Synthesis and Characterization of Carboxylic Acid-Functionalized Polypyrrole–Silica Microparticles

Shuichi Maeda, Roberto Corradi, and Steven P. Armes*

School of Chemistry and Molecular Sciences, University of Sussex, Falmer, Brighton, BN1 9QJ, U.K.

Received October 21, 1994; Revised Manuscript Received January 20, 1995[⊗]

ABSTRACT: 1-(2-Carboxyethyl)pyrrole has been copolymerized with pyrrole in the presence of silica particles to produce stable colloidal dispersions of polypyrrole copolymer–silica microparticles containing carboxylic acid groups. These new dispersions have been extensively characterized in terms of their particle size and chemical composition by transmission electron microscopy, disk centrifuge photosedimentometry, elemental microanalyses, thermogravimetry, and both FTIR and visible absorption spectroscopy. The presence of the carboxylic acid groups at the surface of the microparticles has been confirmed by X-ray photoelectron (XPS) spectroscopy. Visible absorption spectroscopy studies confirmed that the optical absorbance of these carboxylic acid-functionalized polypyrrole–silica microparticles is up to 4 times higher (at a wavelength of 400 nm) than a commercial dyed carboxylated polystyrene latex (Polymer Laboratories' SuperCarboxyl Hi-Dye) of the same solids concentration. Thus these conducting polymerbased microparticles are expected to have biomedical applications as novel "marker" particles in immunodiagnostic assays.

Introduction

Since 1986 various research groups have described the synthesis of colloidal dispersions of air-stable intrinsically conducting polymers such as polypyrrole and polyaniline.[1–4] A wide range of both commercial and tailor-made polymeric stabilizers have been utilized to obtain spherical, rice grain, and needle particle morphologies. Thin conductive films can be easily fabricated from such dispersions; thus this approach can significantly improve the processability of these otherwise intractable materials.

Recently, Tarcha and co-workers at Abbott Laboratories have demonstrated that such polypyrrole colloids (prepared *via* aqueous dispersion polymerization using poly(vinyl alcohol) as a polymeric stabilizer) can be successfully utilized in diagnostic assays for the human pregnancy hormone (hCG), HIV antibody, and hepatitis B surface antigen.[5] The colloidal immunoreagents used in these tests were made by adsorption of the appropriate ligands to the unmodified surface of the latex particles. In this application the electroactivity of the conducting polymer is irrelevant: the "value-added" properties are its intense, intrinsic chromogenicity and its well-defined (narrow size distribution) colloidal dimensions. In order to examine the potential advantages of covalent linkage of specific binding ligands, the Abbott group developed multistep methods for the surface derivatization of poly(vinyl alcohol)-stabilized polypyrrole particles in organic solvents such as *N*-methylpyrrolidone.[5] This derivatization process was followed by extensive purification of the polypyrrole particles by ultrafiltration and/or dialysis in aqueous media prior to their evaluation. Although this approach was successful, there is clearly considerable scope for the development of simpler and less time-consuming syntheses for the production of surface-functionalized polypyrrole particles, particularly on a commercial scale.

Recently, we reported the preparation and characterization of novel polyaniline–silica colloids using small silica particles as *particulate* dispersants in 1.2 M HCl.[6–9] In this approach the silica particles act as a high surface area colloidal substrate for the precipitating polyaniline; the resulting composite particles are stable colloidal dispersions which consist of microaggregates of silica particles "glued" together by the conducting polymer component. We characterized these polyaniline–silica microparticles in terms of their chemical composition and electroactivity by thermogravimetry, CHN microanalyses, FTIR and visible absorption spectroscopy, and dc conductivity measurements.[6] Our transmission electron microscopy (TEM) studies confirmed that the microparticles have an unusual "raspberry" morphology. Subsequent small-angle X-ray scattering experiments in collaboration with Terrill and Crowley were consistent with the conducting polymer dimensions being confined to the 1–10 nm scale; thus these microparticles are true nanocomposite materials.[9]

In 1993 we described the preparation of colloidal dispersions of the analogous polypyrrole–silica microparticles.[10,11] Depending on the synthesis conditions, we showed that these dispersions could contain up to 70% conducting polymer by mass. Furthermore, we found that these new microparticles had relatively narrow particle size distributions and exhibited reasonably high solid-state conductivities (up to 4 S cm^{-1}). More importantly, unlike the polyaniline–silica particles, these polypyrrole–silica dispersions are stable with respect to aggregation at physiological pH. Finally, the polypyrrole–silica microparticles possess "real" surface area (*i.e.*, there is no adsorbed stabilizer layer of surfactant or polymer), which is available for the adsorption of antigens or antibodies of biological interest. Thus these charge-stabilized microparticles are markedly different to sterically stabilized polypyrrole particles, which are completely coated with a solvated, adsorbed layer of a water-soluble polymer such as poly(vinyl alcohol) in their as-synthesized form.

Recently, our X-ray photoelectron spectroscopy studies have confirmed that the surface of the homopolypyrrole–silica microparticles is distinctly silica-rich with respect to the bulk composition of the particles.[12] However, although somewhat depleted relative to the silica component, the conducting polymer is nevertheless present in the first few nanometers of the surface

* Author to whom correspondence should be addressed.
[⊗] Abstract published in *Advance ACS Abstracts,* March 15, 1995.

0024-9297/95/2228-2905$09.00/0

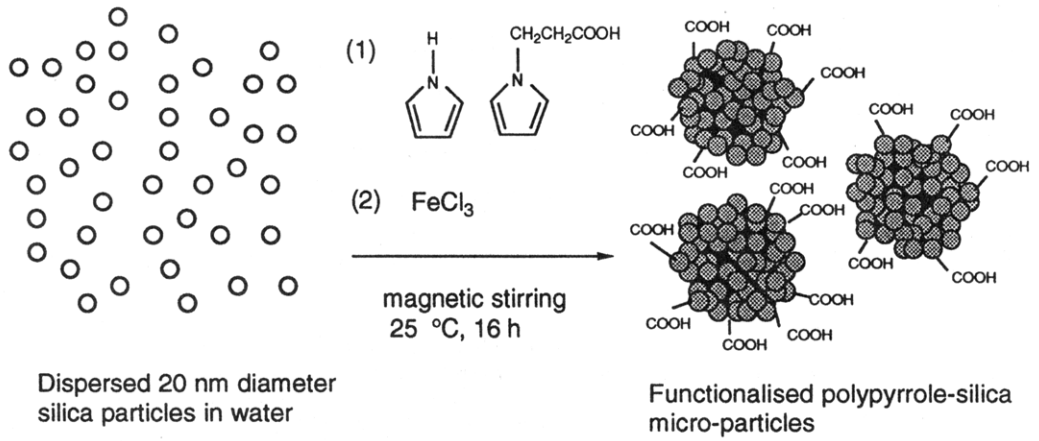

Figure 1. Schematic formation of carboxylic acid-functionalized polypyrrole–silica microparticles.

