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pH-induced Conformational Change and Hydration of Poly(methacrylic acid) Investigated by Analytical Ultracentrifugation

Xiaoyan Wang,[1] Xiaodong Ye,[1,*] Guangzhao Zhang[2]

1) *Hefei National Laboratory for Physical Sciences at the Microscale, Department of Chemical Physics, University of Science and Technology of China, Hefei, Anhui 230026, China;*
2) *Faculty of Materials Science and Engineering, South China University of Technology, Guangzhou, P. R. China 510640*

Abstract: Analytical ultracentrifugation was performed on poly(methacrylic acid) (PMAA) with a series of weight average molar masses (M_w) in aqueous solutions as a function of pH. The scales of sedimentation coefficient (s) and diffusion coefficient (D) to M_w at infinite dilutions were obtained at different pH values, indicating that PMAA chains adopt a collapsed structure at low pH values, and stretches at pH higher than 5.2. Our results show that sedimentation coefficient exhibits a minimum at pH ~ 6, presumably due to the effect of conformational change and hydration state of PMAA chains. When pH increases from 6.0 to 8.5, PMAA chains with a high molar mass shrink a little bit, presumably because the sodium ions act as a bridging agent between nonadjacent carboxylate groups. Furthermore, the weight average molar mass of PMAA at pH 8.5 increases one fold than that at pH 4.0, indicating the condensation of sodium ions and the increase in the number of hydration water molecules around carboxylate groups at high pH value.

Keywords: folding, polyelectrolytes, sedimentation velocity, ultracentrifuge

*To whom correspondence should be addressed

Introduction

Poly(methacrylic acid) (PMAA), one of the weak polyelectrolytes, has received increasing interests not only because of its promising application in cosmetics,[1, 2] pharmaceutics,[3, 4] drug testing[5, 6] and biomedical technology,[7, 8] but also due to its biophysical relevance to the folding and packing process of more complicated biomacromolecules such as proteins and DNA.[9-11] It is known that PMAA exhibits a marked pH-induced conformational transition, that is, PMAA adopts a compact conformation at pH < 4, presumably due to the hydrophobic interactions of the methyl groups and/or the hydrogen bonds between carboxylic acid groups. PMAA chain expands rather sharply at pH 4 – 6 when a critical charge density is attained, where the degree of ionization is about 0.3, whereas poly(acrylic acid) (PAA) chain expands smoothly with the increasing pH.[12-15]

The pH-induced conformational change of PMAA has been studied by different techniques such as viscometry,[14, 16, 17] fluorescence spectroscopy,[18-27] small angle X-ray scattering (SAXS),[28-30] Raman spectroscopy,[31, 32] infrared spectroscopy,[13] laser light scattering[33, 34] and analytical ultracentrifugation.[35] However, it is hard to investigate the conformational change of individual PMAA chains that occur in response to changes in pH because most of the measurements were conducted at a relatively high concentration. Sedimentation velocity (SV) in analytical ultracentrifugation (AUC) is sensitive to detect the hydrodynamic and thermodynamic parameters in aqueous solutions with a high resolution after the development in the data analysis software like SEDFIT program.[36, 37] By use of SV, the conformational change of PMAA chains in aqueous solutions can also be obtained from the scale of sedimentation coefficient (s), diffusion coefficient (D) and hydrodynamic radius (R_h) to weight average molar mass (M_w) in terms of Mark-Houwink-Kuhn-Sakurada (MHKS) equations,[12, 38]

$$s_0 = K_s N^a \tag{1}$$

$$<D_0> = K_D N^{-b} \tag{2}$$

$$<R_{h,0}> = K_R N^c \tag{3}$$

where s_0, $<D_0>$ and $<R_{h,0}>$ are the sedimentation coefficient, diffusion coefficient and hydrodynamic radius of the polymer at infinite dilution, respectively. K_s, K_D and K_R are the scale prefactors, a, b and c are the corresponding scale indexes and $b = c$. For a random coil, $a = 0.4–0.5$ and $b = 0.5–0.6$. For a compact sphere, $a = 2/3$ and $b = 1/3$.[38] Moreover, Raman spectroscopy measurements indicate that PMAA chains exhibit a progressive conformational transition.[39, 40] Yet, other studies reveal that it is cooperative.[13, 41] So it is necessary to examine the pH-induced conformation change of PMAA by the use of SV because of its high resolution size analysis of polymers.[42] For polymers with relatively small molar mass, SV has its advantage over laser light scattering (LLS) because the measured concentration of polymers in LLS should be much higher than that in SV experiments.[33, 34] Besides, the samples does not need labeling in SV measurements.[39] Howard et al.[35] studied the sedimentation and diffusion of PMAA by use of AUC, however due to the lacking of the powerful data analysis software like SEDFIT, the diffusion coefficient and hydrodynamic radius of PMAA at different pH values with high resolution was not obtained at that time.[16]

