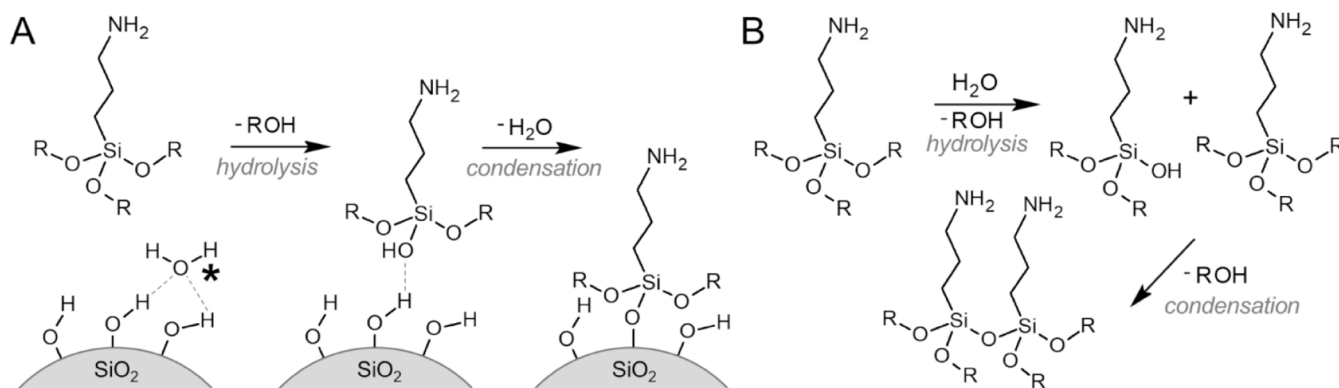


Figure 1. Scheme illustrating (A) the hydrolysis and condensation of aminopropylalkoxysilane (APS) on the hydrated surface of SiNPs (water molecules adsorbed at the silica surface are indicated by *) and (B) the hydrolysis and self-condensation reaction of APS molecules in aqueous solutions resulting in the formation of polymeric silane derivatives (i.e., polycondensation).

Moreover, published research on the amine modification of SiNPs in water shows significant sensitivity to experimental conditions. These studies employ SiNPs of different sizes^{1,2} (ranging from 10 nm¹ to 300 nm²), at varying concentrations^{3,30} (from 2 wt %⁴ to 16 wt %³⁰), with different aminoalkoxysilanes introduced in various quantities^{4,30} (ranging from approximately 3 to 300 aminosilanes per available nanometer of silica surface), and under diverse pH conditions (ranging from acidic^{1,30} to basic^{4,5}). This variability makes it challenging to discern how each physicochemical factor impacts grafting density and morphology. In summary, although amine modification of SiNPs in pure water is commonly performed, a systematic study of the effects of experimental conditions on grafting with a comprehensive characterization of the grafted layer is still lacking.

To fill this gap, the present study was divided in three steps. First, bare SiNPs were subjected to several amine modification protocols in water, each time varying a distinct experimental parameter (particle size and concentration, type and concentration of aminoalkoxysilanes, pH) to identify its influence on grafting efficiency. Second, building on the acquired results, modified SiNPs with “optimal” grafting were prepared and extensively characterized to quantify their grafting density and to investigate their surface morphology. Finally, the long-term stability of the grafting, in particular its sensitivity to hydrolytic degradation, was evaluated.

EXPERIMENTAL SECTION

Materials

Chemicals were obtained from various suppliers and used as received without further purification. Tetraethoxysilane (TEOS) was purchased from ACROS. Ammonium hydroxide (NH₄OH), sodium chloride (NaCl), (3-aminopropyl)-triethoxysilane (APTES), fluorescamine (4'-phenylspiro[2-benzofuran-3,2'-furan]-1,3'-dione) and ethanolamine were obtained from Sigma-Aldrich-Fluka. Additionally, (3-aminopropyl)-trimethoxy-silane (APTMS) was procured from Alfa Aesar, anhydrous ethanol from Biosolve and hydrochloric acid (HCl) from Carl Roth. Ultrapure water (Resistivity ~ 18.2 MΩ·cm) was utilized for all experimental procedures.

Silica Particle Synthesis and Modification

Synthesis of Bare Silica Nanoparticles. Silica nanoparticles (SiNPs) were synthesized using the Stöber method.³⁹ The experimental conditions were adjusted to synthesize three batches of particles with distinct diameters (90, 120, and 145 nm). The reaction, involving the hydrolysis and condensation of tetraethylorthosilicate (TEOS) molecules was conducted by first preparing a

mixture of 400 mL of ethanol (EtOH), 28 mL of Ultrapure water, and different amounts of ammonium hydroxide (NH₄OH) depending on the targeted size. Twenty-four milliliters (mL) of TEOS were then added dropwise to the mixture, which was stirred for 6 h at 350 rpm and different temperatures depending on the targeted size. Table 1 summarizes the experimental conditions selected for achieving the targeted sizes.

Table 1. Experimental Conditions Used to Synthesize SiNPs with Different Targeted Size by the Stöber Method

Target diameter/nm	V(NH ₄ OH) /mL	Temperature (°C)
90	8	30 ± 1
120	8	23 ± 1
145	9.5	23 ± 1

The synthesized nanoparticles were purified by centrifugation (8000 rpm for 20 min at 20 °C) followed by redispersion in Ultrapure water using vortexing and sonication. They were then dialyzed against Ultrapure water using a membrane (Spectra/Por/regenerated cellulose membrane) with a molecular weight cutoff of 6–8 kDa. The water was renewed at least three times over a 24 h period.

The concentration of bare SiNPs after purification was evaluated by introducing small aliquots (of known mass m_{sol}) of the purified solution into three vials. After evaporation overnight in an oven at 80 °C and further drying under vacuum at 70 °C for 1 h, the dry mass m_{dry} was determined. The concentration C (in wt.%) was then obtained as follows:

$$C = 100 (m_{dry}/m_{sol}) \quad (1)$$

Surface Modification of SiNPs with Aminoalkoxysilane

Surface modification of SiNP with amine groups was performed in various conditions to probe the influence of experimental parameters on the grafting density of the modified particles. The generic experimental method is inspired from Lee et al.⁴ and proceeds as follows: a bare SiNP solution (with the desired particle size) was diluted with Ultrapure water to obtain 100 mL of a SiNP solution at the targeted concentration. Then, Ultrapure water (13.33 mL) and the required amount of aminosilane were added dropwise to the SiNP solutions while stirring the solution at 400 rpm. At this stage, the pH was adjusted to the targeted value by adding aliquots of NH₄OH or HCl solutions to the reaction medium. When not specifically mentioned, the pH was set to 10.9 as given in the original method.⁴ Subsequently, the reaction was left under constant stirring (400 rpm) for 16 h in a temperature-controlled bath at 40 °C.