of the particles. Thus, in principle, copolymerization of pyrrole with an appropriate functional pyrrole comonomer should lead to *surface-functionalized* microparticles. Surface functionalization, particularly with certain hydrophilic groups such as carboxylic acids or amines, can be highly desirable in diagnostic assays since it can sometimes minimize the ubiquitous nonspecific binding problems which often result in reduced specificity and/or sensitivity for the analyte of interest.[13] Perhaps more importantly, such functional groups would, in principle, allow covalent attachment of specific binding ligands for the analyte molecules. In the present work we describe the synthesis and characterization of such carboxylic acid-functionalized polypyrrole–silica microparticles. This one-step functionalization is particularly attractive since it is achieved *during the colloid synthesis* (see Figure 1), rather than *via* postpolymerization surface derivatization process(es).

Experimental Section

Monomer Synthesis. 1-(2-Carboxyethyl)pyrrole was synthesized according to procedures described in the literature.[14,15] The commercially starting material, 1-(2-cyanoethyl)pyrrole (Aldrich), was hydrolyzed to 1-(2-carboxyethyl)pyrrole as follows: 50 g of 1-(2-cyanoethyl)-pyrrole was added to 120 mL of 6.7 M KOH. This mixture was heated to reflux under an inert atmosphere of nitrogen until the mixture became amber colored; after 1.5 h a litmus test confirmed that no more ammonia had evolved. This solution was cooled to room temperature and was then acidified to pH 5 using 8 M HCl. The product was extracted five times with ether while maintaining the pH of the aqueous solution at pH 5. The ether was removed using a rotary evaporator, and the resulting beige crystalline product was dried (crude yield 39.6 g; 71%). The crude product was recrystallized twice from boiling *n*-heptane. After removal of the solvent, a colorless needlelike crystalline product was obtained which was dried under vacuum (overall yield 28.4 g; 51%). The compound was confirmed to be 1-(2-carboxyethyl)pyrrole by melting point (57–60 °C), NMR and IR spectroscopy, and CHN elemental microanalyses. Calculated for $C_7H_9NO_2$: C, 60.43; H, 6.47; N, 10.07. Found: C, 60.33; H, 6.32; N, 10.33.

Preparation of Functionalized Polypyrrole–Silica Microparticles. The homopolypyrrole–silica microparticle syntheses have been described in detail in our earlier publications.[10,11] A typical synthesis was carried out as follows: 1.0–3.4 g (dry weight) of silica (Nyacol Products; nominal average particle diameter 20 nm; provided as a 34 or 40 w/w % aqueous dispersion) was added to a solution of $FeCl_3 \cdot 6H_2O$ (9.10 g) or $(NH_4)_2S_2O_8$ (3.84 g) in deionized water (100 mL of total solvent volume) at room temperature. Pyrrole (1.00 mL) was then injected *via* syringe to this stirred solution and the polymerization allowed to proceed for 16 h. This reaction mixture was then centrifuged at 6000 rpm for 30 min, using a Beckman J2-21 centrifuge, and the resulting black sediment was redispersed in deionized water using an ultrasonics bath. This centrifugation–redispersion cycle was repeated twice in order to completely remove the excess small silica particles and (in)organic byproducts from the larger polypyrrole–silica microparticles. Essentially the same procedure was utilized for the carboxylic acid-functionalized polypyrrole–silica microparticles except that the pyrrole monomer was replaced with a mixture of pyrrole and 1-(2-carboxyethyl)pyrrole at various comonomer feed ratios (see below), the mass of silica was increased up to 6.8 g, and the polymerization time was extended to 5–16 h. Finally, some of our more successful polypyrrole copolymer–silica microparticle syntheses were carried out by adding the $FeCl_3$ oxidant *last* to the reaction mixture. For example, pyrrole (0.50 mL) and 1-(2-carboxyethyl)pyrrole (1.00 g) were added to 6.8 g (dry weight) of silica particles in 75 mL of deionized water at 25 °C with constant stirring. A solution of $FeCl_3 \cdot 6H_2O$ (9.10 g) in 25 mL deionized water was then added into this stirred solution and the polymerization allowed to proceed for 16 h.

Chemical Composition and Conductivity. The chemical composition of the functionalized polypyrrole–silica microparticles was quantitatively determined using both CHN elemental microanalyses (Perkin-Elmer 2400 instrument) and thermogravimetric analyses (Perkin-Elmer TGA-7 instrument; scan rate 40 °C/min in air). In the former method the carbon content of the dried microparticles was simply compared to the carbon content of the corresponding polypyrrole bulk powders (*ca.* 55%) synthesized in the *absence* of silica particles. Furthermore, the N/C ratios of the functionalized polypyrrole–silica microparticles were compared to the N/C ratios of both the polypyrrole and poly(1-(2-carboxyethyl)pyrrole) homopolymers. Thus the CHN elemental microanalysis data also enables us to estimate the copolymer composition (*i.e.*, the 1-(2-carboxyethyl)pyrrole:pyrrole comonomer ratio) within the functionalized polypyrrole–silica microparticles. We emphasize that these calculations are only approximate since they assume the same doping level for polypyrrole, poly(1-(2-carboxyethyl)pyrrole) homopolymer, and the polypyrrole copolymers. It is well documented that *N*-substituted pyrrole polymers such as poly(*N*-meth-

ylpyrrole) have somewhat lower doping levels than homopolypyrrole.[16] A more rigorous calculation of the copolymer composition would require microanalytical data for chlorine in order to assess and account for differences in the doping levels between the chloride-doped samples.

