Functional pH-Responsive Polystyrene Microspheres Prepared by Surface Segregation of Diblock Copolymers

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ABSTRACT: We report the preparation of functional polymer latexes by using amphiphilic diblock copolymers as additives during the precipitation polymerization. The crosslinked microspheres with sizes ranging from 1 to 2 μm were prepared by precipitation copolymerization of styrene and divinylbenzene in solvent mixtures of acetonitrile and toluene. Because of the affinity between the polystyrene block and the monomers used for the polymerization, the amphiphilic diblock copolymer polystyrene-*b*-poly(acrylic acid) (PS-*b*-PAA) could be incorporated within the particle. The structure and the chemical composition of the particles were studied by means of SEM, FTIR spectroscopy, and X-ray photoelectron spectroscopy (XPS). Effective surface migration produced either hydrophilic or hydrophobic particles. Exposure of the particles to hot water provoked surface rearrangement and thus the acrylic acid functions to be revealed. On the contrary, the contact with either air or toluene induced migration of the polystyrene chains to the interface. Hence, the chemical composition at the surface can be reversibly modulated. Moreover, the PAA functionalized microspheres have the ability to reversibly change the external charge as a function of the pH by ionization and deionization of the acid functional groups.

Introduction

Functional latexes, i.e., microspheres bearing surface chemical groups, are the center of a large number of studies nowadays.[1−3] Such interest relies on the potential of these particles, when appropriately functionalized, in a wide variety of applications. In particular, they are extensively used in medical and biological applications, e.g., bioseparation, immunoassay and affinity-diagnosis, or as carriers for drug delivery purposes.[4] Also, microspheres have been employed in optical and opto-electrical devices,[5] in catalysis, or micropatterning.[6]

The preparation of functionalized polymer microspheres requires the control of, among other parameters, particle size, nature, and density of the functional groups and colloidal stability. Several methods have been developed for the functionalization of particles that can be summarized into two general approaches: (i) particles prepared by polymerization of a main monomer in the presence of other monomer containing the desired functions, i.e., functional monomers[7] and (ii) methodologies based on the modification of the final polymer latexes either by chemical treatments[8] or by grafting polymers and block copolymers from/onto their surfaces.[9] Both methods have several major drawbacks. The copolymerization of functional monomers is restricted to a limited number of monomers for two main reasons: on the one hand, the different reactivity of the comonomers and/or, on the other hand, chain transfer reactions, if the functional group present has a labile proton.[10] Postmodification of latexes is difficult to perform in terms of reproducibility and generally needs additional steps to obtain the desired functionality.[2]

To overcome several of the drawbacks described above, we designed a novel strategy to prepare functionalized polymeric microparticles. For that purpose, an amphiphilic diblock copolymer was incorporated within the monomer mixture during the precipitation polymerization. The diblock copolymer is designed to contain a block similar in nature to the monomer mixture used during the reaction, i.e., polystyrene, that favors its incorporation during the microgel formation. The second block stands for the surface functionality, e.g., carboxylic functions if poly(acrylic acid) is used. Once the reaction concluded and the particles have been thus formed, simple exposure to water will direct the hydrophilic segment of the block copolymer by surface segregation toward the interface.[11] Equally, annealing either to air or toluene will induce a surface reconstruction in which the lower surface-energy hydrophobic block will be located at the surface. The difference in surface energies of both blocks allows surface rearrangement to occur in response to a change in the environment; thus, the character of the particle can be reversibly changed from hydrophilic to hydrophobic.

Among the methods available for the preparation of polymer particles we have chosen the precipitation polymerization method. This approach leads to rather monodisperse microspheres free of any surfactants or stabilizers that could interact with the surface segregation process. Equally, the use of divinylbenzene leads to highly crosslinked particles stable in shape under harsh temperature and pressure conditions.[12]

Experimental Section

Chemicals. Styrene, divinylbenzene, and *tert*-butyl acrylate were purchased from Sigma-Aldrich. Styrene and *tert*-butyl acrylate were dried under CaH_2 and cryodistilled. Azobisisobutyronitrile (AIBN, 98%) as the initiator was recrystallized from methanol before use. All other solvents were used as received unless otherwise specified.

