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Capillary electrophoresis of ultrasmall carboxylate functionalized silicon nanoparticles

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Capillary electrophoresis is used to separate ultrasmall (~1 nm) carboxylate functionalized Si nanoparticles (Si-np-COO^-) prepared via hydrosilylation with an ω-ester 1-alkene. The electropherograms show a monodisperse Si core size with one or two carboxylate groups added to the surface. On-column detection of their laser-induced fluorescence demonstrates that the individual Si-np-COO^- have narrow emissions (full width at half maximum = 30–40 nm) with a nearly symmetric lineshape. Preparative scale electrophoresis should be a viable route for purification of the Si-np-COO^- for further study and future applications. © *2006 American Institute of Physics*. [DOI: 10.1063/1.2345366]

Luminescent semiconductor nanoparticles (SC-np) have many unique properties that make them well-suited for use in biophysical marking applications. They are optically bright and photostable,[1] with narrow and symmetric fluorescence that is tunable by varying the size of the semiconductor core.[2] Their distinct photophysical properties complement those of organic fluorophores and have led to novel applications in biophysics.[3] Particular interest has been given to the II-VI SC-np such as CdSe and ZnS, but the need to coat them with multiple layers of material requires overall particle dimensions on the order of 5–15 nm,[4] limiting their use mainly to cellular scale applications in mechanics and imaging.[5] To study molecular scale dynamics, particularly at the single molecule level, a smaller and more diverse class of SC-np is desired.

The group IV semiconductor Si is a promising alternative. It has many favorable properties that may help to bridge some of the gaps between the II-VI materials and organic fluorophores. Perhaps most important is the capability for direct coupling with organic species through a strong and stable Si-C linkage, as has been demonstrated for myriad Si surfaces including those derived from bulk Si,[6] porous Si,[7] and Si nanoparticles (Si-np).[8] This allows for a wide range of functionalities and should lead to a diverse array of applications for organically modified Si-np. In addition, given the low inherent biotoxicity of Si,[9] it seems likely that the extensive shielding required for the II–VI semiconductors will not be necessary for Si-np, generally yielding smaller overall sizes and hence, less perturbative effects on the system of study.

We recently reported an example of a multi-functional passivation for Si-np using hydrosilylation with an ω-ester 1-alkene to prepare carboxyl functionalized nanoparticles (Si-np-COOH).[10] The Si-np-COOH were shown to preserve the strong fluorescence of the Si core, have sizes comparable to small organic fluorophores (~1 nm), and to be stable in polar solutions. The carboxyl functionality can also be reacted with primary amines to attach them to various biomolecules. Here, we extend our previous analysis to discrete chemical species by using capillary electrophoresis (CE) to separate the hydrosilylation reaction products and characterize them based on their charge mobility and fluorescence properties. After first discussing the Si-np preparation and functionalization reactions, we show that the purified Si-np-COO^- have a relatively monodisperse Si core size with one or two carboxylate groups added to the surface. In addition, they exhibit narrow fluorescence in the near-UV with a full width at half maximum (FWHM) of 30–40 nm

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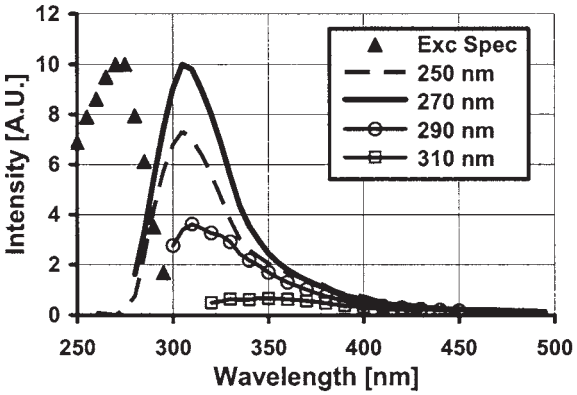

FIG. 1. Fluorescence spectra for the Si-np-H preparation. Excitation spectrum for emission at 310 nm (▲) and emission spectra for excitation at 250–310 nm (see inset). Data from Refs. 10 and 12.