It is well known that hydration is an important event in proteins.[43-45] The hydration of PMAA at different pH values can be considered as a prerequisite for understanding of the protein hydration and worthwhile for further exploration. However, the hydration of PMAA chains and ion binding to carboxylic acid groups of PMAA have received little attention. Pleštil et al.[46] studied the binding of counterions and hydration of PMAA chain in deuterated water by a combination of neutron and X-ray small-angle scattering and their results suggest that the PMAA chain has a hydration shell with 18 molecules of deuterated water per monomer. Ikegami[47, 48] investigated the hydration of PMAA by the method of refractivity measurement in aqueous solutions and stated that there might be two regions of water around the polyelectrolytes, that is, in the first hydration region, water molecules are oriented to the individual charged groups and water molecules are rearranged by the cooperative action of two or more charged groups on the polymer chain in the second hydration region. Chung et al.[49] studied the condensation of sodium ions surrounding the charged PMAA and the water molecules around PMAA by all-atom molecular dynamics simulations. They demonstrated that sodium ions bridge between charged monomers and lead to a more coil-like and locally stretched conformation in dilute solution. When only sodium ions are present in the aqueous solution as the cations, water molecules are adsorbed onto either the condensed sodium ions or the carboxylic acid groups. The hydrogen bond between the absorbed water molecules enhances bridging behavior of monomers. Sulatha and Natarajan[15] also studied the conformation and hydration behavior of PAA and PMAA aqueous solutions using fully atomistic molecular dynamics simulations and found strong interaction and hydrogen bonding between the carboxylate oxygen and water using two different sets of force field parameters. Up to now, there has been no solid experimental data to back such simulation results.

In this study, we have investigated sedimentation and diffusion of a series of narrowly distributed PMAA polymers with weight average molar masses (M_w) ranging from 1.2×10^3 to 3.2×10^5 g/mol as a function of pH by use of SV. Our aim is to understand the conformational change and hydration of individual PMAA chains in aqueous solutions at different pH values.

Experimental Section

Sample Preparation. PMAA standards were purchased from Polymer Standards Service (PSS, USA). Each PMAA sample was lyophilized after dialysis against Milli-Q water (Millipore, resistivity = 18.2 MΩ·cm) for three days prior to use. PMAA aqueous solutions were prepared by dissolving certain amount of PMAA in phosphate buffered saline (PBS) solutions with a constant ionic strength of 100 mM at different pH values which were prepared by dissolving Na_2HPO_4 and NaH_2PO_4 in Milli-Q water. The concentrations of the PMAA solutions for AUC experiments were 0.15, 0.30, 0.45 and 0.60 mg/ml, respectively.

Measurements of the Partial Specific Volume. A DMA4500 densitometer (Anton Paar) was used to measure the density of PMAA aqueous solutions at 20 ˚C with concentrations of 0.2, 0.4, 0.6 and 0.8 mg/ml, respectively. The partial specific volume (ν) of PMAA was determined according to eq.4,

$$v = \frac{1}{\rho_0}\left(1 - \frac{\Delta\rho}{\Delta C}\right) \tag{4}$$

where ρ_0, ρ and C are the solvent density, solution density and the concentration of the solute, respectively.[50] The values of ν at different pH values were (0.683 ± 0.014) ml/g, which is close to the value (v = 0.68 ml/g) reported by Heitz et al.[28] Moreover, as the partial specific volume is almost independent on the molar mass, we used this value for all the PMAA samples.[36] The measurements of the ν of PMAA samples at different pH values can be found in the Supporting Information.

