



Deoxycholate as an efficient coating agent for hydrophilic silicon nanocrystals

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ABSTRACT

Silicon nanocrystals were made hydrophilic by 10-undecenoic acid grafting and were then coated with sodium deoxycholate, a detergent-like compound belonging to the bile acid class which is crucial for absorption of lipids in the small intestine. The resulting silicon nanocrystals have an average diameter of 3–5 nm, can be dispersed in aqueous solutions and show stable photoluminescence. Coating with non-biological surfactants, which are dangerous for cell safety, was investigated for comparison. Results indicate that deoxycholate is a stabilizer of luminescent silicon nanocrystals. Deoxycholate coated nanocrystals appear suitable for applications as multifunctional probes in biomedicine.

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1. Introduction

Nanostructured silicon is attracting a great deal of attention as a bio-investigation tool since it photoluminesces at room temperature and slowly degrades into safe components, such as silicic acid [1], unlike other popular inorganic nanostructures. However a successful application of silicon nanocrystals (Si-nc) to bio-investigation requires some sort of “interfacing”, since we must be able to tune the characteristics of Si-nc, particularly its emission properties, to the biological environment: Si-nc should absorb and emit at the desired wavelength, be brightly luminescent and tolerate contacts with water and oxygen in the experimental time-scale which, for cell studies, is in the range of a few days (Si-nc and pSi are well known to undergo fast oxidation by water and oxygen, which affects their emission wavelength and intensity [2,3]). Lastly, for cell and *in vivo* applications, Si-nc must be targeted to specific action sites. In this case, the Si-nc coating should be tailored and the optimum size established taking into account mechanisms allowing biological barriers to be crossed [4,5]. According to the quantum confinement model, nanocrystal size, together

with surface chemistry effects, contribute to the stability and optical properties of Si-nc [6–8]. However a speculative approach to designing a suitable interface seems to be unrealistic, due to the complexity of the system. Although various empirical strategies have recently been reported, preparation of water-dispersible Si-nc which maintain size-dependent photoluminescence (PL) has proven difficult. Many efforts have focused on chemical stabilization of the surface by alkylation [9–12] or bromine oxidation followed by covalent attachment of alkyl groups [13] which renders Si-nc stable against corrosion. However, the nanoparticles are rather hydrophobic, and large and heterogeneous aggregates form in some cases. Hydrophilic Si-nc are obtained by grafting of carboxyl-terminated alkenes, although the Si-nc surface is still exposed to water and solutes. According to Eckoff et al. [14], a surface density of one or two carboxyl groups per about 3 nm² can be estimated. Water-dispersible propionic acid terminated silicon nanoparticles were synthesized and characterized [15]. Medium-sized (126 nm) silicon nanostructures, with intrinsic near-infrared luminescence, have been fabricated and used for *in vivo* monitoring. However, dimensions of the order of a few nanometers (i.e. comparable to proteins) appear desirable for some applications. In this case, the surface coating should also be designed to avoid small particle aggregation into larger structures [15]. Stabilization by physisorption of organic molecules avoids further reaction steps which may affect the PL emission of Si nanostructures. Studies of stabilization by surfactants, of macroscopic structures containing Si nanocrystals such as pSi [16] or pSi powders [17–19], have been reported and recently reviewed [20], however only few experiments have been made with Si-nc and surfactants and significant

Abbreviations: CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; CTAB, hexadecyltrimethylammonium bromide; DCA, sodium deoxycholate; HEPES, N-(2-Hydroxymethyl)piperazine-N'-(2-ethanesulfonic acid); Si-nc, silicon nanocrystals; MES, 2-(N-morpholino)ethanesulfonic acid; PL, photoluminescence; Si-nc-COOH, functionalized silicon nanocrystals; SDS, sodium dodecyl sulfate; TRIS, tri(hydroxymethyl)aminomethane; RT, room temperature.

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stability improvements are not reported. In particular, surfactant coating has been used for small Si-nc obtained by the bottom-up (chemical synthesis) approach and in this case trioctylphosphine oxide (TOPO) was used [21], although the hydrophobic TOPO layer prohibits direct application of Si-nc in aqueous environment. Conversely, phospholipid micelles yield water-dispersible Si-nc, which have been used successfully for imaging of cancer cells [22]. However these micelles are typically large size (up to 120 nm) and tend to aggregate and form large clumps.

The stability of Si-nc and safe targeting to action sites are still unresolved questions, partly explaining why these systems are not routinely applied in spite of their superior physical properties. Natural detergents belonging to the bile acid class are known to form efficient carrier structures aimed at delivering and protecting insoluble fatty molecules [23] but they are still unexplored as Si-nc delivery systems. In particular sodium deoxycholate (DCA) is released from bile into the human intestine where it forms mixed micelles with fatty acids which diffuse passively across the membranes or are actively taken up by intestinal epithelial cells. The efficient re-absorption of bile acids is suggested to improve the bioavailability of particulates, proteins and drugs [24].