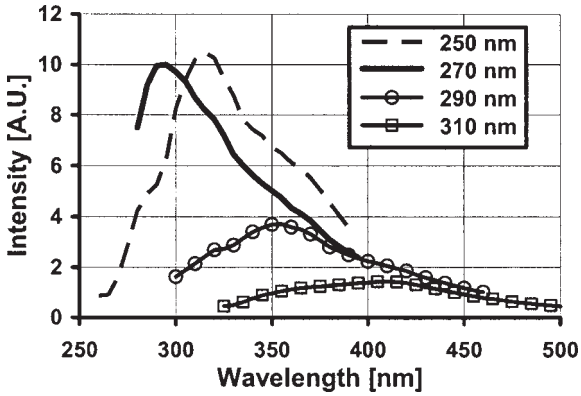

FIG. 2. Fluorescence spectra for the functionalized Si-np-COOH. Emission spectra for excitation at 250–310 nm (see inset).

and a nearly symmetric lineshape with only weak red-tail emissions.

The Si-np were prepared with a H-passivation (Si-np-H) via electrochemical dispersion of a crystalline Si wafer. The method uses HF and H_2O_2 to etch a porous surface layer followed by ultrasonic fractionation to release the trapped Si-np.[11] The presence of Si-H groups on the nanoparticle surface was confirmed using ^{1}H-NMR measurements and size exclusion chromatography shows that the Si-np-H are ultrasmall (~1 nm).[10] Fluorescence spectra for the starting Si-np-H in isopropanol are shown in Fig. 1. The dominant emission is at 305 nm with a FWHM of 43 nm (0.56 eV) and a peak excitation of 275 nm. The red-tail emissions at 335 nm and greater were much more prominent after aqueous treatments and have been attributed to various species of oxidized and aggregated material.[12]

The carboxyl functionalized Si-np-COOH were prepared via hydrosilylation of the Si-np-H with the bi-functional ester methyl 4-pentenoate, $CH_2{=}CHCH_2CH_2C(O)OCH_3$.[10] The reaction proceeds with insertion of the alkene into a thermally activated Si-H bond to form a stable Si-C linkage with the ester exposed to the solvent. The ester is then hydrolyzed in aqueous NaOH to generate the carboxyl functionality, $\equiv Si(CH_2)_4C(O)OH$. Figure 2 shows the fluorescence spectra for the Si-np-COOH. Four distinct features are seen with their emission wavelengths at ~295, 315, 350, and 410 nm, respectively. Clearly, the spectra indicate that we have a heterogeneous mixture of reaction products. In comparison to Fig. 1, the 295–315 nm features are close to the dominant emission for the starting Si-np-H, while the red-shifted emissions at 350–410 nm are much stronger for the Si-np-COOH (i.e., after hydrolysis).

Prior to electrophoretic separation of the reaction mixture, an initial polarity based separation was done to remove species that have unreacted double bonds on their surface. Thermal hydrosilylation with an ω-ester 1-alkene can produce these by-products via backwards reaction of an unprotected ester with the surface or through a free radical mechanism.[10] To remove them, the sample was first dried at basic pH (carboxyl groups ionized) and the solids were redispersed in tetrahydrofuran (THF). Only the less polar species are soluble, including the alkene by-products, so this is referred to as the less polar fraction (LP fr.). After filtration, the remaining solids were then acidified to neutralize the carboxyl groups and the sample was redispersed in THF to extract the previously insoluble species such as those with multiple carboxyl linkers. This is referred to as the more polar fraction (MP fr.) and is the primary product of the synthesis. Segregation of the alkene by-products to the LP fr. was confirmed using Fourier transform infrared (FTIR) analysis.[10]

Electrophoretic separation of the LP/MP fractions was done using a laboratory-built CE system with wavelength-resolved, laser-induced fluorescence (LIF) detection. The method combines the high separation efficiencies and small sample volumes of CE with the low detection limits and high resolution fluorescence spectra obtained from the LIF detection system, which uses a sheath-flow cuvette, spectrograph, and charge-coupled device array.[13] CE with wavelength-resolved fluorescence detection has been used for a wide variety of applications including DNA sequencing, cellular and neurochemical analyses, and rapid clinical assays of biological fluids.[14] The CE-LIF instrumentation was configured for optimal separation of negatively charged species,[13,15] as we anticipate for the carboxylate modified Si-np-COO^- at basic pH. The elution time is a function of electrophoretic mobility (related to the net charge and size of the particles), with the more negative species eluting from the capillary at longer times.

The electropherograms for the MP and LP fractions of Si-np-COO^- are shown in Figs. 3 and 4, respectively. The bottom portion of the figures plots the emission wavelength versus the elution time with the darkness of the bands corresponding to intensity. The top portion plots the total fluorescence intensity integrated from 260–500 nm. One important feature is that all of the bands are quite narrow in terms of elution time, with the base-to-base widths of the individual species being 0.3 min or less. This demonstrates that the Si-np cores are relatively monodisperse in size.