Sedimentation Velocity (SV) Measurements. SV experiments were performed on a Proteomelab XL-A/I analytical ultracentrifuge (Beckman Coulter Instruments) with an An-60 Titanium 4-hole rotor at 20 ˚C. Each of the three cells was assembled by two quartz windows and a double-sector 12 mm length epoxy resin centerpiece. 400 μL of PMAA aqueous solution was loaded in one sector and the sample with 410 μL of PBS in the other sector as the reference. The rotational speed was 60000 rpm for the three PMAA samples with lowest M_w and 56000 rpm for other PMAA samples. The wavelength was set at 220 nm during the experiments. Data was collected using the software provided with the instrument and analyzed by SEDFIT. The absorbance profiles were fitted by the continuous distribution $c(s)$ implemented in SEDFIT using the maximum entropy regularization which followed the CONTIN method provided by Provencher with Lamm equation,[36, 37, 51]

$$\frac{\partial c}{\partial t} = D\left[\frac{\partial^2 c}{\partial r^2} + \frac{1}{r}\frac{\partial c}{\partial r}\right] - s\omega^2\left[r\frac{\partial c}{\partial r} + 2c\right] \quad (5)$$

where c, r, t, ω, s and D are the concentration of the solute, radial distance from the axis of rotation, sedimentation time, angular velocity, sedimentation coefficient and diffusion coefficient, respectively. Note that s is defined as

$$s = \frac{u}{\omega^2 r} = \frac{d\ln r}{\omega^2 \mathrm{d}t} \tag{6}$$

where u is the sedimentation velocity of the solute. The unit of s is Svedberg (S) or 10^{-13} s.

Assuming that all species in solution have the same weight-average frictional ratio, molar mass and diffusion coefficient can be evaluated with a combination of Stokes-Einstein equation and Svedberg equation

$$D = \frac{k_\mathrm{B} T}{6\pi\eta R_\mathrm{h}} \tag{7}$$

$$M = \frac{k_\mathrm{B} N_\mathrm{A} T}{\left(1 - v\rho_0\right)} \frac{s}{D} \tag{8}$$

with the Boltzmann constant k_B, the absolute temperature T, the hydrodynamic radius R_h, the solvent viscosity η, the molar mass M, the Avogadro's number N_A, the solvent density ρ_0 and the partial specific volume of the solute v.

Results and Discussion

SEDFIT program (version 12.1) developed by Schuck was adopted to analyze the absorbance profiles with Lamm equation solutions using the maximum entropy regularization which followed the CONTIN method provide by Provencher. The continuous $c(s)$ distribution model in SEDFIT program could distinguish boundary spreading due to the size heterogeneity from diffusion, so the diffusion coefficient and molar mass of polymer samples can be evaluated based on eq 8. The typical sedimentation coefficient distributions of PMAA samples which are designated as PMAA1–PMAA7 are shown in Figure 1, where the concentrations of PMAA were 0.6 mg/ml and the pH value was 6.0. Figure 1 shows that the sedimentation coefficient increases with the molar mass of PMAA and all of the PMAA samples are narrowly distributed, which is consistent with the information from manufacturer as polydispersity indexes are smaller than 1.2. The characterization data of PMAA samples are summarized in Table 1. The degree of polymerization (*N*) in Table 1 is calculated from the molar mass of PMAA samples at pH = 4.0 determined by AUC because PMAA chains with high M_w aggregate at pH = 3.0 and the M_w increases with the increasing pH due to the hydration of PMAA chains, as we will discuss later.[52] Note that the M_w obtained by AUC is lower than that obtained by SEC, especially for those PMAA samples with highest M_w. This is understandable because SEC gives a relative molecular weight due to the difference between the standards and polymers samples.

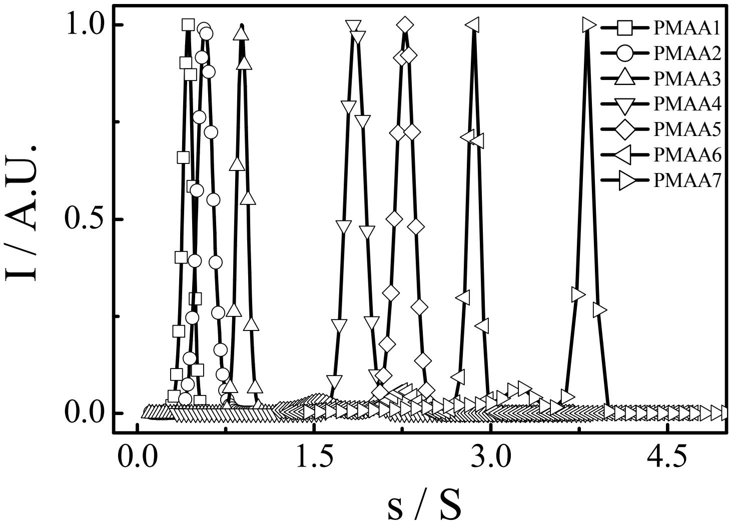

Figure 1. Sedimentation coefficient (*s*) distribution of PMAA samples in phosphate buffer solution at pH 6.0, where the concentration of each PMAA sample is 0.60 mg/ml and ionic strength of the phosphate buffer is 100 mM.

