pH-Responsive Gels of Hydrophobically Modified Poly(acrylic acid)

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ABSTRACT: pH-responsive gels of hydrophobically modified (HM) weak polyacid were prepared from acrylic acid and *n*-alkyl acrylates (n = 8, 12, 18). The HM gels obtained bear up to 20 mol % of *n*-alkyl acrylate units randomly distributed along the network chains. The pH-driven swelling of these gels upon ionization in an aqueous medium was studied. The effect of the fraction and of the side chain length of *n*-alkyl acrylate groups on the equilibrium degree of swelling was examined. It was shown that the swelling transition shifts to alkaline pH with increasing hydrophobicity of the gel. This was explained by the stabilization of the collapsed state of the gel by hydrophobic aggregation of *n*-alkyl side chains. The formation of such aggregates, which break down in the course of gel ionization, was confirmed by the fluorescent probe method with pyrene as a probe and by NMR spectroscopy. Potentiometric titration data of HM poly(acrylic acid) (PAA) gels and of the corresponding linear copolymers evidence that the introduction of hydrophobic repeat units only slightly affects the apparent dissociation constant of PAA, except for the most hydrophobic gels.

Introduction

Polyelectrolyte responsive gels possess some unique properties which make them promising for various practical applications.[1] These gels can change their volume by several hundred times in response to a small variation of different external factors (temperature, pH, electric field, etc.). A number of recent studies concern pH-sensitive polymer gels which are useful, in particular, as potential drug carriers for oral delivery.[2−5]

Most of the gels of weak polybases or polyacids exhibit pH-responsive properties. When immersed in polar media, these gels undergo a conformational transition from the collapsed state to the swollen state upon ionization. The main reason for such a transition is the osmotic pressure exerted by mobile counterions neutralizing the network charges.[6]

It is desirable to find a way to produce responsive gels with any predetermined value of pH at the point of the swelling transition. One of the possible methods to control the pH-responsive properties of the gels is the introduction of a small fraction of hydrophobic repeat units in weak polyacid or polybase gels. In this case hydrophobe groups can aggregate with each other, when the gel is uncharged. These hydrophobic microdomains acting as additional cross-links interfere with the network swelling induced by ionization. Recently, the effect of hydrophobic side chains on the pH-responsive properties of a gel was studied for weakly basic polyelectrolyte gels synthesized by copolymerization of *n*-alkyl methacrylates, (*N*,*N*-dimethylamino)ethyl methacrylate, and divinylbenzene as a cross-linker.[4] These gels are collapsed in alkaline and neutral media, but they swell in an acidic medium. The introduction of hydrophobic groups shifts the swelling transition toward lower pH. The higher the gel hydrophobicity, the larger the shift of transition pH. The same effect is expected for weak polyacid gels as well.

In our previous work,[7] we have prepared a series of hydrophobically modified (HM) weak polyacid gels by copolymerization of hydrophilic (acrylic acid) and hydrophobic (*n*-alkyl acrylate, n = 8, 12, 18) monomers. The fraction of *n*-alkyl acrylate did not exceed 20 mol %. It was shown that HM poly(acrylic acid) (PAA) gel in completely ionized form exhibits an expanded conformation like unmodified PAA gel and that its degree of swelling does not depend markedly on the fraction of hydrophobic units in the network if this fraction is less than 10 mol %. Thus, for fully ionized gels the electrostatic repulsion prevails over the hydrophobic association. At low degrees of ionization the HM PAA gels exist in a collapsed state, in which the network subchains are additionally cross-linked by the hydrophobic aggregates of *n*-alkyl side groups. In this very compact conformation the degree of swelling of the gels decreases with increasing fraction of hydrophobic units. The results obtained suggest that the hydrophobic aggregates existing in an uncharged gel are destroyed during the gel ionization. This process should be determined by an interplay between two oppositely acting forces: the electrostatic repulsion and the attraction of hydrophobic alkyl groups. The role of both these forces can be revealed by varying the degree of ionization and the fraction and the chain length of hydrophobic units. The influence of these factors on the gel swelling/deswelling processes was investigated for HM polybase gels.[4] In the present work, the main attention will be paid to a correlation between macroscopic equilibrium swelling of the gels and the hydrophobic aggregation on a microscopic level. One might suggest that the hydrophobic aggregation can affect the gel swelling because of two reasons: the effective cross-linking of the gel chains by hydrophobic microdomains and a decrease of the dissociation constant of carboxy groups situated in the vicinity of hydrophobic units. We will reveal the role of each of these factors in the peculiarities of the gel swelling.

Thus, the aim of the present paper is to study the effect of the gel hydrophobicity on the pH-driven swell-

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ing transition of HM PAA gels, induced by gel ionization. The fluorescent probe method and NMR spectroscopy will be used to follow the disintergation of hydrophobic aggregates inside the gel during ionization. The possible influence of the hydrophobic groups on the dissociation constant of PAA will be estimated from potentiometric titration data.

Experimental Section

Materials. The following substances were used as comonomers: acrylic acid (AA) (Fluka), *n*-octyl acrylate (Scientific polymer products, Inc.), *n*-dodecyl acrylate (Lancaster synthesis), and *n*-octadecyl acrylate (Scientific polymer products, Inc.). The sodium salt of poly(acrylic acid) ($M = 20\,000$), 2,2′-azobis(isobutyronitrile), and *N*,*N*′-methylenebis(acrylamide) from Fluka were used as received. Pyrene obtained from Aldrich was recrystallized three times from absolute ethanol. Water was purified with a Milli-Q system (Millipore).

Preparation of HM Gels and Linear Copolymers. HM gels were prepared by free radical copolymerization of acrylic acid and *n*-alkyl acrylates in *N*,*N*-dimethylformamide. The fraction of *n*-alkyl acrylate in the monomer mixture was varied from 0 to 20.0 mol %. All copolymerizations were performed at an overall monomer concentration of 2.43 mol/L with 2,2′-azobis(isobutyronitrile) (1.21×10^{-2} mol/L or 0.5 mol %) as initiator and *N*,*N*′-methylenebis(acrylamide) (3.65×10^{-2} mol/L or 1.5 mol %) as cross-linker. Gelation was carried out in cylindrical glass tubes with an inner diameter of 0.40 cm under a nitrogen atmosphere at 63 °C for 24 h. The prepared gels were washed in a large amount of absolute ethanol for 3 weeks to remove unreacted components and the sol fraction.

