

A resonance Rayleigh scattering study of unlabeled and pyrene-labeled poly(acrylic acid) polymers

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ABSTRACT

Three different unlabeled poly(acrylic acid) (PAA) polymers, with molecular weight of 2, 150 and 450 kg/mol, and the correspondent PAA labeled with pyrene (PAAMePy polymers) were investigated, in aqueous solution and in dioxane: water mixtures, using the resonance Rayleigh scattering (RRS) technique. The pH showed to strongly affect the polymer conformation and a decrease of the scattering intensity (I_{RRS}) was observed, which is consistent with an increase of the hydrodynamic radius, reflecting an expanded polymer conformation in solution. The obtained pK_a values were found higher for the labeled by comparison with the corresponding unlabeled polymers. For the PAAMePy polymers the increment of the water content in dioxane:water mixtures leads to an increase of the I_{RRS} intensity; however, for the unlabeled PAA polymers non-significant differences were observed. In general, for the fluorescently labeled polymers, a good agreement between the present RRS results and those previously obtained from fluorescence measurements could be found.

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1. Introduction

Polymers in general and hydrophobically modified polymers (HMP), in particular, are of major importance from industrial and academic points of view. Their interaction with surfactants, are frequently used in pharmaceutical formulations [1,2], cosmetics [1,2] and in all modern water based paints [3,4]. From a pure academic point of view their conformation and the complex formation with other amphiphilic molecules, such as surfactants and cyclodextrins, is currently gaining a renewed interest.

Fluorescence has shown to be a powerful tool to investigate this kind of systems. The photophysical behavior of poly(acrylic acid) randomly labeled with pyrene, with different molecular weights and degrees of labeling, has been extensively studied through absorption and fluorescence techniques [5,6]. Pyrene, due to its unique characteristics, long singlet lifetimes, excimer formation, vibronic structure dependence with polarity [7–9], has a widespread use as a unique probe in the colloidal domain. One of the most useful relations is the excimer-to-monomer ratio (I_E/I_M), which can be directly related with the dynamic behavior of the polymer in solution. For a negatively charged polyelectrolyte, it was shown that by increasing the pH, the polymer conformation changes from a compact to an expanded coil [5,6]. The I_E/I_M ratio variation has also showed to be very useful to detect the complex formation between the PAAMePy polymers and cyclodextrins

[10,11]. Additionally, the vibronically resolved structure of the monomer emission—the ratio between the first and third vibronic band of the monomer emission, the I_1/I_3 ratio—exhibits high sensitivity to changes in the polarity of the local environment felt by the pyrene chromophore and has also been extensively used as a polarity scale [12–18].

Nevertheless, several techniques, besides fluorescence, can be used to study polymer conformation and complex formation, such as conductivity, [19,20] light scattering, [21] NMR, [19,20], etc. Resonance Rayleigh scattering (RRS) is a relatively new technique that has been successfully employed in the study of macromolecular and biological systems. When light interacts with molecules (polymers or chromophores) it can be absorbed or scattered. The scattering process occurs due to the differences in the refractive index of the molecule and the solvent [22,23]. The difference between light scattering and RRS techniques lies in the scattering wavelength; in the case of the light scattering technique, the measurement is performed away from the absorption band whereas in the case of RRS the scattering is observed at, or close to, the molecular absorption band. In the RRS technique the frequency of the absorbed electromagnetic wave is equal to the scattering frequency. Due to the high absorption of light energy by the electron, light will be re-scattered. An increase of the scattering intensity is then observed, constituting the basis of the RRS technique. Among other factors, this increase depends on the molar absorption coefficient [23,24] and on the volume of the scattering object [23]. Examples of applications of RRS can be found in the study of the interaction of heparin with diphenyl naphthylmethane dyes [24] and anthra-

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cycline antibiotics with surfactants [25]. The technique was also successfully applied to the determination of the critical aggregation concentration (cac) of proteins induced by Al(III) [26] and of surfactants in the absence and presence of β -cyclodextrin [27]; and on the interaction between poly(acrylic acid) and SDS and CTAB surfactants [28].

