Scientific Paper

Fluorimetric and Potentiometric Study of the Conformational Transition of Isotactic and Atactic Poly(methacrylic Acid) in Mixed Solvents†

Boštjan Jerman and Ksenija Kogej*

Department of Chemistry and Biochemistry, Faculty of Chemistry and Chemical Technology, University of Ljubljana,
Aškerčeva 5, P. O. Box 537, SI-1000 Ljubljana, Slovenia
E-mail: ksenia.kogej@fkkt.uni-lj.si

Received 08-05-2006

† Dedicated to the memory of Prof. Dr. Davorin Dolar

Abstract

Fluorimetric and potentiometric titrations of isotactic (i-PMA) and atactic poly(methacrylic acid) (a-PMA) were performed in methanol-water and in dioxane-water mixtures with varying contents of organic solvent. Fluorimetric investigation of a-PMA confirmed previous reports that the conformational transition of the atactic chain disappears when organic solvent content exceeds 40 and 20 % of methanol and dioxane, respectively. For the i-PMA chain, potentiometric data revealed that the conformational transition occurs in methanol-water mixtures with up to 60 % of methanol in approximately the same region of degree of neutralization (α_N) as in water. Besides, the irreversibility of this transition does not disappear. On the other hand, the discontinuity in the potentiometric titration curve in dioxane-water mixtures is no longer detectable in 50% dioxane. Concurrently, i-PMA dissolves in 50% dioxane at $\alpha_N = 0$, whereas it is not soluble in unneutralized state in any of the pure solvents. The observed behavior is attributed to high tendency of dioxane for hydrogen bond-formation both with water and with PMA. The nature of the change in chain dimensions upon neutralization for i-PMA in 50% dioxane is discussed. Results are discussed also in view of a high intermolecular association tendency of PMA chains.

Keywords: isotactic poly(methacrylic acid), irreversible conformational transition, mixed solvents, methanol-water, dioxane-water

1. Introduction

Poly(methacrylic acid) (PMA) can be prepared in various isomer forms: as an atactic or heterotactic poly(methacrylic acid), a-PMA, and also as highly regular syndiotactic and isotactic polyacids, s-PMA and i-PMA, respectively. Solution properties of PMA depend strongly on the tacticity of the polymer chain.[1-5] Isotactic PMA is, in contrast to a-PMA or s-PMA, insoluble in water below a certain critical degree of neutralization[2,3] and it behaves as a weaker acid over the whole range of degrees of neutralization, α_N.[1,5] On the other hand, a-PMA and s-PMA display similar solution behavior.[5] The latter becomes obvious if one takes into account that the so-called conventional PMA (or a-PMA) is usually predominantly syndiotactic.[6] All three forms undergo a pH induced cooperative transition between two mean conformations or states.[7,8] The conformation of the chain in aqueous solutions changes from a compact form at low α_N to an expanded one at higher α_N in a rather narrow region of α_N. This conformational transition is irreversible in the case of i-PMA,[1,4] whereas it is completely reversible for the other two isomers. Irreversibility is very clearly demonstrated by a hysteresis loop which is observed in potentiometric acid-base titrations of i-PMA.[4]

Most of the studies dealing with the conformational transition of PMA were performed with the atactic isomer. The stability of the compact form of a-PMA was ascribed to various forces, among them intra- and intermolecular hydrogen bonding,[9,10] van der Waals interactions[11] and to the so-called hydrophobic effect associated with the methyl side-groups.[13,14] Silberberg et al.[9,10] attributed the stability of the compact form of PMA at low pH to intramolecular hydrogen bonding. These intramolecular bonds in the dilute regime should eventually lead to gelation in concentrated a-PMA solutions caused by intermolecular hydrogen bonds between carboxyl groups of different chains.[9,10] Anufrieva et al.[13] reported that the resistance to chain expansion was eliminated when methanol was added to an aqueous a-PMA solution. The conformational transition, which takes place for a-PMA in water, gradually disappears with increasing content of alcohol[13,14] and the behavior of a-PMA becomes similar to that of poly(acrylic acid) (PAA). Based on these observations with mixed methanol-water solvent, it was concluded that the resistance to chain expansion in the case of a-PMA is due to the hydrophobic effect associated with the methyl groups. The absence of the conformational transition in the behavior of PAA could then be explained by the absence of hydrophobic methyl groups in this polyacid. On the other hand, Mandel et al.[11] have concluded on the basis of potentiometric and spectroscopic results obtained with PMA and partly esterified PMA in water and in methanol-water mixtures that the stabilization of the compact state of a-PMA is not primarily due to hydrophobic bonding. By these authors opinion, it should be ascribed to a direct stabilization of certain chain conformations by van der Waals interactions between methyl groups. Atactic PMA was studied also in dioxane-water mixtures,[15] where it was noticed that the compact form of PMA no longer exists beyond 25% of dioxane in water. This finding was attributed to preferential solvation of a-PMA by dioxane[15] and speaks in favor of a presumption that hydrogen bonds are the ones responsible for the stability of the compact PMA coil. Dioxane is namely known to have a high tendency for the formation of hydrogen bonds with other compounds.

It should be pointed out that practically all investigations of the conformational transition of the PMA chain rely in their interpretation on a presumption that this conformational change in dilute aqueous solutions is an intramolecular event. However, a recent extensive light scattering study of a-PMA at low pH revealed that the picture of an intramolecularly bonded compact coil in water should not be taken as totally granted.[6] As pointed out in this research,[6] the compact structure of a-PMA at low degrees of ionization seems to be subjected to a substantial intermolecular association. Strong intermolecular association through cooperative hydrogen bonding was held responsible also for structure (helix) formation of i-PMA in concentrated aqueous solution and for subsequent thermoreversible gelation of the isotactic polymer.[3] The information on possible association between chains can be unequivocally obtained only by techniques, such as light scattering, that provide data on the molar mass or on the radius of gyration of the polymer.[6] Nevertheless, adequate caution should be taken in the interpretation of results obtained by other studies, e.g. potentiometry or fluorimetry, in order not to exclude possible intermolecular association.

