# Organophilic Colloidal Particles with a Synthetic Polypeptide Coating

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Composite colloidal particles with a silica core and a synthetic, homopolypeptide shell have been produced by initiation of benzyl-L-glutamate *N*-carboxyanhydride monomer from primary amine functionalized silica particles. The resulting poly($\gamma$-benzyl-$\alpha$,L-glutamate)-coated spheres were characterized by electron microscopy, dynamic light scattering, infrared spectroscopy, and thermogravimetry. The polypeptide shell accounts for about 20% of the total mass of the particles, which are reasonably uniform in size. Infrared spectra show an $\alpha$-helical secondary structure, but other conformations are not excluded. A geometrical analysis is applied to calculate the maximum number of amino groups that might realistically participate in initiation. The actual shell thickness is smaller than expected on the basis of these geometrical considerations, which reflects undesired termination steps or the conversion of some monomer to unattached polymer by trace initiator impurities.

## Introduction

Uniform particles have been synthesized for a variety of fundamental studies, including colloid stability,[1,2] colloid–polymer interactions,[3–12] and probe diffusion in polymer solutions and gels.[13–24] Such particles also find many practical applications, ranging from coatings to novel optical devices. Typical random coil polymers, such as polystyrene or poly(dimethylsiloxane),[25,26] are often attached to the surface of colloidal particles to provide steric stabilization[27] against the natural tendency toward aggregation.

Colloidal particles coated with homopolypeptides have not been studied extensively, which is surprising given the special properties of these polymers. In addition to a very versatile chemistry, polypeptides feature well-defined secondary structures. In the $\alpha$-helical conformation, the polymers form extended, stiff rods. Close approach of particle pairs and the resultant destabilization could be prevented efficiently with a minimal coating of stiff rods. It is possible to produce nearly uniform polypeptides of large size, and the biotechnology to produce perfectly monodisperse peptides of small size continues to develop.[28] Thus, the minimum distance between cores might be set precisely by a polypeptide coating. In addition to being among the most rigid of polymers, homopolypeptides can also be among the most responsive of polymers; their secondary structure can change with temperature, pH, or salt. Such changes might be used to control stability phenomena or to make active colloids or colloid-based materials. Finally, the chiral nature of the chains may be useful for various separation processes.

We report the preparation and characterization of nearly uniform, organophilic colloidal silica coated with poly($\gamma$-benzyl-$\alpha$,L-glutamate). The overall strategy, Scheme 1, is to prepare silica cores by the classical method of Stöber,[29] followed by surface amino functionalization with 3-aminopropyltrimethoxysilane. The free primary amino groups initiate *N*-carboxyanhydride (NCA) ring-opening polym-

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## Scheme 1.  Preparation of Polypeptide−Silica Composite Colloids (Not Drawn to Scale)
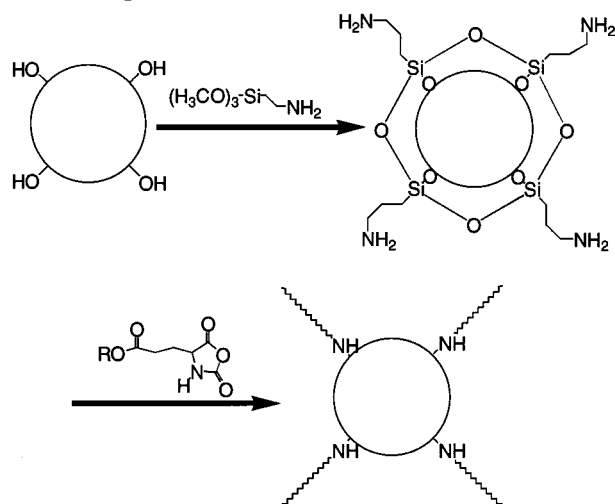


erization[30] to produce a "living" polypeptide shell. The polypeptide−silica colloidal composites are characterized by dynamic light scattering, thermal gravimetry, transmission electron microscopy, and infrared spectroscopy. We are not the first to initiate NCA's with colloidal particles. Previous synthetic work described the attachment of polypeptides to carbon black particles[31] and functionalized fumed silica.[32] Here, we place greater emphasis on particle uniformity and characterization. Both previous studies and the present work are closely related to the growing literature devoted to the attachment of linear homopolypeptides to flat surfaces.[33−43]