2. Experimental

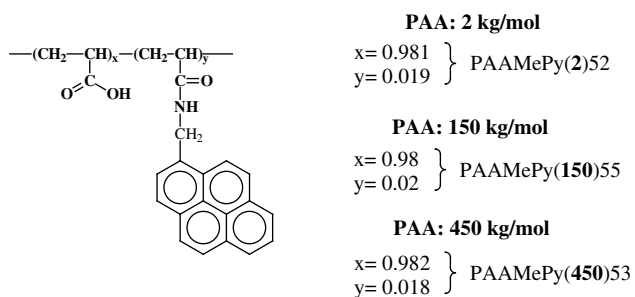
2.1. Polymers

The unlabeled poly(acrylic acid) polymers with nominal weight $M_n = 2000, 150,000$ and $450,000$ g/mol (Aldrich) were used as received. Their acronyms are based on their molecular weight, i.e., PAA(2), PAA(150) and PAA(450), where 2, 150 and 450 stands for the 2000 g/mol, 150,000 g/mol and 450,000 g/mol polymers, respectively. The PAA polymers randomly labeled with 1-pyrenylmethylaminehydrochloride (Aldrich) were synthesized according to the procedure described elsewhere [5,6]. All reagents and solvents used in the synthesis were obtained from commercial sources and used as received. The pyrene (Py) labeling content was determined by UV spectroscopy by comparison with the parent compound 1-pyrenylmethylamine ($\epsilon = 37070 \text{ M}^{-1} \text{ cm}^{-1}$ in methanol). The nomination of the polymers was again based on the molecular weight of the PAA polymer and on the Py content of the polymers. The polymers here investigated are denoted as PAAMePy(2)52, PAAMePy(150)55 and PAAMePy(450)53 where 52, 55 or 53 corresponds to number of PAA monomer units per Py chromophore, respectively (Scheme 1).

The solvents used in the polymer solutions were of spectroscopic or equivalent grade. Water used for the polymer solutions was twice distilled and passed through a Millipore apparatus. The measured pH values were obtained with a Crison microPH 2000 and adjustments of the hydrogen ion concentration of the solutions were made with dilute HCl and NaOH solutions.

2.2. Experimental techniques

The fluorescence and RRS spectra were recorded with a Horiba-Jobin-Ivon SPEX Fluorog 3-22 spectrometer and were corrected for the instrumental response. The Fluorolog consists in a modular spectrofluorimeter with double grating excitation (range 200–950 nm, optimized in the UV and with a blazed angle at 330 nm) and emission (range 200–950 nm, optimized in the visible and with a blazed angle at 500 nm) monochromators. The excitation source consists of a ozone-free 450 W Xenon lamp and the emission detector is an Hamamatsu R928 Photomultiplier (200–900 nm range), cooled with a Products for Research thermoelectric refrigerated chamber (model PC177CE005), or a Hamamatsu R5509-42 (900–1400 nm range), cooled to 193 K in a liquid nitrogen chamber (Products for Research model PC176TSCE-005-) and a photodiode as the reference detector. For the RRS experiments, the



Scheme 1.

excitation and emission spectra were recorded in the 200–700 nm range with synchronous scanning at $\lambda_{ex} = \lambda_{em}$ (i.e., $\Delta\lambda = 0$ nm); the slit widths are of 2.0 nm/2.0 nm (Ex/Em) and 3.0 nm/2.0 nm (Ex/Em) for the labeled and unlabeled systems, respectively.

3. Results and discussion

3.1. Influence of the molecular weight of the PAAMePy polymers on the RRS spectra

3.1.1. Aqueous solutions

Three different molecular weight poly(acrylic acid) polymers (2000, 150,000 and 450,000 g/mol) labeled with pyrene, PAAMePy(2)52, PAAMePy(150)55 and PAAMePy(450)53, are the subject of the current investigation. The RRS spectra of the PAAMePy polymers in aqueous solution are presented in Fig. 1. From the spectra in Fig. 1 five peaks at $\sim 294, \sim 304, \sim 318, \sim 336$ and ~ 376 nm can be observed, and shown to be independent of the molecular weight of the PAA polymer. The absorption spectra of the pyrene-labeled polymers display the characteristic vibronically resolved spectra of this chromophore but are red-shifted (8–10 nm) relative to pyrene itself (see the inset in Fig. 1 for an example of the absorption spectra of pyrene-labeled polymer).

For a polymer concentration of 0.1 g/l, the intensity of the peak at 375 nm is approximately constant and independent of the molecular weight of the PAA polymer. However, the peak with a maximum scattering wavelength at ~ 294 nm significantly increases upon going from the low ($M_w = 2000$ g/mol) to the high ($M_w = 450000$ g/mol) molecular weight PAA polymer, showing that by increasing the polymer M_w an increase of the RRS scattering intensity, I_{RRS} , is observed. Indeed, the excess of Rayleigh ratio promoted by the concentration fluctuations in a solution of small independent particles ($n \sim n_0$) is given by the following equation [29]

$$\Delta R_\theta = \left[\frac{4\pi^2 n_0^2 (\partial n / \partial c)}{N_A \lambda^4} \right] \cdot M c = K M c \quad (1)$$

where $\partial n / \partial c$ is the refractive index increment, M is the molar mass, N_A is Avogadro's number, λ is the wavelength of light, K is the optical constant and c the concentration. Thus, in a diluted solution the increase of the molecular weight of the solute, or in this case of the polymer, leads to an increase of the scattered light and consequently of its intensity.