Table 1: Characterization data of PMAA samples

Sample	M_w (g/mol) [a]	M_w (g/mol) [b]	M_w (g/mol) [c]	N [c]	M_w (g/mol) [d]
PMAA1	1250	990	1300	15	1720
PMAA2	3150	2510	2480	29	3590
PMAA3	8210	6540	4980	58	8130
PMAA4	34700	27600	18200	211	36400
PMAA5	76800	61200	38000	442	82600
PMAA6	163000	129800	95600	1110	176600
PMAA7	311000	247600	154000	1790	274500

[a] M_w of the ionized PMAA (polymethacrylic acid sodium salt) calculated from M_w of the parent poly(*t*-butyl methacrylate) with the factor of 0.76 by the manufacturer. [b] M_w of the unionized PMAA calculated from M_w of the parent poly(*t*-butyl methacrylate) with the factor of 0.61. [c] Determined by AUC at pH 4.0. [d] Determined by AUC at pH 8.5.

Figure 2 shows the concentration dependence of s, which is the sedimentation coefficient of the PMAA sample at 20 °C in aqueous solution. When M_w is lower than 18200 g/mol (PMAA1–4), s is almost independent on the concentration. However, when M_w is higher than 18200 g/mol (PMAA5–7), s decreases with the concentration. The facts indicate that interactions between PMAA chains increase with M_w, which is common phenomenon of hydrodynamic interactions for neutral polymers and polyelectrolytes.[35, 53-55] Since the measurements were performed in very dilute solutions, equation $s = s_0(1-k_s c)$ can be used for the extrapolation, where s_0 is the sedimentation coefficient at infinite dilution and k_s is the concentration coefficient.

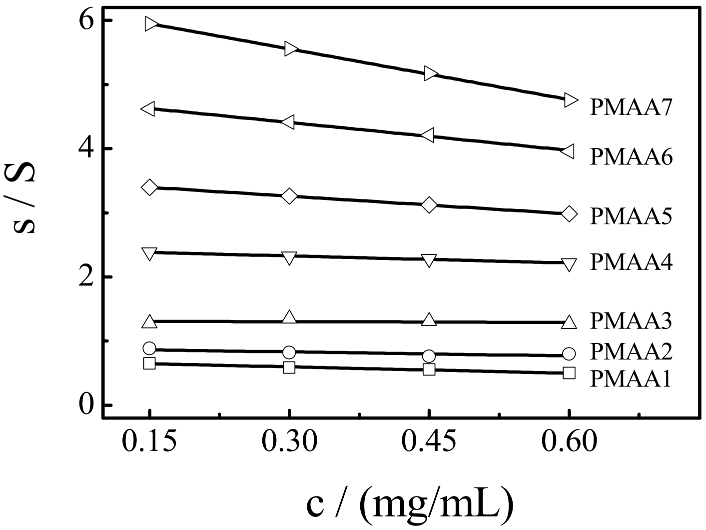

Figure 2. Concentration dependence of sedimentation coefficients of PMAA at pH 8.5, where the concentrations are 0.15, 0.30, 0.45, 0.60 mg/ml, respectively.

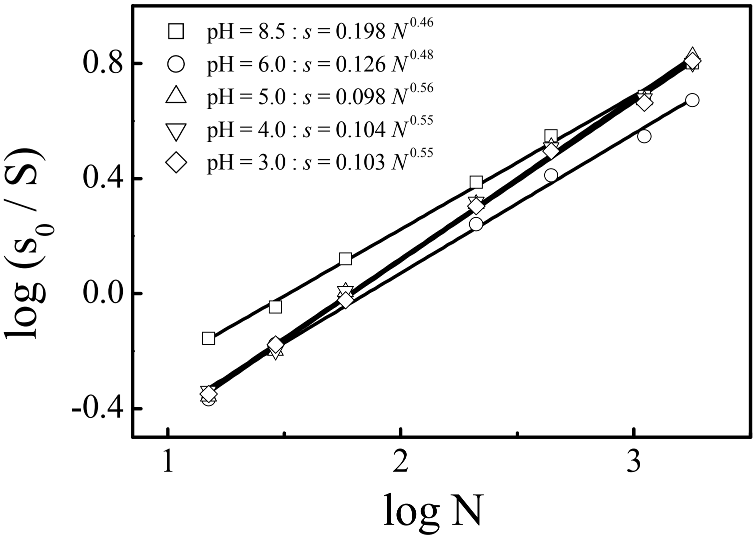

Figure 3. Degree of polymerization (N) dependence of PMAA sedimentation coefficient at infinite dilution (s_0) at different pH values.