The present work focuses on DCA coating of Si-nc. In our approach, the Si-nc were first made completely water soluble by covalent binding of an alkyl chain bearing a carboxyl group on the tail (Si-nc-COOH). The hydrophilic Si-nc-COOH were then coated with DCA and their properties were found to be comparable or even better than those of other surfactants such as SDS or Triton X 100. The latter cannot be used for cell or *in vivo* applications because of their toxicity [25,26] (a detailed documentation is available at the US National Library of Medicine web site, <http://ncbi.nlm.nih.gov>). The experimental conditions required for stable Si-nc aqueous suspensions were set-up, and surfactant effects on light emission were investigated.

2. Materials and methods

All chemicals were purchased from SigmaAldrich and used as received.

2.1. Preparation of porous silicon (pSi)

Microporous pSi was obtained by electrochemical etching of p-silicon (resistivity: 10–20 Ω cm, purchased from Universitywafers.com) in 48% HF and ethanol 1:2, at 80 mA/cm² for 5 min. (Caution: HF-based etchants are extremely hazardous if not properly stored and handled). This experimental protocol was set-up to provide a porous layer efficiently removed by sonication in toluene/ethanol (see Section 2.2).

2.2. Preparation of Si-nc and Si-nc-COOH

Hydrophobic Si-nc were prepared by sonication of microporous pSi with a UNISSET AC2 (Emmegi) ultrasonic bath. About 1 cm² of pSi was sonicated in 20 mL toluene/ethanol 1:1 under an Ar atmosphere for 30 min. The Si-nc thus prepared were hydrogen-terminated. Hydrophilic Si-nc-COOH were obtained from microporous pSi by sonication in the presence of 10-undecenoic acid according to a light-driven silylation reaction [25]. Briefly, freshly etched pSi was put into a 20 mL solution of 0.7 M 10-undecenoic acid in toluene and ethanol 1:1, previously bubbled with N₂, and sonicated for 30 min, under an Ar flow and white illumination (ELC bulb, 250 W). After sonication, the solution was kept under Ar and illumination and stirred for 2 h. The solvents were then removed in a rotavapor under vacuum (Heidolph LABOROTA 4000 pump) and the Si-nc-COOH were suspended in ethanol containing 1 mM

HCl. The Si-nc were purified from non-reacted 10-undecenoic acid by ten filtration–suspension cycles. Each washing cycle was performed by concentrating aliquots of 2 mL of Si-nc-COOH suspension to 0.5 mL by centrifugation on Centricon filters (with regenerated cellulose membranes, 100 kDa cut-off, Millipore), and the retentate (0.5 mL) was then adjusted again to 2.0 mL with ethanol–HCl. FTIR spectra of the filtrate confirmed that no 10-undecenoic acid was detectable after five filtration cycles (within the sensitivity of the instrument). At the end of the last cycle, the aliquots were collected together and the final volume adjusted to 20 mL with ethanol–HCl. This solution is henceforth called the concentrated Si-nc-COOH ethanol solution. The concentration of Si-nc-COOH in this solution was estimated by gravimetry, assuming that the pSi layer removed by sonication was entirely recovered as Si-nc-COOH. The weight of this layer was measured by a precision balance (Sartorius, precision: 10 μ g) by measuring the dried pSi sample before and after sonication. The average pSi weight was (500 \pm 50) μ g. Since the average diameter of the Si-nc-COOH was about 4 nm (see TEM image in the Supporting information, SI), assuming that Si-nc-COOH were spherical in shape and that their density was the same as that of macroscopic silicon (2.330 g/cm³), each Si-nc-COOH on average weighs 7×10^{-13} μ g. In this way we estimated a Si-nc-COOH concentration of the order of 10^{14} nanocrystals mL⁻¹. Although this is an overestimation (due to the hypotheses considered above and to the fact that the experimental Si-nc leakages were not taken into consideration) it matches calculation based on PL emission of Si-nc-COOH in solution, which leads to an estimation of 10^{13} nanocrystals mL⁻¹ (see SI for details). This concentrated solution was stable for several months (PL fluctuations were less than 2–3% and comparable to our fluorescence measurements experimental error; data not shown) and was diluted before experiments.

The concentrated Si-nc-COOH ethanol solution and all the diluted solutions prepared starting from it were left in contact with atmospheric oxygen.