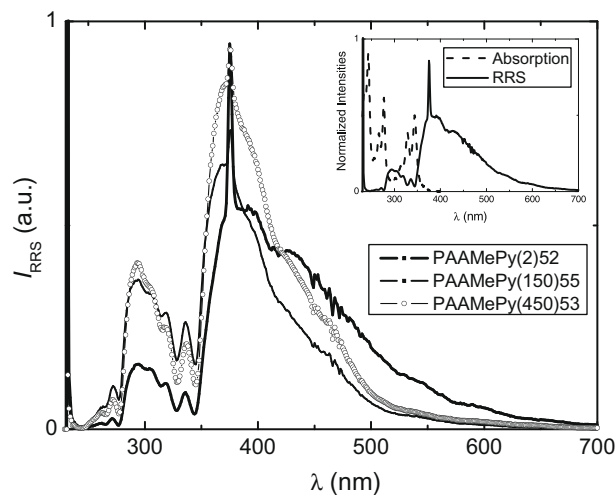


Fig. 1. RRS spectra of the PAAMePy(2)52, PAAMePy(150)55 and PAAMePy(450)53, at pH 3. As inset is shown the absorption and the RRS spectra of the PAAMePy(2)52 polymer, at pH 3.

Fig. 2A shows the effect of the pH on the RRS spectra of the PAAMePy(150)55 polymer. A general decrease of the I_{RRS} is observed with the pH; however, the ratio between the maximum scattering intensity and the other peak intensities also varies with pH, Fig. 2A (inset), suggesting that this ratio is probing the conformational changes suffered by the polymer. The dependence of the ratio between the intensities at $\lambda \sim 294$ nm and $\lambda \sim 375$ nm, defined as I_B/I_F (R) is for the PAAMePy(2)52, PAAMePy(50)55 and PAAMePy(450)53 polymers, presented in Fig. 2B. For the long M_w polymers [PAAMePy(150)55 and PAAMePy(450)53] a decrease of the I_B/I_F ratio with the pH, which levels on up to $\text{pH} \sim 7$, is observed. The use of an intensity ratio instead of the absolute RRS intensity at a given and particular wavelength (I_{RRS}) constitutes an internal correction that was introduced to avoid some of the dispersion that occurs in the RRS intensity values, due to slightly different experimental conditions: e.g., concentration, pH, temperature, etc., which could not be avoided if, instead, we had only considered the I_{RRS} signal.

At a given and specific wavelength the RRS intensity depends on the absorption at this same wavelength, i.e., to a maximum absorption value usually corresponds a higher scattering intensity [23]. For a constant absorption value (concentration), the RRS technique can be used to follow the conformational changes of the polymer chain. The relationship between the RRS intensity and the size of the polymer chain was recently described by Li et al. [28] which showed that the RRS intensity is reciprocally proportional to the hydrodynamic radius (R_H) of the molecule. Both I_{RRS} and I_B/I_F ratio decrease with the increasing pH; this decrease reflects the augment of the R_H for the long size chain PAAMePy polymers at high pH values. At low pH, the pyrene–pyrene interactions, within the

PAA chain, lead to the adoption of a coiled conformation (high I_{RRS} and I_B/I_F values) [5,6]. The increase of the pH leads to the progressive ionization of the carboxylic groups and the electrostatic repulsion between the carboxylate groups competes with the hydrophobic interactions between adjacent pyrene groups [5,6]. At alkaline pH values the former forces become dominant and an expansion of the PAA chain is then observed which is mirrored by the decrease of the I_{RRS} and I_B/I_F ratio values. Indeed, similar conclusions were previously drawn from fluorescence studies [5,6]. As stated in the experimental section, the adjustments of the pH were made with dilute HCl and NaOH solutions. In previous fluorescence studies on a similar PAAMe polymer labeled with naphthalene [30] the dependence of the excimer-to-monomer ratio (I_E/I_M) ratio with the pH was performed with two different acids [HCl and perchloric acid (HClO_4)], showing that the same effect was obtained with different counterions and that these do not contribute significantly to the change in the polymer conformation. Consequently, it is not likely that the observed changes in the RRS spectra result from the effect of the chlorine ions on the conformation adopted by the polymer coil, as they did not for analogous polymers.