In the thermogravimetry experiments the microparticles were heated to 800 °C and the observed weight loss was attributed to the quantitative pyrolysis of the conducting polymer. A small correction was made to allow for the weight loss due to surface dehydration of the silica particles when calculating the conducting polymer content of the microparticles.

Fourier transform infrared (FTIR) spectroscopy studies were carried out on the dried microparticles dispersed in KBr disks using a Perkin-Elmer FTS-40 instrument (100 scans per spectrum at 8 cm^{-1} resolution). Visible absorption spectra of functionalized polypyrrole–silica dispersions diluted to 10 mg dm^{-3} in various buffer solutions (pH 3–12) were recorded using a UNICAM UV2 UV/visible absorption spectrophotometer. All conductivity measurements were made on compressed pellets of dried powders using the conventional four-point probe technique at room temperature.

Particle Size and Colloidal Stability. The particle size and pH-induced aggregation of the functionalized polypyrrole–silica microparticles were examined using a Brookhaven Instruments disk centrifuge photosedimentometer at 25 °C operating in the external line-start mode.[17] The sample preparation protocol was as follows: the microparticles were first diluted to 0.1–0.5 w/v % using pH 3–12 buffer solutions which contained 20 v/v % methanol. A small aliquot (0.20 mL) of each of these diluted dispersions was injected in turn *via* syringe into the disk cavity which contained the spin fluid. This spin fluid comprised 14.0 mL of the appropriate pH buffer solution with an additional 1.0 mL of methanol to create the "density gradient" which leads to fractionation of the particles within the sample. We selected a centrifugation rate of 6000–7000 rpm, which corresponded to run times of approximately 10–20 min. It was assumed that the highly absorbing microparticles had the same extinction coefficient correction factor as carbon black. Standard deviations were calculated assuming normal statistics for the particle size distributions. The microparticle densities were calculated from the combined elemental microanalyses and thermogravimetry data assuming simple additivity and taking the density of the functionalized polypyrrole copolymers to be approximately the same as that of the chloride-doped polypyrrole homopolymer (1.46 g cm^{-3}). The density of the ultrafine silica sol was determined to be 2.17 g cm^{-3} using a Micromeritics Accu-Pyc 1330 helium pycnometer. This instrument was also used to directly determine the density of several samples of the dried microparticles: these values were in reasonably good agreement with those densities calculated on the basis of additivity.

Morphology. Transmission electron microscopy (TEM) studies were made on diluted dispersions dried down onto carbon-coated copper grids (3 mm diameter, Agar Scientific Ltd.) using a JEOL 100C instrument.

Surface Composition Studies. The surface composition of the functionalized polypyrrole–silica microparticles was examined using X-ray photoelectron spectroscopy (XPS) using a JEOL JPS-80 photoelectron spectrometer. The samples were prepared as follows; a *ca.* 5 × 5 mm piece of double-sided adhesive tape was used to stick a *ca.* 10 × 10 mm piece of Sellotape to the sample stub with the Sellotape being the adhesive side up. A microspatula tip-full of the sample was then deposited in the center of the Sellotape and pressed down gently. Any loose material was carefully removed using an air duster. A separate control experiment was carried out to confirm that the Sellotape was purely hydrocarbon-based and contained no silicon-based contaminants which might otherwise affect the results. The sample analysis area was a circle of approximately 6.0 mm diameter.

Table 1. Effect of Synthesis Conditions on the Colloid Formation of Carboxylic Acid-Functionalized Polypyrrole–Silica Microparticles

sample no.	init comonomer feed mol ratio pyrrole/carboxyl pyrrole	init silica concn (w/v %)	oxidant type	reacn time (h)	colloid formation
1	100/0	1.1	$FeCl_3{\cdot}6H_2O$	16	yes
2	100/0	3.4	$(NH_4)_2S_2O_8$	16	yes
3	75/25	1.1	$FeCl_3{\cdot}6H_2O$	16	no
4	75/25	3.4	$FeCl_3{\cdot}6H_2O$	16	yes
5	75/25	6.8	$(NH_4)_2S_2O_8$	16	no
6	50/50	6.8	$FeCl_3{\cdot}6H_2O$	16	no
7	50/50	6.8	$FeCl_3{\cdot}6H_2O$	5	yes
8	50/50	6.8	$(NH_4)_2S_2O_8$	5	no
9	25/75	6.8	$FeCl_3{\cdot}6H_2O$	5	no
10	0/100	6.8	$FeCl_3{\cdot}6H_2O$	5	no
11	50/50	6.8	$FeCl_3{\cdot}6H_2O$[a]	16	yes
12	50/50	6.8	$(NH_4)_2S_2O_8$[a]	16	no

[a] Oxidant added *last* to stirred reaction solution.

Results and Discussion

The effect of synthesis conditions on the formation of colloidal dispersions of carboxylic acid-functionalized polypyrrole–silica particles is presented in Table 1. In our homopolymerization control experiments (samples 1 and 2) we obtained stable dispersions of polypyrrole–silica microparticles using both the $(NH_4)_2S_2O_8$ and $FeCl_3$ oxidants at relatively low silica concentrations. In contrast, use of the $(NH_4)_2S_2O_8$ oxidant in our copolymer syntheses (samples 3–12) produced only macroscopic precipitates, with little or no colloid formation. Stable colloidal dispersions of functionalized polypyrrole–silica microparticles could *only* be obtained with the $FeCl_3$ oxidant. This observation may be related to the known kinetic differences between these two oxidants: Bjorklund has reported that the rate of polymerization of pyrrole is much faster with the $(NH_4)_2S_2O_8$ oxidant than the $FeCl_3$ oxidant.[17] In addition, it is evident from Table 1 that a significantly higher initial silica concentration is required for the quantitative formation of functionalized polypyrrole–silica microparticles (3.4–6.8 wt %; samples 4, 7, and 11) compared to homopolypyrrole–silica microparticles (1.1 wt % for samples 1 and 2).

We also found that long reaction times can be detrimental to efficient colloid formation. For example, we obtained stable carboxylic acid-functionalized polypyrrole–silica colloids instead of macroscopic precipitates simply by reducing the reaction time from 16 to 5 h (see samples 6 and 7).