2.3. PL of the Si-nc-COOH aqueous solutions

PL experiments in aqueous solutions were carried out by diluting 0.05 mL of the concentrated Si-nc-COOH ethanol solution into 0.95 mL of various buffered aqueous solutions in the presence and absence of surfactants. After vigorous shaking for a few seconds, the PL of these Si-nc-COOH aqueous solutions was recorded with a Cary Eclipse (Varian) fluorimeter, being the excitation wavelength set at 350 nm. The PL was then followed for 7–12 days after Si-nc-COOH dilution. PL intensity was measured at the wavelength of maximum emission. Since the absorption band of Si-nc is typically broad, in some control experiments various excitation sources and longer excitation wavelengths (up to 532 nm) were used, but no significant changes in PL relative values or time-courses were observed. To rule out the possible effect of the 5% ethanol still present in the final aqueous solutions, some experiments were also performed by drying 0.05 mL of concentrated ethanol solution under vacuum and re-suspending the nanocrystals in 1 mL of aqueous solution. Si-nc-COOH behavior was found to be independent of the mode of transfer in the aqueous environment. PL data were always normalized to the intensity of the corresponding Si-nc-COOH solution in ethanol–HCl, obtained by diluting 0.05 mL of the concentrated Si-nc-COOH ethanol solution in 0.95 mL ethanol–HCl. This solution was considered as the reference.

Quantum yield measurements of Si-nc-COOH in ethanol solution exposed to air were performed using 361 nm excitation line of a SpectraPhysics UV-extended Argon ion laser and SOPRA monochromator interfaced with a visible Photo Multiplier Tube,

with spectral resolution of 1 nm. A red LED (MV5052 from Fairchild Semiconductors) was used to calibrate the collection system.

The time resolved decays of PL of Si-nc-COOH were recorded in aqueous solution (0.3 mL of the concentrated Si-nc-COOH ethanol solution diluted in 0.7 mL of water). Measurements were carried out using as an excitation source the third harmonic (355 nm, 6 ns pulse width, 10 Hz repetition rate) of a Niodimium:Ytterbium (Nd:YAG) laser with the corresponding flux of approximately 10^{25} photons/(sxc m^2) on the sample. CCD Streak camera interfaced with a spectrometer (10 ns of time resolution, 1 nm of spectral resolution) was used as detection system.

2.4. Surfactants and buffers

Surfactant solutions were prepared in various buffers (10 mM MES, pH 6, 100 mM HEPES, pH 7, and 100 mM TRIS, pH 9) and were left to equilibrate at room temperature (RT, 21 °C) for 24 h. The following surfactants were tested: sodium deoxycholate monohydrate (DCA, anionic), hexadecyltrimethylammonium bromide (CTAB, cationic), sodium dodecyl sulfate (SDS, anionic), 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS, zwitterionic) and Triton X-100 (non-ionic). Typically surfactant concentration was about twice the critical micellar concentration (CMC) at RT, i.e.: 2 mM CTAB, 12 mM DCA, 18 mM SDS, 16 mM CHAPS, 0.5 mM Triton X 100, except DCA and SDS which were also used at lower concentrations (equal to and below the CMC).

2.5. FTIR of Si-nc-COOH

In order to check the covalent binding of 10-undecenoic acid to Si-nc, 2 mL of concentrated Si-nc-COOH solution were further concentrated to 0.5 mL (on Centricon filters), deposited, drop by drop, on a clean transparent Silicon support, and dried under a mild vacuum. In order to measure the background, an ethanol–1 mM HCl solution was put through the filtration–suspension cycles on Centricon filters as for Si-nc-COOH purification and deposited on another silicon support. FTIR measurements were performed in transmission mode (spectral resolution: 8 cm $^{-1}$, 32 accumulations for each spectrum) with a Nicolet iN10 MicroFTIR (ThermoFisher), and a nitrogen cooled MCT detector.

2.6. TEM on Si-nc-COOH

TEM analysis was conducted on Philips CM12 transmission electron microscope equipped with a W filament and operating at 120 kV. Droplets of ethanol solutions of Si-nc-COOH were deposited onto a holed carbon film of a Cu grid and were left to dry under gentle argon flow at RT.

2.7. XPS on Si-nc-COOH

X-ray Photoelectron Spectroscopy (XPS) measurements of Si-nc-COOH were carried out on a Scienta ESCA 200 instrument equipped with a hemispherical analyser and a monochromatic Al K (1486.6 eV) X-ray source. The emission angle between the analyser axis and the sample surface was 90°, corresponding to a sampling depth of approximately 10 nm. Quantification of relative elemental percentage was obtained, after Shirley background subtraction, by using the integrated area of the deconvoluted core lines together with atomic sensitivity factors. The Si-nc-COOH were deposited from the concentrated ethanol solution, drop by drop on a flat, clean gold surface. The solvent was left to evaporate in a fume cupboard equipped with dust filters and then inserted into the XPS high-vacuum chamber.