The dependence of the I_B/I_F ($=R$) ratios with the pH presented in Fig. 2B, were fitted to a sigmoidal Boltzman-like equation according to the following equation [5]

$$R = I_0 + \frac{I_1 - I_0}{1 + e^{\frac{\text{pH} - \text{pK}_a}{\text{d pH}}}} \quad (2)$$

from which the pK_a could be estimated. The obtained values, for the PAAMePy(150)55 and PAAMePy(450)53 polymers, were, respectively, ~ 5.1 and ~ 5.2 . From previous spectral and photophysical investigations on the PAAMePy(150)55 [6] and PAAMePy(450)53 [5] polymers the determination of the pK_a values of these polymers could be obtained from the peak-to-valley (P_A) ratio of the $S_0 \rightarrow S_2$ absorption band and found to be, respectively, 5.8 and 5.5. From these results it can be seen that a good agreement between the values obtained from absorption and RRS techniques could be obtained.

In the case of the PAAMePy(2) polymers, a different behavior was found. As can be seen from Fig. 2B, there is first an increase of the I_B/I_F ratio with pH until $\text{pH} \sim 4$, and only above this value the I_B/I_F ratio progressively decreases to the initial values. This unusual feature was also observed with the dependence of the I_E/I_M ratio with the pH, where the I_E/I_M ratio increases up to values close to the pK_a value, followed by a slight decrease of the I_E/I_M ratio [5]. The initial increase of the I_B/I_F ratio (and I_E/I_M ratio) [5] can be explained by a contraction of the PAAMePy(2)52 polymer chain, i.e., the pyrene–pyrene interactions are promoted by the introduction of some charges in the system and the polymer adopts a “micelle-like” conformation where the (essentially two) pyrenes are located [5]. A further increase of the alkalinity of the media, leads to a predominance of the (repulsive) electrostatic forces with an expansion of the polymer chain with the consequent loss of the “micelle-like” structure. In the case of the short chain polymer the inflexion in the I_B/I_F ratio with pH is found between $\text{pH} 4$ and 5 , which is, within the experimental error, in very good agreement with the maximum observed in the I_E/I_M ratio as a function of the pH [5]. However, it should be stressed that the I_B/I_F ratio is not related with the monomer–excimer kinetics, i.e., with the I_E/I_M ratio. The I_E/I_M and I_B/I_F are two different ratios (obtained from different experiments) that give complementary information on the conformational changes of the pyrene-labeled polymers submitted to different external factors. It is also worth noting that the I_B/I_F ratio is not present in the unlabeled PAA polymers.

Another important observation is the fact that when the wavelength of Rayleigh scattering is located at, or close to, the absorp-

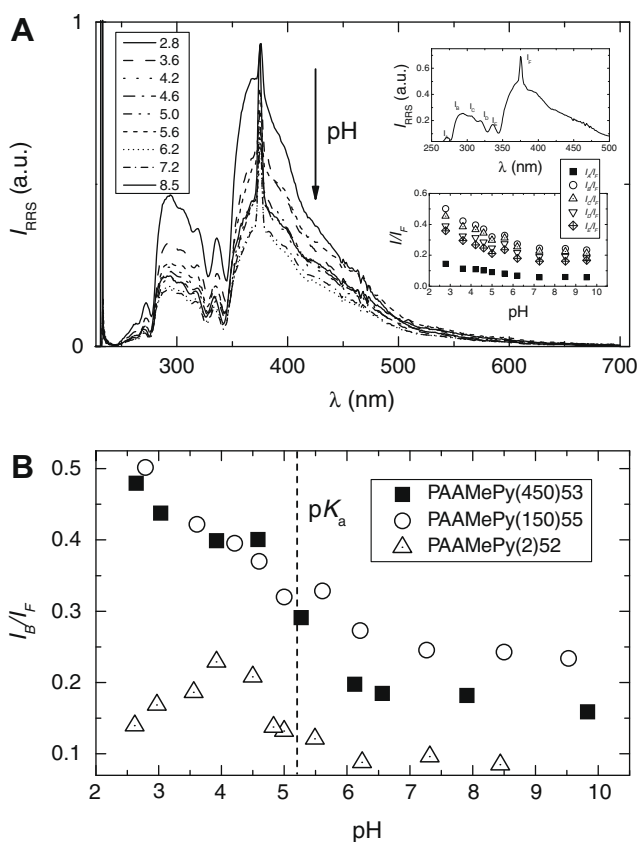


Fig. 2. (A) RRS spectra at different pH values for the PAAMePy(150)55 polymer, $c = 0.1$ g/l. Shown as inset are the I_A/I_F , I_B/I_F , I_C/I_F , I_D/I_F and I_E/I_F ratios as a function of the pH. (B) Dependence of the I_B/I_F ratio obtained from the RRS spectra on the pH, for three different polymers: PAAMePy(2)52, PAAMePy(150)55 and PAAMePy(450)53.