From what was indicated above, it is obvious that the origin of forces responsible for the stabilization of the compact coil and the nature of the compact state itself are quite controversial even for a-PMA, not to say anything about i-PMA. In fact, no precise studies of i-PMA that would shed more light on the irreversible conformational transition and on the nature of the compact state of this stereoisomer in dilute solution seem to have been conducted. In this preliminary study we try to address the nature of forces that contribute to the stability of the i-PMA state at low degrees of neutralization, whereas investigation of intermolecular association (in dilute solution conditions) is left for future work. We have performed potentiometric titrations of i-PMA in mixtures of water and an organic solvent. Two solvents were chosen for this purpose, methanol and dioxane, for reasons given above, and measurements were performed for solvent mixtures containing up to 60% of the organic component. In addition to potentiometric titrations, the conformational change in mixed solvents was followed also by fluorescence emission spectroscopy based on the use of pyrene as the external fluorescence probe. This fluorescence technique was used previously to follow the conformational transition of a-PMA chain in aqueous solutions,[16] but to our knowledge it has never been used in mixed solvents.

2. Experimental

2.1. Materials

Isotactic poly(methacrylic acid) was prepared by the hydrolysis of isotactic poly(methyl methacrylate) (i-PMMA). I-PMMA (a gift from Prof. H. B.) was synthesized at room temperature in toluene according to the procedure reported in the literature.[3,17] The molar mass of the polymer was obtained by size-exclusion chromatography. The number- and weight-average molar masses of the starting ester form were 136 and 257 kg/mol, respectively. Triad content was determined by ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy. The sample contains 96% of isotactic and around 4% of atactic triads. This polymer was converted into the acid form by hydrolysis in oxygen-free 96% H_2SO_4, followed by hydrolysis in 1 M NaOH according to the procedure reported in the literature.[2-4,17,18] For this purpose, 3 g of i-PMMA were added to 150 mL of H_2SO_4 at room temperature and stirred for 24 hours under constant flow of N_2. The temperature was raised to 60 °C. After 5 hours at 60 °C, when the polymer completely dissolved, the solution was cooled to 0 °C and poured into 3 L of ice-cold distilled water. The lightly yellow precipitated polymer was filtered and dissolved in water by adding solid NaOH until the pH of the solution was around 9. After heating the polymer at 60 °C for approximately 30 minutes, i-PMA was precipitated by a drop-wise addition of concentrated H_2SO_4. For further purification of i-PMA, the dialysis was used. The polymer was first dialyzed against 0.02 M HCl to obtain i-PMA at $\alpha_N = 0$ and then against water. The pure product was dried by lyophilization. The degree of hydrolysis of the final product was determined from the ^{1}H NMR spectrum in D_2O by adding just enough solid NaOH so that the polymer dissolved. The spectrum has revealed that more than 92% of the ester groups were hydrolyzed.

The atactic form of PMA, a-PMA, was obtained by polymerization of methacrylic acid using a standard procedure and was characterized previously by light scattering measurements.[6] The resulting value for the weight-average molar mass of this a-PMA sample was M_w = 131 kg/mol and the polydispersity index was 2.44.[6] The sample is predominately syndiotactic:[6] it contains around 49% of syndiotactic, 39% of atactic, and 12% of isotactic triads. This is a typical composition of triads when PMA is obtained by direct polymerization of methacrylic acid. The polymer was purified by dialysis and stored in the refrigerator as a concentrated stock solution.

Spectroscopically pure 1,4-dioxane and methanol p.a. (both from Merck) were used without further purification. For the preparation of water-organic solvent mixtures, triple distilled water was used and mixtures were prepared by volume. Solutions of i-PMA in these solvent mixtures were prepared by weighing an appropriate amount of solid i-PMA and adding the desired volume of solvent so that the concentration of i-PMA was around 5×10^{-3} mol COOH/L. Potentiometric titrations were carried out with standardized aqueous NaOH and HCl solutions with concentrations 0.1 mol/L. Fluorescence measurements were performed also with a-PMA at the same polymer concentration as for i-PMA.

I-PMA at $\alpha_N = 0$ is not soluble in any of the pure solvents used in this study. In water and in methanol-water mixtures, it dissolves at α_N larger than approximately 0.2. It is soluble, however, in 50% dioxane-water already at $\alpha_N = 0$, whereas in 20% dioxane-water it dissolves after the addition of the first volume-increment of NaOH (at α_N approximately 0.02).

2.2. Fluorescence Measurements.

Pyrene (Aldrich, optical grade) was used as the external fluorescence probe to monitor the conformational transition in pure a-PMA and i-PMA solutions in methanol-water and in dioxane-water mixtures. The preparation of water saturated with pyrene was as reported previously.[19] Mixed solvents with added pyrene were prepared by dissolving a small amount of pyrene in the solvent mixture so that the concentration of pyrene was approximately the same as in saturated water solution (*i.e.* around 2×10^{-6} mol/L[19]). These solvents were stirred for approximately 24 hours before they were used for the preparation of PMA solutions. The composition of solvents for fluorescence measurements was the following: water, 20 and 40% methanol-water, and 10 and 20% dioxane-water mixtures. Measurements were performed for α_N values below approximately 0.53 in the direction of increasing α_N (by the addition of aqueous NaOH) and also in the direction of decreasing α_N (by the addition of aqueous HCl). In the case of a-PMA, the change in α_N was performed by adding the titrant to the solution directly in the measuring cuvette and spectra were recorded after 10 minutes of thermal equilibration. In the case of i-PMA, only the titration in the forward direction (i.e. with NaOH) was carried out with the same titration technique, whereas solutions with decreasing α_N were prepared individually into Erlenmeyer flasks. The time needed for the pyrene fluorescence ratio (see below) to stabilize after each addition of NaOH to i-PMA solutions was considerably longer (up to 1 hour) than in the case of a-PMA.

The fluorescence emission spectra of pyrene were recorded on a Perkin-Elmer model LS-50 luminescence spectrometer at 25 °C following the experimental details reported in our previous studies.[19] From the spectra, the ratio of intensities of the first (I_1) and the third (I_3) vibrational peak of pyrene or the so-called pyrene fluorescence ratio (I_1/I_3) was calculated. These values in pure water and in pure methanol were around 1.8 and 1.3, respectively, in good agreement with literature data.[20] The I_1/I_3 in pure dioxane was determined in this study for the first time and it was around 1.23. This is close to the value found in tetrahydrofurane (I_1/I_3 = 1.2[20]).