3. Results

3.1. Surface modification by covalent grafting of 10-undecenoic acid

10-Undecenoic acid was grafted on Si-nc by hydrosilylation reaction performed in toluene/ethanol by stirring under white light [27].

XPS analysis was performed on Si-nc-COOH ethanol solution deposited drop by drop on a flat, clean gold surface. The XPS survey spectrum is shown in the SI together with atomic percentage (Supporting information, Fig. S1). The Au signal was absent in the sample area where Si-nc-COOH were present, indicating that only the Si-nc-COOH layer was sampled. Only peaks corresponding to C, O, and Si were observed, matching the elemental composition of the undecenoic acid-grafted Si-nc. A ratio C/O of about 2.9 indicated that oxygen was partly due to Si oxidation which was confirmed by the Si 2p spectrum (Fig. S2 shown in SI) where combined composition of Si–OH and SiO $_2$ (Supporting information, Figs. S2 and S3 components respectively in Fig. S2) was present. Si oxidation was expected since all Si-nc-COOH manipulations were made under ambient atmosphere, after the functionalization step.

The efficacy of the reaction was verified by FTIR on freshly prepared Si-nc-COOH deposited on a silicon support and the relevant functionalities were assigned according to the literature [5,27–37]. Fig. 1 shows the spectrum of deposited Si-nc-COOH (A), together with that of 10-undecenoic acid in a KBr pellet (B), for comparison.

Spectrum A shows peaks due to both C–H (2925, 2854 and 1360 cm $^{-1}$) and C=O (1740 cm $^{-1}$) vibrations, whereas the C=C stretching vibration near 1645 cm $^{-1}$ is not observed. This suggests that the carboxylic acid present on Si nanocrystals is mostly chemically bound, the C=C being responsible for the surface reaction, and –COOH groups are exposed. This is further confirmed by peaks due to Si–C bonds (1460 and 1250 cm $^{-1}$). Si–H vibrations (also back-bound to oxygen) at 2135 and 2250 cm $^{-1}$ were still present, together with a relatively strong peak assigned to Si–O at 1000–1200 cm $^{-1}$, and a weak, flat OH band near 3200 cm $^{-1}$. These latter peaks indicate silicon oxidation, probably occurring mainly after the functionalization procedure (Si-nc-COOH drying in air after deposition on silicon support).

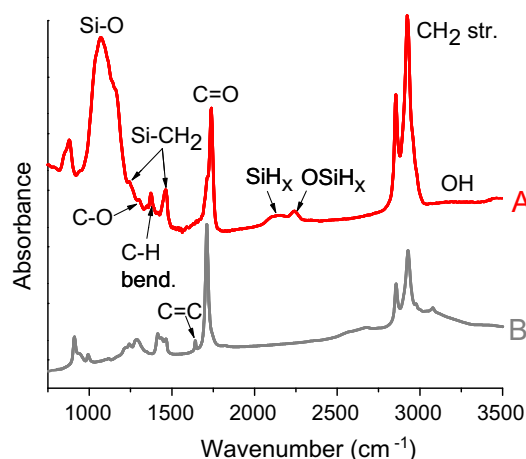


Fig. 1. FTIR spectra Si-nc-COOH after covalent grafting of 10-undecenoic acid. Si-nc-COOH deposited from ethanol–HCl solution on a clean and IR-transparent silicon support (A); 10-undecenoic acid powder in a KBr pellet (B). Spectrum (A) was obtained accumulating 32 scans and taking as background the spectrum of the solvent (ethanol–HCl) processed and deposited as Si-nc-COOH solution, with spectral resolution of 8 cm $^{-1}$. The measurements were performed under a mild N $_2$ flux.

3.2. Si-nc-COOH in aqueous solutions: effect of DCA

The ethanol concentrated Si-nc-COOH solution was diluted 1:20 (or dried and re-suspended, see experimental section) in aqueous solutions at various pHs (6.0, 7.0, 9.0), in the presence and absence of DCA. Clear colloidal suspensions were obtained independently of DCA, indicating the favorable interaction of Si-nc-COOH with water. Conversely it was not possible to dilute or re-suspend non-grafted Si-nc in aqueous solutions, either in the presence or absence of DCA, as the Si-nc either aggregated or were physisorbed on the vessel. The colloidal suspensions of Si-nc-COOH emitted in the red region. Fig. 2 shows representative PL spectra of freshly prepared Si-nc-COOH (ethanol concentrated solution diluted 20 times) in ethanol (A) and in 6 mM DCA solution containing 100 mM HEPES at pH 7.0 (B).