Linear HM copolymers were synthesized in the same conditions as HM gels but without cross-linking agent. The prepared copolymers were isolated from the reaction mixture by precipitation into diethyl ether and purified by two successive precipitations from ethanol into diethyl ether. Then the copolymers C8-20% and C18-10% were dried at 60 °C for 24 h. The other copolymers were dissolved in water, dialyzed against pure water, and freeze-dried.

Linear copolymers and gels are identified in terms of the comonomer identities and molar contents. For example, C8-10% indicates a copolymer sample containing 10 mol % of *n*-octyl acrylate (C8) and 90 mol % of acrylic acid repeat units.

Characterization of HM Gels and Linear Copolymers. The weight fraction of the polymer in the swollen network β was determined by the formula $\beta = m_0/m_{sw}$, where m_0 is the mass of the dry gel and m_{sw} is the mass of the swollen gel. Most of the prepared gels swollen in ethanol have similar values of β ($\beta = 0.041 \pm 0.002$). This allows us to suggest that the efficiency of the cross-linking reaction is not appreciably affected by the amount of *n*-alkyl acrylates and their chain length.

The composition of the prepared HM gels and linear copolymers was characterized by ^{1}H NMR. NMR measurements were performed on a Bruker ARX 250 spectrometer. The mixture of deuterium oxide, 99.9 atom % D (Spectrometrie Spin et Techniques), and deuterated methanol, 99.6 atom % D (Spectrometrie Spin et Techniques), was used as solvent. It was shown that for all the gels and linear copolymers under investigation the composition of the copolymer is identical to the composition of the initial monomer mixture. The distribution of hydrophobic units along the polymer chains was characterized by ^{13}C NMR. It was shown that the *n*-alkyl acrylate units are randomly distributed along the polymer chains.

Swelling Experiments. To study the gel swelling at different pH-values, the gel samples (dry or swollen in water) were put in aqueous solutions of HCl or NaOH of the desired pH (4 mL of solution per 1 mg of the dry gel). The swelling experiments were performed in sealed bottles in a nitrogen atmosphere in order to avoid the uptake of CO_2. The gel samples remain in the solutions at 25 °C for the time required to attain equilibrium (3 weeks). When equilibrium was attained, the gel samples were weighed and the pH of the exterior solution was measured.

The degree of swelling of the gel samples equilibrated at different pH-values was characterized by the $(m - m_0)/m_0$ ratio, where m is the mass of the sample at the equilibrium state and m_0 is the mass of the dried gel sample. The values of the degree of neutralization α were determined from the quantity of NaOH taken up by the gel.

Potentiometric Measurements. Measurements of pH were made with Tacussel TT Processeur 2 and Mettler Delta 350 pH-meters. The pH-meters were standardized with a two-point calibration method. All measurements were conducted at 22 °C under nitrogen atmosphere.

Potentiometric titrations of linear copolymers were performed with Tacussel Automatic Titration Equipment. The solutions for the titration experiments were prepared by dissolving the copolymer in the acid form in water with the calculated amount of 0.1 N NaOH required to neutralize completely the copolymer carboxylic groups. The copolymer concentration was 2×10^{-2} mol of AA units/L, and the initial volume of the solution before the titration was 25.0 mL. Titrations were performed with 0.1 N HCl obtained by dilution from Merck's Titrisol ampules. Each system was titrated at least twice, and it was shown that the results coincide very closely. Only the solutions of PAA and C8-2.5% remain transparent during the titration. The solutions of other copolymers become turbid in acidic media at pH-values lower than 2.23−2.25, and the most hydrophobic copolymers (C8-20%, C12-5%, C12-10%, C18-5%, and C18-10%) precipitate from solution at these pH-values.

The apparent dissociation constants pK_{ap} of the linear copolymers were calculated from potentiometric titration curves using the following relation:

$$pK_{ap} = pH - \log \frac{\alpha}{1 - \alpha}$$

with the degree of ionization α defined by

$$\alpha = \alpha' + \left(\frac{[H^+]}{C_p}\right)$$

where α' is the degree of neutralization, $[H^+]$ is the proton concentration (deduced from the pH), and C_p is the concentration of AA units. In our conditions, assuming a dissociation constant $K_a = 1.8 \times 10^{-5}$ for an AA acid unit in pure water,[8] the degree of dissociation of acrylic units is very low ($\alpha \approx 0.03$) at zero degree of neutralization α'.

Fluorescence Measurements. Fluorescence spectra were recorded with a Shimadzu RF-5000 spectrofluorimeter using 5-nm and 1.5-nm band-pass settings for excitation and emission, respectively. All measurements were performed at 22 °C with air-saturated samples. The excitation wavelength was 334 nm. The ratio of the intensities of the third (384 nm) to first (373 nm) vibronic peak of the fluorescence spectrum of monomer pyrene was used as an estimate of the polarity of the pyrene microenvironment.

The gel samples for fluorescence measurements were prepared by pipetting a microquantity of pyrene stock solution in absolute ethanol (2×10^{-4} mol/L) into a flask containing a gel sample equilibrated in NaOH solution of the definite concentration required to attain the desired degree of gel ionization. The solutions were allowed to stay, after mixing for at least 2 days, in the dark, before fluorescence measurements were made. The final concentration of pyrene was equal to 8×10^{-7} mol/L. NMR spectra showed that at such low concentrations pyrene molecules do not affect the hydrophobic aggregation of *n*-alkyl chains in the gel. To perform the fluorescence measurements, the gels were cut to a suitable size to fill completely the quartz cell.