Synthesis. The synthesis of the diblock copolymers obtained by atom transfer radical polymerization (ATRP) has been already described elsewhere.[13] The preparation of the particles has been carried out by precipitation polymerization as follows (procedure

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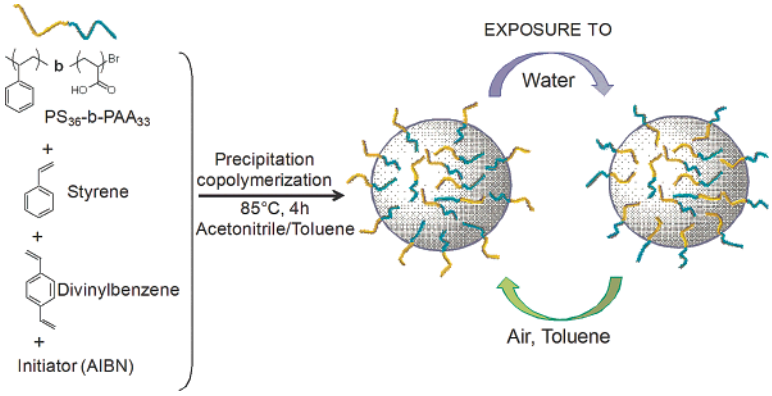

Figure 1. Schematic of the functional particles synthesis by precipitation copolymerization.

for a targeted crosslinking density of 40%): 0.79 mL of styrene (Sty), 0.79 mL of divinylbenzene (DVB), and 72 mg of azobisisobutylonitrile (AIBN) where dissolved in 30 mL of an acetonitrile/toluene (95%/5%) mixture. In a separate step, the diblock copolymer (10% related to the total amount on Sty/DVB) was dissolved in the same solvent mixture and added to the previous solution. The polymerizations were carried out at a constant temperature of 85 °C during 4 h. After the polymerization was accomplished, the soluble polymer was separated from the insoluble fraction by vacuum filtration. The insoluble polymer was extensively washed with tetrahydrofuran.

Annealing. The reversibility in terms of surface functionality has been evidenced by annealing the particles to different environments. Dispersed particles were annealed to hot water (90 °C for 2 days), which induced the hydrophilic block to migrate to the interface polymer/water. On the contrary, annealing to dry air (100 °C, 2 days) or to toluene (room temperature, 2 days), which is a good solvent for polystyrene, brought about a surface rearrangement where the hydrophobic polystyrene chains moved preferentially toward the interface.

Particle Size and Potential ζ Analysis. Particle size, size distributions, and surface charge were measured using a Zetasizer 3000 (Malvern Instruments). All DLS measurements were carried out at a wavelength of 633 nm, 25 °C, and an angle detection of 90°. For the ζ potential measurements, the sample concentration was 1 mg/mL and the pH was varied between 3 and 10.

SEM. The morphological characterization of the functionalized microspheres was carried out with a scanning electron microscope (SEM, JEOL JSM-5200 scanning microscope). The particles were dropped onto a sample holder, placed under vacuum at room temperature and gold-coated prior to examination.

X-ray Photoelectron Spectroscopy (XPS) Experiments. XPS spectra were recorded with a 220i-XL ESCALAB from VG. The particles, supported on indium, were put under ultrahigh vacuum (UHV) to reach the 10^{-8} Pa range. The nonmonochromatized Mg X-ray source was used at 100 W, as well as a flood gun to compensate for the nonconductive samples. The spectra were calibrated in relation to the C 1s binding energy (284.6 eV), which was applied as an internal standard. Fitting of the high-resolution spectra was provided through the AVANTAGE program from VG. Sputtering depth profiling was achieved by irradiation of the polymer surface with low-energy Ar^+ ions (3 kV). The sputtering rate, used throughout all the experiments, was estimated to be 0.12 nm/s by using a Ag/silicon wafer surface reference.