Remarkably, we found that the *order of addition* of the reagents could sometimes be important for successful colloid formation. Under the reaction conditions utilized for sample 6, only a macroscopic precipitate was obtained, whereas stable functionalized polypyrrole–silica microparticles were formed in an identical synthesis provided that the $FeCl_3$ oxidant was added *last* to the solution (see sample 11). Adding the oxidant last to the reaction mixture may also have a beneficial effect on the long-term colloid stability of the microparticles:

Table 2. Copolymer Composition, Copolymer Content, and Particle Size of Carboxylic Acid-Functionalized Polypyrrole–Silica Microparticles

sample no.	comonomer mol ratio pyrrole/carboxyl pyrrole[a]	polymer content[a] (wt %)	TEM particle size[b] (nm)	DCP particle size[c] (nm)
1	100/0	69.5	200–300	160 ± 20
2	100/0	37.9	80–120	110 ± 20
4	77/23	76.0	120–200	
7	56/44	56.6	50–110	
11	60/40	66.1	70–130	100 ± 30

[a] As determined by elemental microanalysis (confirmed by thermogravimetry). [b] Number-average diameter as determined by transmission electron microscopy. [c] Weight-average diameter as determined by disk centrifuge photosedimentometry.

sample 11 is reasonably stable with respect to flocculation for several months, whereas samples 4 and 7 were visibly flocculated within 1 month. We have no explanation for these unexpected observations at the present time.

Homopolymerization of 1-(2-carboxyethyl)pyrrole functional monomer in the presence of the silica particles was also attempted. We anticipated that, if successful, this synthesis would yield functionalized microparticles with a high concentration of surface carboxylic acid groups; unfortunately, only á macroscopic precipitate was obtained.

A summary of our experiment data for the chemical composition and particle size of the functionalized polypyrrole–silica microparticles is presented in Table 2. In general, their copolymer content is pyrrole-rich compared to the composition expected from the comonomer feed ratios. This is not surprising since there is literature evidence to suggest that *N*-substituted pyrrole monomers generally polymerize more slowly than pyrrole.[16] We conclude that some of the 1-(2-carboxyethyl)-pyrrole comonomer probably remains unreacted after the copolymerization.

We have previously shown that, in the synthesis of homopolypyrrole–silica microparticles, increasing the initial silica concentration from 1.0 to 3.4 w/v % results in a moderate reduction in the polypyrrole content [from 67.5% down to 61.3% using the $FeCl_3$ oxidant and from 54.4% down to 37.9% for the $(NH_4)_2S_2O_8$ oxidant[10,11]]. Considering the rather high initial silica concentrations (3.4–6.8 w/v %) employed in the present study, the copolymer contents of the functionalized polypyrrole–silica microparticles are relatively high. For example, despite an increase of more than sixfold in the initial silica concentration, the copolymer content of sample 11 is comparable to the homopolymer content of sample 1.

Our IR spectroscopy studies on the microparticles yielded useful, albeit qualitative, information. The IR spectrum of the commercial silica particles (see Figure 2a) had two major bands at 802 cm^{-1} (scissor deformation) and 1117 cm^{-1} (three superimposed bands due to the Si–O stretch mode). Examination of the IR spectrum of the poly(pyrrole-*co*-1-(2-carboxyethyl)pyrrole) bulk powder (see Figure 2b) confirmed the presence of carboxylic acid and carboxylate ion (bands at 1724 and 1389 cm^{-1}) in addition to bands characteristic of doped polypyrrole (at 1561, 1467, 1304, 1203, 1052, and 940 cm^{-1}).[1a,19] As expected, the IR spectrum of the dried, functionalized polypyrrole–silica microparticles (sample 11) exhibited bands attributable to both the functionalized polypyrrole copolymer (1707, 1562, 1462, 1385, 1300, 1216, 1054, and 934 cm^{-1}) and silica (1117 and 789 cm^{-1}) components (see Figure 2c).

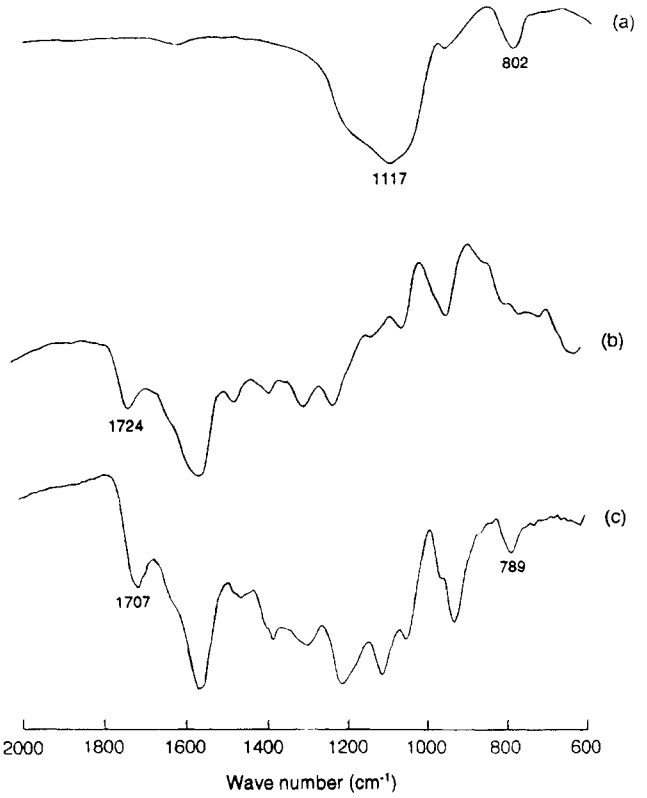

Figure 2. IR spectra of (a) the pristine silica sol, (b) a "bulk powder" poly(pyrrole-*co*-1-(2-carboxyethyl)pyrrole) prepared in the absence of silica particles and (c) carboxylic acid-functionalized polypyrrole–silica microparticles. Note the extra peak at 1707 cm^{-1} in spectrum c which is characteristic of the carboxylic acid group.