NMR Spectra. ^{13}C NMR measurements were performed with proton decoupling on a Bruker ARX 250 spectrometer operating at 62.9 MHz. A spectral width of 15 000 Hz and a flip angle of 90° were used. The acquisition and delay times were 1 and 10 s, respectively, and the total number of scans

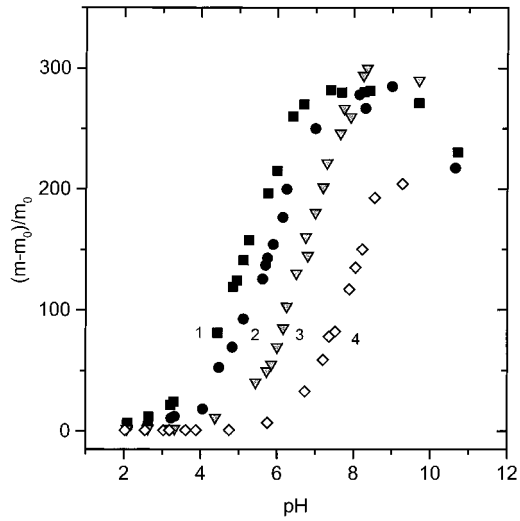

Figure 1. Dependences of the degree of swelling of PAA gel (1) and of the HM PAA gels C8-2.5% (2), C8-10% (3), and C8-20% (4) on the pH of the solution surrounding the gel sample.

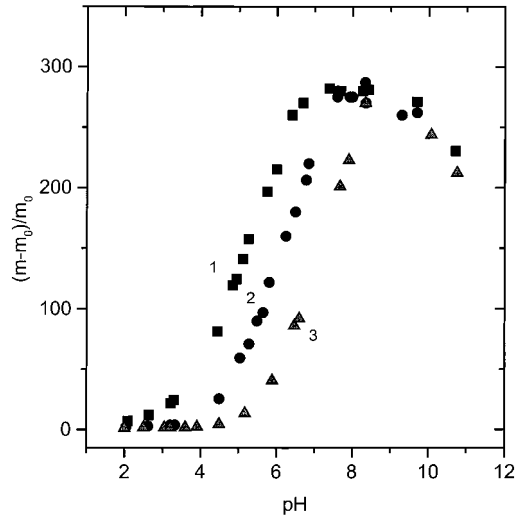

Figure 2. Dependences of the degree of swelling of PAA gel (1) and of the HM PAA gels C8-5% (2) and C12-5% (3) on the pH of the solution surrounding the gel sample.

recorded ranged between 1 500 and 10 000 depending on the sample concentration. The solutions of linear copolymers or gels (used under powder form at solid state) were prepared at least 1 week prior to analysis at a given concentration and degree of ionization in a H_2O/D_2O mixture (60/40 w/w). Trace of methanol was added to the solutions and used as internal reference ($\delta[CH_3OH] = 49.9$ ppm).

Results and Discussion

Swelling Behavior of HM PAA Gels in the Course of Titration. Figures 1 and 2 show the typical dependences of the degree of swelling of HM PAA gels on the pH of the solution surrounding the gel sample. From these figures it is seen that the gels are shrunken in an acidic medium; increasing pH leads to gel swelling. This effect is related to the gel ionization. The gel swelling upon ionization in polar media is known to be due mainly to the osmotic pressure exerted by mobile counterions.[6] At pH-values higher than 10 a moderate deswelling is observed (Figures 1 and 2). At high pH, when ionization is complete, the added sodium hydroxide acts like a low molecular weight salt. It screens electrostatically the network charges and increases the osmotic pressure of the external solution. Both these factors force the gel to shrink.

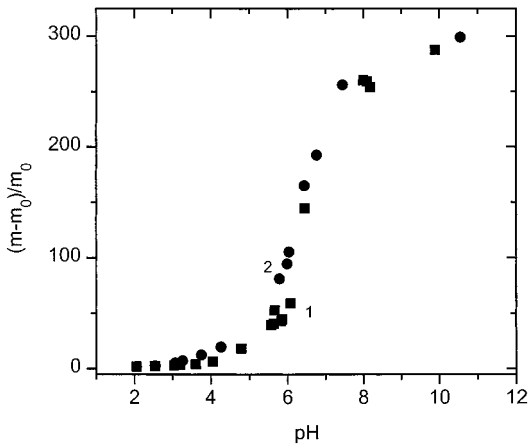

Figure 3. Swelling curves of the HM PAA gel C18-2.5% as a function of the pH of the exterior solution obtained in two ways: by swelling dry gel (1) and by contracting swollen gel (2).

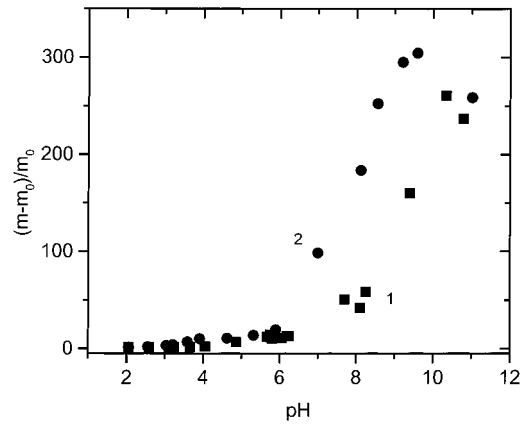

Figure 4. Swelling curves of the HM PAA gel C18-5% as a function of the pH of the exterior solution obtained in two ways: by swelling dry gel (1) and by contracting swollen gel (2).

To check whether the pH-isotherms represent the equilibrium states, they were obtained in two ways: by swelling dry gel and by contracting swollen gel. The corresponding typical swelling curves are depicted in Figures 3 and 4. It was shown that for most of the gels these curves are quite identical (Figure 3). Only for two gel samples (C18-5% and C18-10%) some hysteresis was observed (Figure 4). This can be a consequence of the crystallization of hydrophobic domains consisting of *n*-octadecyl acrylate groups in the dried gel. Such crystallization for the gels of copolymers of acrylic acid and *n*-octadecyl acrylate was studied in ref 9. On the other hand, it was shown[9] that for the copolymer gel of acrylic acid and *n*-dodecyl acrylate the crystallized structure is not formed.