Fig. 2 shows that both, ethanol and aqueous solutions are brightly photoluminescent. The quantum yield of Si-nc-COOH in ethanol solution was measured as described in the experimental section and was about 30%. Fig. 2 also shows that the half-height linewidth is of the order of 100 nm which suggests particle-size

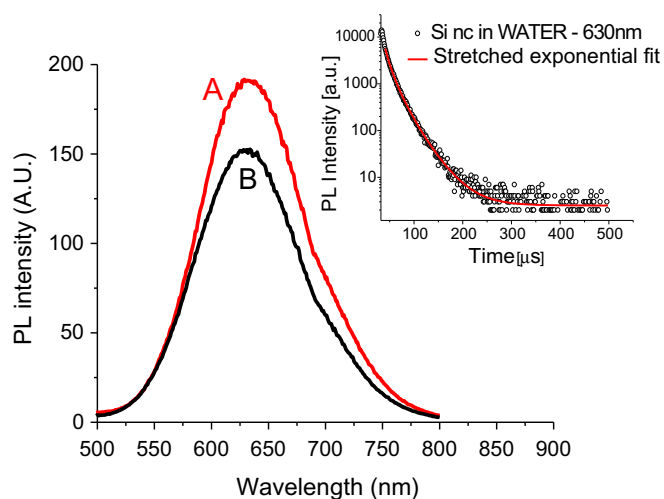


Fig. 2. Photoluminescence spectra of Si nanocrystals after covalent grafting of 10-undecenoic acid. Undecanoic acid-coated Si-nc were suspended and purified in ethanol (Si-nc-COOH concentrated ethanol solution) and then diluted 1:20 in ethanol (spectrum A) or 100 mM HEPES pH 7.0 containing 6 mM DCA (spectrum B). Excitation was set to 350 nm. Inset: PL time decay of an aqueous solution of Si-nc-COOH. Other experimental conditions as reported in the text.

dispersion. The TEM image (see Supporting information, Fig. S3 reported in the SI, together with Supporting information, Fig. S4, showing the size distribution histogram) of Si-nc-COOH suspended in ethanol and then deposited on the holed carbon film of a Cu grid indicates that the Si-nc-COOH are mainly discrete, with an estimated size of 6 nm, standard deviation 2 nm. The TEM image also shows a few larger structures, with the diameter under 20–30 nm.

For better insights regarding the origin of PL and separation between different nanocrystals, the time resolved PL spectrum of Si-nc-COOH in aqueous solution was measured by monitoring the emission at 630 nm using an excitation wavelength of 355 nm. The experimental PL decay is shown in Fig. 2 (inset) and was satisfactorily fitted to a stretched exponential function:

$$f(t) \propto e^{-\left(\frac{t-t_0}{\tau}\right)^\beta}$$

where $f(t)$ is PL during decay, τ is decay time; dispersion factor β represents a measure of the deviation of PL decay from a single exponential. The result of the fit gave τ value of about 15 μ s and β close to 0.85. A τ value in μ s range is compatible with the hypothesis of the quantum confinement origin of PL signal [38]. Very high values of β also suggest that exciton internanocrystal transport is negligible and that Si-nc-COOH are mainly discrete [39].

The time-course of PL spectrum of DCA coated Si-nc-COOH was followed and the observed behavior was compared with that of other surfactants: cationic (CTAB), anionic (SDS), zwitterionic (CHAPS) and non-ionic (Triton X 100). Exposure of Si-nc-COOH to an aqueous environment resulted in the non-monotonic kinetic behavior of PL intensity and in a shift of the wavelength of maximum PL toward the blue region. Fig. 3 shows the time-course of PL intensity and maximum PL peak position at pH 7.0.

In solutions buffered at pH 7.0, the aqueous environment induced initial luminescence quenching with respect to the ethanol solution, both in the presence and absence of surfactants. In buffer or in the presence of anionic, non-ionic or zwitterionic surfactants, this quenching was about 25–35% of the initial PL, and was followed by PL recovery lasting about 24 h. However in CTAB (cationic) the quenching progressively increased to 100% of initial PL. PL recovered completely only in the presence of anionic surfactants, i.e. DCA and SDS. In the presence of these two surfactants, PL is then stable for about 8–10 days. Conversely, in buffer alone, progressive PL instability was observed. A comparable blue shift of the wavelength of maximum emission was observed in all samples, except in Si-nc-COOH dispersion in aqueous CTAB and in ethanol–HCl. In CTAB, a sudden shift toward shorter wavelength was observed. In ethanol–HCl the wavelength of maximum emission