The resulting amino-functionalized nanoparticles (SiNP-NH₂) were purified by centrifugation (8000 rpm for 20 min at 20 °C) followed by redispersion in acidic water (pH ~ 3, adjusted with HCl),

using vigorous vortexing (5 min at maximum speed on a Vortex Genie 2 apparatus from Scientific Industries) and sonication (5 min in a S30 Elmasonic ultrasonic bath from Elma). They were then dialyzed against acidic water (pH $\sim$ 3) using a regenerated cellulose membrane with a molecular weight cutoff of 6–8 kDa. The water was renewed four times within a 24 h period. Purification was performed in acidic conditions in order to promote the colloidal stability of the amine-grafted particles (at acidic pH, amine groups are protonated thereby introducing surface charges and subsequent electrostatic repulsion between particles) while delaying the hydrolysis of grafted aminosilanes.⁴⁰ The concentration of SiNP-NH₂ was evaluated as described for bare SiNP.

Characterization of Silica Nanoparticles

Dynamic Light Scattering (DLS). DLS measurements were performed using a Malvern DTS Nano zetasizer device operating with a HeNe laser at 173° (λ = 633 nm). The measurements were carried out at 25 °C and the samples were previously diluted with Ultrapure water to a concentration of 0.05 wt.% so as to avoid multiple scattering. The pH of the samples prepared for DLS varied depending on the type of particles: pH $\sim$ 8.5 for bare SiNPs samples and pH $\sim$ 4.0 for SiNP-NH₂ due to purification in acidic conditions.

The data analysis was performed by fitting the autocorrelation function with a third order polynomial equation (using the method of cumulants)⁴¹ to extract the average hydrodynamic diameter (referred to as D_h in this manuscript) and the polydispersity index (PDI).

The error bars ΔD_h further shown in graphs starring DLS data was calculated on the basis of the measured mean diameter and the diameter distribution function PDI as

$$\Delta D_h = \frac{D_h \cdot \sqrt{PDI}}{2} \quad (2)$$

that represents the width of the size distribution if the distribution is Gaussian (often referred to as PDI width).⁴²

ζ -Potential Measurements. ζ -Potential measurements were performed on a Malvern DTS Nano zetasizer using folded capillary Zeta cells (DTS1070). Samples were prepared by first diluting particle solutions with Ultrapure water to achieve a final concentration of 0.05 wt.%. The pH of each sample was then adjusted (between 4 and 9.5) by the addition of aliquots of NaOH or HCl solutions. And finally, NaCl salt was added to reach a concentration of 10 mM so as to normalize the ionic strength in all samples. The data analysis was conducted with the Malvern Zetasizer software using the Smoluchowski approximation to calculate the ζ -potential from the measured electrophoretic mobility. The error on the ζ -potential values (illustrated by error bars in the ζ -potential graphs throughout the manuscript) corresponds to the average of the standard deviation measured for each 3 consecutive measurements.

Transmission Electron Microscopy (TEM). Samples for TEM were prepared by depositing droplets of dilute SiNP suspensions onto glow-discharged carbon-coated TEM grids. After a few minutes, the liquid in excess was blotted with filter paper, and the preparation was allowed to air-dry. Images were recorded with a JEOL JEM-2100-Plus microscope operating at 200 kV and equipped with a Gatan Rio 16 digital camera. The size distribution of the nanoparticles was then extracted from the TEM images using the image analysis software Fiji. To this end, images at different magnifications were treated with the 'analyze particles' plugin after thresholding (the threshold was adjusted manually to correctly highlight the particles while avoiding black holes inside the particles) and noise removal with the "Despeckle" tool.

The roughness factor R of the nanoparticles was evaluated from TEM images to quantify deviations from an ideal smooth surface. R is defined as the ratio of the actual surface area A (accounting for irregularities, protrusions, and pores) to the projected surface area A_0 of a perfect sphere with the same dimensions. Using Fiji software, the particle boundaries were manually contoured to measure the true perimeter P (Figure S1–A). Assuming the 2D perimeter scales with the 3D surface area, the actual surface area was estimated as $A = \pi D^2$, where the diameter D was derived from $P = \pi D$. The roughness factor

was then calculated as $R = A/A_0$, where $A_0 = \pi D^2$ for an ideal sphere (Figure S1–B). R values are ≥ 1 , with higher values indicating rougher surfaces.

Quantification of Amine Grafting Density

Fluorescence Spectroscopy. The "Fluorescamine assay" was used to quantify the surface grafting density of amine groups on the silica surface as is commonly performed in the literature (Figure S2).^{43–49} Fluorescamine is inherently nonfluorescent; however, upon reaction with primary amines, it exhibits fluorescence, with absorption and emission peaks around 288 and 490 nm, respectively. A calibration curve (Figure S2–C) was first constructed by measuring the fluorescence resulting from the reaction between ethanolamine at varying concentrations (0.01 to 0.4 mM) and fluorescamine (1 mM). Sample preparation consisted in mixing 2.7 mL of ethanolamine solution (previously adjusted to pH 6 by adding aliquots of NaOH or HCl solutions, as the reaction is pH-dependent) with 0.3 mL of fluorescamine in ethanol. The mixture was stirred at 300–500 rpm for 1 min and transferred in 2 mL quartz cuvettes with optically transparent faces. Fluorescence measurements were made exactly 10 min after mixing, with a Fluorimax-4 fluorometer (HORIBA Scientific). The wavelength selected to construct the calibration curve was 487 nm, corresponding to the maximum emission intensity of fluorescamine-ethanolamine adduct. Fluorescence emission spectra were recorded at room temperature from 400 to 700 nm, with excitation wavelengths set to 365 nm. The fluorescence of a reference sample consisting of 2.7 mL water and 0.3 mL of fluorescamine in ethanol was subtracted to the measured fluorescence.

For the fluorescent measurements of samples containing SiNP-NH₂, the particle solutions were first diluted 20 times in Ultrapure water and then submitted to the exact same procedure as the one described for ethanolamine solutions. The fluorescence of two reference samples was subtracted to the measured fluorescence: (i) a sample consisting of SiNP-NH₂ without fluorescamine (in order to remove the scattering of SiNP), and (ii) a sample consisting of 2.7 mL water and 0.3 mL of fluorescamine as "blank".

Thermogravimetric Analysis (TGA). TGA measurements were performed using four instruments—a TGA-DSC2 (Mettler Toledo), a Setline TGA (Setaram), a THEMYS TGA (Setaram), and a TGA Discovery 550/55 (TA Instruments)—to validate data reproducibility across different setups. Measurements were performed up to 1000 °C using a heating rate of 10 °C/min under oxygen. Samples for TGA were obtained by drying particle solutions at 80 °C and by grinding the resulting dry extract to a fine powder. TGA was performed on 15 mg of powder.