Let us consider the effect of the gel hydrophobicity on the pH-responsive swelling behavior of PAA gel. Figure 1 demonstrates the equilibrium swelling curves for C8 gels with different fractions of *n*-octyl acrylate units, while in Figure 2 the content of *n*-alkyl acrylate units is fixed, but their *n*-alkyl chain length is varied. From these figures it is seen that the pH of the transition becomes higher with increasing fraction and chain length of hydrophobic units. This is consistent with the data obtained for HM polybase gels[4] and for linear HM polyacids.[10−14]

Thus, increasing the hydrophobicity of the HM PAA gel leads to the shift of the swelling transition toward

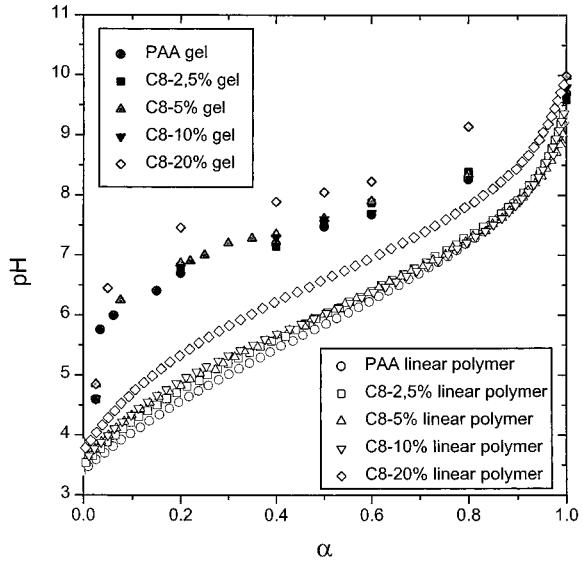

Figure 5. Potentiometric titration curves of PAA gel and of the HM PAA gels C8-2.5%, C8-5%, C8-10%, and C8-20% and of the corresponding linear copolymers.

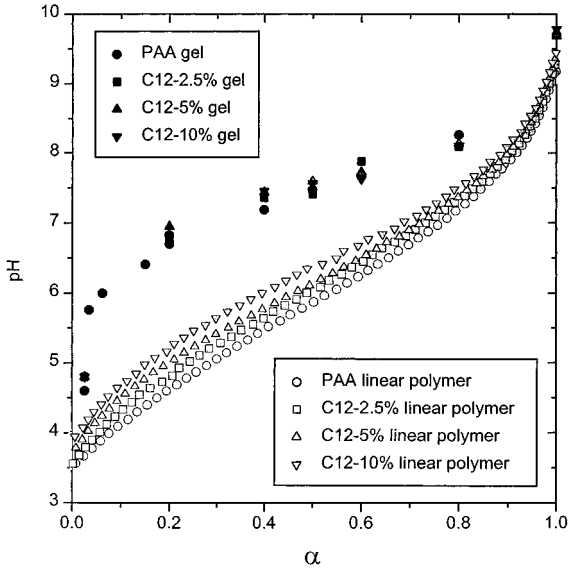

Figure 6. Potentiometric titration curves of PAA gel and of the HM PAA gels C12-2.5%, C12-5%, and C12-10% and of the corresponding linear copolymers.

more alkaline pH. Two possible reasons may be responsible for this effect. First, the hydrophobic groups leading to the effective decrease of the local dielectric constant near the network chain could influence the value of the dissociation constant of acrylic acid units. In this case the same equilibrium pH of the surrounding solution will correspond to a lower degree of ionization for a more hydrophobic gel. The second reason can be related with the formation of hydrophobic domains including *n*-alkyl groups belonging to different network subchains. These domains stabilize the compact structure of a nonionized gel, and a higher degree of ionization is required to destroy these domains. The role of the first reason can be estimated from potentiometric titration data, while the formation of hydrophobic aggregates can be demonstrated by the fluorescent probe method and NMR spectroscopy.

As to the effect of the hydrophobicity on the gel deswelling at high pH, we did not examine it because of the possibility of hydrolysis of ester bonds of HM PAA gels in alkaline medium.

Potentiometric Titration of HM PAA Gels and Linear Copolymers. Typical titration curves demonstrating the equilibrium pH of the external solution as a function of the degree of ionization α of HM PAA gels are presented in Figures 5 and 6. From these figures it is evident that the curves do not depend significantly on the degree of hydrophobicity with the exception of the C8-20% gel. For this gel the potentiometric curve is shifted to higher pH. This can be attributed to a lower dielectric constant inside the C8-20% gel which makes deprotonation more difficult. Thus, the hydrophobic groups affect the ionization of PAA units only when their amount is sufficiently high. Hence, the shift of the swelling transition on pH-isotherms cannot be explained by the lower apparent dissociation constants of HM PAA gels. Analogous results were obtained for the corresponding linear copolymers (Figures 5 and 6). The titration curves are very close for most HM copolymers except for the most hydrophobic ones (C8-20% and C18-10%).

The influence of hydrophobicity can be evidenced more clearly by plotting the pK_{ap} versus α (Figure 7). We used here the values obtained from potentiometric titration of linear HM copolymers (see Figure 5), but

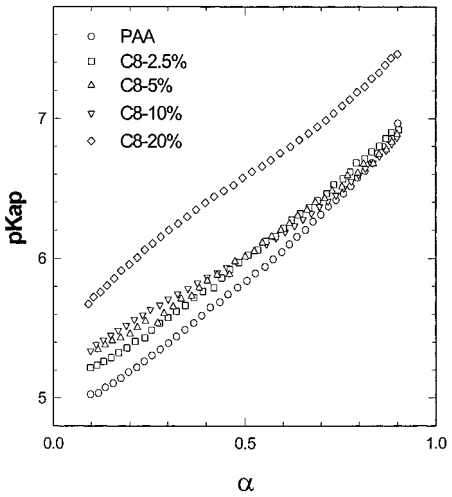

Figure 7. Apparent dissociation constant as a function of the degree of ionization for the linear polymers PAA, C8-2.5%, C8-5%, C8-10%, and C8-20%.

the same tendency holds true for the gels. As we can see from Figure 7, the main difference concerns the C8-20% copolymer which displays pK_{ap} values 0.6−0.7 units higher compared to the PAA ones. The other copolymers, even C8-10%, are close to the PAA reference. A slight splitting of the pK_{ap} curves with alkyl content occurs only at low α values, when hydrophobic interactions overcome the repulsive forces and favor the formation of nonpolar clusters. Although the deviations at low α are small and lie within the limits of experimental error, they look systematic. These deviations are more pronounced for more hydrophobic copolymers.