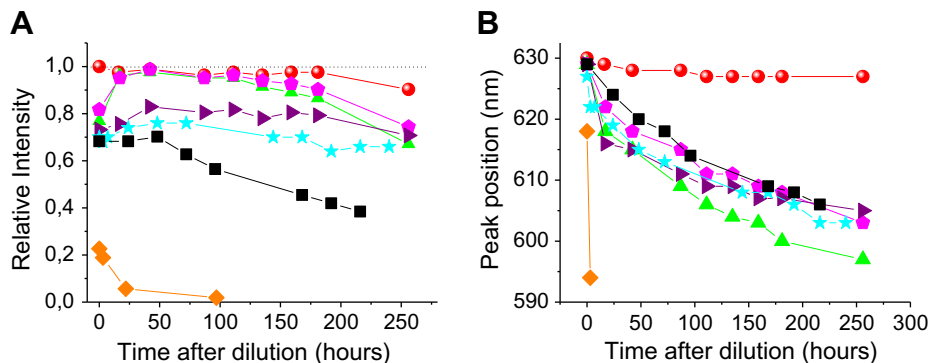


Fig. 3. Time dependence of PL intensity (panel A) and maximum peak position (panel B) of the nanocrystals dispersed in surfactant aqueous solution (at pH 7.0). Si-nc-COOH functionalized by 10-undecenoic acid and dispersed in ethanol after purification were diluted in an aqueous solution (1:20) containing HEPES 100 mM at pH 7.0 (black squares), in the presence of 18 mM SDS (magenta pentagons), DCA 12 mM (green upward triangles), 1 mM Triton X 100 (purple rightward triangles), 16 mM CHAPS (cyan stars) and 2 mM CTAB (orange rhombi). Red circle-traces: behavior of the Si-nc-COOH diluted 1:20 in ethanol containing 1 mM HCl (reference). The spectra were acquired at 350 nm exciting wavelength. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