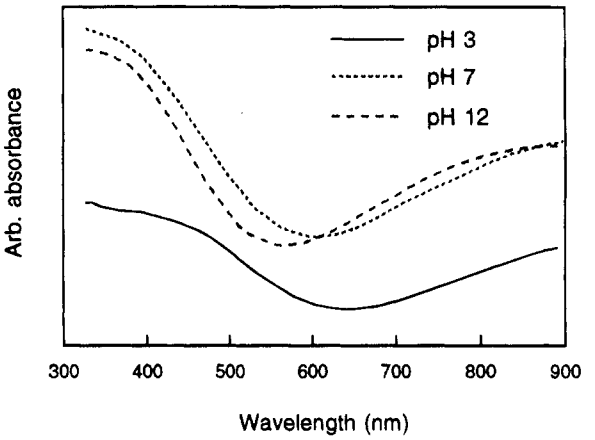

Figure 3. Visible absorption spectra of carboxylic acid-functionalized polypyrrole–silica microparticles (sample 11) dispersed in various pH buffer solutions.

The visible absorption spectra of functionalized polypyrrole–silica microparticles diluted in buffer solutions in the range of pH 3–12 are shown in Figure 3. This technique only "sees" the functionalized polypyrrole component since the ultrafine silica particles are poor light scatterers due to their small particle size. At pH 7 and pH 12 our spectra are very similar to those reported by Pei and Qian for electrochemically synthesized homopolypyrrole films soaked in various buffer solutions.[20] Thus the functionalized microparticles absorb strongly across the entire visible spectrum both at physiological pH and also when partially dedoped. The rather low absorbance of the microparticles in the pH 3 buffer solution is probably due to their rapid sedimentation in the sample cell during the measure-

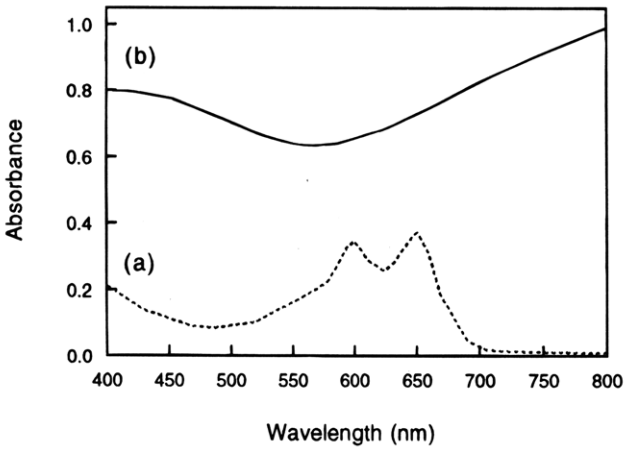

Figure 4. Visible absorption spectra of (a) a 125 nm diameter extrinsically dyed, carboxylic acid-functionalized commercial polystyrene latex (SuperCarboxyl Hi-Dye, Polymer Laboratories) and (b) a 100 nm diameter carboxylic acid-functionalized polypyrrole–silica dispersion (sample 11). Note that the color intensity of the conducting polymer-based microparticles is significantly higher at all wavelengths in the visible absorption spectrum at the same solids concentration (40 mg dm^{-3}).

ments; our disk centrifuge data confirm that the microparticles are appreciably flocculated under these conditions. Pei and Qian observed a distinct absorbance peak at *ca.* 450–470 nm which is characteristic of highly doped polypyrrole when their films were immersed in acidic media (pH 1–3). This peak is present only as a broad shoulder in our spectra, which suggests that the doping level of the polypyrrole copolymer component of the microparticles is somewhat lower than that of conventional homopolypyrrole. It is well documented that the incorporation of *N*-substituted pyrrole comonomers into the polypyrrole backbone lowers both the doping level and conductivity (see below) of this material.[16]

The "marker" particles used in most conventional visual diagnostic assays are colored polystyrene latex particles. An example is Polymer Laboratories, Super-Carboxyl Hi-Dye, an extrinsically dyed carboxylated polystyrene latex of 125 nm diameter with an intense blue coloration. We have compared the color intensity of this blue latex with that of our black carboxylic acid-functionalized polypyrrole–silica microparticles at the same solids concentration of 40 mg dm^{-3} using visible absorption spectrophotometry (see Figure 4). It is clear that the conducting polymer-based dispersion outperforms the commercial latex at all wavelengths in the visible spectrum. For example, at a wavelength of 400 nm the former system has an optical absorbance approximately 4 times that of the latter. We conclude that these polypyrrole–silica particles have considerable potential for the development of diagnostic assays of increased sensitivity for the analyte of interest.

A typical transmission electron micrograph of a diluted, dried carboxylic acid-functionalized polypyrrole–silica dispersion (sample 11) is shown in Figure 5. The microparticles are approximately spherical with relatively little evidence for the silica component. On the other hand, the homopolypyrrole–silica microparticles clearly contain the original small silica particles within the microparticles since they have a distinctive "raspberry" morphology, particularly at lower polypyrrole loadings (see Figure 6). Thus this difference in particle morphology may be due to the relatively high conducting polymer content in the functionalized microparticles. Alternatively, preferential incorporation of the more hydrophilic functional comonomer at the surface of the microparticles toward the end of the

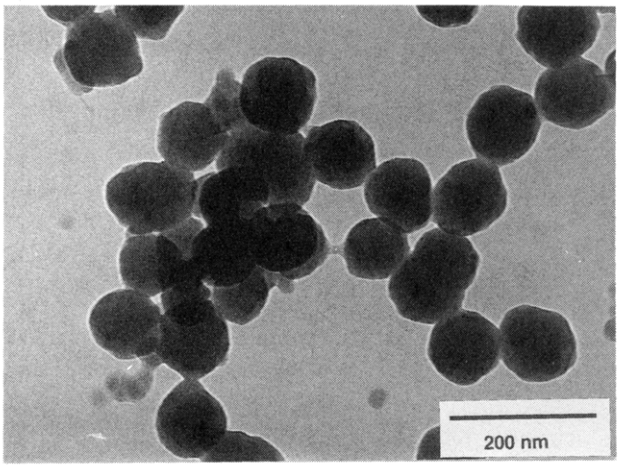

Figure 5. Typical transmission electron micrograph of the carboxylic acid-functionalized polypyrrole–silica microparticles (sample 11).