Table 2： The scale prefactors and corresponding scale indexes of MHKS equations

pH	$s_0 = K_sN^a$		$\langle D_0\rangle = K_DN^{-b}$		$\langle R_{h,0}\rangle = K_RN^c$	
	K_S (S)	a	K_D ($\times 10^{10}$ m^2/s)	b	K_R (nm)	c
3.0	0.103	0.55	5.43	0.38	0.367	0.38
4.0	0.104	0.55	8.65	0.44	0.237	0.44
5.0	0.098	0.56	9.66	0.49	0.212	0.50
6.0	0.126	0.48	11.4	0.59	0.180	0.59
8.5	0.198	0.46	9.14	0.54	0.223	0.54

The degree of polymerization dependence of s_0 of PMAA in a double logarithmic plot is shown in Figure 3. It is clear that there is a scaling relationship between s_0 and the degree of polymerization, i.e. $s_0 = K_sN^a$. The fitting parameters K_s and a at different pH values are summarized in Table 2. It is known that for a uniform and compact sphere, $a \sim 2/3$ and for a linear flexible random coil chain, $a \sim 0.4 – 0.5$.[38] The value of a is 0.46 at pH = 8.5, and ~ 0.56 when pH is lower than 5.0, indicating that the conformation of the polymer chain is random coil at high pH and is collapsed but not a compact one at low pH since a is smaller than 2/3.

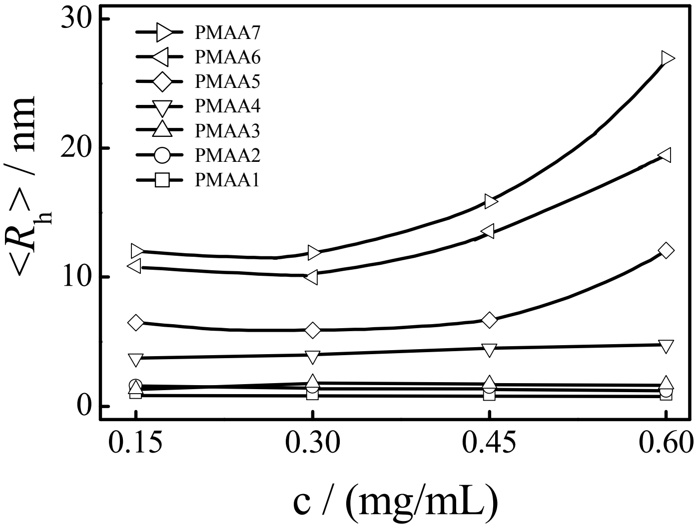

Figure 4. Concentration dependence of hydrodynamic radius (<R_h>) of PMAA at pH = 8.5.

It is known that not only sedimentation coefficient but also diffusion coefficient (<D>) and hydrodynamic radius (<R_h>) can be obtained in SV experiments with the help of SEDFIT program. Figure 4 shows that <R_h> increases with the concentration when the weight average molar masses of PMAA samples are higher than 18200 g/mol (PMAA5–7) at high pH such as 8.5. Note that Ruiz-Pérez et al.[34] reported that hydrodynamic radius of PMAA with the M_w of 80000 g/mol was 15 nm at pH > 6 by use of dynamic light scattering, where the ionic strength of the solution was 0.01 M and the concentration of PMAA was 1.0 mg/mL. From Figure 4 we know that there are still nonnegligible interactions between PMAA chains with M_w higher than 18200 g/mol when the concentration is higher than 0.30 mg/mL. But the hydrodynamic radii of PMAA samples do not change when the concentration of PMAA is lower than 0.30 mg/mL. Note that interaction between PMAA chains is largest at pH = 8.5 as PMAA chains are fully ionized.

Thus, in this study the <R_h>s of PMAA at different pH values with the concentration of 0.15 mg/mL are used thereafter to eliminate the effect of concentration on the hydrodynamic radius at all pH values.