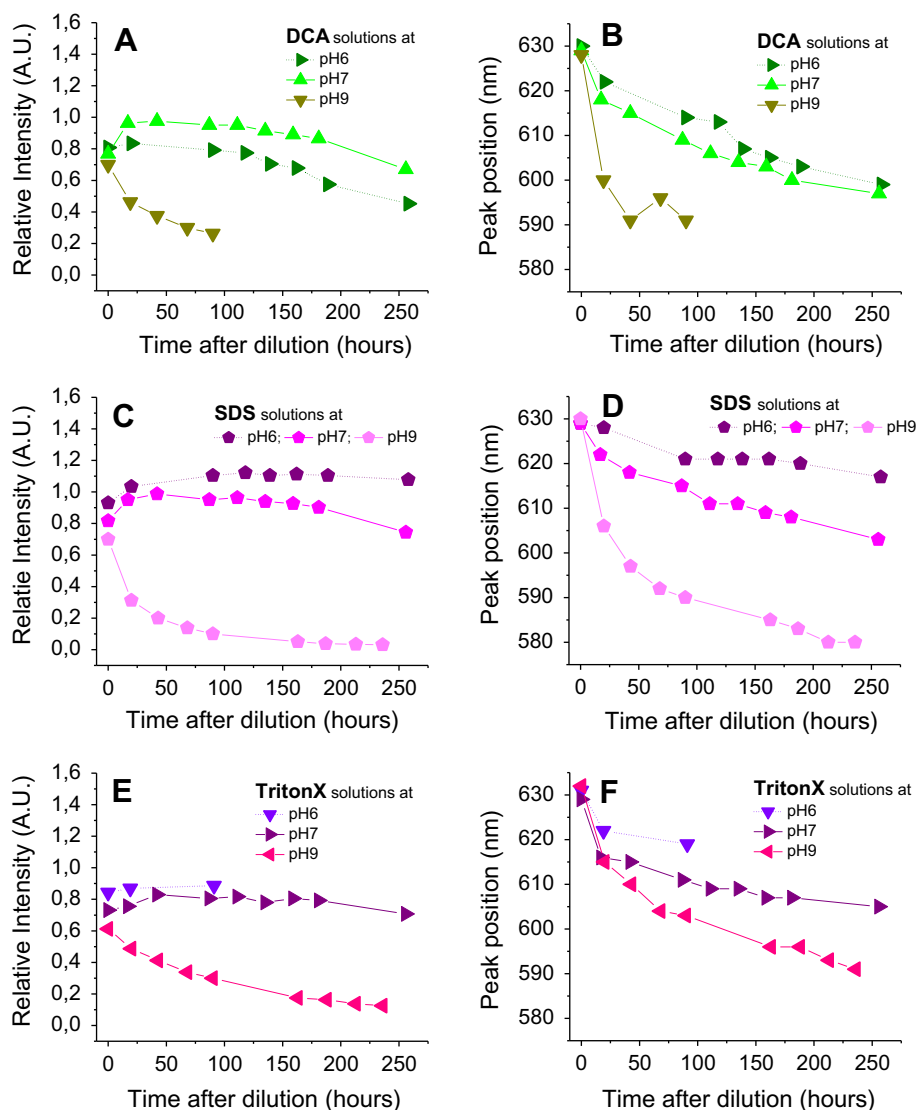


Fig. 4. Time dependence of PL intensity (panels A, C and E) and maximum peak position (panels B, D and F) of Si-nc-COOH dispersed in surfactant aqueous solution at various pHs. The concentrated ethanol Si-nc-COOH solution was diluted 1:20 in each buffered aqueous solution containing the surfactant at twice the respective CMC. Buffered solutions were 100 mM HEPES (pH 7.0), 10 mM MES (pH 6.0), 100 mM TRIS (pH 9.0). Surfactants were added at twice the CMC to each solution. Panels A and B: 12 mM DCA, at pH 6 (▶), pH 7 (▲) and pH 9 (▼); panels C and D: 18 mM SDS at pH 6 (●), pH 7 (◆) and pH 9 (◇); panels E and F: 0.5 mM Triton X 100 at pH 6 (▼), pH 7 (▶) and pH 9 (◀) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

shifted initially by about 5 nm and then stabilized at around 623 nm. The experiments of Fig. 3 were repeated with three Si-nc-COOH preparations and the same behavior was observed.

Some experiments were carried out at three pHs, to investigate the influence of pH on PL in the presence of DCA, SDS and Triton X 100. In particular the wavelength of maximum emission and normalized PL intensity at this wavelength were monitored over time in aqueous solutions at pH 6.0, 7.0 and pH 9.0, (Fig. 4, panels A–F).

Fig. 4 shows that PL evolution is strongly affected by pH. In particular, at pH 7.0 and 6.0, DCA induced the fast recovery of PL which then remained stable for a few days. Lastly, slow PL decay was observed. The behavior of SDS and Triton X 100 was similar to that of DCA, although slightly better PL stability was observed in SDS and Triton X 100 at pH 6.0 than at pH 7.0, i.e. the opposite behavior to that observed in DCA solutions. Since the ionization constant of the carboxyl group of DCA is about 6.58 [40], at pH 6.0 DCA behaves predominantly as a non-ionic surfactant. At pH 9.0, fast, continuous PL decay and wavelength shift are observed with all the tested surfactants.

Experiments were also performed varying DCA concentration in the range 1–12 mM (Fig. 5 panels A and B). For comparison, the SDS concentration was varied in the range 118 mM (Fig. 5 panels C and D). The concentration range explored was centered around the CMC of the two surfactants (the CMC of DCA is 6 mM and that of SDS is 7–10 mM at RT [41]).

Fig. 5 shows that the stability of PL emission increased, with the surfactant concentration, and this increase persisted even at surfactant concentrations higher than their respective CMC.

4. Discussion

The striking features of the time evolution of PL emission in the presence of DCA emerge from data obtained at pHs close to the physiological ones: the instantaneous decrease of PL intensity upon Si-nc-COOH transfer from ethanol to water, PL recovery in the presence of DCA, and a decrease in the maximum wavelength emission in aqueous solutions, even if DCA is present (although not