## Experimental Methods

**Synthesis.** $\gamma$-Benzyl L-glutamate was prepared using the method of Blout and Karlson.[44] This was done through an esterification reaction by heating benzyl alcohol, hydrochloric acid, and L-glutamic acid slowly until the mixture became homogeneous (2−3 h). After the solution cooled to room temperature, the resulting slurry was poured into a large volume of acetone and stored in a refrigerator for 3 days. The mixture was then filtered, and the precipitate was dissolved in an icy slush. The pH of the slush was brought up to 7, and the foam was filtered. The foam was then crystallized in hot water twice, and the $\gamma$-benzyl-L-glutamate crystals were dried in a vacuum oven. (Recently, we have found that it is better to transfer the foam

to cold water and repeat the neutralization step before performing the two recrystallizations.) The $\gamma$-benzyl-N-carboxy-L-glutamate anhydride was prepared in the following manner using dried glassware, starting reagents, and solvent. $\gamma$-Benzyl-L-glutamate was added to a flask along with tetrahydrofuran, THF. After the mixture was brought to reflux, triphosgene crystals were added quickly to catalyze the formation of the NCA ring. A clear solution subsequently formed, and it was refluxed for an additional 40 min. Afterward, the solution was sparged with dry nitrogen for several hours to remove the HCl. Dry hexane was slowly poured into the clear solution to initiate crystallization, and the solution was then fully crystallized in a refrigerator overnight. The NCA crystals were filtered and washed with hexane. Colloidal silica was prepared by the method of Stöber.[29] The particle size can be controlled by the concentration of ammonia and by the amount of water. Ethanol (800 mL) was added to concentrated ammonium hydroxide (37 mL). Then 40 mL of tetraethyl orthosilicate (TEOS) was added rapidly to ensure size uniform particle growth. The solution was allowed to stir overnight, and the colloidal dispersion was subsequently washed several times with water from a Barnstead Nanopure device, through centrifugation and redispersion. Silanation took place overnight with 3-aminopropyl-trimethoxy silane in ethanol. The resultant amino-terminated spheres were washed three times by resuspension/centrifugation. The pellets were then washed twice with dried and distilled dimethylformamide. The pelletized, amino-terminated spheres were resuspended in dry DMF by sonication and subsequently poured into a Schlenk flask, which was sonicated for about 2 h. The amino-terminated sphere solution was dried with a generous amount of 4 Å molecular sieves through gentle swirling of the Schlenk flask for 3 days. Freshly prepared NCA monomer was polymerized in N,N-dimethylformamide, DMF. The amino-terminated silica cores were added, and the NCA solution was allowed to stir for 3 days. The solution was kept under a dry environment using a drying tube containing anhydrous $CaSO_4$.

For comparison, alkylated organophilic spheres were prepared by the method of Iler.[45] The silica colloids were coated with C18 chains supplied by 1-octadecanol. The amount used was usually four times the weight of the Stöber spheres. The 95% ethanol/Stöber sphere dispersion was added in small increments into a reaction vessel containing 100% ethanol, and the azeotrope was then distilled off until the dispersion was anhydrous. Addition of octadecanol was followed by heating of the mixture to ensure coupling of the hydrocarbon chains to the surfaces of the colloidal particles. The residual octadecanol was removed by washing the dispersion with THF through centrifugation and redispersion.