Let us compare the titration curves for HM PAA gels and the corresponding linear copolymers (Figures 5 and 6). Figures 5 and 6 show that the titration curves for the gels are shifted to the alkaline medium. The main reason for this may be the confinement of most of the counterions inside the gel (due to the electroneutrality condition). As a result the pH inside the gel differs essentially from that of the external solution (for the discussion of this problem see also refs 15 and 16).

The representative swelling curves plotted as a function of the degree of ionization of the gel α are shown

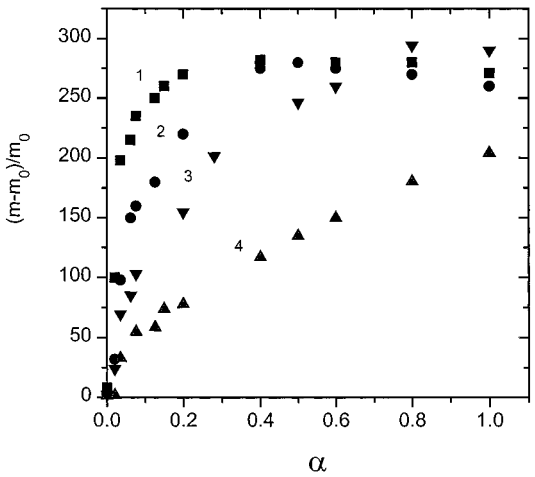

Figure 8. Dependences of the degree of swelling of PAA gel (1) and of the HM PAA gels C8-5% (2), C8-10% (3), and C8-20% (4) on their degree of ionization α.

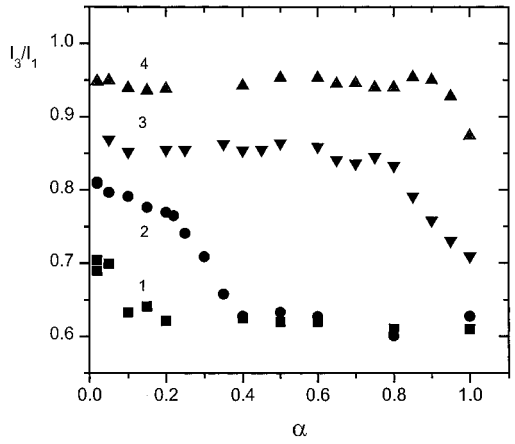

Figure 9. Variation in I_3/I_1 ratio in the fluorescence spectra of pyrene embedded in PAA (1), C8-5% (2), C8-10% (3), and C8-20% (4) gels as a function of the degree of ionization α of the gels.

in Figure 8. From this figure it is seen that for unmodified PAA gel the most dramatic changes of the gel volume take place at very low α and the maximum swelling is reached at $\alpha \sim 0.2$. In a highly charged polymer gel counterions are condensed in the vicinity of the gel chains by a strong electric field, which reduces their osmotic activity.[15−17] Another reason for the flatness of the swelling above $\alpha \geq 0.2$ can be due to full extension of the network subchains.[15,17]

The introduction of only 5 mol % of *n*-octyl acrylate units in PAA gel leads to significantly lower swelling of the gel at small degrees of ionization ($\alpha < 0.2$), at higher α the swelling curve of the C8-5% gel practically coincides with that of the PAA gel. This may indicate that the hydrophobic aggregates which hinder the gel swelling break up, when the degree of ionization exceeds 0.2. This supposition will be confirmed by fluorescence data (see below).

For the more hydrophobic C8-10% gel nonpolar interactions between *n*-alkyl chains maintain the network at a lower level of swelling compared to that for an unmodified gel until the degree of ionization exceeds 0.8.

When the fraction of hydrophobic units is high enough (C8-20% gel), the electrostatic effect cannot overcome the hydrophobic aggregation, and even for the completely ionized gel the degree of swelling is lower than can be expected in the absence of hydrophobic aggregates. The hydrophobic microdomains effectively increase the cross-linking density of the gel.

Fluorescence of Pyrene in HM PAA Gels in the Course of Gel Ionization. To follow the influence of the degree of ionization of a gel on the side chain aggregation, the fluorescence probe method was employed. Pyrene was used as a probe. The ratio of the third to the first vibronic peaks in fluorescence spectra of pyrene, I_3/I_1, is known to be quite sensitive to the polarity of the microenvironment of the probe (e.g., in dodecane I_3/I_1 is ca. 1.67, in ethanol I_3/I_1 is ca. 0.91, and in water I_3/I_1 is ca. 0.63).[18] The formation of hydrophobic microdomains by *n*-alkyl side chains and the penetration of pyrene in these domains should lead to the increase of the values of I_3/I_1.

Figure 9 demonstrates the changes in the I_3/I_1 ratio with ionization of the gels. From this figure it is obvious that the character of this dependence is determined by the hydrophobicity of the gel. For the unmodified PAA gel at $\alpha > 0.05$ the I_3/I_1 ratio shows a little variation with the degree of the gel ionization remaining close to that for pyrene in water (ca. 0.63). This indicates the highly hydrophilic microenvironment of pyrene molecules in the unmodified gel. These results are quite similar to those for linear PAA.[19,20] In a slightly charged PAA gel (with $\alpha < 0.05$) the I_3/I_1 ratio equals 0.70, which suggests a somewhat more hydrophobic microenvironment of pyrene. This may be due to the location of the probe in the vicinity of the uncharged network chains.