Assessing the Time Dependent Hydrolysis of Grafted Amine Groups. To investigate the potential degradation of the aminosilane coating by hydrolysis overtime, acidic solutions of SiNP-NH₂ (as obtained directly after dialysis; pH $\sim$ 3–4) were stored either at room temperature (20 ± 2 °C) or in the fridge (5 ± 2 °C) for 4 weeks. Acidic conditions were selected to guarantee the colloidal stability of the amine-grafted particles while delaying the hydrolysis of grafted aminosilanes.⁴⁰ At different time points during this storage (0, 7, and 28 days), the size, surface charges and grafting density of the SiNP-NH₂ were assessed by DLS, ζ -potential and fluorescence spectroscopy respectively. The measurement at time 0 corresponded to the characterization of the freshly prepared SiNP-NH₂ (i.e., just after dialysis). After 7 and 28 days, a specific volume of the stored solutions (around 10 mL) was first centrifuged (12000 rpm for 20 min at 20 °C) to remove the molecules potentially "detached" by hydrolysis, and redispersed in acidic water (pH $\sim$ 2.5), before being subjected to DLS, ζ -potential and fluorescence measurements.

RESULTS AND DISCUSSION

Effect of Synthesis Parameters on the Amine Grafting Density

The first part of this work focuses on a qualitative evaluation of the influence of different physicochemical parameters (aminoalkoxysilane type and concentration, SiNP size and concen-

tration, pH) on the amine grafting. In this initial step, modified SiNPs were characterized with DLS and ζ -potential measurements to assess size, colloidal stability, and provide a rapid and qualitative measure of grafting density.

Influence of the Type of Aminoalkoxysilane

The grafting of two distinct aminoalkoxysilanes, namely APTMS (3-aminopropyltrimethoxysilane) and APTES (3-aminopropyltriethoxysilane), was first tested (see Figure 2-

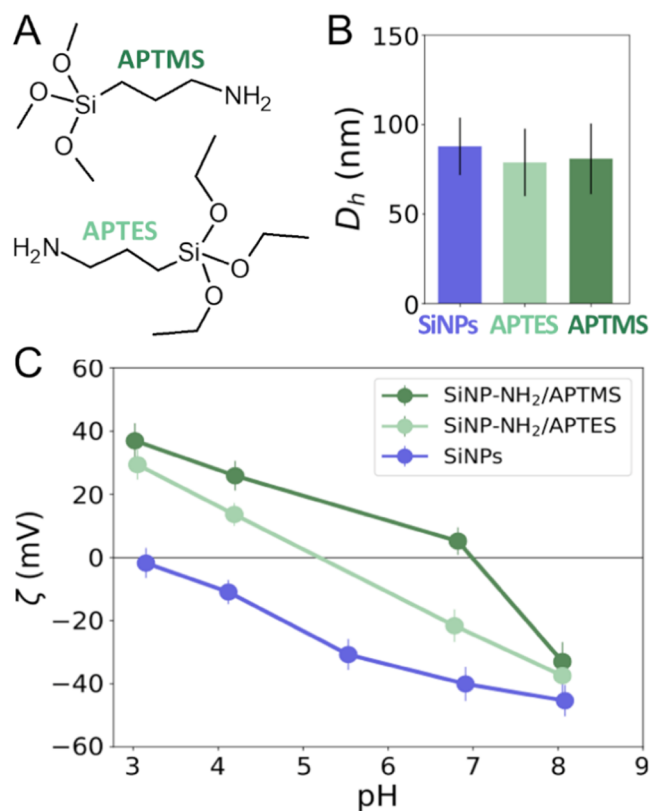


Figure 2. Influence of silane derivatives on the amine modification of SiNPs. (A) Formula of the two alkoxy silane used APTMS and APTES. (B) Mean hydrodynamic diameter (D_h) obtained by DLS for bare (blue) and modified (green) SiNPs. Error bars are calculated from the Polydispersity Index (see Experimental Section). (C) ζ -potential as a function of pH for SiNP-NH₂ modified with APTMS (dark green symbols) and SiNP-NH₂ modified with APTES (light green symbols), compared with those of bare SiNPs (blue symbols).

A). These two silane derivatives were selected as they are commonly used for the modification of SiNPs^{4,30,50} due to their low cost³⁴ and high reactivity. Indeed, their primary amine group in the propyl position can catalyze both intra- and intermolecular formation of siloxane bonds, which accelerate the grafting kinetics.^{51,52}

The surface modification with these two coupling agents was conducted on SiNPs of ~90 nm diameter (at a concentration of 0.5 wt.%) under basic aqueous conditions (pH = 10.9, as detailed in the Experimental Section). A large excess of alkoxy silane was introduced (corresponding to ~270 aminosilane per nm² of silica) as was used in the literature.⁴ The modified particles were then characterized by DLS and ζ -potential measurements (see Figure 2-B and 2-C).

DLS data (Figure 2-B) reveals no significant change in hydrodynamic diameter after modification with APTES and APTMS compared to bare SiNPs. This is expected since the

thickness of an alkoxy silane monolayer should not exceed a few angstroms⁵² and is thus not detected by DLS. On the other hand, ζ -potential measurements (Figure 2-C) first show that bare SiNPs display a negative potential at all tested pH values. This is consistent with the literature⁵³ and is attributed to the presence of deprotonated silanol groups (Si-O⁻) introducing negative charges on the silica surface.

In contrast, SiNPs modified with APTES (light green symbols) and APTMS (dark green symbols) exhibit higher (i.e., more positive) ζ -potential values compared to bare SiNPs, indicating successful surface modification with amine groups. Below its pK_a (~10.5),^{54,55} the primary amine in the propyl position is protonated (NH₃⁺) and thus brings positive charges to the silica surface leading to an increase in the ζ -potential values.

Notably, SiNPs modified with APTMS show higher potential values than those grafted with APTES. Specifically, the isoelectric point (IEP)—the pH at which the particle is virtually uncharged (ζ -potential = 0)—is significantly higher for APTMS-modified SiNPs (~7) compared to APTES-modified SiNPs (~5). Given that the pK_a values of APTMS and APTES are expected to be similar for these propylamine molecules (typically around 10.5),^{54,55} this difference in IEP suggests a higher amine grafting density when APTMS is used. This is likely due to the faster hydrolysis rate of methoxy groups compared to ethoxy groups^{52,56} which may accelerate the modification kinetics (see reaction route in Figure 1).

Given that APTMS provides higher grafting efficiency, it was chosen as silane precursor for all subsequent studies.

Effect of SiNP Size

To assess the impact of SiNP size on amine grafting density, three SiNP solutions (0.5 wt.%) with diameters of 90, 120, and 145 nm were prepared, modified with APTMS (~270 APTMS groups per nm²) and characterized by DLS and ζ -potential.