**Dynamic Light Scattering.** The custom built apparatus used laser radiation of 6328 Å and an ALV-5000 digital autocorrelator. Measurements were made in homodyne mode[46] at multiple scattering angles from $\theta = 30$ to $90°$. The apparent diffusion coefficient is defined as the quotient of the decay rate, $\Gamma$, of the electric field autocorrelation function, $g^{(1)}(t)$, and the squared scattering vector magnitude, $q^2$:

$$D_{app} = \Gamma/q^2 \tag{1}$$

where $q$ is given by $q = 4\pi n \sin(\theta/2)/\lambda_o$ with $n$ being the solvent refractive index, $\theta$ the scattering angle and $\lambda_o$ the wavelength in vacuo. In the limit of zero concentration and zero $q$, $D_{app}$ becomes the diffusion coefficient $D_o$, which is inversely related to the particle hydrodynamic radius, $R_h$, through the Stokes−Einstein relation

$$D_o = \frac{kT}{6\pi\eta_o R_h} \tag{2}$$

where $k$ is Boltzmann's constant, $T$ is the absolute temperature, and $\eta_o$ is the solvent viscosity. Bare spheres were measured in

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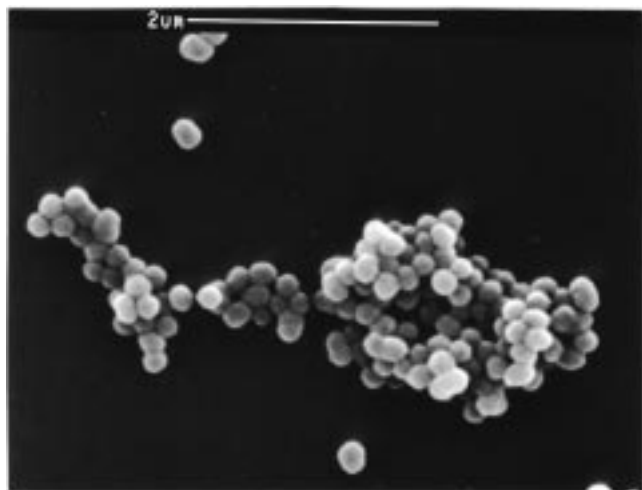(46) Berne, B.; Pecora, R. *Dynamic Light Scattering*; Wiley: New York, 1976.

**Figure 1.** Scanning electron micrograph of polypeptide colloidal silica composite particles.

water at 25 °C ($\eta_o = 0.8904$ cP). Polypeptide-coated spheres were measured in DMF at 25 °C ($\eta_o = 0.802$ cP). The spheres coated with $C_{18}H_{37}$ were measured in THF at 25 °C ($\eta_o = 0.453$ cP). Particle concentrations were typically less than 2 $\mu$g/mL. The adequacy of this degree of dilution was ascertained, in selected cases, by diluting the samples still further.

**Titration.** We attempted to determine the surface density of amino groups on the colloidal initiators by titration. A dilute HCl solution ($\sim$0.035 M) was standardized against 1 M NaOH. A small sample of the amino-terminated spheres was then titrated using the acid. The amino-terminated spheres in water were stirred continuously to achieve full and rapid neutralization of the terminal amino groups.

**Spectroscopy.** Infrared spectra were recorded with a Perkin-Elmer 1760 FT-IR using GRAMS 386 software. Dry samples were prepared as KBr pellets. A Bruker 200 MHz NMR was used to follow the formation of the NCA ring from the reaction between glutamic acid and triphosgene. Ring formation leads to a slight shift upfield for the hydrogen attached to the nitrogen from 6.9 to 6.5 ppm.

**Thermogravimetric Analysis.** TGA was performed using a Seiko EXSTAR 6000 system. The sample was heated under nitrogen and air (essentially no difference) at a rate of 5 °C/min to an upper limit of 1000 °C.