tion band, the scattering intensity is enhanced by several orders of magnitude and the scattering intensity, that is, the resonance Rayleigh scattering (RRS) intensity, deviates from the Rayleigh law ($I \propto 1/\lambda^4$) [24]. For this reason, the RRS spectra is also influenced by the different electronic transitions [24]; consequently, several components contribute to the observed spectra, namely the absorption and fluorescence of the chromophore, the solvent and also the overlap between the excitation and emission spectra [31]. In our study all the RRS spectra were corrected for the instrumental response of the equipment (such as the Xe lamp profile, monochromator and detector response, etc.); however, as can be seen from the inset in Fig. 1, and particularly in the wavelength region between 250 and 400 nm, the RRS bands clearly results from the contributions of the pyrene chromophore absorption and fluorescence (the peak at ~ 376 nm corresponds to the maximum emission wavelength of the pyrene monomer). However, if one observes the red region of the spectra (>385 nm, outside the absorption and emission region of the pyrene chromophore) a decrease of the RRS intensity with the incident wavelength is clearly seen. Hence, we performed the correction of the obtained RRS spectra by taking into account the solvent contribution and we neglected the intensity values below 380 nm. The I_{RRS} obtained at 385 nm is plotted as a function of the pH, and it is presented in Fig. 3. From the normalization of the I_{RRS} intensity at 385 nm with the I_B/I_F ratio, it can be seen that they are in agreement with one another. A clear indication of the two conformational regimes (coil and expanded) is present in both data, proving that the I_B/I_F ratio (which includes the contributions from fluorescence and absorption processes) can also be used to monitor the changes of the polymer conformational changes.

3.1.2. Dioxane:water mixtures

In dioxane:water mixtures significant differences, relative to the situation in water, were observed in the absorption and emission spectra of the PAAMePy polymers. The PAAMePy polymers are amphiphilic molecules and in aqueous solution they can orient themselves in a way that they expose their hydrophilic groups toward water and simultaneously protect their hydrophobic (pyrene) groups from the contact with water and, consequently, they are able to form hydrophobic microdomains. Dioxane is a good solvent to the pyrene groups being completely miscible with water. Therefore, the presence of dioxane in an aqueous solution significantly increases the quality of the solvent to the hydrophobic probe. The pyrene-solvent contacts become more favorable and the polymer adopts a structure of a random coil, which can be mirrored by

a decrease in the intramolecular interactions occurring between the pyrene groups (decrease of the I_E/I_M ratio). A decrease of the overall scattering intensity (Fig. 4A) and of the I_B/I_F ratio (Fig. 4B) was also observed for the long chain polymers.

In the case of the short-size chain polymer, PAAMePy(2)52, a slight decrease of the I_B/I_F ratio was also observed; however, the decrease of the I_B/I_F ratio and of the overall I_{RRS} is not as high as in the case of the long-sized chain polymers labeled with pyrene. The reason for the less abrupt decrease on the scattering intensity lies, not only on the lower molecular weight of the polymer (minor changes in the R_H), but also, and essentially, in the fact that 59% of the PAAMePy(2)52 chains are unlabeled, 31% chains are single-labeled and only 8.4% and 1.6% chains possess two and three pyrene units, respectively [5]. As a consequence, the gradual increment of the good solvent contribution has a small effect on the unlabeled or single-labeled polymer chains. It is likely that only the PAAMePy(2)52 polymer chains with two or more pyrene groups will show a detectable change in their conformation in solution. The dependence of the I_E/I_M ratio with the molar fraction of dioxane showed, however, a different behavior. Indeed, the excimer emission reaches a maximum at a molar fraction of dioxane (x_{Dx}) equal to 0.083 decreasing (with the increase in x_{Dx}) from thereon. The initial increase in the I_E/I_M ratio is probably due to the existence of van der Waals forces between the pyrene groups. This increase, of the intramolecular interactions between the pyrene groups of the PAAMePy(2)52 polymer, was not detected through the RRS spectra due to the fact that no significant changes in the polymer conformation could be observed. The increase of the I_E/I_M ratio is mainly promoted by small movements of the PAA chain and not by a contraction (decrease of the R_H) of the polymer. Up to $x_{Dx} \sim 0.083$, the dioxane content in solution is high and favorable for the pyrene probe to overcome the contributions from the van der Waals interactions between the pyrene groups, consequently leading to a decrease of the pyrene-pyrene interactions, i.e., to an expansion of the polymer chain. A parallel behavior was found with the analogous polymer ($M_w = 150$ kg/mol) randomly labeled with naphthalene, Np, in organic solvent:water mixtures, [32] in aqueous solution [30] and in the presence of surfactants [33].

3.2. Influence of the labeling on the RRS spectra

Being the PAAMePy polymers randomly labeled with pyrene, they allow the study of the dynamics of the polymer in a situation where only intramolecular interactions are found to be present.