DLS shows no significant changes in hydrodynamic diameter after modification for all sizes (Figure 3-A). However, the surface potential of the modified particles is found to increase with particle size (Figure 3-B), along with a rise in the IEP from 6.8 (for the 90 nm particles) to 9.2 (for the 145 nm particles). This trend suggests a higher amine grafting density on larger particles. A similar observation was reported by Iijima et al.,⁵⁷ who studied the grafting of hexyltrimethoxysilane (structurally similar to APTMS) on fumed silica nanoparticles in methylethylketone. This phenomenon is attributed to the lower curvature of larger particles, which should bring silanol groups closer together. The proximity of these groups enhances polarization via hydrogen bonding (see the schematic in Figure 3-C) and thus increases the electrophilicity of the Si atom in the silanol groups. This polarization facilitates nucleophilic attack by the aminosilane, which improves grafting efficiency. In contrast, the higher curvature of smaller particles hinders the interactions between neighboring silanol groups. In conclusion, denser amine grafting is observed on larger particles. Therefore, SiNPs with diameter around 145 nm were selected in the following studies.

Effect of the Alkoxy silane Concentration

In the literature, amine modification of SiNPs is usually carried out with varying amounts of aminosilane ranging from 4 to 300 aminosilane molecules per available nm² of silica.^{1,4,30} For comparison, the grafting of a monolayer of APTMS on SiNPs corresponds to a surface density of 2–5 APTMS molecules per nm².^{20,45,50} To qualitatively assess the effect of aminosilane

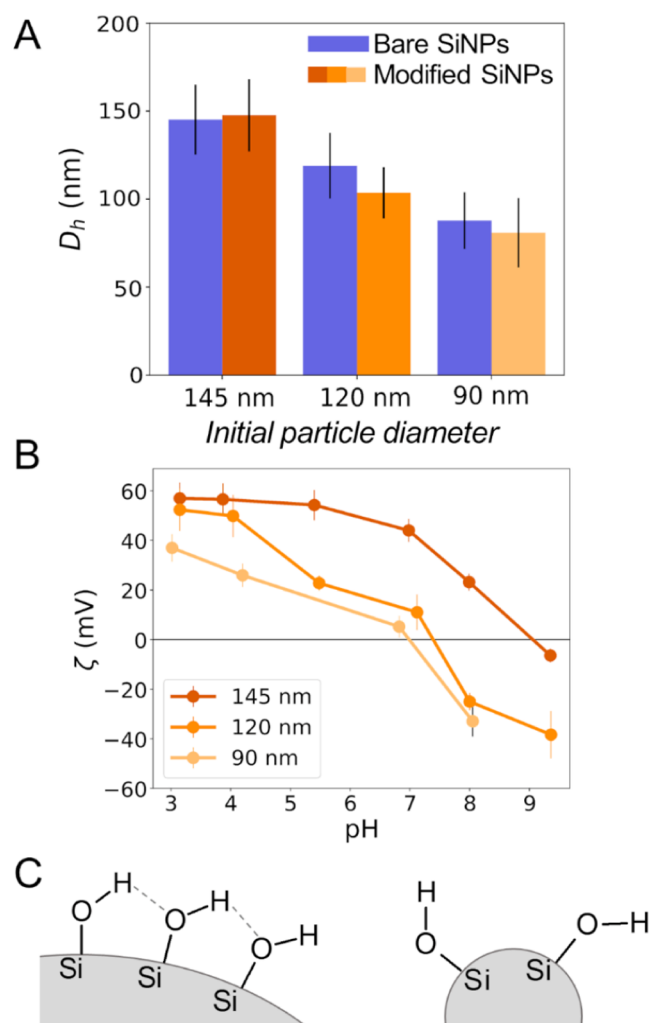


Figure 3. Influence of SiNP size on the amine modification. (A) Mean hydrodynamic diameter (D_h) obtained by DLS for bare (blue) and modified (shades of orange) SiNPs. Error bars are calculated from the Polydispersity Index (see Experimental Section). (B) ζ -Potential as a function of pH for modified particles with different initial diameters: 90 nm (light orange), 120 nm (orange) and 145 nm (dark orange). (C) Scheme illustrating the effect of surface curvature on the interaction between adjacent silanol groups.

concentration on the amine grafting density, three batches of ~ 145 nm SiNPs, were modified with different amounts of APTMS corresponding to 5, 20, and 270 APTMS molecules per accessible nm^2 .

The DLS results show no significant differences in D_h between the different batches (Figure 4-A). In contrast, the ζ -potential data (Figure 4-B) reveal larger potential values at high pH for the batch with 270 APTMS/ nm^2 , which also exhibits the highest IEP ($> \text{pH } 9.5$). In contrast, the other batches show an IEP around pH 8. While a quantitative analysis of the grafting density will be provided in Section 3.2, these qualitative data already indicate that a higher amine grafting density is achieved with a large excess of APTMS.

Consequently, for the subsequent experiments, we chose to systematically use 270 APTMS molecules per nm^2 .

Effect of SiNP Concentration

To study the effect of the initial SiNP concentration on grafting efficiency, three solutions of SiNPs ($D_h \sim 145$ nm) at

different concentrations (0.5 wt.%, 1.2 wt.% and 1.8 wt.%) were modified with APTMS.

As with the previous modifications, the hydrodynamic diameters of the three batches of modified particles are consistent with those of bare SiNPs (Figure 4-C). However, the measured ζ -potential is higher when the modification is performed with a more diluted solution (0.5 wt.%, see Figure 4-D), showing an IEP around 9, compared to ~ 8 for more concentrated solutions. This suggests denser APTMS grafting in the more diluted SiNP batch. To our knowledge, such concentration-dependent effect on grafting efficiency has not been previously reported. It may be linked to the improved dispersion of diluted suspensions, which are less prone to aggregation⁵⁸ and expose more silanol groups for reaction with APTMS. Regardless, since the highest grafting density was achieved with diluted SiNP solutions, all subsequent functionalizations were performed at this low concentration (0.5 wt.%).

Role of pH

The literature reports that amine modification of SiNPs in water can be achieved either in basic⁴ or acidic conditions,¹ but the impact of pH on grafting efficiency remains unclear. To address this, SiNPs ($D_h \sim 145$ nm) were modified at three different pH: 10.9 (being the pH used in all the previous sections), 9 and 5. These pH values were adjusted prior to introducing APTMS by adding aliquots of NH_4OH or HCl solutions.