FTIR (transmission mode) spectra were taken in KBr pellets containing dispersed particles with a concentration of 1:100, at room temperature. The IR spectra were recorded at 20 ± 1 °C in the spectral range of 650−4000 cm^{-1} using a Perkin-Elmer Spectrum One spectrometer.

Load and Release of Methylene Blue (MB). The particles were submerged in a buffered MB solution at pH 7.0 with a concentration of MB of 10^{-3} M. Note that at neutral pH values, the acrylic acid groups are negatively charged and hence establish electrostatic interactions with the positively charged MB (load). After 1 h, the solutions were dialyzed against water at pH 7.0 for 30 min. A second dialysis against an acidic water solution (pH 3.0) allowed the MB to be released. Finally, the quantity of MB was estimated by UV spectroscopy (Lambert−Beer law).

Results and Discussion

The preparation of microspheres by precipitation methods has important advantages, including narrow size distribution or the absence of steric or ionic stabilizers within the reaction mixture, and is currently well documented.[14] To date, the synthesis of functional microspheres by precipitation is mainly carried out either by copolymerization of different monomer mixtures or postmodification. However, they require either the fine adequacy of the monomers or additional, and in general tedious, modification steps after the formation of the particle.[15] We describe, in this article, an alternative approach concerning the surface segregation methodology, largely used for planar interfaces,[16] to introduce the functional groups at the particle surface.

The preparation of functional particles by using this method improves several main aspects: given that the diblock copolymer used as an additive is prepared in a separate step by controlled radical polymerization,[17] chain lengths of the PAA block that will form the particle shell can be easily modified. Additionally, controlled radical polymerization methods allow the preparation of diblock copolymers with narrow molecular weight distributions (MWD). Hence, the particle shell layer is formed by PAA chains having all similar lengths. More importantly, the density of functional groups, crucial for many applications and difficult to control following the postreaction approach, can be controlled by the quantity of diblock added during the polymerization step.

The synthetic route for the preparation of functional microspheres having a PAA shell is outlined in Figure 1. The copolymerization reaction takes place in a homogeneous solution containing the initiator (AIBN), the monomers (styrene and divinylbenzene), and the diblock copolymer (PS_{36}-*b*-PAA_{33}) in an acetonitrile/toluene mixture. Toluene was used as a cosolvent both to enhance the stability of growing particles and to facilitate the solubilization of the block copolymers. The structure of the diblock copolymer was chosen to be symmetric in order to reduce desorption or solubilization during exposure either to water or to toluene. During the crosslinking copolymerization, partially soluble microgels are first formed. When the critical precipitation point is attained (specific crosslinking ratio and chain length), the microgels aggregate to produce colloidally stable particles. Steric stabilization is provided by the solvated polymer chains located at the slightly swollen surface of the particles preventing, thus, the aggregation. The gradual incorporation of oligomer microgel conducts the growth of particles as long as polymerization occurs. Moreover, the amphiphilic