**Electron Microscopy.** Approximately 5 $\mu$L of the sample dispersion was pipetted onto a collodion-coated copper specimen grid, which was then coated with carbon by resistance vaporization in a Balzers/Union MED010 deposition system. The grid was attached to an aluminum mounting stub with double-sided tape and conductive paint, sputter coated with gold/palladium in an Edwards 5150 sputter coater, and examined and photographed with a Cambridge 260 Stereoscan SEM.

### Results and Discussion

The particle suspensions were stable for several weeks, but precipitates did form eventually. Electron micrographs of the particles from partially resuspended mixtures appear in Figure 1. The round particles often appeared in clusters, making accurate size determinations difficult. Meaningful average sizes were obtained from dynamic light scattering on the fresh suspensions; see below.

Figure 2 shows infrared spectra for bare silica spheres and the composite particles. The spectra leave no doubt that polymer is attached to the composite particles. It is difficult to prove that the attachment is *covalent.* That is certainly the expectation, since primary amine initiation of NCA's does generally result in attachment of the
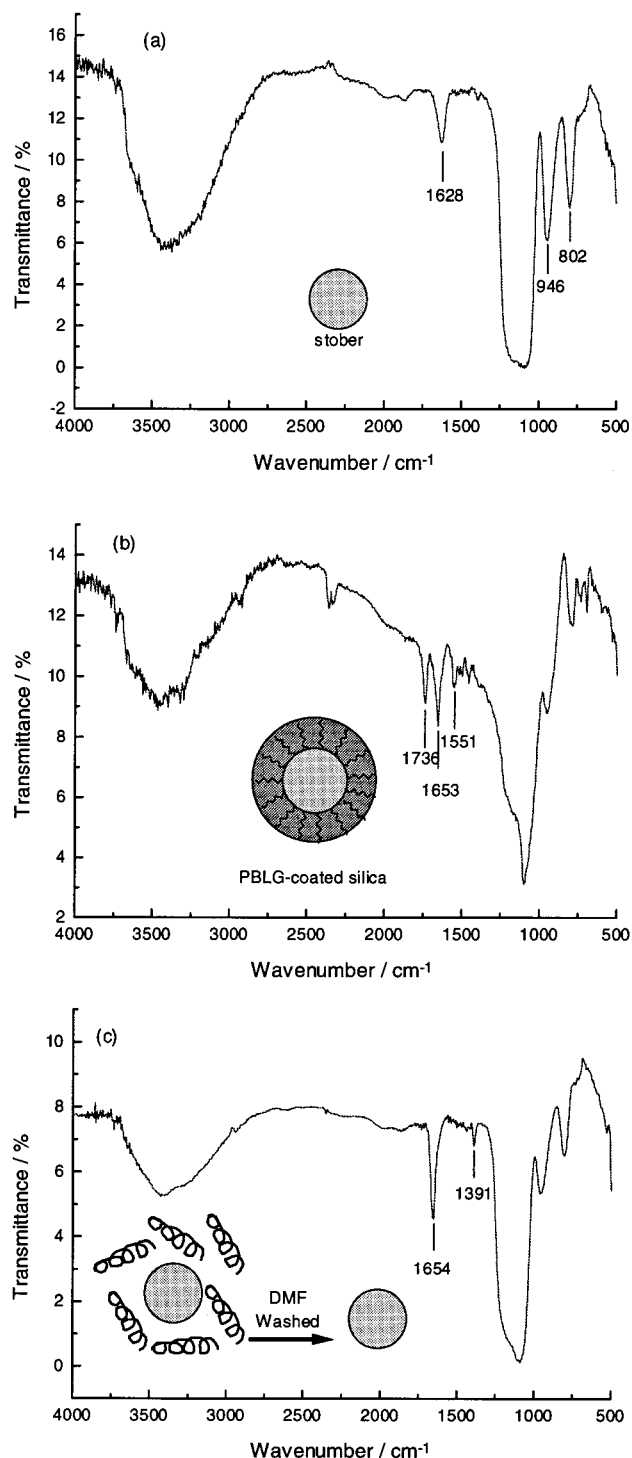