DLS shows similar sizes for the pH 5 and 10.9 batches (141 ± 24 nm and 141 ± 26 nm, respectively), which are close to the size of bare SiNPs (137 ± 23 nm, see Figure 4-E). The pH 9 batch shows a slightly larger size (170 ± 33 nm), though still within the error margin. This difference may be attributed to mild aggregation, likely occurring during the modification process, since the particles are weakly charged at pH 9 (ζ -potential < 15 mV at pH 9, see Figure 4-F). Regarding the ζ -potential, the trends for modified SiNP prepared at pH 9 and 10.9 are similar, remaining positive across the entire pH range (Figure 4-F). On the contrary, SiNPs modified under acidic conditions exhibit lower potential at higher pH values (> 7), with an IEP around pH 8. These results suggest that basic conditions promote higher grafting density. This is consistent with the findings of Brochier Salon et al.,⁴⁰ who studied the hydrolysis and condensation of silane derivatives by NMR in the presence of deuterated water (20 wt.%). They reported that basic conditions efficiently catalyzed silane hydrolysis while significantly accelerating self-condensation, leading to the formation of polymeric silane derivatives. In our case, this behavior could result in the grafting of polymeric amino-silanes displaying multiple amine groups on the surface of SiNPs, thus increasing the amine grafting density. In contrast, Brochier Salon et al. showed that, although acidic conditions catalyzed hydrolysis, they resulted in stable silanol entities with slower condensation rates. This slower condensation would likely lead to lower grafting efficiency on SiNPs, which is consistent with the lower ζ -potential observed for SiNPs modified at pH 5 (Figure 4-F).

To achieve dense amine grafting, we selected the basic condition of pH 10.9 for future modifications.

Characterization of SiNP- NH_2 with High Grafting Density

The screening study outlined above identified the optimal conditions for achieving high-density amine grafting on SiNPs using water as the primary solvent. These conditions include a

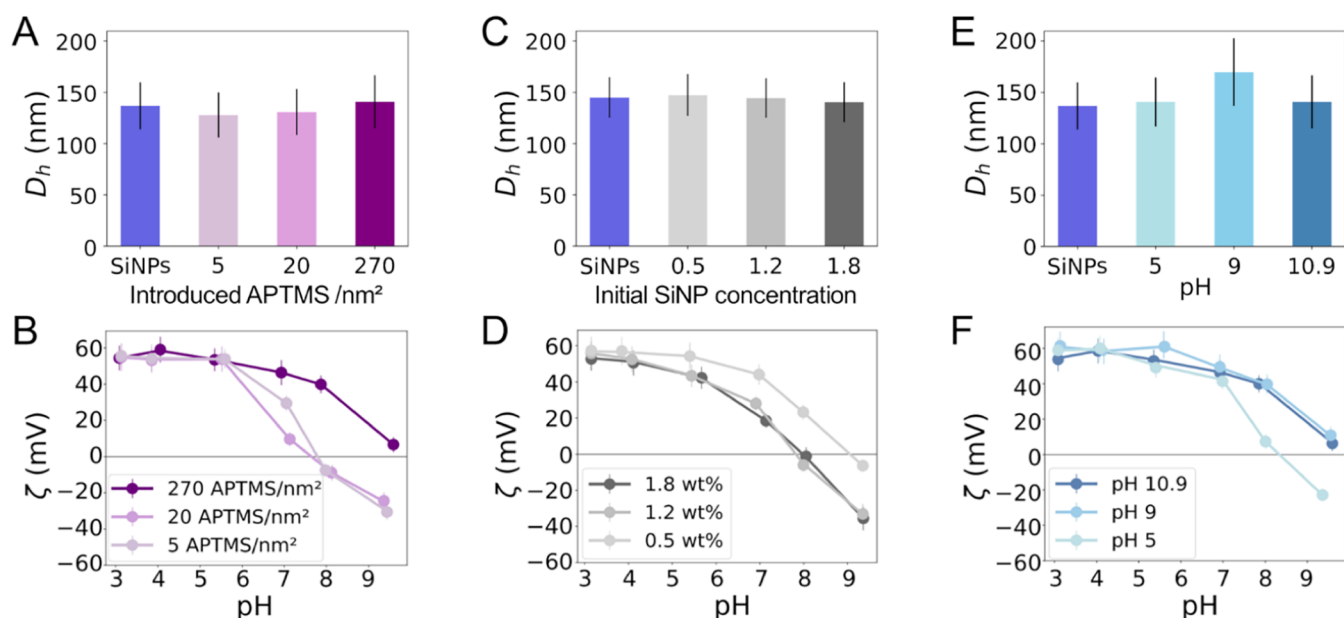


Figure 4. Effect of initial APTMS concentration used for modification (A, B), effect of initial particle concentration (C, D) and effect of pH (E, F) on the size and surface charges of amine modified SiNPs. (A) Hydrodynamic diameter (D_h) obtained by DLS for bare SiNPs (blue) and SiNPs modified with various amounts of APTMS (shades of purple). (B) ζ -Potential of SiNPs modified with various amounts of APTMS. (C) Hydrodynamic diameter (D_h) of bare SiNPs (blue) and SiNPs modified from different initial particle solutions with various concentrations (shades of gray). (D) ζ -Potential of SiNPs modified from different initial particle solutions with various concentrations. (E) Hydrodynamic diameter (D_h) of bare SiNPs (blue) and SiNPs modified at different pH (shades of turquoise). (F) ζ -Potential of SiNPs modified at different pH.