For the most hydrophobic C8-20% gel the I_3/I_1 ratio is also practically independent of the degree of ionization (Figure 9). But in this case the value of I_3/I_1 (ca. 0.94) indicates that pyrene is located in a relatively low polarity microenvironment. This can be attributed to the solubilization of the probe in hydrophobic microdomains which appear as a result of the aggregation of *n*-alkyl side chains of the HM gel. It should be pointed out that although the I_3/I_1 ratio indicates the existence of hydrophobic aggregates in a whole range of the degree of ionization, the swelling curve shows gel expansion by two orders of magnitude during ionization (Figure 8). The same I_3/I_1 values for the collapsed uncharged gel and for the highly swollen ionized gel evidence that the low polar microenvironment of pyrene cannot be explained simply by the effective decrease of the average polarity of the medium inside the gel, but it is mainly due to the incorporation of pyrene molecules inside the hydrophobic microdomains. The local environment of the probe embedded in these microdomains is more polar than that in pure hydrocarbons but resembles that of pyrene in surfactant micelles (e.g. in sodium laurate micelles I_3/I_1 is ca. 0.96[18]).

As to the I_3/I_1 vs α curve for a less hydrophobic C8-5% gel, it consists of three distinct regions. In the first one the I_3/I_1 ratio is relatively high (ca. 0.80) and changes only slightly with α. In the second region ($\alpha = 0.2-0.4$) the value of I_3/I_1 drops to 0.63. In the third region ($\alpha > 0.4$) the I_3/I_1 ratio remains constant with further increase of α. In this region the value of I_3/I_1 is close to that of pyrene in water. The variation in the polarity parameter of pyrene embedded in a C8-5% gel indicates that initially in the nonionized gel there are hydrophobic aggregates which break up with gel ionization at $\alpha = 0.2-0.4$. A similar behavior of the I_3/I_1 ratio at the gel ionization was observed for the C8-10% gel. But for this gel the drop of the I_3/I_1 value indicating a disruption of hydrophobic aggregates takes place at much higher α ($\alpha = 0.8-0.9$).

The hydrophobicity of the local environment around pyrene probes in microdomains increases with increasing fraction of *n*-octyl chains: $I_3/I_1 = 0.80$ for the C8-5% gel, $I_3/I_1 = 0.86$ for the C8-10% gel, and $I_3/I_1 = 0.94$ for the C8-20% gel. Taking into account that the observed I_3/I_1 ratio is a measure of the polarity averaged over the various environments experienced by the pyrene probe (i.e. in aqueous solution, in hydrophobic aggregates, etc.), the lower value of I_3/I_1 may be related to a smaller concentration of hydrophobic microdomains. Alternatively, one might imagine that the hydrophobic aggregates in C8-5%, C8-10%, and C8-20% gels differ in structure. In the C8-20% gel the *n*-alkyl side chains could be more densely packed inside the microdomains, which provides more hydrophobic sites for solubilized pyrene molecules.

Thus, the fluorescence measurements revealed that in the most hydrophobic C8-20% gel the hydrophobic aggregates exist over the whole range of the degree of ionization of AA units, while in less hydrophobic C8-5% and C8-10% gels the microdomains are completely destroyed with gel ionization at $\alpha = 0.2-0.4$ and $\alpha = 0.8-0.9$, respectively.

This behavior is quite reminiscent of that for alternating linear copolymers of maleic acid and *n*-alkyl vinyl ethers.[21] It was shown that for slightly hydrophobic copolymers with short *n*-alkyl side chains ($n = 1-3$) the I_3/I_1 ratio is close to that in water independent of α. For the copolymers with intermediate size alkyl groups ($n = 4-8$) a transition from a microdomain-containing conformation to an expanded, hydrated conformation was observed with ionization.[14] For highly hydrophobic copolymers of decyl vinyl ether the I_3/I_1 ratio is equal to 1.03 independent of the degree of ionization of maleic acid units, which indicates the existence of hydrophobic microdomains in the entire range of α.

Although the fluorescence properties of alternating copolymers are very similar to those of HM PAA gels, their swelling and potentiometric behavior is quite different. In the potentiometric data the main difference arises for the copolymers exhibiting a transition from a microdomain-containing conformation to an expanded conformation. For alternating copolymers of maleic acid with *n*-alkyl vinyl ethers (with $n \geq 4$) the potentiometric curves plotted as pH vs α start out more steeply, then flatten out, and finally join the curves of methyl and ethyl copolymers, which represent normal polyacid behavior.[11-14] The initial steepness of the curves is consistent with the compact conformation which hampers the deprotonation, while the flattening is indicative of a conformational transition from a hypercoiled conformation to an expanded conformation. In contrast to this, the disruption of hydrophobic aggregates in HM PAA gels, which is evident from fluorescence data, is not reflected so evidently in potentiometric titration curves. In HM PAA gels the deviations of the titration curves from that of the reference PAA gel are quite small (Figures 5 and 6). This may be ascribed to a low fraction of *n*-alkyl acrylate units in the HM gels under study. Probably, the hydrophobic group should be located in the immediate vicinity of the carboxy groups to affect their dissociation constants. Moreover, the absence of influence of the adjacent hydrophobic units on the potentiometric curve at high α may indicate that only the carboxy groups on the surface of hydrophobic aggregates have different dissociation constants. The amount of such COOH groups should be much higher in the alternating copolymers, where each carboxy group has one adjacent hydrophobic unit.