**Figure 2.** Infrared spectra of (a) bare silica spheres, (b) composite PBLG-coated silica spheres, and (c) bare silica spheres exposed to 5% solution of premade PBLG and then isolated.

initiator,[30,47] even large initiators.[31,32] Unfortunately, the literature dealing with functionalized flat surfaces for peptide initiation is inconclusive; this is troubling because powerful surface characterization techniques were applied. Wieringa and Schouten [48] showed that methyl glutamate NCA could be attached to flat silicon oxide surfaces, producing a $\beta$-sheet and an $\alpha$-helix. In contrast, Chang, von Esbroeck, and Frank[33,34] found that the amino-

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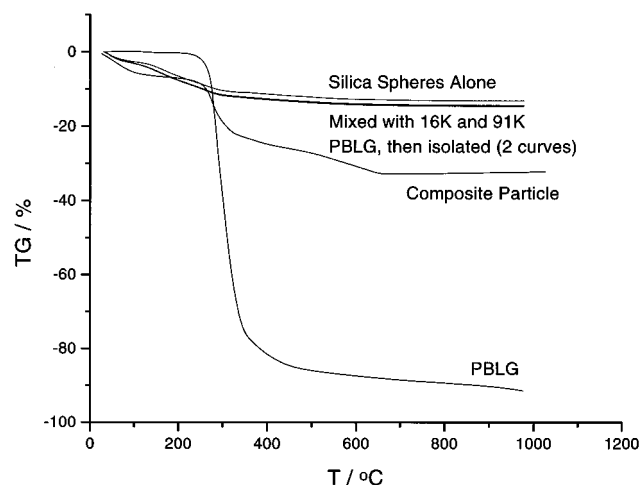
**Figure 3.** Five thermogravimetric curves. From top to bottom: bare silica spheres; silica spheres exposed to 5% solution of premade PBLG ($M = 16\,000$); silica spheres exposed to 5% solution of premade PBLG ($M = 91\,000$); composite polypeptide-silica particles; untethered, linear PBLG.



**Figure 4.** Apparent diffusion coefficients for bare, $C_{18}H_{37}$-coated and PBLG-coated, particles at several scattering vectors.

functionalized 100 surface of silicon oxide was a poor initiator for BLG−NCA, producing mostly oligomers that tended to adopt the $\beta$-sheet conformation. These authors suggest that premade polymers should be attached, instead of trying to initiate monomers from flat surfaces. That route has not been considered here because one objective is the facile production of colloids with living, reactive polymer surfaces. The terminal amino groups of premade polypeptides often would be deactivated during isolation steps, normally involving nonsolvent addition. In contrast, composite colloids are easily isolated by centrifugation.

During the initiation by the functionalized spheres, some untethered PBLG is also produced, probably due to unwanted trace initiators. These can include water, dimethylamine that is almost always present in DMF due to decomposition, or an amino-functionalized silane agent liberated from any unremoved physisorbed multilayer on the colloidal particles.[49] The question arises whether these linear polymers can bind to the silica surfaces and therefore account for some of the polymer in the IR spectrum. In a control experiment, Figure 2c, silica spheres lacking the amino function were mixed with 5% solutions of premade PBLG ($M = 91\,000$) and rinsed three times with DMF and once with THF. The spectrum is virtually indistinguishable from that of silica spheres not exposed to PBLG, proving the weakness of the physical interaction between polymer in DMF and the *unfunctionalized* spheres. Addition of premade polymers to amino-functionalized spheres would not be a meaningful experiment to test for physical adsorption, since such spheres might bind through the carboxy terminus of the polypeptide. While it is not possible to prove absolutely that PBLG produced by stray initiators does not bind to the amino-functionalized spheres, repeated washing, precipitation, and resuspension of the composite polypeptide-coated colloids did not diminish the intensity of the IR bands associated with polymer coating. The PBLG shell is either covalently attached or *very* strongly adsorbed to the amino-functionalized colloidal core.