2.3. Potentiometric Titrations.

Potentiometric titrations were performed with a pH meter Iskra MA 5740 by using a combined electrode from Mettler-Toledo (InLab 406). The electrode was calibrated with two aqueous buffers having pH values 6.865 and 9.18 at 25 °C. The titration vessel was thermostated at 25 °C and a constant flow of nitrogen was lead through the solution. Titrants (standardized aqueous solutions of NaOH and HCl with concentrations 0.1 mol/L) were added with a micro-syringe burette. Potentiometric titrations of i-PMA were carried out in pure water, in 20, 40 and 60% methanol-water mixtures and in 20, 40 and 50% dioxane-water mixtures, both in the direction of increasing (by the addition of NaOH; the forward titration) and decreasing α_N (by the addition of HCl; the backward titration). Because titrants were aqueous solutions, the composition of mixed solvent changed in such titration cycle. This change was the largest (around 23%) in 60% methanol-water and somewhat smaller in 50% dioxane-water mixture. A 23%-change means that in the case of a 60%

methanol-water mixture, the content of methanol dropped to around 46% at the completion of the cycle. Although the pH values in mixed solvents cannot be interpreted in a simple way,[7,11,14] the titration curves showed some important common features, which are the basis for discussion. For comparison reasons, titration in water in the forward direction was performed also with a-PMA. The concentration of a-PMA was 0.01 mol COOH/L.

From the measured pH of solutions, degree of ionization (α) of carboxyl groups on the polyion was calculated from the following relation:

$$\alpha = \alpha_N + \frac{[H^+]-[OH^-]}{c_p} \tag{1}$$

α_N is obtained from the known concentration of the polyelectrolyte, c_p, and the amount of added NaOH (or HCl), and $[H^+]$ and $[OH^-]$ are the activities of hydrogen and hydroxide ions calculate from the measured pH.

4. Treatment of potentiometric titration curves

The potentiometric titration of a weak polymeric acid is usually treated in terms of the negative logarithm of the apparent dissociation constant ($pK_a = -\log K_a$). The dependence of pK_a on degree of ionization is for weak polymeric acids quite different from the one for low molar-mass weak acids. pK_a is practically independent on α for monomeric acids, whereas it increases in the case of polymeric acids. This increase in pK_a is ascribed to the increasing difficulty of dissociating an acidic group on the macromolecular chain with increasing charge on this chain.[21] In the case of PAA, for example, the values of pK_a increase proportionally with α, indicating the absence of any conformational change, *i.e.* they point to a continuous increase in chain dimensions, which is caused by the repulsion between charged groups on the polyion. For polyacids such as PMA and poly(glutamic acid), on the other hand, the plot of pK_a against α shows a non-monotonic increase: pK_a first increases, attains more or less constant values in the intermediate α region and then increases again. This contrasting behavior of PAA and PMA was interpreted as reflecting the cooperative conformational transition of the PMA chain from a compact to an extended form.[4,22]

The values of pK_a are usually expressed by the Henderson-Hasselbalch equation[23]

$$pK_a \equiv pH + \log\frac{1-\alpha}{\alpha} \quad \text{or}$$

$$pH = pK_a - \log\frac{1-\alpha}{\alpha} \tag{2}$$

Equation 2 describes well the pH versus $\log(1 - \alpha)/\alpha$ curves for monobasic acids, the slope of these curves being -1.[7,23] It has been shown[7,8,11] that the corresponding slope for the polymeric acid approaches -2. The titration curve of polymeric acids is thus formally described by an extension of equation 2:

$$pH = pK_a - n\log\frac{1-\alpha}{\alpha} \tag{3}$$

in which n is the experimental slope of the pH versus $\log(1 - \alpha)/\alpha$ curve and pK_a is the sum of two terms

$$pK_a = pK_0 + 0.4343\frac{\Delta G_{el}}{RT} \tag{4}$$

pK_0 is the intrinsic ionization constant that refers to the ionization of the first ionizable group on the uncharged polymer, α is the fraction of ionized carboxyl groups (or the degree of ionization), R is the gas constant, T is the absolute temperature, and ΔG_{el} is the electrostatic free energy required to remove a hydrogen ion from the charged polyion. pK_a and n are usually practically independent of the degree of polymerization of the polyelectrolyte, but may depend on the solvent composition and on the simple salt concentration. In our case, the dependence on the solvent composition will be evaluated.

3. Results and Discussion

It was pointed out in the Introduction, that intermolecular association between PMA chains should not be neglected in the interpretation of the results obtained by methods, such as the ones used in this paper, that are unable to give information on molecular size-parameters. The intermolecular effects seem to be even more important for i-PMA[3] than for a-PMA.[6] In view of this important characteristics of PMA solutions, the term "compact coil", which will be used in the interpretation of the experimental results herein, should be looked upon more as an entanglement of several chains and not just as a certain conformation of one macromolecular chain. Whenever possible, we will try to explain the results by allowing chain-aggregation.

3.1. Fluorescence

Results of fluorescence measurements are shown in Figures 1a and 1b for a-PMA in methanol-water and in dioxane-water mixtures, respectively, and in Figure 2 for i-PMA in both mixed solvents. The I_1/I_3 values obtained in aqueous a-PMA solutions in the course of increasing α_N show a sharp increase from a value around 0.9 to a value around 1.8, which takes place in the region $0.05 \le \alpha_N \le 0.2$. This change is shifted to somewhat lower α_N in the backward direction and stabilizes at slightly higher value for $\alpha_N \approx 0$ (that is at $I_1/I_3 \approx 0.96$). Although the forward and the backward fluorimetric titrations suggest, that the conformational transition of the a-PMA chain may be irreversible to a certain extent, results of potentiometric measurements show[1,4] that this is probably not the case.