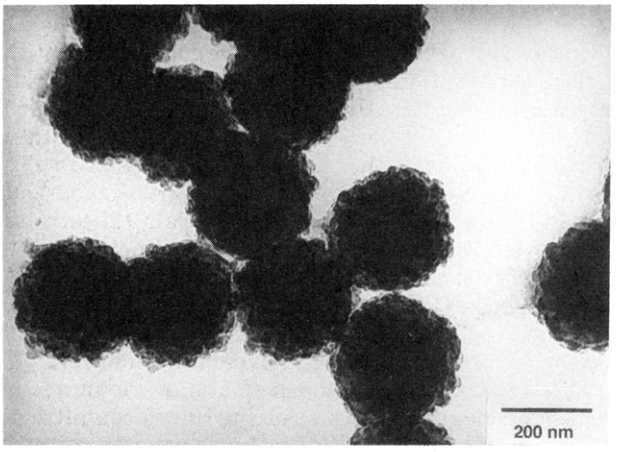

Figure 6. Typical transmission electron micrograph of the homopolypyrrole–silica microparticles (sample 1) showing the distinctive "raspberry" morphology.

copolymerization may be responsible for their more spherical appearance.

Typical weight-average particle size distributions of a dispersion of functionalized polypyrrole–silica microparticles (sample 11, diluted in pH 9 buffer solution) and two polystyrene calibration standards (particle diameters 502 ± 4 and 890 ± 8 nm; Duke Scientific Corp.) obtained using our disk centrifuge are shown in Figure 7. Ideally, we would have liked to compare our microparticles to a polystyrene standard of similar particle size. Unfortunately, it is rather difficult to size polystyrene particles of <150 nm diameter using the disk centrifuge due to the low particle density of this material (1.05 g cm^{-3}). On the other hand, there is no problem in sizing polypyrrole–silica microspheres of *ca.* 100 nm diameter using this instrument because these latter particles are relatively dense (1.6–1.8 g cm^{-3}) compared to the aqueous spin fluid. The particle size distribution of the functionalized polypyrrole–silica microparticles is surprisingly narrow and unimodal, particularly in view of the relatively simple preparation techniques utilized in its synthesis. The particle sizes of the functionalized polypyrrole–silica microparticles are generally smaller than the homopolypyrrole–silica microparticles (compare sample 11 with 1 and sample 7 with 2 in Table 2). Finally, we note that the number-average and weight-average particle diameters determined using the disk centrifuge are in reasonably good agreement with our electron microscopy observations.

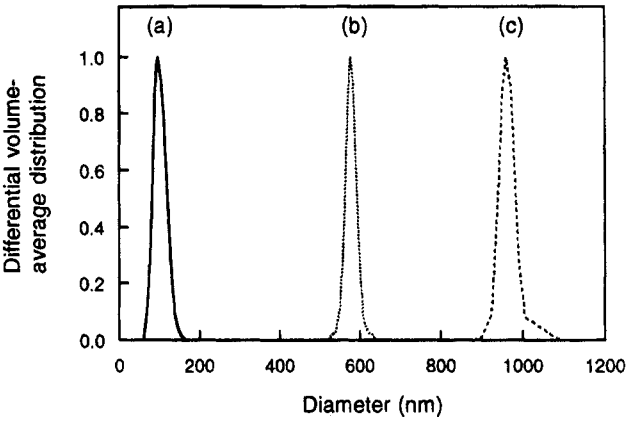

Figure 7. Weight-average particle size distribution curves obtained with the Brookhaven disk centrifuge for (a) carboxylic-acid functionalized polypyrrole–silica microparticles (sample 11), (b) 502 ± 4 nm polystyrene latex particles and, (c) 890 ± 8 nm polystyrene latex particles. Latexes b and c are certified calibration standards from Duke Scientific Corp.

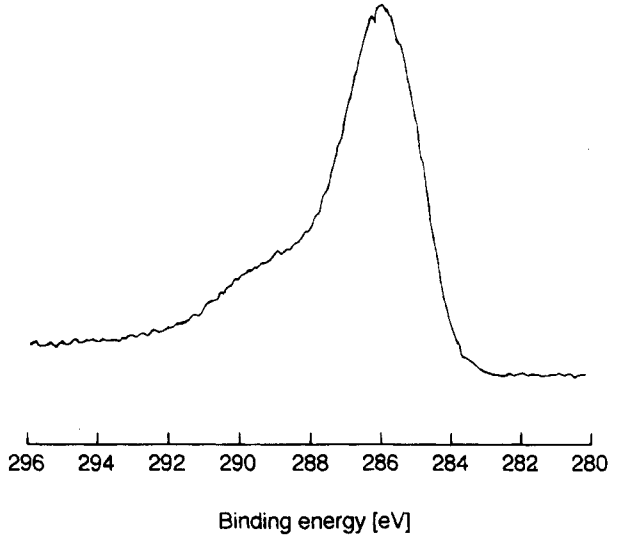

Figure 8. C_{1s} core-line X-ray photoelectron spectrum of the carboxylic acid-functionalized polypyrrole–silica microparticles (sample 11). Note the shoulder at approximately 289.5 eV due to the carbonyl carbon of the surface carboxylic acid groups.

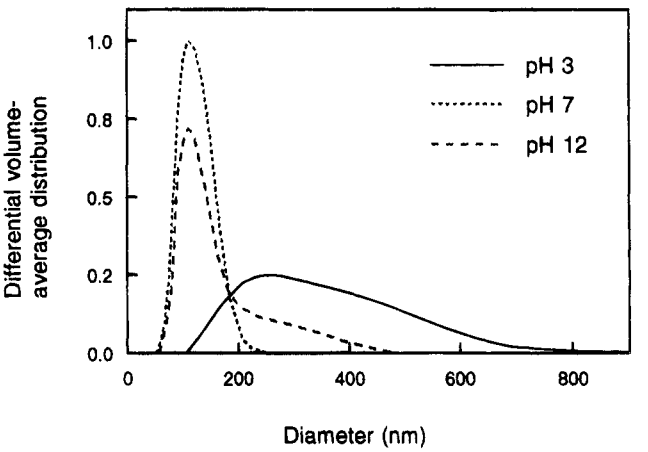

Figure 9. Colloid stability studies using the disk centrifuge. The carboxylic acid-functionalized polypyrrole–silica microparticles were dispersed in a pH-buffered aqueous spin fluid prior to analysis. The apparent increase in particle diameter is interpreted as an increase in the degree of flocculation. Thus these functionalized microparticles are flocculated at pH 3 but have good colloid stability at pH ≥ 7.