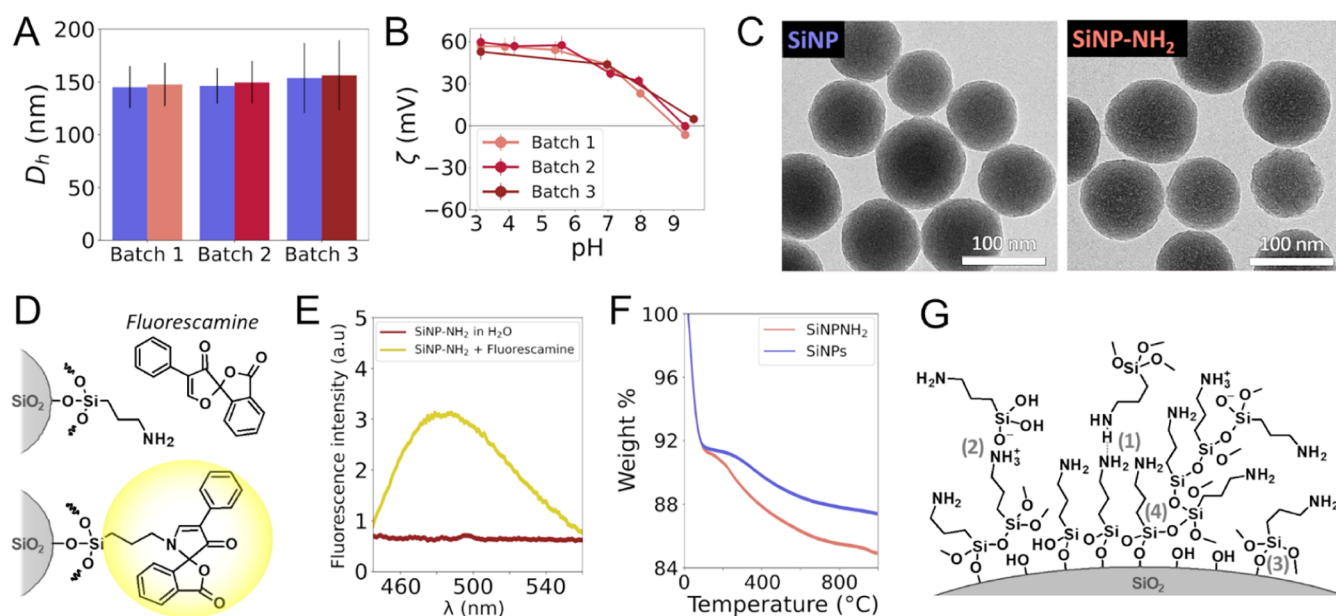


Figure 5. Characterization of SiNPs modified in conditions ensuring a dense amine grafting according to Section 3.1. (A) Mean hydrodynamic diameter D_h for bare SiNPs (blue) and three batches of SiNPs modified in "optimal" conditions (shades of red). (B) ζ -Potential of three batches of SiNPs modified in "optimal" conditions. (C) TEM images of bare SiNPs (left) and SiNPs modified in "optimal" conditions (right). (D) Schematic representation of the principle of the fluorescamine assay. (E) Fluorescence emission spectra of modified SiNPs with (yellow) and without fluorescamine (red). (F) TGA curves of bare SiNPs (blue) and SiNPs modified in optimal conditions (red). (G) Schematic representation of the grafting of APTMS molecules on the SiNP surface. APTMS can bind the surface through (1) hydrogen bond, (2) electrostatic attraction, (3) covalent bond with surface silanol groups, (4) polymerization with other APTMS (i.e., polycondensation).

large excess of APTMS (270 molecules/ nm^2), relatively large SiNPs ($D_h \sim 145$ nm), and a low nanoparticle concentration (0.5 wt.%) under basic conditions (pH 10.9). In the following section, we present a detailed analysis of the morphology and quantitative grafting density of the SiNPs modified under these optimized parameters.

Reproducibility and Morphology

First, the reproducibility of the functionalization method was evaluated by synthesizing three batches under identical conditions. DLS analysis (Figure 5-A) showed consistent hydrodynamic diameters (~ 150 nm) and relatively low

polydispersity. ζ -potential measurements (Figure 5-B) also revealed nearly identical results for the three batches, confirming the reproducibility of the surface grafting.

Second, the morphology of modified SiNPs was investigated using TEM and compared to that of bare SiNPs (Figure 5-C). Corresponding images taken shortly after purification (less than 20 h for bare SiNPs and less than 3 h for SiNP-NH₂) revealed similar morphologies for both types of particles, with spherical shapes and slight surface roughness, indicating the presence of nanoporosities typical of Stöber silica nanoparticles.⁵⁹ The size distribution obtained from TEM images (see Figure S3) showed an average particle diameter of approximately 100 nm for both particle types (100 ± 12 nm for SiNPs and 103 ± 13 nm for SiNP-NH₂). This is smaller than that estimated from DLS (154 ± 34 nm for SiNPs and 156 ± 35 nm for SiNP-NH₂): this can be attributed to the inherent tendency of DLS to detect larger particles and aggregates, especially when using cumulant analysis that yields intensity-weighted sizes.⁶⁰ A similar discrepancy was already reported in the literature for Stöber particles.⁶⁰ Nevertheless, the key observation is that the size and morphology of the SiNPs are not significantly affected by the APTMS functionalization. This suggests homogeneous grafting with a limited degree of silane polymerization on the particle surface. It is important to note that polysiloxane layers based on APTMS, which have a composition similar to silica, are visible by TEM and exhibit comparable contrast in TEM images.⁶¹

Quantitative Grafting Density

The amine grafting density on the surface of modified SiNPs was quantified using two distinct methods.

First, a fluorescamine-based method was employed, in which fluorescamine, an inherently nonfluorescent molecule, reacts with grafted amines to form a fluorescent compound (Figure 5-D). This method is commonly used for quantifying amines grafted on silica surfaces.^{43–49} The reaction of fluorescamine with our modified SiNPs resulted in the expected fluorescence emission peak at 490 nm (Figure 5-E). By comparing the fluorescence intensity to a calibration curve obtained with ethanolamine (Figure S2–C), the amine grafting density was estimated to be approximately 14 amine groups per nm² of silica surface (see Table 2). This result was obtained by

Table 2. Amine Grafting Density of the Modified SiNPs Evaluated by Different Analytical Methods^a

	Fluorescamine Method (N = 4, b = 4)	TGA (N = 4, b = 1)
Grafting density (NH ₂ per nm ² of silica surface)	14 ± 2	10 ± 2

^aThe particle size used for calculating the grafting density is that obtained by the analysis of TEM images (*i.e.*, diameter ~ 100 nm). *N* is the number of sample measured from a number *b* of distinct SiNP-NH₂ batches.

averaging four measurements led on different batches of SiNP-NH₂ (Table S1). Considering the precision of the calibration curve (Figure S2–C), the error margin for this value is estimated to be around ±20%, in accordance with the uncertainty typically reported in the literature for this method.^{48,49} However, fluorescamine assay results may sometimes underestimate the grafting density, as the relatively large size of fluorescamine molecules can hinder access to all grafted amine groups.⁶²

Hence, to confirm the values obtained by fluorescence, grafting density was also evaluated by a second method: thermogravimetric analysis (TGA). TGA was performed on both bare and modified SiNPs to estimate the relative composition of silica and the grafted organic aminopropyl layer (Figure 5-F).^{50,63,64} The TGA curves show two distinct weight loss steps. The first occurs below 120 °C and is attributed to the evaporation of physically adsorbed water. The second occurs between 120 and 1000 °C and is more pronounced for SiNP-NH₂ (see Figure 5-F). For bare SiNPs, this second weight loss is related to the dihydroxylation of residual silanol groups.⁵⁰ By taking the weight loss of bare SiNPs as a reference, the additional weight loss observed for SiNP-NH₂ can be attributed to the decomposition of the grafted aminopropyl groups. Based on the difference in weight loss (2.33 ± 0.34%, as determined by averaging four separate measurements, Table S2), a grafting density of 10 ± 2 aminopropyl groups per nm² was calculated (see Table 2). This result is consistent with the grafting density value obtained from the fluorescamine method. It is worth noting that grafting density values, both in this work and in the cited literature, are most often based on the assumption of a smooth silica surface, neglecting the effects of surface roughness and the corresponding increase in effective surface area.