The region $0.05 \le \alpha_N \le 0.2$ corresponds to the pH-induced conformational transition of the a-PMA chain from a compact conformation to an extended one,[1,5,8] which is accompanied by a release of pyrene from the hydrophobic environment of rather low polarity inside the coil ($I_1/I_3 < 1.0$) to very polar aqueous surroundings ($I_1/I_3 > 1.8$).[16] In potentiometric titration curves, one usually observes this transition in a somewhat higher pH-region[1,5,8] ($0.1 \le$ pH ≤ 0.3; see also results of potentiometric measurements reported below). It is possible, however, that the method based on pyrene fluorescence is more sensitive to detect the loosening up of the compact coil than the potentiometric one.[24]

The change of I_1/I_3 in aqueous i-PMA solutions (Figure 2) is less sharp than the one in the a-PMA case: the initial I_1/I_3 is around 1.17 whereas the final one is around 1.6. The increase takes place in a broader α_N region, between $\alpha_N = 0.05$ and $\alpha_N = 0.3$ and it is clearly irreversible; I_1/I_3 values are considerably larger in the backward direction (compare values 1.17 for the forward and 1.52 for the backward directions, both at $\alpha_N \approx 0.02$). This pronounced difference may be attributed to strong intermolecular association,[3] which leads to different intermediate chain conformations in the direction of decreasing charge on the polyion. Higher I_1/I_3 values could then be explained by associates between i-PMA chains in a relatively more extended conformation (possibly locally helical[3,6]) in the backward than in the forward direction at the same α_N values. Such association would be less compact and would therefore offer a less effective protection of pyrene against the polar aqueous environment. In conformation of this scenario, differences in chain-conformations were proposed for i-PMA in various states, *i.e.* for the precipitated polymer, for amorphous films prepared from dimethyl formamide (DMF) or dimethyl sulpohxide (DMSO), for the water-treated films, or for the structure formed in aqueous solution at $\alpha_N = 0.28$.[3,25] This last case is of special interest, since it is the basis for the formation of thermoreversible elastic gels in aqueous i-PMA solutions.[3] The gel state was achieved at $\alpha_N = 0.28$ in a 10% (w/w) solution by the "back titration method" and the authors ascribed this gelation to the formation of a helix conformation and to the subsequent intermolecular association through strong hydrogen bonding.

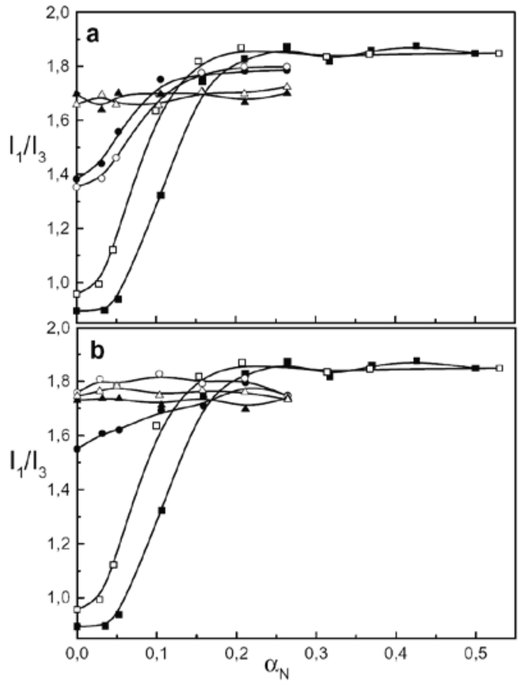

Figure 1: The I_1/I_3 ratio of pyrene in dependence on degree of neutralization (α_N) for a-PMA in mixed solvents ($c_p = 5 \times 10^{-3}$ mol COOH/L): a) methanol-water mixtures: 20% methanol (●,○), 40% methanol (▲,△); b) dioxane-water mixtures: 10 % dioxane (●,○), 20% dioxane (▲,△); full symbols: the forward titration; open symbols: the backward titration. Measurements in water (■,□) are plotted in both figures.

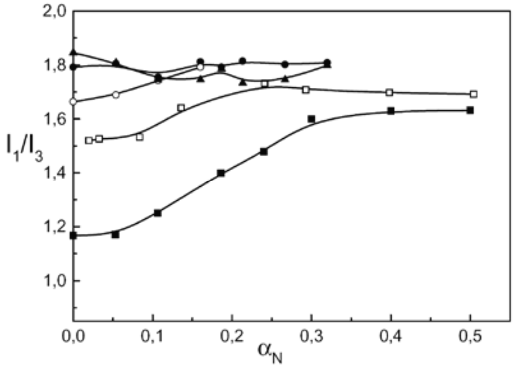

Figure 2: The I_1/I_3 ratio of pyrene in dependence on degree of neutralization (α_N) for i-PMA in mixed solvents ($c_p = 5 \times 10^{-3}$ mol COOH/L): water (■,□), 20% methanol (●,○), 10% dioxane (▲,△); full symbols: the forward titration; open symbols: the backward titration.

The I_1/I_3 values obtained for both PMA forms in water point to some important differences in the micropolarity that is detected by pyrene in aqueous a-PMA solutions on one hand and in the i-PMA ones on the other, both in the compact and in the extended conformations of the polymer chain. First, the interior of the compact coil in the case of i-PMA is more polar (less hydrophobic) than in the case of a-PMA. Second, in the extended conformation the i-PMA chain makes the microenvironment that is sensed by pyrene less polar than is the case with the a-PMA chain. More polar interior of the i-PMA compact coil could be explained by presuming that the hydrophobic methyl groups on the isotactic chain, due to their highly regular orientation, which makes the chain more rigid,[24] cannot hide as effectively into the compact coil as can the ones on the atactic chain. As a consequence of this, a larger number of CH_3 groups cover the surface of the i-PMA coil in comparison with the atactic form, whereas more COOH groups reside in the interior. This makes the compact state of i-PMA, as compared with a-PMA, more hydrophobic from the outside and more hydrophilic from the inside and explains the higher value of I_1/I_3 in the unneutralized state. Along with this, the insolubility of i-PMA at $\alpha_N = 0$ can be attributed to its predominantly hydrophobic surface.[2,3,24] On the other hand, when the polyion expands ($\alpha_N > 0.3$), the i-PMA chain seems to be more hydrophobic than the a-PMA one.[24] This is ascribed to the possibility of a locally helical conformation of the i-PMA chain in aqueous solution (see also above).[3,24]

The conformational transition of a-PMA is observed also when 20% of methanol is added to the water (I_1/I_3 changes from around 1.35 to almost 1.8 and there is practically no difference between values measured in the forward and in the backward directions), whereas it is not detected by the pyrene fluorescence ratio in 40% of methanol (I_1/I_3 is approximately constant at around 1.7). The constancy of I_1/I_3 does not necessarily imply the absence of the conformational change. The conclusion on its absence can be reached by taking into consideration potentiometric studies of a-PMA in methanol-water mixtures.[13] According to Braud et al.,[13] the behavior of a-PMA becomes similar to that of PAA in solvent mixtures that contain 40 or 50% of methanol, *i.e.* there is no longer a detectable discontinuity in titration curves.