One more difference arises for highly hydrophobic samples. The more hydrophobic alternating copolymers show at all α the same values of I_3/I_1 for pyrene solubilized in microdomains (as the C8-20% gel).[21] But these alternating copolymers retain the compact conformation over the whole titration range, while the C8-20% gel swells by two orders of magnitude during ionization. One of the possible reasons for this consists in a partial disruption of hydrophobic microdomains in the C8-20% gel in the course of titration. In spite of this disruption the pyrene molecules can prefer to reside in the remaining microdomains rather than in water, which is reflected in almost constant I_3/I_1 values. In order to confirm this supposition, it is important to determine the fraction of *n*-alkyl groups involved in the aggregation as a function of the degree of ionization of the gel.

NMR Measurements of the Fraction of Hydrophobic Groups Included in Microdomains in HM Gels. The fraction of hydrophobic side chains residing in microdomains can be estimated from ^{13}C NMR spectra. NMR provides a very interesting tool to investigate the formation of hydrophobic clusters in aqueous solutions. As a matter of fact, the chemical shift of atoms belonging to a hydrophobic chain depends on their specific environment, which can vary appreciably when moving from a highly polar medium (hydrated chain) to a low polarity one (hydrophobic cluster). For instance, ^{19}F and ^{13}C NMR were extensively applied in the last 30 years to study the micellization of fluorinated and hydrogenated surfactants.[22-24] Above the critical micelle concentration (cmc), the surfactant molecules exhibit very fast exchange between the free and the micellar forms (with respect to the NMR characteristic time), and only a single signal is obtained for the CF_3 (or CH_3) group. This peak, which is moved upfield compared to that of the free surfactant (below the cmc), corresponds to an intermediate value between those of the free and associated forms. More recently, Petit et al.[25] applied successfully ^{19}F NMR to investigate the aggregation process in aqueous solutions of poly(sodium acrylate) grafted with 8 mol % of $CH_2C_7F_{15}$ side chains. Above the critical aggregation concentration (cac), two signals were obtained for the CF_3 group, corresponding to the aggregated and the free forms, respectively. The same feature was also evidenced by ^{13}C NMR with a poly(sodium acrylate) grafted with 10 mol % of *n*-dodecyl chains. Here the splitting is observed for both the penultimate CH_2 and the CH_3 groups.[26] The bands at 14.5 and 14.8 were shown to be due to nonaggregated and aggregated penultimate CH_2 groups, while the bands at 23.1 and 23.5 were ascribed to nonaggregated and aggregated CH_3 groups, respectively.[26] Compared to that of the surfactants, this behavior was attributed to the slowing down of the association dynamics due to polymer backbone which limits the molecular motions of the pendent alkyl chains. In that case, since the dynamics of exchange of hydrophobic groups between the associated and free states is slow, the integration of the two peaks provides a quantitative picture of the ratio between bound and free alkyl chains.

In the present work, ^{13}C NMR was first applied to the study of hydrophobic aggregation in HM gels. We followed by ^{13}C NMR the splitting behavior of the bands of *n*-alkyl groups during ionization for the C8-20% gel.

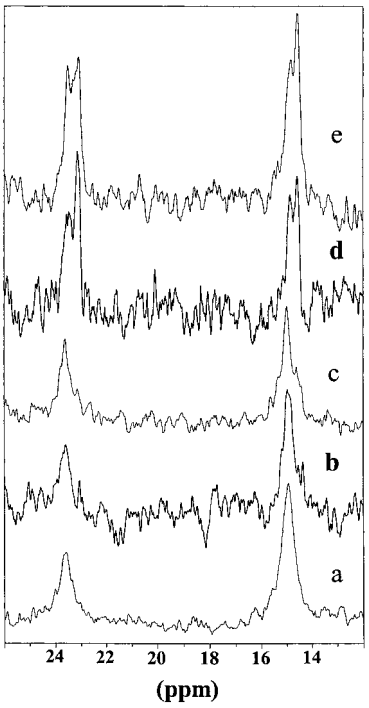

Figure 10. ^{13}C NMR spectra of C8-20% gels swollen to equilibrium in water at different degrees of ionization α: 0.05 (a), 0.2 (b), 0.4 (c), 0.6 (d), 1.0 (e).

The NMR spectra reported in Figure 10 display the region of the chemical shifts of both penultimate methylene and terminal methyl groups (δ = 13.5−24.5 ppm). To fulfill the comparative conditions with the equilibrium swelling curves and the fluorescence data, the gel samples for NMR measurements were prepared, at given α values, at equilibrium degree of swelling of the gel. At these conditions the concentration of polymer chain units in the gel is relatively low; as a consequence, even at a high number of scans the background can be quite noisy. This fact does not allow us to calculate the exact fraction of *n*-alkyl chains residing in hydrophobic microdomains, and we will consider the obtained results only semi-quantitatively.

The first two spectra in Figure 10 (a and b), corresponding to the C8-20% gel at $\alpha \leq 0.2$, show only one peak for each carbon group ($\delta[CH_2]$ = 23.5 ppm and δ-$[CH_3]$ = 14.9 ppm). According to ref 26, these peaks can be attributed to the aggregated form of *n*-alkyl chains. At a further increase of α to 0.4, 0.6, and 1.0 (Figure 10 c−e) new bands corresponding to nonaggregated CH_2 and CH_3 groups[26] appear at 23.1 and 14.5 ppm, respectively. Increasing the degree of ionization leads to an increase of the relative intensity of these bands. Thus, the gel ionization results in a partial disruption of hydrophobic aggregates. But even at α = 1, we can estimate that ca. 40% of the *n*-alkyl chains of the C8-20% gel remain embedded inside the hydrophobic clusters. At the same time, for the less hydrophobic C8-10% gel full ionization results in a complete disruption of hydrophobic aggregates (Figure 11). The NMR spectrum of this gel contains only peaks corresponding to the nonaggregated forms of CH_2 and CH_3 groups (at 14.4 and 22.9 ppm, respectively). Thus, the NMR results correlate well with those obtained from swelling, potentiometry, and fluorescence measurements.

Conclusions

To reveal the role of hydrophobic interactions in the swelling behavior of the gel upon ionization, a series of HM PAA gels with a controlled degree of hydrophobicity was prepared. The variation of the content of ionic and hydrophobic groups in these gels allows us to examine the opposing effects of hydrophobic and electrostatic interactions on the behavior of polyelectrolyte gels.