Nevertheless, the grafting densities obtained using these two different methods are consistently around 12 amine groups per nm². Given that 270 APTMS molecules per nm² of silica were initially introduced, the grafting yield is calculated to be about 4.5%.

This reported density is significantly higher than what is typically observed for surface modifications performed in organic solvents. Indeed, reported grafting densities for SiNP modifications in organic solvents generally range from 0.5 to 5 amine groups per nm²,^{21,24,26,50} even when a large excess of aminosilane is used (>200 aminosilane molecules/nm², comparable to our introduced APTMS concentration).²⁴

To explain this phenomenon, one must first consider that a typical silica surface presents approximately 4–5 silanol groups per nm².⁶⁵ In case of an ideal APTMS monolayer—where each molecule is covalently grafted to a single silanol group—the expected grafting density would lie in the range of 4–5 amine groups per nm²,^{20,45} consistent with observations in organic solvents. Therefore, the remarkably high amine density observed in our study suggests a denser grafting than a monolayer.

This could be attributed to several phenomena. One possibility is the adsorption of APTMS molecules onto the modified SiNP surface through noncovalent interactions such as hydrogen bonding (Figure 5-G (1)) or electrostatic interactions (Figure 5-G (2)). However, this explanation is less likely, as the particles undergo extensive purification (centrifugation and repeated dialysis against Ultrapure water) before characterization, which should remove any weakly adsorbed APTMS molecules. A more plausible explanation is the covalent grafting of polymerized silane onto the silica surface (Figure 5-G(4)), resulting in multiple amine functions bound to the same surface silanol group. Given the assumed surface silanol group density of 4–5 per nm², the measured amine densities (~12 NH₂/nm², Table 2) suggest the presence of grafted silane polymers with an average degree of polymerization of approximately 2–3 (*i.e.*, dimers or trimers).

This behavior aligns with previous studies that have reported the grafting of silane polymers during amine modification of

flat silica surfaces in the presence of water.^{35,38} For example, APTMS deposition from a 3 mM toluene solution containing 1 vol % water resulted in considerable surface roughness (~ 10 nm), attributed to the formation of long polysiloxane chains.³⁵ However, in our case, no significant surface roughness is observed despite employing a much higher APTMS concentration (~ 30 mM, corresponding to ~ 270 molecules per nm^2). This is consistent with the work of Paquet and coworkers,⁶⁶ who demonstrated that the polycondensation kinetics of aminosilanes are considerably faster in ethanol/water mixtures than in pure water. They observed that after 16 h of reaction—corresponding to the duration of our surface modification—aminosilane species in pure water largely remain as monomers and dimers, whereas in mixed organic–aqueous solvents, these species are rapidly consumed within minutes to form longer polysiloxanes or three-dimensional networks. This difference in polymerization behavior likely accounts for the shorter silane chains and lower surface roughness observed in our aqueous system.

Taken together, these results suggest that pure water can serve as an effective solvent for the surface modification of SiNPs with APTMS, enabling the homogeneous grafting of short polymerized aminosilane chains without significantly altering the particle size or surface roughness. This grafting yields a high density of surface amine groups ($\sim 12 \text{ NH}_2/\text{nm}^2$), which is among the highest reported in the literature.

Evaluation of Hydrolytic Stability

As mentioned above, the amine group of APTMS in the propyl position is able to catalyze the formation and hydrolysis of siloxane bond both intra- and intermolecularly (see Figure 6-A(i)).^{51,52} Although this accelerates the grafting kinetics (as discussed in Section 3.1), it can also promote the hydrolysis of siloxane bonds at the silica surface and result in the degradation of the coating (see Figure 6-A(ii)).^{34,52,67} Accordingly, it is important to quantify the hydrolytic stability of the APTMS graft over long storage time in water.

To this aim, a batch of SiNPs was modified with our optimized functionalization method and stored for a long time (28 days) in acidic water at 2 different temperatures (20 ± 2 °C and 5 ± 2 °C). Acidic conditions were selected for two reasons: first to guarantee the colloidal stability of the positively charged amine-grafted particles for the duration of the study and second because acidic conditions are known to delay the hydrolysis of grafted aminosilanes.⁴⁰ At different time points during this storage (0, 7, and 28 days), the size, surface charges and grafting density of the SiNP- NH_2 were assessed by DLS, ζ -potential and fluorescence spectroscopy respectively (see Figures S4, S5 and Figure 6-B and 6-C).

As illustrated in Figure S4, particles stored under acidic pH conditions retained their colloidal stability, as evidenced by the constant size measured by DLS. However, ζ -potential measurements revealed a progressive loss of surface charge over time. Specifically, when stored at 20 °C (Figure 6-B), the isoelectric point (IEP) decreased from approximately 10 to 8.5 after 7 days and further to 7 after 28 days. This suggests a degradation of the grafted layer.

This is confirmed by quantitative assessment of grafting density using the fluorescamine assay (Figure 6-C). At 20 °C, the amine density (NH_2/nm^2) declined steeply from ~ 15 to ~ 8 within the first week, followed by a more gradual decrease to ~ 4 over the subsequent 3 weeks—effectively reducing the grafting density by a factor 3–4 over 28 days. Interestingly, this