In dioxane-water solutions of a-PMA, the disappearance of the step-wise change in I_1/I_3 is observed at even lower content of the organic solvent (see Figure 1b). A small change in I_1/I_3 can be seen in 10% dioxane in the course of increasing α_N, whereas I_1/I_3 is constant (at around 1.7) in 20% dioxane. It can be concluded that these fluorescence results point to the absence of the conformational transition for a-PMA in 20% dioxane. This conclusion is in agreement with report of Vorreux et al.[15] who found out by potentiometric titrations of a-PMA in dioxane-water mixtures that beyond 25% of dioxane the conformational change of the a-PMA chain no longer takes place.

For the isotactic PMA, fluorescence measurements were performed only in 20% methanol-water and in 10% dioxane-water mixtures (Figure 2), because no detectable change in I_1/I_3 could be seen already at these low contents of organic solvents (the I_1/I_3 is between 1.7 and 1.8). One cannot make any final conclusion on the absence or presence of the conformational transition in the i-PMA case on the basis of fluorescence data alone. It will be demonstrated by potentiometric titration curves given below that the conformational transition of the i-PMA chain is still observed at much higher content of organic solvents (for example, it is still present in 60% methanol, but almost gone in 40% dioxane). Moreover, the pyrene-based fluorescence method gives information only on the hydrophobicity sensed by the probe, but is unable to identify eventual intermolecular association.

3.2. Potentiometric Titrations

The titration cycles for i-PMA (the forward and the backward titrations) are shown in Figures 3a and 3b for 20 and 60% methanol and for 20 and 50% dioxane, respectively, both in comparison with pure water. Other solvent compositions are omitted in order to avoid crowding of the curves. The purpose of these plots is to demonstrate the irreversibility in potentiometric titration curves of i-PMA in investigated solvent mixtures, which was in solutions of pure water observed before.[1,4] It can be clearly seen that the forward and the backward titration paths are not reversible also in water-organic solvent mixtures. The fact that in 60% methanol and in 50% dioxane the titration curves do not coincide may be attributed to some extent to the change in solvent composition because the titrants were aqueous solutions (see Experimental). The larger part, however, comes from the irreversible change in the conformation of the chain. In view of what was pointed out above for water solutions (see discussion of fluorescence data), one may propose that intermolecular association, simultaneously with the intramolecular one, plays an important role also in mixed solvents. Unfortunately, pH titration data, so as the fluorimetric ones, cannot shed much light on the intermolecular processes.

The conformational transition corresponds to the region of approximately constant (or less steeply changing) pH values. It clearly exists still in 60% methanol in the forward direction, but it disappears in the back-titration (see the smooth decrease of pH, similar to the one found for PAA[25]). On the other hand, the course of the titration curves in 50% dioxane suggests that the conformational transition may have disappeared in this solvent both in the forward and in the backward direction (this will be more clearly seen from the pK_a versus α curves given below). At the same time, the equivalence point at $\alpha_N = 1$, when complete neutralization is reached, is in 50% dioxane the most clearly expressed (see Figure 3b). This may be a consequence of the fact that 50% dioxane is actually a good solvent for i-PMA (see Experimental).

Figures 4a and 4b show the pK_a values plotted against α in the forward titration for all solvent compositions. For comparison, the curve for a-PMA in water at c_p = 0.01 mol COOH/L is also included. All curves for i-PMA, except the ones for 40 and 50% dioxane, show a well-expressed minimum at $\alpha_N \approx 0.2$. This minimum coincides with the solubility limit of i-PMA in these solvents (see Experimental). The preceding decrease of pK_a with increasing α partially overlaps with the plateau region of approximately constant pK_a values in the case of a-PMA. Note that the constancy of pK_a values in the a-PMA case is attributed to the conformational transition of the polymer chain.[4] It is proposed therefore, that the dissolution process of the i-PMA interferes with the conformational transition of the chain in the region $0 < \alpha_N < 0.2$. The decrease of pK_a at $\alpha_N < 0.2$ thus includes both events. The dissolution process makes an important contribution to pK_a values when solvent mixture contains predominantly water. In 40 and 60% methanol, for example, the minimum is rather shallow, and only an inflection similar to the a-PMA case can be traced in 40% dioxane.

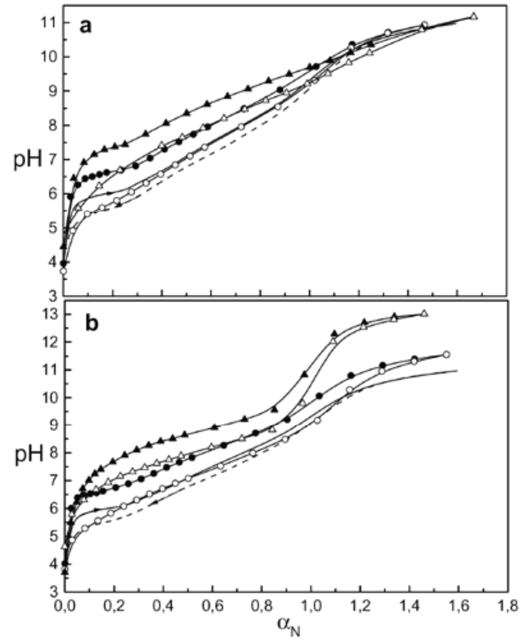

Figure 3: Potentiometric titration curves (pH versus α_N) for i-PMA in mixed solvents ($c_p = 5 \times 10^{-3}$ mol COOH/L: a) 20% methanol-water (●,○), 60% methanol-water (▲,△); b) 20% dioxane-water (●,○), 50% dioxane-water (▲,△); full symbols: the forward titration; open symbols: the backward titration. For comparison, the titration curves for the same i-PMA sample in water are also presented: solid line (the forward titration); dashed line (the backward titration).