Our earlier XPS experiments have confirmed that the surface composition of the homopolypyrrole–silica microparticles is distinctly silica-rich, at least within the XPS sampling depth of 1–5 nm.[12] Thus, in the context of colloidal stability, the homopolypyrrole–silica microparticles can, to a first approximation, be considered to be "large" silica particles; *i.e.*, they are almost certainly charge-stabilized like the original ultrafine silica sols. Essentially the same experimental strategy was adopted in the present XPS study: the silicon atoms in the silica particles and the nitrogen atoms in the polypyrrole copolymer were utilized as elemental "markers" for sample 11. Since these "marker" atoms are unique for each component, simply measuring the silicon/nitrogen surface atomic ratio by XPS allows a semiquantitative assessment of the silica/polypyrrole surface composition. This surface atomic ratio was then compared with the bulk silicon/nitrogen atomic ratio calculated from our macroscopic chemical composition data for sample 11. Thus, the surface silicon/nitrogen atomic ratio was determined to be 0.70 ± 0.07 by XPS, while the bulk silicon/nitrogen atomic ratio was calculated to be 0.40 ± 0.04. This difference between the surface and bulk compositions of the carboxylic acid-functionalized microparticles is rather less than those observed for the homopolypyrrole–silica microparticles[12] and is consistent with the spherical and raspberry particle morphologies observed for these two systems (see above).

The presence of carboxylic acid groups at the surface of the functionalized polypyrrole–silica microparticle samples was also confirmed by our XPS studies. The high-resolution C_{1s} XPS spectrum of sample 11 is shown in Figure 8. A distinct shoulder characteristic of carboxylic carbon is discernible at a binding energy of approximately 289.5 eV.[21] We stress that this shoulder is absent in all of the homopolypyrrole–silica samples examined by XPS in our laboratory to date. Unfortunately, we were not able to carry out satisfactory deconvolution analyses of the C_{1s} envelope due to software limitations. However, since the XPS technique is acknowledged to be highly surface-specific, we conclude that the carboxylic acid groups must be present at (or within the first few nanometers of) the surface of the functionalized polypyrrole–silica microparticles. This observation is consistent with our aqueous electrophoresis measurements[22] which also indicate the presence of surface carboxylic acid groups in these functionalized polypyrrole–silica microparticles.

The disk centrifuge is a useful instrument for assessing the degree of dispersion of the microparticles over a range of solution pH. In these experiments a shift in the weight-average particle size distribution to larger particle diameter is interpreted as an increase in the degree of flocculation of the dispersion rather than an actual increase in particle diameter. We found that our carboxylic acid-functionalized polypyrrole–silica particles (sample 11) could be reversibly flocculated by adjusting the solution pH. Thus the particles are quantitatively aggregated at pH 3, whereas they have a high degree of dispersion at pH 7 and remain reasonably dispersed up to pH 12 (see Figure 9). This behavior is markedly different from that of the homopolypyrrole–silica microparticles. This latter system is stable from pH 1 up to pH 9 but becomes more flocculated in more alkaline media. Clearly, these colloid stability differences must be related to differences in the surface chemistry between these two systems. In view of our XPS results (see above), we attribute the relatively high degree of dispersion at higher pH exhibited by the functionalized polypyrrole–silica microparticles to the

ionization of their surface carboxylic acid groups. Such ionization would be expected to result in a higher (negative) surface charge and hence provide additional charge stabilization for these microparticles. We anticipate that these functionalized polypyrrole–silica microparticles will be excellent candidates for new "marker" particles in improved diagnostic assays since these dispersions have optimum colloid stability around physiological pH.

The conductivities of our functionalized polypyrrole–silica microparticles were too low ($<10^{-6}$ S cm^{-1}) to be measured by our four-point probe apparatus. This is not surprising, since it is well known that copolymerizing *N*-substituted pyrrole monomers with pyrrole dramatically reduces the conductivity of the resulting polypyrrole copolymers.[16,23] However, we stress that high electrical conductivities are not a prerequisite for diagnostic assays: this application simply requires a *conjugated* polymer to provide the intense coloration. On the other hand, for certain other applications such as electrochromatography it may be desirable to have highly conductive polypyrrole–silica microparticles.[24]

Conclusions

Copolymerization of 1-(2-carboxyethyl)pyrrole with pyrrole in the presence of ultrafine silica particles can yield stable colloidal dispersions of surface-functionalized polypyrrole–silica particles. However, these copolymer syntheses are not as robust as the analogous homopolypyrrole–silica syntheses. In particular, colloidally stable microparticles are obtained with the $FeCl_3$ oxidant but not the $(NH_4)_2S_2O_8$ oxidant and significantly higher initial silica concentrations are required. Furthermore, under certain conditions the order of addition of the principal reagents (the $FeCl_3$ oxidant and the two pyrrolic comonomers) and the reaction time can also influence the success of the microparticle syntheses. In this context, stable dispersions are more likely if the oxidant is added last and the reaction time is reduced. The copolymer composition is always richer in pyrrole than the initial comonomer feed ratio, which suggests that the 1-(2-carboxyethyl)pyrrole copolymerizes at a slower rate than pyrrole.

These new functionalized polypyrrole–silica microparticles have relatively narrow particle size distributions and exhibit good colloid stability at physiological pH. Our XPS studies have confirmed that the surface of these functionalized microparticles contain carboxylic acid groups. They are significantly more chromogenic than a commercial sample of an extrinsically dyed, carboxylic acid-functionalized polystyrene latex (Polymer Laboratorie's SuperCarboxyl Hi-Dye) across the entire 400–800 nm range of the visible spectrum. In view of their facile synthesis we anticipate that these functionalized polypyrrole–silica microparticles will be excellent candidates for a new generation of "marker" particles for use in diagnostic assays.