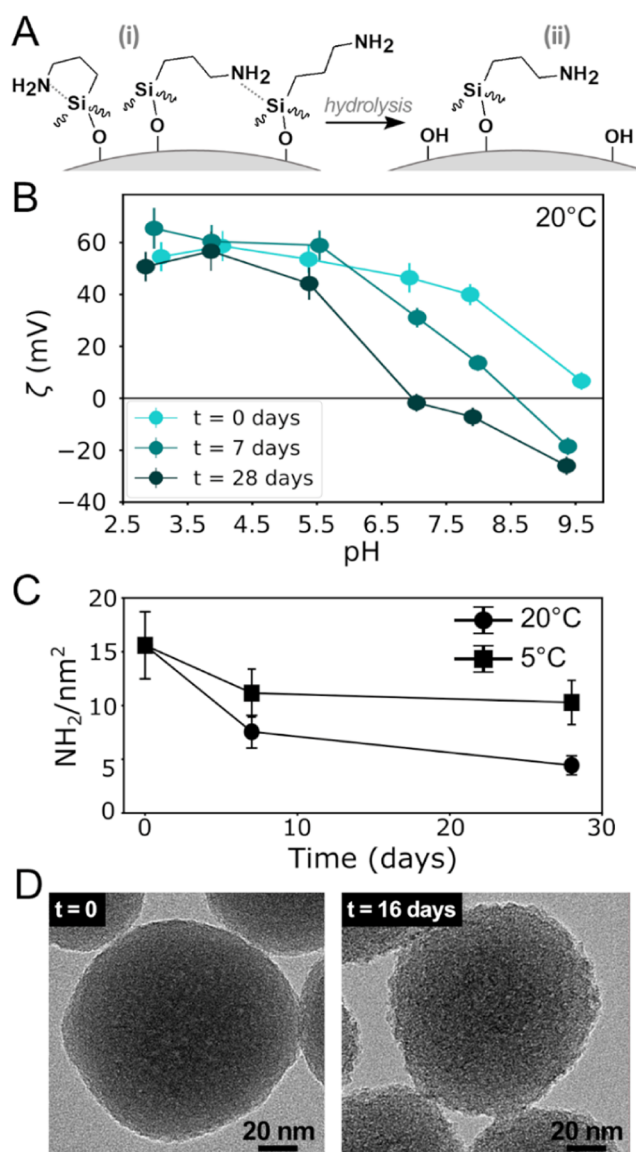


Figure 6. (A) Schematic representation of surface grafting degradation via hydrolysis of siloxane bonds, catalyzed by the amine groups of APTMS. (B) ζ -Potential of modified SiNPs as a function of pH, measured immediately after preparation (light turquoise), after 7 days (turquoise), and after 28 days (dark turquoise) of storage in acidic water at 20 °C. (C) Amine grafting density of modified particles, determined by the fluorescamine assay, as a function of storage duration in acidic water. (D) TEM images of modified SiNPs immediately after preparation (left) and after 16 days of storage in acidic water at 20 °C (right).

degradation was less pronounced at lower temperatures, where the amine density decreased to $\sim 11 \text{ NH}_2/\text{nm}^2$ after 7 days and remained relatively stable ($\sim 10 \text{ NH}_2/\text{nm}^2$) over the following 3 weeks. Similarly, limited variations of ζ -potential values were recorded over 28 days for the sample stored at 5 °C (Figure S5).

In addition, transmission electron microscopy (TEM) images of modified SiNPs after prolonged storage (16 days at 20 °C; Figure 6-D) revealed a significant increase in surface roughness compared to freshly prepared particles. Importantly, such roughness was not observed in bare SiNPs, even after extended storage (~ 48 days at 20 °C; Figure S6), suggesting that it arises from degradation of the grafted aminosilane layer.

To quantify this effect, the roughness factor (R) of SiNPs and SiNP-NH₂ was evaluated from TEM images (detailed in the “Characterization of Silica Nanoparticles” Section - subsection TEM) over extended storage periods (Figure S7). For bare SiNPs, R increased only marginally, from 1.2 ± 0.1 to 1.3 ± 0.2 over more than 40 days, underscoring the stability of the bare silica surface. In contrast, for SiNP-NH₂, R rose from 1.2 ± 0.1 to 2.1 ± 0.1 , clearly demonstrating the formation of porosities on the modified silica surface over time. Additionally, the depth of these surface porosities (2–3 nm) is consistent with the detachment of short polysiloxane chains ($D_p \sim 3$ –4 given the size of an APTMS molecule is typically 8 Å)⁵² from the silica surface. This observation aligns well with the grafting morphology previously inferred from fluorescence and TGA measurements.

Collectively, these results clearly demonstrate that the APTMS graft formed via aqueous SiNP modification undergoes hydrolytic degradation over time. The relatively rapid degradation observed—particularly at 20 °C—can be attributed to the hydrolysis of surface siloxane bonds leading to the detachment of grafted polysiloxane chains. In this process, the cleavage of a single Si–O bond results in the loss of multiple amine groups, which explain the rapid decrease in amine grafting density.

Direct comparison of the reported hydrolytic stability with the literature is challenging. Although the hydrolytic stability of grafted aminopropylsilane layers has been investigated on both flat surfaces^{34,52,67,68} and nanoparticles,⁶⁹ quantitative comparisons are hindered by variations in substrate geometry, experimental conditions (e.g., solvent, temperature) and analytical methods (e.g., NMR, ellipsometry, AFM, colorimetric, and fluorescence assays) across studies. Nonetheless, a common observation is that short-chain aminoalkylsilanes, such as APTMS or APTES, are particularly prone to hydrolysis, primarily due to their propensity for self-catalyzed degradation (as schemed in Figure 6-A).^{52,67} For instance, complete loss of attached silane was observed on flat surfaces functionalized with APTES in toluene after immersion in water at 40 °C for 24 h.³⁴ One commonly proposed mitigation strategy involves the use of bulkier^{34,52} or longer-chain⁶⁷ aminosilanes, which are structurally less prone to intramolecular catalysis. However, this increased hydrolytic stability often comes at the cost of slower grafting kinetics and reduced grafting density, highlighting an inherent trade-off between stability and surface coverage.

Regardless, despite the susceptibility of our densely grafted SiNPs to hydrolytic degradation, our results indicate that storage at low temperature under acidic conditions preserves a high amine surface density (≥ 10 NH₂/nm²) for at least 28 days.

CONCLUSION

In this work, we have performed a systematic investigation of the surface modification of silica nanoparticles (SiNPs) by aminoalkoxysilanes in aqueous media. A qualitative study first established that, similar to behavior in organic solvents, high amine grafting densities can be achieved in water by employing a large excess of (3-aminopropyl)trimethoxysilane (APTMS) on dilute SiNP suspensions (0.5 wt %) with relatively large hydrodynamic diameters (~ 150 nm), under basic conditions (pH 10.9).

The resulting APTMS-modified SiNPs were extensively characterized and possessed highly reproducible grafting

densities of approximately 12 NH₂ nm⁻² (among the highest reported to date when assuming a smooth SiNP surface). These results suggest the relatively homogeneous grafting of small polysiloxane chains with a degree of polymerization around 3–4. This contrasts with modification in organic solvents, which typically results in the formation of an APTMS monolayer and yields significantly lower grafting densities (≤ 5 NH₂/nm²).

The hydrolytic stability of these densely grafted layers was also examined. At 20 °C, the graft was found to undergo gradual degradation, likely due to the detachment of polysiloxane chains, each releasing multiple amine groups. However, storage at reduced temperatures (~ 5 °C) significantly improved stability, maintaining a grafting density of ~ 10 NH₂/nm² over several weeks.

Overall, this study provides a comprehensive understanding of the key parameters influencing amine grafting of SiNPs in water, and offers practical guidelines for achieving dense, stable surface functionalization. This is an important progress for applications ranging from colloidal stabilization to biofunctionalization and nanomaterials engineering. Future work could extend this understanding to diverse types of SiNPs with varying surface chemistries and topologies, such as fumed silica or mesoporous silica, for targeted applications.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.langmuir.6c03900>.

Evaluation of the roughness ratio R , details of the fluorescamine assay, particle size distribution from TEM images, temporal evolution of particle size during long-term storage in acidic media, temporal evolution of particle zeta potential during long-term storage at 5 °C, evolution of particle surface roughness during long-term storage, tables summarizing grafting densities measured by the fluorescamine assay and TGA (PDF)

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