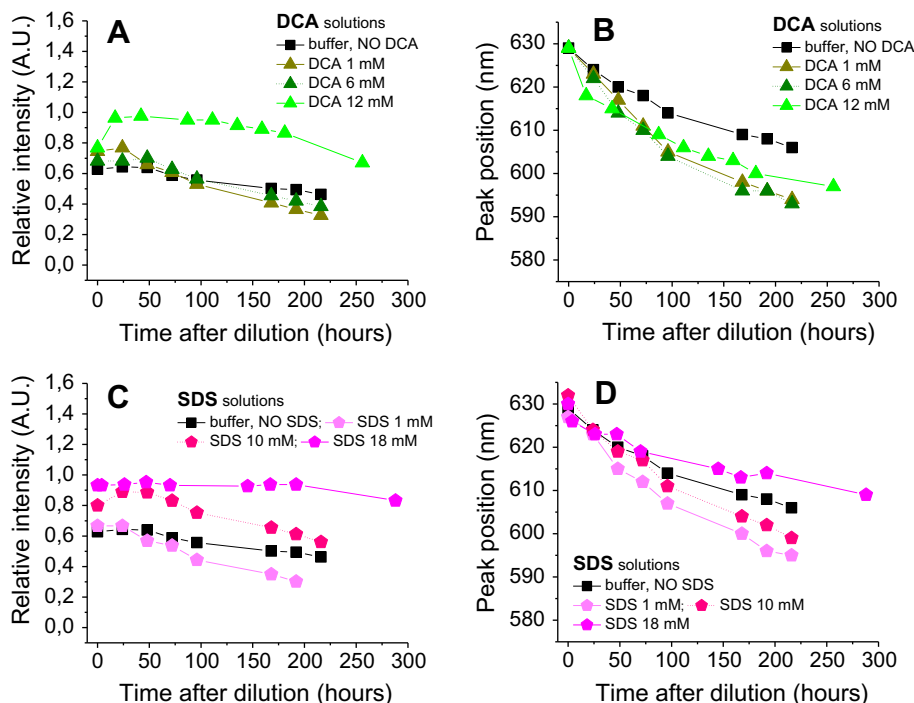


Fig. 5. Time dependence of PL intensity (panels A and C) and maximum peak position (panels B and D) of Si-nc-COOH dispersed in aqueous solutions containing variable anionic surfactant concentration. The concentrated Si-nc-COOH ethanol solution was diluted 1:20 in the aqueous surfactant solution containing 100 mM HEPES pH 7.0. Panels A and B: DCA 1 mM (dark yellow), 6 mM (dark green), 12 mM (green) was added. Panels C and D: SDS 1 mM (light magenta), 10 mM (pink), 18 mM (magenta) was added. Panels report also the behavior of Si-nc-COOH in 100 mM HEPES at pH 7.0 without surfactants (black). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

in ethanol). These features were compared with those observed when SDS (anionic and similar to DCA as regards interaction with Si-nc-COOH) or Triton X 100 (neutral) were used.

In this work we dealt with single, or agglomerates of a few, silicon nanocrystals which, when suspended in an aqueous solution, expose the whole surface to water, unlike pSi and silicon powder, in which molecular diffusion inside nanopillars is hindered. Fast water-mediated oxidation of the Si-nc surface consequently takes place, and the chemical nature of the resulting native silicon oxide is poorly controlled [42]. In addition, the organization Si-nc-COOH at the nanometer length scale when dispersed in DCA solutions probably also contributes to the observed behavior.

The instantaneous initial decrease of PL observed after suspending Si-nc-COOH in aqueous solutions (see Figs. 2 and 3), can be ascribed to the change in solvent polarity [43]. Chemical modification of surface due to fast hydration processes may also contribute. The progressive core oxidation in aqueous solution induces the blue shift of PL [44], which, conversely, is not observed in ethanol (Fig. 3). The blue shift is independent of the nature of the surfactant. In this regard, the fast, continuous decay of PL and the blue shift observed at pH 9 are strong indications that OH^- plays a fundamental role in the oxidation of Si-nc-COOH in aqueous solutions.

The PL recovery in the presence of anionic and neutral surfactants (though less effective in the latter case) may be due to shielding of the Si-nc-COOH by organic molecules, with a consequent decrease in dielectric constant (Fig. 3), although other effects may also occur [45]. The PL emission of pSi in the presence of surfactants has been recently reviewed [20] and various mechanisms are suggested to play a role. In particular Chattopadhyay and Bohn [16] observed similar PL enhancement by anionic surfactants on pSi which was interpreted within the hole-depletion layer model. This layer is non-emissive and contracts in the presence of an anionic surfactant inducing PL recovery. Conversely the fast,

continuous decrease of PL, observed in the presence of a cationic surfactant (CTAB) may be ascribed to expansion of the depletion layer. In addition, the dramatic blue shift (Fig. 3) observed with CTAB suggests that CTAB-catalysed Si oxidation also occurs in colloidal suspensions of Si-nc-COOH as in pSi [46].

Si-nc (not previously grafted by 10-undecenoic acid) were completely insoluble in aqueous environment even in the presence of surfactants, supporting an alkyl chain-mediated interaction between surfactants and Si-nc-COOH. In this regard, the protective effect of surfactants still increases above the CMC where the concentration of monomeric surfactant molecules available for Si-nc-COOH coating does not increase further (Fig. 5), suggesting that insertion of Si-nc-COOH into micelles increases PL stabilization. In particular deoxycholate has one hydrophobic and one hydrophilic side bearing hydroxyl and carboxyl groups. This “sidedness” permits the formation of small micelles which probably locate around the Si-nc-COOH.

5. Conclusions

The chemical grafting of 10-undecenoic acid makes Si-nc-COOH water soluble and introduces an alkyl chain which favors physical coating by surfactants. Some of tested compounds were efficient Si-nc-COOH stabilizers although they have a denaturing effect on biostructures such as proteins and membranes and are therefore toxic for cells. In this paper, we demonstrate for the first time that DCA is an efficient stabilizer of Si-nc-COOH. This compound is characterized by a low toxicity being a natural detergent containing a free carboxyl group which may increase the biocompatibility of nanostructures [47]. In humans, DCA is synthesized in the liver, secreted into bile, and delivered to the small intestine, where DCA micelles function as fatty acid carriers. These characteristics,

together with stabilization of colloidal Si-nc-COOH properties in addition to maintenance of Si-nc-COOH PL, by this surfactant, suggest that a DCA-based delivery system can be designed for *in vivo* administration of nanosized Si-based particulate for imaging and therapeutic applications [22,48].

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.jcis.2011.03.006](https://doi.org/10.1016/j.jcis.2011.03.006).

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