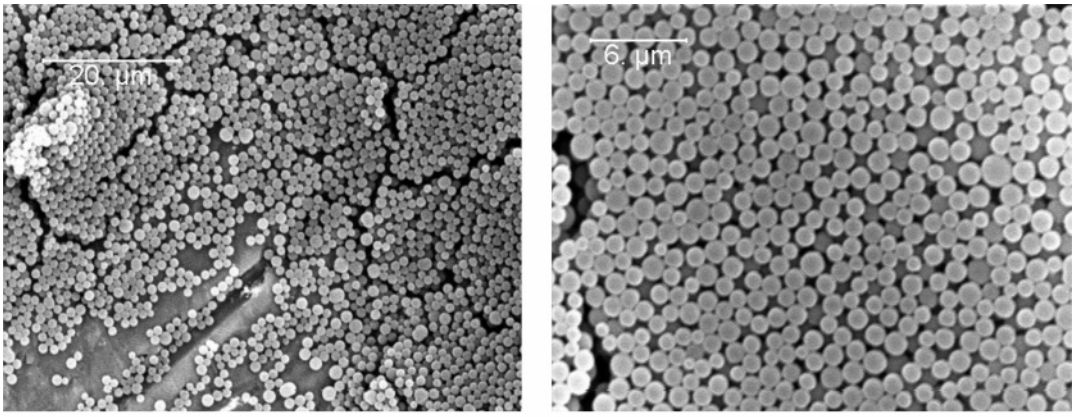

Figure 2. Representative scanning electron micrographs (SEM) of the styrene/divinylbenzene microspheres using 10% of diblock copolymer $PS_{36}-PAA_{33}$ as an additive during the precipitation polymerization (targeted crosslinking density 40%). The scale bars are 20 (a) and 6 μm (b) in length.

diblock copolymers are incorporated simultaneously within the microgels provided the affinity between the latter and the polystyrene block. Whereas the polymerization parameters, temperature, solvent mixture, amount of diblock copolymer (10%), and reaction time (4 h), were maintained invariable throughout all the experiments, the targeted degree of crosslinking was modified. For that purpose, the relation styrene/divinylbenzene (Sty/DVB) introduced in the initial mixture was varied between 0.6 and 0.25 for targeted crosslinking between 40 and 80%, respectively. Targeted crosslinking densities below 40% will not produce a precipitate or will afford particles with a reduced stability that can be damaged during the annealing treatments. On the contrary, the use of DVB exclusively in the initial feed will lead to highly crosslinked particles that will reduce the incorporation of diblock copolymer.

The morphology of the particles was characterized by scanning electron microscopy (SEM) that provides a first insight into the regularity and the size of the particles. Representative images of the particles obtained using 10% of the diblock copolymer PS_{36}-*b*-PAA_{33} are depicted in Figure 2. Rather regular in terms of shape, the microspheres have an average diameter size between 1 and 2 μm.

To determine whether the diblock copolymer was successfully incorporated within the microspheres, FTIR spectroscopy analyses were performed. The infrared absorption of the acrylic acid functions with a characteristic band at 1720 cm^{-1} are present exclusively in the diblock copolymer additive and can be easily identified. In Figure 3 are depicted the IR spectra for the particles prepared with different targeted crosslinking densities varying between 40 and 80%. An additional spectrum of crosslinked PS particles without diblock copolymer in the initial mixture was used as reference. For targeted crosslinking densities between 40 and 55%, the carbonyl signal at 1720 cm^{-1} is clearly observed and evidenced the presence of the block copolymer within the particles. Above 60%, the intensity of the carbonyl signal decreases significantly and no appreciable difference could be observed between the reference and those particles crosslinked up to 80%. With no doubt, excessive increase of the crosslinking density make the incorporation of the diblock within the particle difficult during the polymerization step. It has to be noted that since after the precipitation polymerization, the particles were washed with THF, i.e., a good solvent both for polystyrene and poly(acrylic acid), and the detected PAA results on the FTIR spectra correspond to diblock copolymer incorporated within the particle rather than adsorbed on the surface.