With the many bands seen in Figs. 3 and 4, it is helpful to divide them into distinct classes in order to discuss their origins. We will focus first on the major species. The labels A, B, and C at the top of Fig. 3 denote the three strongest bands in the MP fraction. These species have elution times of

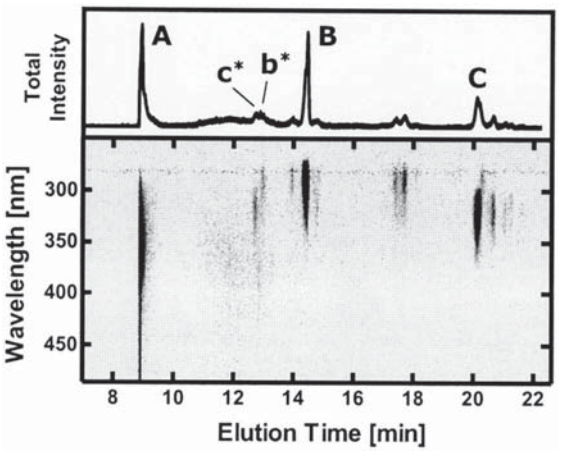

FIG. 3. Electropherogram showing the wavelength-resolved fluorescence emission as a function of elution time for the MP (more polar) fraction of Si-np-COO^-. The top portion plots the total intensity integrated from 260–500 nm. A, B, and C label the three most prominent bands. c^* and b^* label minor bands discussed in the text.

(A) 8.9 min, (B) 14.4 min, and (C) 20.1 min. Calibrations of the system using a range of organic fluorophores indicate that the 8.9 min band corresponds to neutral or near-neutral species, while the remaining bands at longer elution times fall in the ranges typical of small organics with one or two negative charges.[13] For example, the fluorescein dianion elutes at ~22 min under the same conditions.

Fluorescence spectra for the major bands are shown in Fig. 5. Looking first at band A, its peak emission is at 356 nm and the fluorescence is broad with a shoulder ~300 nm and strong red-tail emissions up to ~600 nm. We attribute this band to a collection of neutral species that are not sufficiently separated from one another. There appears to be multiple species when zooming in closely on Fig. 3. Neutral by-products could include any unreacted, oxidized, or aggregated material, consistent with the mixture of emissions seen in Fig. 5 (spectrum A) and also helping to explain the strong fluorescence redshift in comparison to the starting material.

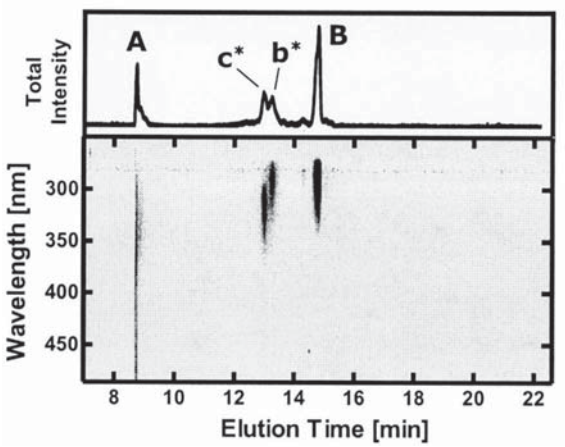

FIG. 4. Electropherogram showing the wavelength-resolved fluorescence emission as a function of elution time for the LP (less polar) fraction of Si-np-COO^-. The top portion plots the total intensity integrated from 260–500 nm. A, B, c^*, and b^* label the corresponding bands from Fig. 3.

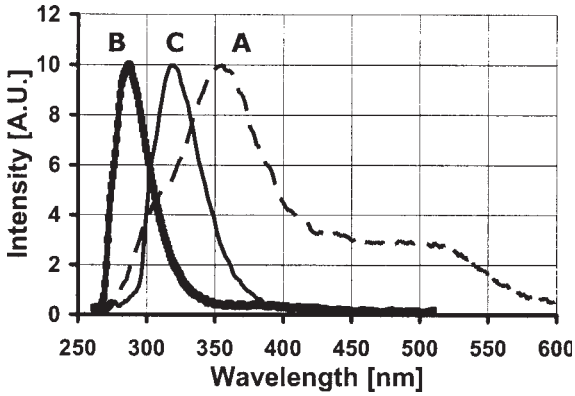

FIG. 5. Fluorescence emission spectra for the major bands (A, B, and C) from the MP fraction of Si-np-COO^- (see Fig. 3). Excitation is at 257 nm.