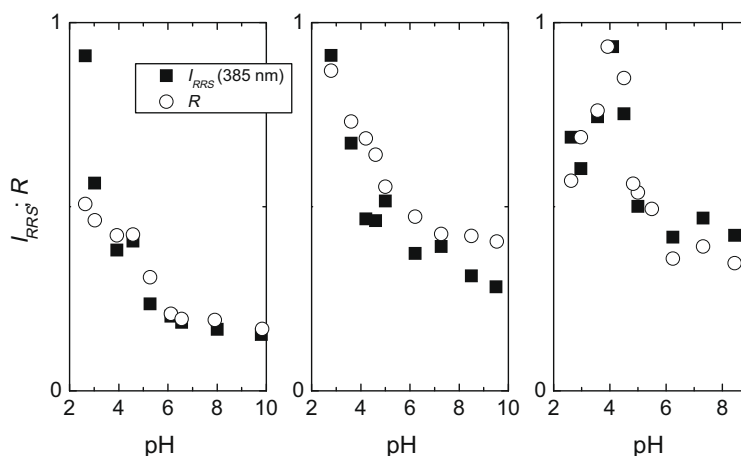


Fig. 3. Dependence of the RRS intensity at 385 nm [$I_{RRS}(385\text{ nm})$] and of the R ratio with the pH, for the PAAMePy(450)53, PAAMePy(150)55 and PAAMePy(2)52 polymers, $c = 0.1$ g/l. The $I_{RRS}(385\text{ nm})$ and the R ratio were normalized in order to allow a direct comparison of the data.

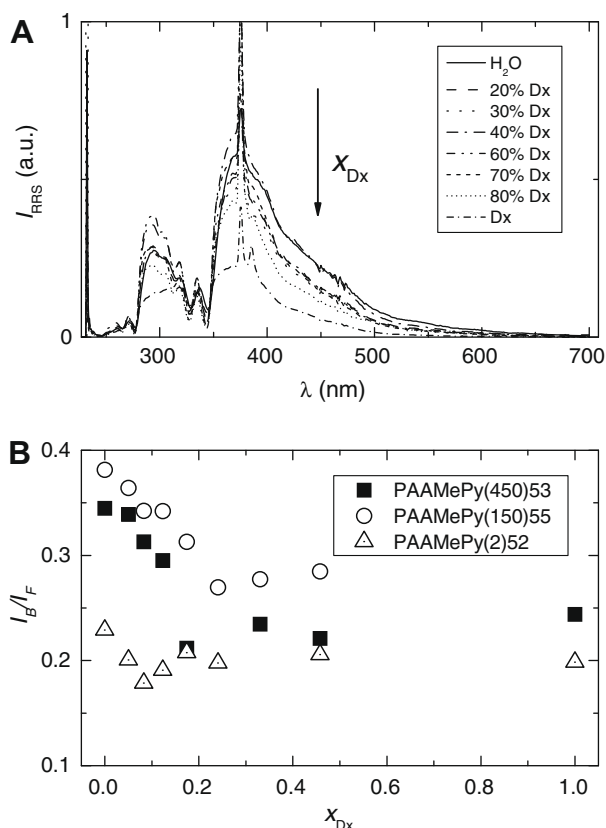


Fig. 4. RRS spectra of the PAAMePy(450)53 polymer in dioxane:water mixtures, $c = 0.1$ g/l. Dependence of the I_B/I_F ratio obtained from the RRS spectra on the molar fraction of dioxane, for three different polymers: PAAMePy(2)52, PAAMePy(150)55 and PAAMePy(450)53.

The RRS technique can be used to follow the conformational changes of the PAA polymers with and without labeling.

In this section, we wish to investigate the effect of the probe (labeling) on the PAA conformation in solution as seen by RRS. Fig. 5 shows the RRS spectra of three different poly(acrylic acid) polymers: PAA(2), PAA(150) and PAA(450). Comparison of the unlabeled with the correspondent labeled PAA polymers (presented in Fig. 1), shows that the introduction of pyrene groups onto

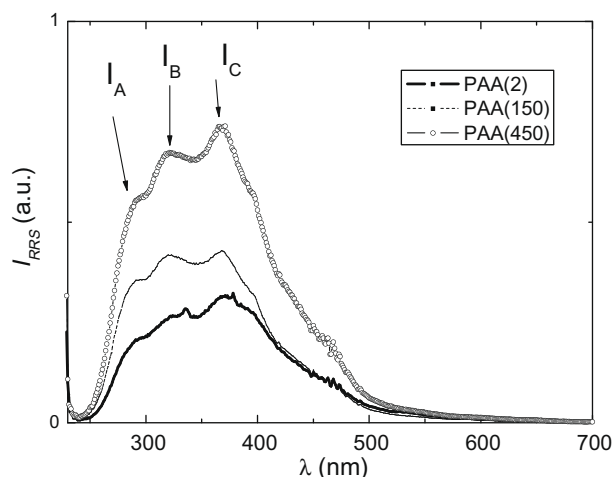


Fig. 5. RRS spectra of PAA(2), PAA(150) and PAA(450) polymers in aqueous solution, at pH 3.5 and $c = 0.1$ g/l.