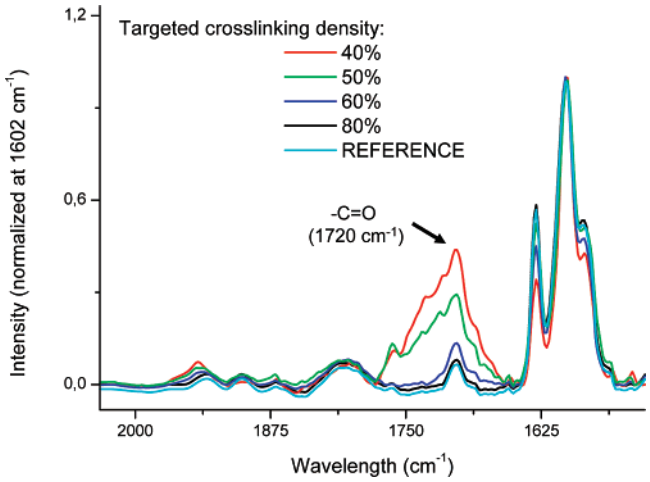

Figure 3. FTIR spectra of particles obtained for different targeted crosslinking densities between 40 and 80%. The intensity of the −C═O absorption band decreases with increasing crosslinking density. This fact evidences the difficulty of incorporation in the particles having a highly crosslinking network.

Surfaces of binary polymer blends can be enriched in one of the components depending both on the entropy[18] and enthalpy[19] of the components but also on the environment of exposure. Polymer blends annealed to air exhibit surfaces enriched in the lower energy groups, such as fluorocarbons.[20] On the contrary, in contact with water or water vapor, surface reconstruction generally leads to surfaces in which high-energy surface groups are preferred.[21] The particles behave as a binary blend in which the compatibility is assured by the chemical similarity between the PS segment of the diblock copolymer and the chemical structure of the PS crosslinked particle. Hence, the microspheres can be functionalized at the surface by segregation of the PAA block toward the interface under the appropriate conditions. To induce the migration of the diblock to the interface, the particles were dispersed in water and heated during 2 days at 90 °C. After drying under vacuum, the average surface chemical composition was analyzed by using X-ray photoelectron spectroscopy (XPS). Figure 4 shows the XPS O_{1s} spectra of the particles (targeted crosslinking density 40%) either treated to air or to water. In addition, to determine whether the diblock is present within the particles, samples exposed to water were depth profiled. The higher intensity of the oxygen peak (O_{1s}) in those particles annealed to hot water evidenced the increase of the diblock copolymer concentration at the surface. On the

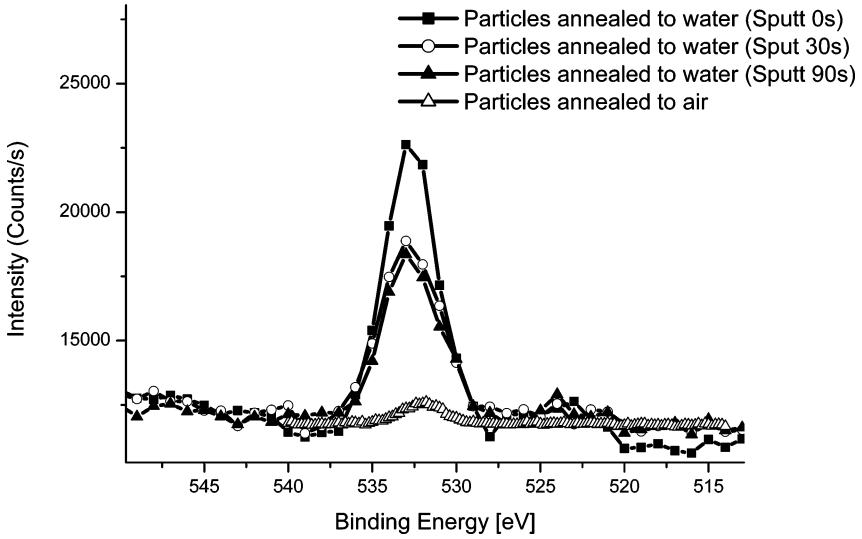

Figure 4. Oxygen (O_{1s}) signal from XPS spectra of the precipitated particles having PS_{36}-*b*-PAA_{33} as an additive. The four spectra correspond to samples annealed to water at variable sputtering times, 0, 30, and 90 s, and the particles annealed to air. Approximate sputtering rate: 0.12 nm/s.