The five thermogravimetric analysis traces of Figure 3 corroborate this interpretation. Stöber spheres lose 14% of their mass, probably indicating some moisture or solvent
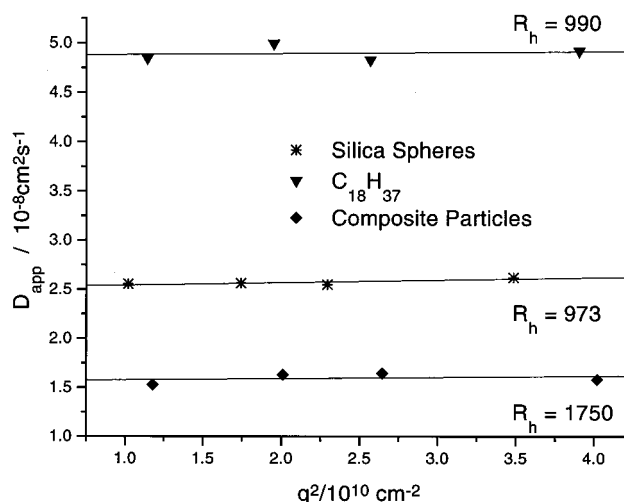
adsorption. Spheres exposed to 5% solutions of premade linear PBLG scarcely differ. The composite particles lose a little mass below 200 °C, but unlike pure spheres or spheres exposed to linear polymer, they lose substantial mass at about 300 °C, the same temperature where pure, linear PBLG decomposes. About 20% of the total mass can be ascribed to PBLG. Assuming the sub-200 °C losses are solvent or adsorbed water, the attached polymer comprises about 23% of the dry mass of the particles. Above 600 °C, the composite particle trace is flatter than that of pure, linear PBLG. This probably reflects that a thin PBLG coating on the composite particles has been efficiently thermolyzed, while charring of the comparatively thick PBLG layers in the bulk polymer may retard thermolysis. Later, we shall return to the TGA data to compute the effective polymer surface density.

Figure 4 shows DLS results for bare, C18-coated, and PBLG-coated spheres. The diffusion coefficient at $q = 0$ was extracted from each plot and converted to $R_h$. The more rapidly diffusing particles may be larger than the slower diffusers, due to the viscosities of the different solvents. The core is nondraining, and it will be demonstrated that the shell is also rather dense and therefore probably also nondraining. We thus take $R_h$ as a valid particle radius. Figure 5 defines the important dimensions of the composite spheres. The core radius is $R_c$, and the total radius with the polymer layer attached is $R_t$. The shell thickness, $t = R_t − R_c$, of the PBLG coating is about 800 Å. The plots of $D_{app}$ vs $q^2$ in Figure 4 are reasonably flat, as expected for monodisperse, uniformly round particles. A polydisperse preparation containing some large particles exhibits an upward slope because large particles contribute less scattering as $q$ increases, which renders the smaller, faster particles relatively more visible. Even monodisperse particles can exhibit positive slopes if they are not round and if they are sufficiently large. In such cases, DLS senses rotation or, if the particles are flexible, internal motions.[46] The weakness of these effects for the large particles in this study implies good uniformity and spherical shape. Further evidence for the uniformity of the particles comes from cumulants analysis.[50] The electric field autocorrelation function can be written ln-$(g^{(1)}(t)) = -\Gamma t + \mu_2 t^2 +$ higher terms. The dimensionless ratio $\mu_2/\Gamma^2$ is zero for perfectly monodisperse, round spheres. The measured values were typically 0.05, just slightly higher than latex standards of the best quality.