the PAA chain promotes significant differences in the RRS spectra. Indeed, the RRS spectra of the PAAMePy polymers corresponds to the RRS pattern found for the pyrene probe itself (data not shown). Contrary to what was observed in the RRS spectra of the Py labeled PAA polymers, where five peaks were found, for the unlabeled PAA polymers only three relevant peaks, with maximum intensities at 292 (I_A), 320 (I_B) and 366 nm (I_C), could be observed. Li et al. [28] have recently performed a study on the influence of the pH on the RRS spectra of a similar unlabeled PAA polymer ($M_w = 240,000$ g/mol) using the RRS technique. The RRS spectra, obtained for that polymer, showed a maximum scattering wavelength at 401 nm, with two less intense scattering peaks at 471 and 536 nm, respectively, which is in contrast with the RRS spectra shown in Fig. 5. Also from the data presented in Fig. 5, the shape of the RRS spectra should be independent of the molecular weight of the PAA polymer. This difference could again be potentially explained by the different experimental conditions, namely on the correction factors introduced, such as the monochromators bandpass, Xe lamp profile, etc. [31] on the RRS spectra.

The comparison of the pH and solvent effect on the PAA and PAAMePy polymers will be here presented, as an illustrative example, for the high molecular weight PAA(450) and PAAMePy(450)53 polymers, since the same behavior was found with the other investigated polymers. The changes in the PAA(450) polymer will be presented in terms of the ratio between the intensity at 320 (I_B) and 366 nm (I_C), I_B/I_C ratio, since these two seem to show the more straightforward trends with pH.

Fig. 6 shows the dependence of the I_B/I_C and I_B/I_F ratios as a function of the pH for, respectively, the unlabeled PAA(450) and for the correspondent pyrene-labeled polymer [PAAMePy(450)53]. In the two cases, a decrease of the R ratio is observed, consistent with an expansion of the polymer chain. A more pronounced effect is observed with the pyrene-labeled PAA chain due to the presence of attractive hydrophobic interaction, within the PAAMePy(450)53 polymer, leading to a more compact coil at acidic pH values. A decrease of the I_B/I_C ratio was also found for the PAA(2) polymer (not shown). Comparison between the labeled, PAA(2), and the unlabeled, PAAMePy(2)52, polymers (Fig. 2B) shows that in the case of these short-size chain polymers, the presence of the probe affects the behavior of the PAA chain in solution. This shows, as stated above, that the intramolecular interactions between the pyrene groups are responsible for a higher level of contraction of the polymer, observed at acidic pH values. When the attractive hydropho-

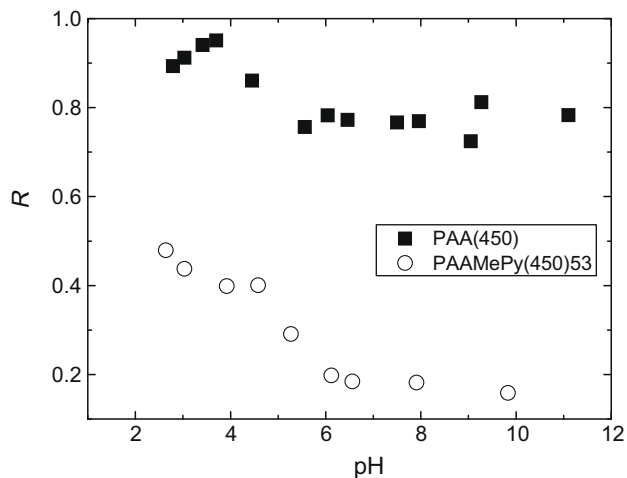


Fig. 6. Plot of the R ratio as a function of the pH for the PAA(450) and PAAMePy(450)53 polymers. The R ratio consists in the I_B/I_C and the I_B/I_F ratios for the PAA(450) and PAAMePy(450)53 polymers, respectively.

bic interactions, within the polymer chain, are absent, the short chain polymers behave as normal polyelectrolytes.