The course of all curves at α > 0.2 is similar to that for the a-PMA, except that they are shifted to higher pK_a values with increasing content of the organic solvent (an exception is the curve for i-PMA in pure water that runs at somewhat higher pK_a values; this is attributed to the fact that the concentration of a-PMA is higher than that of i-PMA; see Experimental). Higher pK_a values imply that i-PMA is becoming an increasingly weaker acid. This observation can be explained by taking into account the dielectric constants of methanol and dioxane (32.7 and 2.21,[20,26] respectively). The decrease of the dielectric constant in mixed solvents increases the electrostatic attraction between hydrogen ions and the carboxylate anion and thus increases the pK_a.

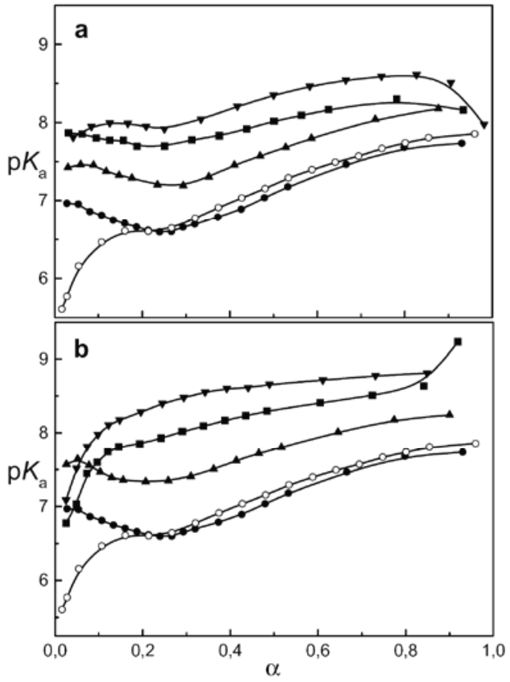

Figure 4: The change of pK_a with α for i-PMA ($c_p = 5 \times 10^{-3}$ mol COOH/L) in mixed solvents in the forward titration: a) methanol-water mixtures: 0% methanol (●), 20% methanol (▲), 40% methanol (■), 60% methanol (▼); b) dioxane-water mixtures: 0% dioxane (●), 20% dioxane (▲), 40% dioxane (■), 50% dioxane (▼). For comparison, the titration curve for a-PMA (c_p = 0.01 mol COOH/L) in water is also presented (○).

When approaching zero degree of neutralization in the course of the backward titration path, the precipitation of the polymer does not take place immediately. At these low polymer concentrations (note that the concentration of i-PMA was 5×10^{-3} mol COOH/L in all titration experiments), crystallization is delayed for several hours or even days. The minima in the pK_a versus α curves in the backward direction (not shown) are thus seen only in water and in 20% organic solvent-water mixtures.

Another way of representing potentiometric titration curves is in the form of Henderson-Hasselbalch plots (see Eq. 3).These plots are presented in Figures 5a and 5b for water mixtures with methanol and dioxane, respectively. Again, the a-PMA case in pure water is included for comparison. The curve for a-PMA clearly displays two linear regions with almost the same slopes separated by a transition region around $0.1 < \alpha_N < 0.3$ (compare this with the region obtained from fluorescence measurements, see above). In contrast to a-PMA, the slopes of curves for i-PMA after the transition region (the low pH region) are considerably lower than the ones before the transition (the high pH region). We believe that this is partly related to the insolubility of i-PMA under these conditions (low pH) because the differences in slopes are not so pronounced for the titrations in the reverse direction (Henderson-Hasselbalch plots for the back-titration are not shown).

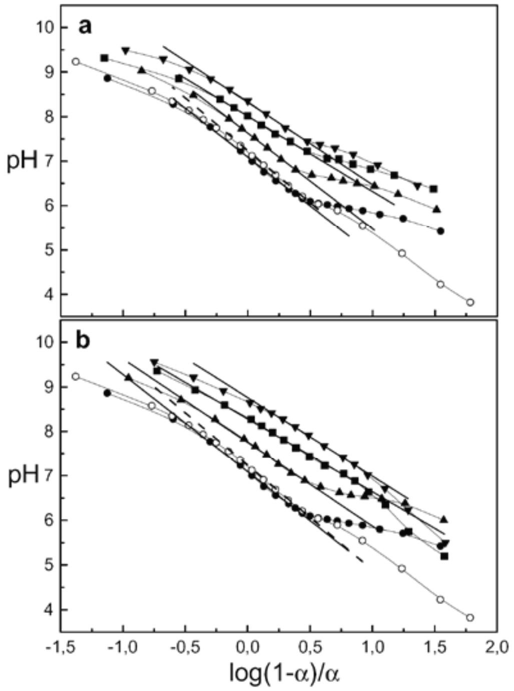

Figure 5: Henderson-Hasselbalch plots for i-PMA ($c_p = 5 \times 10^{-3}$ mol COOH/L) in mixed solvents in the forward titration: a) methanol-water mixtures: 0% methanol (●), 20% methanol (▲), 40% methanol (■), 60% methanol (▼); b) dioxane-water mixtures: 0% dioxane (●), 20% dioxane (▲), 40% dioxane (■), 50% dioxane (▼). For comparison, the plot for a-PMA (c_p = 0.01 mol COOH/L) in water is also presented (○). Straight lines indicate the slopes of the linear regions in these plots in the region of high pH.

The slopes of the Henderson-Hasselbalch plots in the region of high pH values are reported in Table 1 for both mixed solvents. It can be seen that the absolute values ($|n|$) of these slopes decrease with increasing content of the organic solvent up to 40% of the non-aqueous component. In 60% methanol and in 50% dioxane, they show a small increase again. Lower slopes in dioxane-water mixtures are in agreement with previous findings for a-PMA in such solvent mixtures.[7] The slopes in the literature report[7] were evaluated for 0, 30, and 60% dioxane and reached rather low absolute values, even lower than 1, which is the slope for the monobasic acid (Eq. 2). In 30% dioxane, for example, the slope for a-PMA was 1.62,[7] which compares well with the value 1.64 for i-PMA in 40% dioxane in our case.