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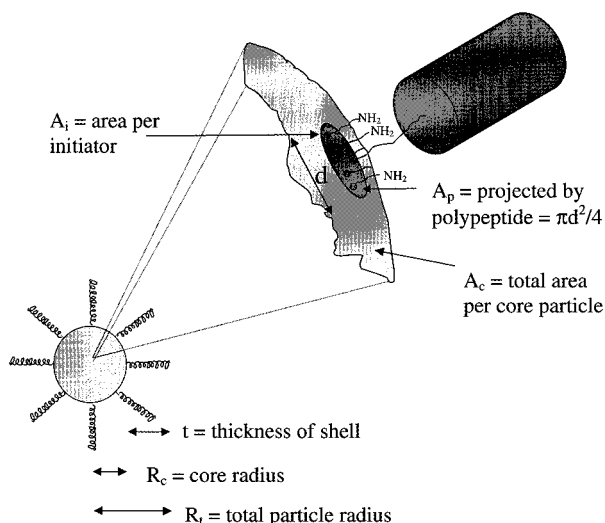(50) Koppel, D. E. *J. Chem. Phys.* **1972**, *57*, 4814−4820.

**Figure 5.** Definition of some parameters of the silica–polypeptide composite particles.

**Table 1. Properties of Silica–Polypeptide Composite Colloids**

| Core | |
| --- | --- |
| radius from DLS = $R_c$ | 970 Å |
| mass | $7.48 \times 10^{-15}$ g |
| MW | $4.50 \times 10^{+9}$ g/mol |
| number of particles/g | $1.33 \times 10^{+14}$ |
| surface area/particle | $1.18 \times 10^{+7}$ Å$^2$ |
| surface area/g | $1.56 \times 10^{+21}$ Å$^2$/g = 15.6 m$^2$/g |
| **PBLG Shell** | |
| radius from DLS | 1750 Å |
| thickness from DLS = $t$ | 780 Å (i.e., 1750−970 Å) |
| volume | $1.86 \times 10^{-14}$ cm$^3$ |
| calculated mass assuming solid PBLG, $\rho = 1.26$ g/mL | $2.35 \times 10^{-14}$ g |
| mass according to TGA | $2.23 \times 10^{-15}$ g |
| apparent PBLG weight % in solvated shell | 9.5% |
| expected shell thickness assuming effective number of initiators follows eq 9 | 11 500 Å |

Table 1 displays other particle parameters. The mass of the silica core can be computed as

$$m_c = (4\pi/3)R_c^{3}\rho_c \tag{3}$$

where $\rho_c$ is the density of colloidal silica core, estimated as 1.960 g/mL.[51] The surface area of the bare silica core is

$$A_c = 4\pi R_c^{2} \tag{4}$$

The number of particles in the titration sample can be computed as the total mass of particles divided by $m_c$. In principle, one might determine the number of surface amino groups per particle by titration. This proves difficult, probably due to residual hydroxyl groups on and within the particles. These groups probably contribute to the remarkably low isoelectric points of amino-functionalized silica spheres.[49] We believe the surfaces to be heavily coated with amino groups, because 3-aminopropyltri-methoxysilane was added in large excess. Initiation by residual silanol groups should minimal; if it did occur, the polymer would still be expected to be covalently bound.

(51) Uniform Particles Certificate of Analysis. Dec 1, 1994. Bangs Laboratories.

Polymerization of NCA's upon primary amine initiation normally proceeds without terminating steps[30,47] to produce living polymers that are characterized by a sharp, Poisson distribution of mass. The expected length and corresponding molar mass, if all −NH$_2$ groups were to participate in initiation and no monomers were lost to unwanted trace initiators, may be calculated from the $\alpha$-helix axial length per monomer, 1.5 Å, the monomer mass, 219 g/mol, and the monomer/initiator ratio:

$$L/\text{Å} = 1.5 N_{\text{NCA}}/N_{\text{NH}_2} \tag{5a}$$

$$M = 219 N_{\text{NCA}}/N_{\text{NH}_2} \tag{5b}$$

$N_{\text{NCA}}$ and $N_{\text{NH}_2}$ are the number of NCA and −NH$_2$ groups present in the reaction mixture. However, it is likely that many −NH$_2$ groups cannot participate as initiators, due to steric hindrance. Referring again to Figure 5, the projection of a rod perpendicular to the surface is