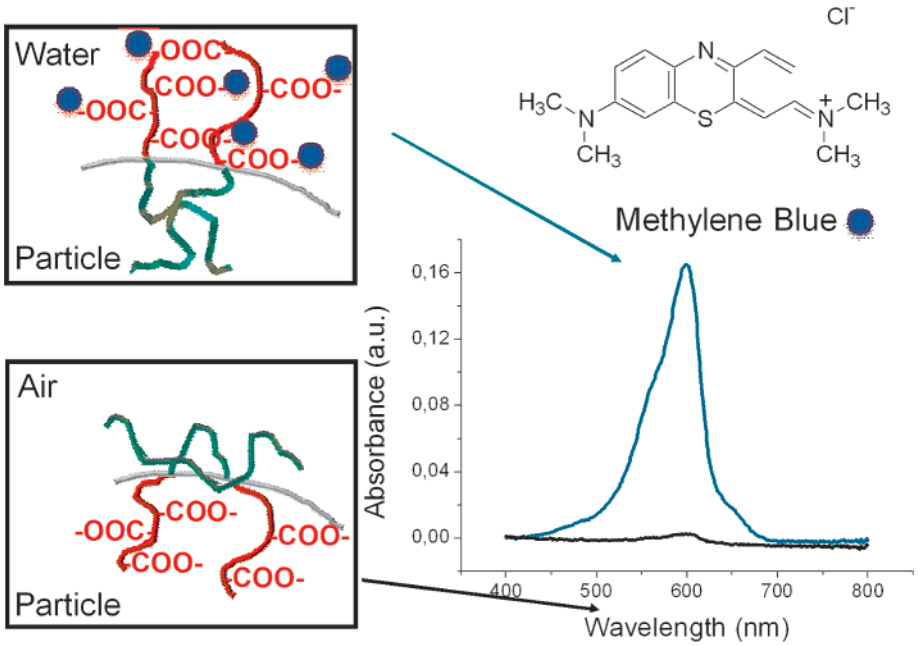

Figure 5. Scheme of the surface rearrangement in the microspheres annealed to water and air. Whereas annealed particles are able to absorb methylene blue due to the presence of PAA at the surface, in the air annealed particles, the PAA chains are hidden beneath the surface.

contrary, the rather small oxygen signal intensity in particles annealed either to air or in toluene supports the surface rearrangement of PAA blocks beneath the surface. Moreover, successive environmental changes by exposure to water and air/toluene afford particles with polar carboxylic acid functions or a hydrophobic corona, respectively. Reversible surface reconstruction upon exposure to different environments has been already highlighted as a potential delivery mechanism in which a specific functionality can be easily brought to the surface by environmental changes.[22]

XPS analysis by sputtering the surface with a photoelectron beam allows a depth profile of the compositional variations of water annealed particles. The control of the sputtering time on the polymer surface permits the study of the composition at different depths. XPS spectra carried out at sputtering times of 30 and 90 s demonstrated a decrease of the concentration in oxygen compare to the initial surface composition. On the other hand there is no significant reduction of the oxygen density between 30 and 90 s. It indicates that the surface segregation, i.e., the enrichment of diblock copolymer at the interface, occurs exclusively at the extreme surface (~10−15 nm). Since a large proportion of oxygen is present inside the particle, this result clearly proves that the diblock copolymer has been incorporated during the precipitation polymerization and the idea of a possible absorption of the diblock copolymer at the surface of the particles can be discarded.