Table 1: Absolute values of slopes ($|n|$) obtained from the Henderson-Hasselbalch plots for a-PMA in water (c_p = 0.01 mol COOH/L) and for i-PMA ($c_p = 5 \times 10^{-3}$ mol COOH/L) in water, in methanol-water and in dioxane-water mixed solvents.

polymer	solvent	$\|n\|$ (Eq. 3)
a-PMA	water	2.35
i-PMA	water	2.19
	20% methanol	2.15
	40% ”	1.69
	60% ”	1.85
	20% dioxane	1.88
	40% ”	1.64
	50% ”	1.77

Two exceptions are noted where (1) no transition is observed in the Henderson-Hasselbalch plot and (2) the slope of the plot in the low pH region is higher than the one in the high pH region: these exceptions are 40 and 50% dioxane.

The first observation agrees with the previous conclusion that the conformational transition disappears with increasing dioxane content. One may go a step further and propose that i-PMA in “good” solvents (like 40 or 50% dioxane) is initially (at low α) in a comparatively more extended conformation than in “bad” solvents like pure water. With increasing α, it gradually transforms into a more collapsed state and not into a more extended one as usually proposed for the aqueous medium. This type of a change in chain dimensions would explain larger slopes of the Henderson-Hasselbalch plots in the low pH region. The reason for the opposite direction of the conformational change may be two-fold. First, ion-condensation becomes very large in the presence of dioxane and considerably reduces the effective charge on the chain. This results in reduced electrostatic repulsion between carboxyl groups and leads to a more coiled chain conformation. Second, dioxane is probably not a good solvent for PMA in the ionized state, whereas it proved to be a good one (in combination with water, see results of this study) for the unionized i-PMA. A reasonable conclusion is also that the change in chain-dimensions is for i-PMA in 40 and 50% dioxane a continuous one, because there is no observable transition region in the corresponding Henderson-Hasselbalch plot.

The second finding on increased slope in the Henderson-Hasselbalch plot is quite exceptional. Usually, nearly no change, like for a-PMA, or a decrease are observed.[26] This latter type of behavior is believed to be a consequence of the conformational change from a compact form of the PMA chain at low α to a more extended one at high α. Increased slopes in the case of i-PMA were observed only in solvent mixtures with a rather large amount of dioxane in water, and not at all in the ones with methanol and water. In an attempt to explain this difference, one has to take into account the following important facts concerning both organic solvents. The dielectric constant of dioxane is considerably lower than the one of methanol (compare 2.21 with 32.7,[20,27] respectively). In addition, dioxane is known to exhibit a high tendency for the formation of hydrogen bonds, whereas methanol is recognized to be a worse competitor for their formation than water.[13] In a system PMA/water/dioxane, hydrogen bonds can be formed between all components. Specific hydrogen bonds were found to exist between water and dioxane, leading to the formation of a 3-1 complex between solvent molecules[15] (a 3-1 complex corresponds to a 61 volume% of dioxane[15]). Furthermore, it has been concluded on the basis of refractive index increment measurements[15] that the a-PMA compact coil is preferentially solvated by dioxane near the methyl groups (inside the coil) and by the solvent 3-1 complex (or by water) near the outer carboxyl groups. The maximum in preferential solvation of a-PMA by dioxane was found near 70% dioxane. The observed phenomena were attributed to the formation of hydrogen bonds between dioxane and water and also to the ones between dioxane and carboxyl groups on a-PMA.[15]

Preferential solvation of COOH groups by water (or by the 3-1 solvent complex) and of CH_3 groups by dioxane may play an important role also in the case of i-PMA. First, this phenomenon can explain the previous finding that i-PMA at $\alpha_N = 0$ is soluble in 50% dioxane but does not dissolve in pure dioxane or in pure water. The compact state of i-PMA is different from the one of a-PMA (see discussion of fluorescence results). On the surface, i-PMA is largely covered with methyl groups[24] that are preferentially solvated by dioxane. When compared with a-PMA, a larger number of hydrophilic carboxyl groups are forced inside the coil, although some of them are still situated on its surface. These are preferentially solvated by water or by the solvent 3-1 complex. Consequently, both water and dioxane are needed for effective solubilization of i-PMA in the unneutralized state. Water is not excluded form the interior of the i-PMA coil at $\alpha_N = 0$. On the contrary, the polarity inside the i-PMA coil may be rather large not only because of the presence of COOH groups but also on the account of the present water (see I_1/I_3 values for i-PMA in water at $\alpha_N < 0.1$, Figure 2). Such distribution of water and dioxane on the micro-level could explain why nearly no difference in the pyrene fluorescence ratio in dependence on α was found for i-PMA in 10% dioxane (see Figure 2). Additional complication with pyrene may result if the probe itself specifically interacts with dioxane.

4. Conclusions

Our main interest in this study was the highly regular isotactic poly(methacrylic acid). We have compared it with the usual and most studied form, which is the atactic poly(methacrylic acid). Consequently, some studies that were not reported in the literature so far, in particular fluorescence measurements in mixed solvents were performed also with a-PMA. The experimental information obtained for i-PMA in the chosen mixed solvents allows us to draw the following conclusions:

1) Water and methanol or their mixtures are not good solvents for i-PMA in the unneutralized state. The conformational transition of the i-PMA chain and its irreversible character persist up to 60% of methanol. Besides, i-PMA at $\alpha_N = 0$ does not dissolve in pure methanol. These results indicate that effects due to the presence of methyl groups on the chain may not be the most important ones for the stabilization of the compact structure of the i-PMA coil at low α_N and for the subsequent conformational transition.

2) The only solvent that dissolves i-PMA at $\alpha_N = 0$ is a 50% dioxane-water mixture and not pure dioxane. In this solvent mixture, the conformational change, which takes place for i-PMA in water, disappears. This observation can be explained by taking into account findings on hydrogen bond formation in PMA/water/dioxane system. Hydrogen bonds can form between all components in the system and contribute to selective solubilization of COOH groups by the water-dioxane complex or by water alone and of CH_3 groups by dioxane. This is by our opinion a forceful argument in favor of a presumption that hydrogen bonds are also the ones that stabilize the compact structure of i-PMA.