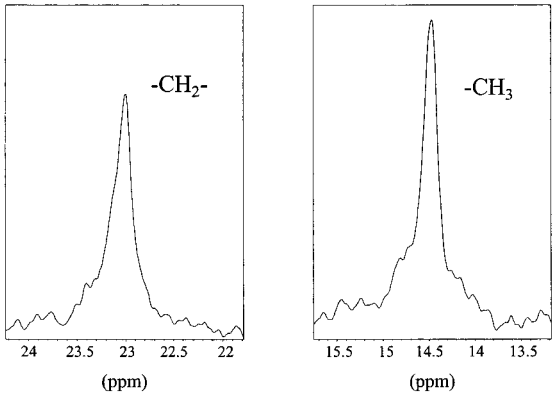

Figure 11. ^{13}C NMR spectrum of fully ionized C8-10% gel swollen in water (α = 1.0).

The swelling transition of HM PAA gels upon ionization was studied. It was demonstrated that the hydrophobic aggregation does in fact play an important role in the swelling behavior of the gels. Increasing the fraction and chain length of *n*-alkyl side chains leads to a systematic shift of the pH of the swelling transition to alkaline medium which is due to the stabilization of the collapsed state of the gel by hydrophobic forces. It was shown that the introduction of hydrophobic groups in polyelectrolyte gel allows us to obtain gels with readily variable transition pH-values.

The formation of hydrophobic aggregates was monitored by means of the fluorescent probe method and NMR spectroscopy. It was shown that in the gels of intermediate hydrophobicity the aggregates of *n*-alkyl side chains break up with gel ionization. The higher the hydrophobicity of the gel, the higher the degree of ionization α needed to destroy these aggregates. In a highly hydrophobic C8-20% gel the hydrophobic aggregation can effectively overcome the electrostatic repulsion, and in this gel the hydrophobic domains exist in the entire range of α. The competition of hydrophobic and electrostatic forces in this gel manifests itself in a decrease of the fraction of aggregated *n*-alkyl side chains with gel ionization. Thus, the hydrophobic microdomains in HM PAA gels may be reversibly destroyed or created by changes in the pH of the medium.

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References and Notes

(1) See, for example: *Adv. Polym. Sci.* **1993**, *109*, *110*.
(2) Brannon-Peppas, L.; Peppas, N. A. *J. Controlled Release* **1989**, *8*, 267−274.
(3) Dong, L.-C.; Hoffman, A. S. *J. Controlled Release* **1991**, *15*, 141−152.
(4) Siegel, R. A. *Adv. Polym. Sci.* **1993**, *109*, 233−267.

(5) Keszler, B.; Kennedy, J. P. *J. Polym. Sci., Part A: Polym. Chem.* **1994**, *32*, 3153−3160.
(6) Khokhlov, A. R.; Starodubtzev, S. G.; Vasilevskaya, V. V. *Adv. Polym. Sci.* **1993**, *109*, 123−171.
(7) Philippova, O. E.; Hourdet, D.; Audebert, R.; Khokhlov, A. R. *Macromolecules* **1996**, *29*, 2822−2830.
(8) Brannon-Peppas, L.; Peppas, N. A. *Polym. Bull.* **1988**, *20*, 285−289.
(9) Matsuda, A.; Sato, J.; Yasunaga, H.; Osada, Y. *Macromolecules* **1994**, *27*, 7695−7698.
(10) Fenyo, J. C.; Delben, F.; Paoletti, S.; Crescenzi, V. *J. Phys. Chem.* **1977**, *81*, 1900−1905.
(11) Strauss, U. P.; Schlesinger, M. S. *J. Phys. Chem.* **1978**, *82*, 571−574.
(12) Martin, P. J.; Strauss, U. P. *Biophys. Chem.* **1980**, *11*, 397−402.
(13) Strauss, U. P. In *Microdomains in Polymer Solutions*; Dubin, P., Ed.; Plenum Press: New York, London, 1985; pp 1−12.
(14) Strauss, U. P. In *Interactions of Surfactants with Polymers and Proteins*; Goddard, E. D., Ananthapadmanabhan, K. P., Eds.; CRC Press: Boca Raton, Ann Arbor, London, Tokyo, 1992; pp 277−294.
(15) Katchalsky, A.; Michaeli, I. *J. Polym. Sci.* **1955**, *15*, 69−86.
(16) Hasa, J.; Ilavsky, M.; Dusek, K. *J. Polym. Sci., Polym. Phys. Ed.* **1975**, *13*, 253−262.
(17) Silberberg-Bouhnik, M.; Ramon, O.; Ladyzhinski, I.; Mizrahi, S.; Cohen, Y. *J. Polym. Sci., Part B: Polym. Phys.* **1995**, *33*, 2269−2279.
(18) Kalyanasundaram, K.; Thomas, J. K. *J. Am. Chem. Soc.* **1977**, *99*, 2039−2044.
(19) Maltesh, C.; Somasundaran, P. *Colloids Surf.* **1992**, *69*, 167−172.
(20) Magny, B.; Iliopoulos, I.; Zana, R.; Audebert, R. *Langmuir* **1994**, *10*, 3180−3187.
(21) Binana-Limbele, W.; Zana, R. *Macromolecules* **1987**, *20*, 1331−1335.
(22) Muller, N.; Birkhahn, R. H. *J. Phys. Chem.* **1967**, *71*, 957−962.
(23) Muller, N.; Platko, F. E. *J. Phys. Chem.* **1971**, *75*, 547−553.
(24) Persson, B. O.; Drakenberg, T.; Lindman, B. *J. Phys. Chem.* **1976**, *80*, 2124−2125.
(25) Petit, F.; Iliopoulos, I.; Audebert, R. *Polymer*, submitted.
(26) Petit, F. Etude du comportement de polyélectrolytes associatifs en solution et caractérisation de leurs agrégats hydrophobes. Ph.D Thesis, University of Paris 6, 1996.

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