The presence of carboxylic functions at the periphery has been also identified by means of the methylene blue method.[23] Methylene blue is a cationic dye capable of establishing electrostatic interactions with the negatively charged carboxylic functions, thus at neutral pH values. These electrostatic interactions are pH dependent and can be disrupted by reducing the pH of the solution where the carboxylic groups are neutralized. The quantity of methylene blue adsorbed, directly related to the carboxylic acid functions, can be estimated after release in acidic water by means of UV−vis spectroscopy. Figure 5 illustrates particles treated with water and air. Exposed to a 10^{-3} M MB solution and both dialyzed at neutral pH in order to

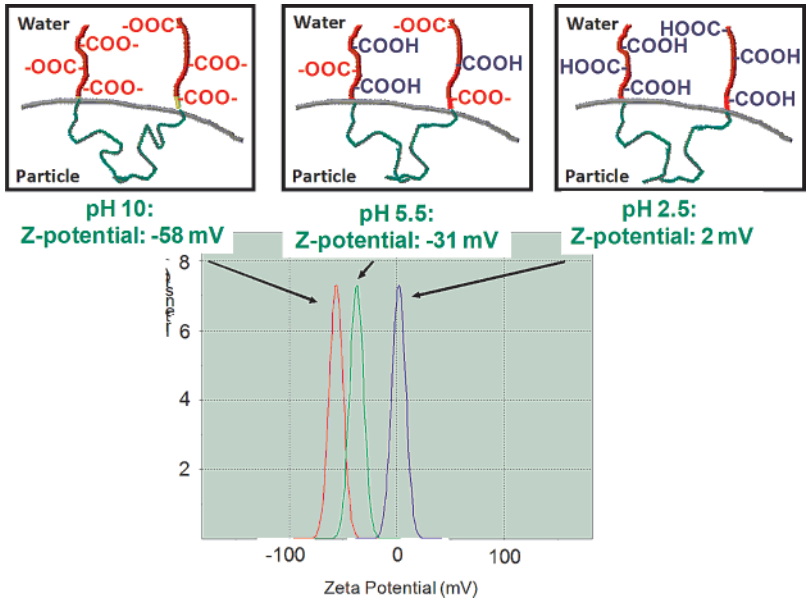

Figure 6. ζ potential measurements of the particles annealed to water at different pH values, 10, 5.5, and 2.5. The charge density increases with increasing pH due to the deprotonation of the acrylic acid functional groups.

remove the excess of cationic dye, the surface of those particles annealed to water exhibit a blue color and demonstrated the presence of carboxylic acid functions. On the contrary, those particles annealed to air remain white. The UV−vis spectra obtained after release of MB from those previous two cases are depicted in Figure 5. From this experiment we can conclude that whereas those samples exposed to air have almost no carboxylic functions since the quantity of MB delivered is rather low, those exposed to water have a large quantity of PAA chains at the surface.

The microspheres prepared have additionally the ability to respond to pH. Even thought the MB method already served to prove this concept, the pH response has been complementarily analyzed by potentiometric measurements (see Figure 6). For that purpose, the particles treated with water have been dispersed in solutions at different pH. At low pH values (pH 2.5), the particles have the carboxyl groups protonated and the global charge of the particle is close to zero. Increasing the pH slightly above the pK_a of the PAA (∼4.8) gives partially negatively charged particles. Finally, increase of the solution basicity (pH 10) lead to particles containing the PAA segment completely charged. Such behavior has been probed to be reversible after successive additions of acid and base to the solution particles.

Conclusions

We developed an alternative method for the design of functionalized polymeric microspheres. Instead of using tedious postreaction treatments, we incorporated during the precipitation polymerization an amphiphilic diblock copolymer designed to have a compatible part with the particles (in this case polystyrene) and a second block that will give the functionality at the surface, e.g., PAA. The precipitation of the particles is produced due to the high crosslinking density of the particles and the stability is thus assured against harsh thermal conditions. The particles surface composition was reversibly modified by exposure to water or to air/toluene where the carboxylic acid functions are either revealed or hidden beneath the surface. The PAA functionalized particles have, in addition, the ability of reversibly changing the surface charge from neutral to negative depending on the solution pH.

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