The fit of the dependence of the I_B/I_C ratio with the pH, using Eq. (2), allows the determination of the pK_a values for the PAA polymers. The obtained value is ~ 4.6 for the PAA(150) and PAA(450) polymers, in agreement with the literature values (ca. 4.7 at 25 °C) [34]. For the unlabeled polymers, the pK_a values are lower than those of the correspondent labeled polymers, thus showing that the introduction of the pyrene probe increases the apparent pK_a of the PAA polymer. The hydrophobic interaction, between the pyrene groups, leads to a more compact conformation than that adopted by the unlabeled polymer. The potentiometric curves obtained for the labeled polymers [5] have a similar behavior to that found with poly(methacrylic acid), [35,36] but much less intense in the former case. In the case of poly(methacrylic acid) the anomalous behavior was explained by a cooperative globule-to-coil transition at low degrees of ionization, followed by a further expansion of the polymer chain, consequence of the coil-to-expanded conformation transition [35,36]. In the case of PAAMePy(150)55 and PAAMePy(450)53 polymers, the anomalous behavior was attributed to the formation of hydrophobic microdomains, within the polymer chains, at low pH values, which lead to an increase of the pK_a value relative to the unlabeled polymer.

In the case of short-sized chain polymer, the pK_a value could not be obtained. A decrease of the I_B/I_C ratio with the pH was also observed, but due to the experimental error associated to the obtained values, no accurate pK_a value could be determined. In the case of the labeled PAAMePy polymers the pK_a value was determined even with for the 2000 g/mol M_w polymer. The higher sensitivity of the RRS technique for the pyrene-labeled polymer, when compared with the correspondent unlabeled polymer, is explained by the higher molar extinction coefficient (ϵ) of the former polymer, which is directly related with the RRS intensity [23].

In dioxane:water mixtures, the R ratio seems, for the unlabeled polymer, and within the experimental error, to display rougher constant values (Fig. 7). However, with the labeled polymer(s) there is a change in the R ratio (see Figs. 4B and 7) which shows that the expansion of the PAA chain, of the PAAMePy polymers, with the increment of the dioxane content in the mixture is, again, due to the presence of hydrophobic groups covalently bound to the PAA chain. In the absence of pyrene groups this effect is not observed and minor changes in the PAA polymer chain are promoted by an increase of the dioxane content in the mixture.

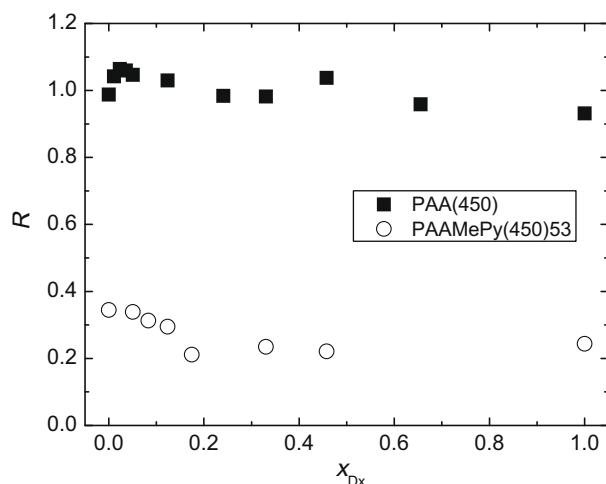


Fig. 7. Plot of the R ratio as a function of the molar fraction of dioxane for the PAA(450) and PAAMePy(450)53 polymers. The R ratio consists in the I_B/I_C and the I_B/I_F ratios for the PAA(450) and PAAMePy(450)53 polymers, respectively.

4. Conclusions

A RRS study on low and high M_w PAA labeled (with pyrene) and unlabeled polymers was performed showing that identical conclusions could be drawn from those obtained from previous fluorescence investigations. The RRS technique showed to be very useful to compare the behavior of these polymers in solution in the absence and presence of tagged pyrene groups. In the case of the high M_w polymers, the same behavior was found for both the labeled and unlabeled polymers in aqueous solution: predominance of the associative behavior at low pH (high I_{RRS} values) and predominance of the repulsive electrostatic repulsions at high pH (low I_{RRS} values). For the low M_w polymers the presence of the pyrene probe tagged to the PAA(2) chain affects their behavior in solution: the unlabeled polymer shows a typical behavior of a polyelectrolyte, i.e., the increase of the pH leads to an expansion of the polymer chain due to the predominance of repulsive electrostatic interaction between the deprotonated groups, whereas in the case of the labeled polymers the adoption of a “micelle-like” conformation was detected at intermediate pH values. In dioxane:water mixtures, the dioxane content in the mixture has little effect on the conformation adopted by the unlabeled polymers, whereas in the case of labeled polymers an expansion of the PAAMePy polymers was observed due to the increase of the dioxane content of the mixture. The overall study showed that the RRS technique can be easily performed in a spectrofluorimeter (where the steady-state fluorescence experiments are also obtained), prompting for the importance of this approach as a useful and complementary technique for the investigation of macromolecular structures.

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