$$A_p = \pi d^2/4 \tag{6}$$

where the cross-sectional diameter of PBLG is $d = 16$ Å.[52] Arranged hexagonally, each rod occupies an area

$$A_{p,\text{hex}} = d^2\frac{\sqrt{3}}{2} \tag{7}$$

The number of effective initiation sites per particle is therefore limited to

$$I_{\text{eff}} = \frac{2 A_c}{d^2 \sqrt{3}} \tag{8}$$

On this basis, the expected PBLG length, and shell thickness, is

$$L/\text{Å} = 1.5\frac{d^2\sqrt{3}N_{\text{NCA}}}{2A_c N_{\text{sphere}}} \tag{9}$$

where $N_{\text{sphere}}$ is the number of colloidal particles in the reaction mixture. By this estimate, we expect $L = 11\,500$ Å. The observed thickness, $\sim 800$ Å, is about 14 times lower than expected from eq 9. Failure to achieve the expected thickness may indicate some chain termination leading to a number of short chains on a crowded surface. It is also consistent with the previously discussed parasitic initiation of monomer by trace impurities.

We can now return to the TGA data to compute the density of PBLG in the shell. By TGA, the polypeptide shell accounts for about 23% of the dry mass of the composite particles—i.e., $m_s \cong 2.23 \times 10^{-15}$ g/particle. The effective PBLG density in the shell can be computed as

$$\rho_{s,\text{eff}} = \frac{3 m_s}{4\pi(R_t^{3} - R_c^{3})} \tag{10}$$

The resulting effective shell density, 0.12 g/mL, is considerably lower than the density of solid PBLG, about 1.26 g/mL. This suggests a solvated shell containing about 9.5% PBLG.

Now that the PBLG content of the surface is described, we return to the IR spectra, Figure 2, which also contain

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information about the polypeptide conformation.[30] For random coils one expects absorptions at 1660 and 1535 $cm^{-1}$. For $\alpha$-helices, absorptions occur at 1660 and 1550 $cm^{-1}$. Absorptions at 1630 and 1535 $cm^{-1}$ correspond to $\beta$-sheets. The observed spectra do not adhere exactly to any of these expectations. All exhibit a very distinctive, broad and intense peak at around 1100 $cm^{-1}$ due to $SiO_2$. The 1630 $cm^{-1}$ peak expected if a $\beta$-sheet were present is obscured by the 1628 $cm^{-1}$ peak from the silica core. The 1535 $cm^{-1}$ peak expected for the $\beta$-sheet or random conformations is less pronounced than the clearly obvious 1550 $cm^{-1}$ (1551 $cm^{-1}$ observed) $\alpha$-helix peak. The large thickness of the polypeptide shell also favors the presence of substantial amounts of extended $\alpha$-helix. The simultaneous presence of other conformational states would not be surprising for a crowded surface. Although we found no evidence for binding of the unattached PBLG produced by initiator impurities, a recent atomic force microscopy study by Kitaev, Schillen, and Kumacheva[53] finds that PBLG binds to flat, freshly cleaved mica surfaces, possibly with surface-induced conformational changes. The length of the fibrillar structures formed exceeds the diameter of our silica particles. Less adsorption occurred when C18-coated mica was exposed to solutions in highly polar solvents, suggesting a route to passivation of the spaces between amino groups on more lightly functionalized colloidal particles.

## Conclusion

A facile synthesis of reasonably uniform polypeptide-coated silica spheres has been demonstrated. It seems very likely that the polypeptide shells are covalently attached to the surface. Controlling the thickness of the shells will require a lower surface density of initiators, reduction of noncolloidal trace initiators, and possibly passivation of the silica surface between initiators. Better methods for estimating the surface functionality of the colloidal surfaces may also be helpful.