Acknowledgment. We wish to thank Mr. H. Miyagawa and Mr. A. Hiroe of the New Oji Paper Co., Ltd., Japan, for assistance with the XPS studies. We thank the New Oji Paper Co., Ltd., for generous financial support of this project in the form of a studentship for S.M. Abbott Laboratories Diagnostic Division, North Chicago, IL, is acknowledged for partial financial support of the microparticle syntheses. The SERC is acknowledged for a capital equipment grant for the purchase of the disk centrifuge (GR/H93606). S.P.A. wishes to thank the Royal Society for travel funds for a study visit to the New Oji Paper Co., Japan. We acknowledge Nyacol Products, Inc., for the kind donation of the colloidal silica sol and Dr. M. J. J. Roberts for providing a sample of Polymer Laboratories's SuperCarboxyl Hi-Dye latex.

References and Notes

(1) (a) Bjorklund, R. B.; Liedberg, B. *J. Chem. Soc., Chem. Commun.* **1986**, 1293. (b) Armes, S. P.; Vincent, B. *J. Chem. Soc., Chem. Commun.* **1987**, 288.
(2) (a) Armes, S. P.; Aldissi, M. *Polymer* **1990**, *31*, 569. (b) Cawdery, N.; Obey, T. M.; Vincent, B. *J. Chem. Soc., Chem. Commun.* **1988**, 1189. (c) Epron, F.; Henry, F.; Sagnes, O. *Makromol. Chem., Macromol. Symp.* **1990**, *35/36*, 527. (d) Odegard, R.; Skotheim, T. A.; Lee, H. S. *J. Electrochem. Soc.* **1991**, *138*, 2930. Digar, M. L.; Bhattacharyya, S. N., Mandal, B. M. *J. Chem. Soc., Chem. Commun.* **1992**, 18.
(3) (a) Armes, S. P.; Aldissi, M. *J. Chem. Soc., Chem. Commun.* **1989**, 88. (b) Vincent, B.; Waterson, J. W. *J. Chem. Soc., Chem. Commun.* **1990**, 683. (c) Liu, J.-M.; Yang, C. *J. Chem. Soc., Chem. Commun.* **1991**, 1529. (d) Gospodinova, N.; Mokreva, P.; Terlemezyan, L. *J. Chem. Soc., Chem. Commun.* **1992**, 923.
(4) (a) Armes, S. P.; Aldissi, M.; Agnew, S. F.; Gottesfeld, S. *Langmuir* **1990**, *6*, 1745. (b) Armes, S. P.; Aldissi, M.; Agnew, S. F.; Gottesfeld, S. *Mol. Cryst. Liq. Cryst.* **1990**, *190*, 63. (c) Tadros, P.; Luk, S. Y.; Armes, S. P. *J. Mater. Chem.* **1992**, *2*, 125. (d) DeArmitt, C.; Armes, S. P. *J. Colloid Interface Sci.* **1992**, *150*, 134.
(5) Tarcha, P. J.; Misun, D.; Finley, D.; Wong, M.; Donovan, J. J. in Polymer Latexes: Preparation, Characterisation and Applications; Daniels, E. S., Sudol, E. D., El-Aassar, M. S. Eds.; ACS Symp. Ser. 492; American Chemical Society: Washington, DC, 1992; p 347.
(6) Gill, M.; Mykytiuk, J.; Armes, S. P.; Edwards, J. L.; Yeates, T.; Moreland, P. J.; Mollett, C. *J. Chem. Soc., Chem. Commun.* **1992**, 108.
(7) Gill, M.; Armes, S. P.; Fairhurst, D.; Emmett, S. N.; Idzorek, G.; Pigott, T. *Langmuir* **1992**, *8*, 2178.
(8) Gill, M.; Baines, F. L.; Armes, S. P. *Synth. Met.* **1993**, *55–57*, 1029.
(9) Terrill, N. J.; Crowley, T.; Gill, M.; Armes, S. P. *Langmuir* **1993**, *9*, 2093.
(10) Maeda, S.; Armes, S. P. *J. Colloid Interface Sci.* **1993**, *159*, 257.
(11) Maeda, S.; Armes, S. P. *J. Mater. Chem.* **1994**, *4*, 935.
(12) Maeda, S.; Gill, M.; Armes, S. P.; Fletcher, I. W., submitted to *Langmuir*.
(13) Kawaguchi, H. In *Microspheres for Diagnosis and Bioseparation*; Tsuruta, T., Hayashi, T., Kataoka, K., Ishihara, K., Kimura, Y., Eds.; CRC Press: London, 1993; p 294.
(14) Blume, R. C.; Lindwall, H. G. *J. Org. Chem.* **1945**, *10*, 255.
(15) (a) Allcock, H. R.; Dodge, J. A.; Van Dyke, L.; Martin, C. R. *Chem. Mater.* **1992**, *4* (4), 780. (b) Wolowacz, S. E.; Yon, H. B. F. Y.; Lowe, C. R. *Anal. Chem.* **1992**, *64*, (14), 1541.
(16) (a) Neoh, K. G.; Kang, E. T.; Tan, T. C.; Tan, K. L. *J. Appl. Polym. Sci.* **1989**, *38*, 2009. (b) Kiani, M. S.; Mitchell, G. R. *Synth. Met.* **1992**, *46*, 293.
(17) Holsworth, R. M.; Provder, T.; Stansbrey, J. J. In Particle Size Distribution: Assessment and Characterization; Provder, T. Ed.; ACS Symp. Ser. 332, American Chemical Society: Washington, DC, 1987; p 191.
(18) Bjorklund, R. B. *J. Chem. Soc., Faraday Trans. 1* **1987**, *83*, 1507.
(19) Lei, J.; Liang, W.; Martin, C. R. *Synth. Met.* **1992**, *48*, 301.
(20) Pei, Q.; Qian, R. *Synth. Met.* **1991**, *45*, 35.
(21) Beamson, G.; Briggs, D. *High resolution XPS of Organic Polymers*; Wiley: Chichester, 1992; pp 110–112.
(22) Butterworth, M. D.; Maeda, S.; Lascelles, S.; Johal, J.; Corradi, R.; Armes, S. P. *J. Colloid Interface Sci.*, in press.
(23) Diaz, A. F.; Kanazawa, K. K. In *Extended Linear Chain Compounds*; Miller, J. S., Ed.; Plenum: New York, 1982; Vol. 3, p 417.
(24) Ge, H.; Teasdale, P. R.; Wallace, G. G. *J. Chromatogr.* **1991**, *544*, 305.

MA946006O