The remaining bands at longer elution times correspond to negatively charged species. Based on the calibrations mentioned above, we assign bands B and C to Si-np with one and two carboxylate linkers, respectively. This assignment is consistent with the expected mobility for an ~1 nm Si-np with one or two negative charges. Relative to the neutral band A (8.9 min), note that band C (20.1 min) elutes with about twice the time-delay of band B (14.4 min). This assignment is also logical in terms of the chemistry involved. In particular, we expect to have a continuous distribution of linker coverage, so attributing the major bands, for example, to zero, two, and four linkers, is not reasonable since in that case we would also expect to see bands attributable to one and three linkers.

In contrast to the neutral band, the fluorescence spectra for the major carboxylate species (bands B and C) show rather narrow emissions with only minor red-tail fluorescence (Fig. 5). The one-carboxylate species (spectrum B) emits at 287 nm with a FWHM of 31 nm (0.46 eV), while the two-carboxylate species (spectrum C) emits at 318 nm with a FWHM of 42 nm (0.51 eV). Compared to the starting Si-np-H (Fig. 1), the spectra are both narrower and more symmetric. An interesting observation is that the emission redshifts from 287 nm to 318 nm in going from one to two carboxylate linkers. It is not clear whether this is due to the increased charge on the Si-np or perhaps related to minor structural alterations accompanying the formation of the Si-C linkages on the surface.

Among the many minor bands seen in the electropherograms, we will now discuss the species eluting at ~13 min (labeled b^* and c^* in Figs. 3 and 4). These two bands are weak in the MP fr. (Fig. 3), but much stronger in the LP fr. (Fig. 4). Their fluorescence spectra are shown in Fig. 6. The spectra are similar to those for the major carboxylate species (Fig. 5), except rather than having a single narrow peak, they appear to contain a mixture of emissions. This is highlighted by the inset to Fig. 6, which shows the difference between each of these minor spectra and the corresponding major spectra. The magnitudes of the difference spectra suggest that ~20% of the intensity can be attributed to secondary emissions.

The increased prominence of the b^*/c^* bands in the LP fr. (Fig. 4) provides a second clue to their origin. As dis-

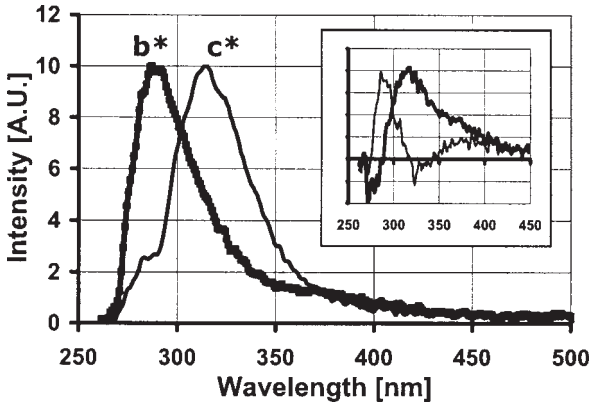

FIG. 6. Fluorescence emission spectra for the minor bands (b^* and c^*) from the LP fraction of Si-np-COO^- (see Fig. 4). Inset shows the difference between these spectra and the corresponding major spectra (B and C) in Fig. 5.

cussed above, the LP fr. was seen to contain some amount of alkene by-products (with unreacted double bonds on their surface) that were not present in the MP fr. Such species can cross-link with other Si-np through the hydrosilylation mechanism (i.e., alkene+Si-H bond). Thus, a reasonable assignment for these bands is species consisting of two Si-np cores cross-linked together with one carboxylate group among them. If the two cores are separated by a multi-atom spacer preventing direct electronic communication between them, then the units can emit independently, providing an explanation for the mixture of emissions seen in Fig. 6. Similarly, the two minor bands at ~17.5 min in the MP fr. (Fig. 3, not labeled) may be due to two Si-np cores cross-linked together, but with two carboxylate groups total.

The final class of minor bands to be discussed is the side bands located very close to the major bands B and C. In Fig. 3, this includes a minor band on each side of band B (~14–15 min) and four minor bands just to the right of band C (~20.5–22 min). The fluorescence spectra for these bands have almost identical lineshape and wavelengths as the corresponding major species (i.e., there is no apparent mixing of emissions). This suggests that they are also due to single Si-np cores instead of cross-linked aggregates. One possibility is that they originate from minor structural or conformational differences among the Si-np-COO^-. For example, the minor bands close to the two-carboxylate species (Fig. 3, four weak bands to the right of band C) may arise from differing relative locations of the two linkers on the surface, which can cause minor changes in their mobilities due to changes in pK and diffusion rate.