3) The analysis of the potentiometric titration curves suggests that the nature of the change in polymer chain dimensions, which is induced through ionization, is different in 50% dioxane on one hand and in pure water on the other. In "good" solvents like 50% dioxane, i-PMA is initially (at low α_N) in a more extended conformation than in "bad" solvents like pure water. With increasing α_N, the chain gradually transforms into a more collapsed state and not into a more extended one as usually proposed for the aqueous medium. Similarly to the present case, it was concluded for PAA in pure methanol in the presence of sodium ions that

the PAA chain, rather surprisingly, does undergo a conformational change in this medium, which takes place in the opposite direction.[26]

Obviously, the role of dioxane in PMA solutions is a very complex one. It would be of interest to study other strongly hydrogen bonding solvents, for example DMF or DMSO. It has been found that these are the only pure solvents in which i-PMA is soluble in its unneutralized state.[25] Moreover, one has to admit that the present findings may be to a considerable extent influenced by intermolecular association between polymer chains. Unfortunately, the intrinsic limitations of the experimental results do not allow making any definite conclusion on this point.

5. Acknowledgments

This work was supported by the Slovenian Research Agency through Physical Chemistry Research Program No 0103-0201. The authors thank Prof. Hugo Berghmans for providing the i-PMMA sample.

6. References

1. V. Crescenzi, *Adv. Polymer Sci.* **1968**, *5*, 358–386, and references cited herein.
2. E. M. Loebl, J. J. O'Neill, *J. Polym. Sci.* **1960**, *45*, 538–540.
3. E. van den Bosch, Q. Keil, G. Filipcsei, H. Berghmans, H. Reynaers, *Macromolecules* **2004**, *37*, 9673–9675.
4. J. C. Leyte, H. M. R. Arbouw-van der Veen, L. H. Zuiderweg, *J. Phys. Chem.* **1972**, *76*, 2559–2561.
5. M. Nagasawa, T. Murase, K. Kondo, *J. Phys. Chem.* **1965**, *69*, 4005–4012.
6. K. Kogej, H. Berghmans, H. Reynaers, S. Paoletti, *J. Phys. Chem.* **2004**, *108*, 18164–18173.
7. A. Katchalsky, P. Spitnik, *J. Polym. Sci.* **1947**, *2*, 432–446.
8. Leyte, J. C.; Mandel, M. *J. Polym. Sci.* Part A **1964**, *2*, 1879–1891.
9. J. Eliassaf, A. Silberberg, *Polymer* **1962**, *3*, 555–564.
10. A. Silberberg, J. Eliassaf, A. Katchalsky, *J. Polym. Sci.* **1957**, *23*, 259–284.
11. M. Mandel, J. C. Leyte, M. G. Stadhouder, *J. Phys. Chem.* **1967**, *71*, 640–649.
12. A. M. Liquori, G. Barone, V. Crescenzi, F. Quadrifoglio, V. Vitagliano, *J. Macromol. Chem.* **1966**, *1*, 291–305.
13. E. V. Anufrieva, T. M. Birshtein, T. N. Nekrasova, O. B. Ptitsyn, T. V. Sheveleva, *J. Polym. Sci.*, Part C **1968**, *16*, 3519–3531.
14. C. Braud, G. Muller, J.-C. Fenyo, E. Selegny, *J. Polym. Sci., Polym. Chem.* **1974**, *12*, 2767–2778.
15. G. Vorreux, M. Morcellet, C. Loucheux, *Makromol. Chem.* **1982**, *183*, 711–720.
16. Chu, D.; Thomas, J. K. *J. Am. Chem. Soc.* **1986**, *108*, 6270–6276.
17. W. E. Goode, F. H. Owens, R. F. Fellmann, W. H. Snyder, J. E. Moore, *J. Polym. Sci.* **1960**, *46*, 317.
18. E. Klesper, D. Strasilla, W. Regel, *Makromol. Chem.* **1974**, *175*, 523–534.
19. Kogej, K.; Škerjanc, J. *Langmuir* **1999**, ***15***, 4251–4258.
20. D. S. Karpovich, G. J. Blenchard *J. Phys. Chem.* **1995**, *99*, 3951–3958.
21. F. Oosawa *Biopolymers* **1968**, *6*, 135–144.
22. R. Arnold *J. Colloid Sci.* **1957**, *12*, 549–556.
23. P. W. Atkins: Physical Chemistry, Oxford University Press, Oxford, **1994**, 5th Edition, pp. 300–304.
24. N. Vlachy, J. Dolenc, B. Jerman, K. Kogej *J. Phys. Chem.* **2006**, *110*, 9061–9071.
25. E. van den Bosch, H. Berghmans *Polym. Bull.* **2006**, in press, available on-line.
26. N. Klooster, F. van der Touw, M. Mandel *Macromolecules* **1984**, *17*, 2070–2078.
27. K.Kalyanasundaram, J. K. Thomas *J. Am. Chem. Soc.* **1977**, *99:7*, 2039–2044.

Povzetek

Izvedli smo fluorimetrične in potenciometrične titracije izotaktične (i-PMA) in ataktične polimetakrilne kisline (a-PMA) v mešanicah metanola in vode ter dioksana in vode z različno vsebnostjo organskega topila. Fluorimetrične raziskave a-PMA so potrdile prejšnje izsledke, da konformacijski prehod pri ataktični verigi izgine, ko je metanola v mešanici več kot 40% in dioksana več kot 20%. Za verigo i-PMA pa so potenciometrični podatki pokazali, da pri tem polielektrolitu do konformacijskega prehoda pride tudi še v 60% metanolu in to v približno enakem območju stopnje nevtralizacije (α_N) kot v vodi. Poleg tega ireverzibilnost tega prehoda ne izgine. V primeru mešanic dioksana in vode nezveznosti v potenciometrični krivulji i-PMA ni več zaslediti, ko je dioksana okrog 50%. Hkrati s tem se i-PMA v 50% dioksanu raztopi že pri $\alpha_N = 0$, medtem ko v nenevtaliziranem stanju ni topna v nobenem od čistih topil. Opaženo obnašanje lahko pripišemo veliki tendenci, ki jo izkazuje dioksan za tvorbo vodikovih vezi z vodo in s PMA. Podan je možen način spremembe v dimenzijah verige i-PMA, do katerega pride pri nevtralizaciji v 50% dioksanu. Rezultati so interpretirani tudi s stališča velike tendence verig PMA po medmolekulski asociaciji.