In conclusion, CE separation of carboxylate functionalized Si-np-COO^- was used to isolate and analyze the spectral properties of individual species within the hydrosilylation reaction products. Several important elements were demonstrated. First, it confirms that the hydrosilylation protocol developed previously was successful in attaching carboxylate groups to the Si-np surface.[10] Second, it shows that the individual carboxylate species will be purifiable via preparative scale electrophoresis to remove neutral and cross-linked by-products. Finally, it demonstrates that the major Si-np-COO^- species have narrow emissions in the near-UV with only minimal red-tail fluorescence.[16] Additional work will focus on preparative scale isolation of the various bands, allowing for detailed analysis of the individual species. Studying the minor species should also be of considerable help in optimizing the hydrosilylation reaction mechanism.

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[1] F. Chen and D. Gerion, Nano Lett. **4**, 1827 (2004).
[2] B. O. Dabbousi, J. Rodriguez-Viejo, F. V. Mikulec, J. R. Heine, H. Mattoussi, R. Ober, K. F. Jensen, and M. G. Bawendi, J. Phys. Chem. B **101**, 9463 (1997).
[3] T. Q. Vu, R. Maddipati, T. A. Blute, B. J. Nehilla, L. Nusblat, and T. A. Desai, Nano Lett. **5**, 603 (2005).
[4] D. Gerion, F. Pinaud, S. C. Williams, W. J. Parak, D. Zanchet, S. Weiss, and A. P. Alivisatos, J. Phys. Chem. B **105**, 8861 (2001).
[5] W. C. W. Chan, D. J. Maxwell, X. Gao, R. E. Bailey, M. Han, and S. Nie, Curr. Opin. Biotechnol. **13**, 40 (2002).
[6] T. Böcking, M. James, H. G. L. Coster, T. C. Chilcott, and K. D. Barrow, Langmuir **20**, 9227 (2004).
[7] M. P. Stewart and J. M. Buriak, Comments Inorg. Chem. **23**, 179 (2002).
[8] L. Wang, V. Reipa, and J. Blasic, Bioconjugate Chem. **15**, 409 (2004).
[9] A. H. Mayne, S. C. Bayliss, P. Barr, M. Tobin, and L. D. Buckberry, Phys. Status Solidi A **182**, 505 (2000).
[10] E. V. Rogozhina, D. A. Eckhoff, E. Gratton, and P. V. Braun, J. Mater. Chem. **16**, 1421 (2006).
[11] G. Belomoin, J. Therrien, A. Smith, S. Rao, R. Twesten, S. Chaieb, M. H. Nayfeh, L. Wagner, and L. Mitas, Appl. Phys. Lett. **80**, 841 (2002).
[12] D. A. Eckhoff, J. D. B. Sutin, R. M. Clegg, E. Gratton, E. V. Rogozhina, and P. V. Braun, J. Phys. Chem. B **109**, 19786 (2005).
[13] X. Zhang, J. N. Stuart, and J. V. Sweedler, Anal. Bioanal. Chem. **373**, 332 (2002).
[14] J. N. Stuart, A. B. Hummon, and J. V. Sweedler, Anal. Chem. **76**, 120A (2004); L. Ciobanu, S. S. Rubakhin, J. N. Stuart, R. R. Fuller, A. G. Webb, and J. V. Sweedler, Anal. Chem. **76**, 2331 (2004); J. N. Stuart, J. D. Ebaugh, A. L. Copes, N. G. Hatcher, R. Gillette, and J. V. Sweedler, J. Neurochem. **90**, 734 (2004).
[15] The CE separation used a running buffer of 50 mM boric acid/sodium borate at pH 8.8. The capillary was 75 cm in length with a 50 μm inner diameter. The applied field was 300 V/cm. Fluorescence excitation was via a frequency-doubled argon ion laser at 257 nm. The LIF detection system included a sheath-flow cuvette, reflective microscope objective, imaging spectrograph, and liquid N_2 cooled, charge-coupled device array detector. Fluorescence was collected from 260–720 nm. See also Ref. 13.
[16] If UV transitions are not appropriate for a particular application, visible wavelengths can be obtained by starting with a larger Si core size or by developing an appropriate surface modification to redshift the transitions. See also Refs. 11, 12, and 17.
[17] A. Puzder, A. J. Williamson, J. C. Grossman, and G. Galli, J. Chem. Phys. **117**, 6721 (2002).