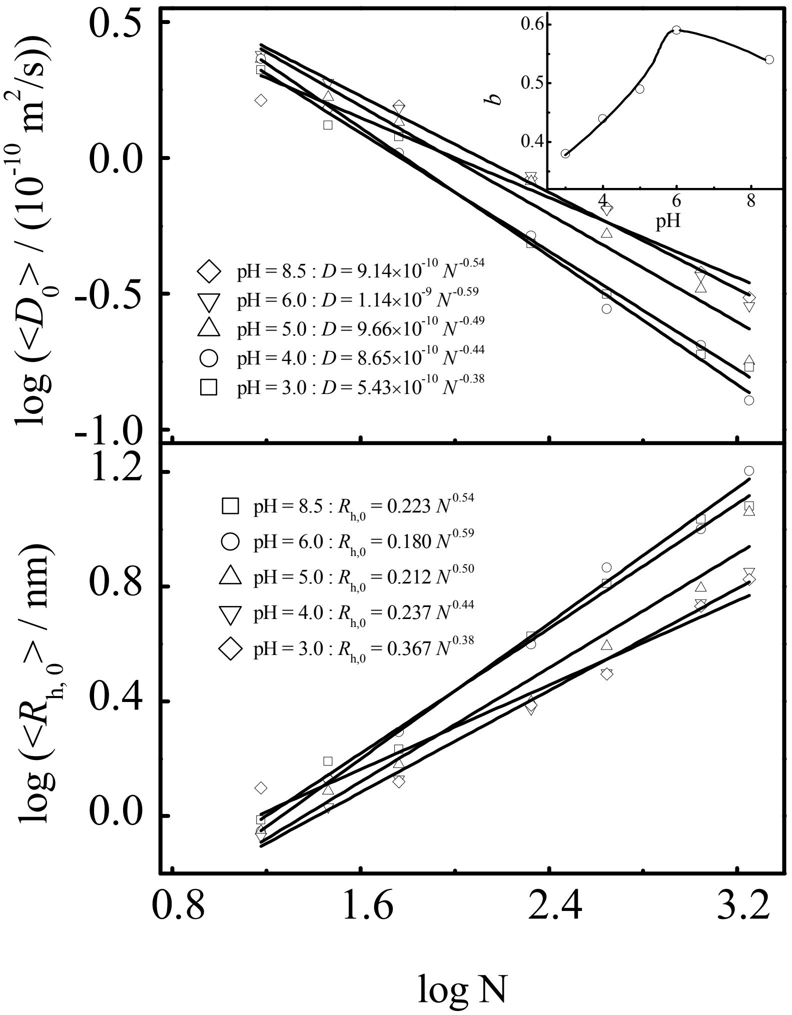

Figure 5. Degree of polymerization dependence (*N*) of diffusion coefficients (<D_0>) and hydrodynamic radii (<$R_{h,\,0}$>) of PMAA samples at different pH values, where the <*D*> and <R_h> of PMAA with the concentration of 0.15 mg/mL are used as <D_0> and <$R_{h,0}$>. The inset shows the pH dependence of *b*.

Figure 5 shows the N dependence of $<D_0>$ and $<R_{h,\,0}>$ in a double logarithmic plot, where $<D_0>$ and $<R_{h,0}>$ are the values of $<D>$ and $<R_h>$ of PMAA with the concentration of 0.15 mg/mL. It shows that $<D_0>$ decreases with the weight average molar mass which is similar to that for neutral polymer.[55] Besides, there is also a scaling relationship between $<D_0>$ or $<R_{h,0}>$ and the degree of polymerization, i.e. $<D_0> = K_D N^{-b}$ and $<R_{h,0}> = K_R N^c$. The fitting parameters including the scale prefactors (K_D, K_R) and the corresponding scale indexes (b and c) are summarized in Table 2. As we know that $D \propto 1/R_h$, so the scale index b is equal to c. As discussed above, for a compact sphere, $b \sim 1/3$; for a random coil chain, $b \sim 0.50 - 0.60$.[38] The scale index b indicates that the conformation of PMAA in aqueous solution at low pH value is collapsed but not a compact sphere and the PMAA polymer expands to random coil chain at pH > 6.0. The inset shows that b value increases with pH, reaches a maximum at pH = 6.0, and then decrease from pH = 6.0 to 8.5, indicating that the PMAA chain is more stretching at pH = 6.0 than at 8.0, which will be discussed later.

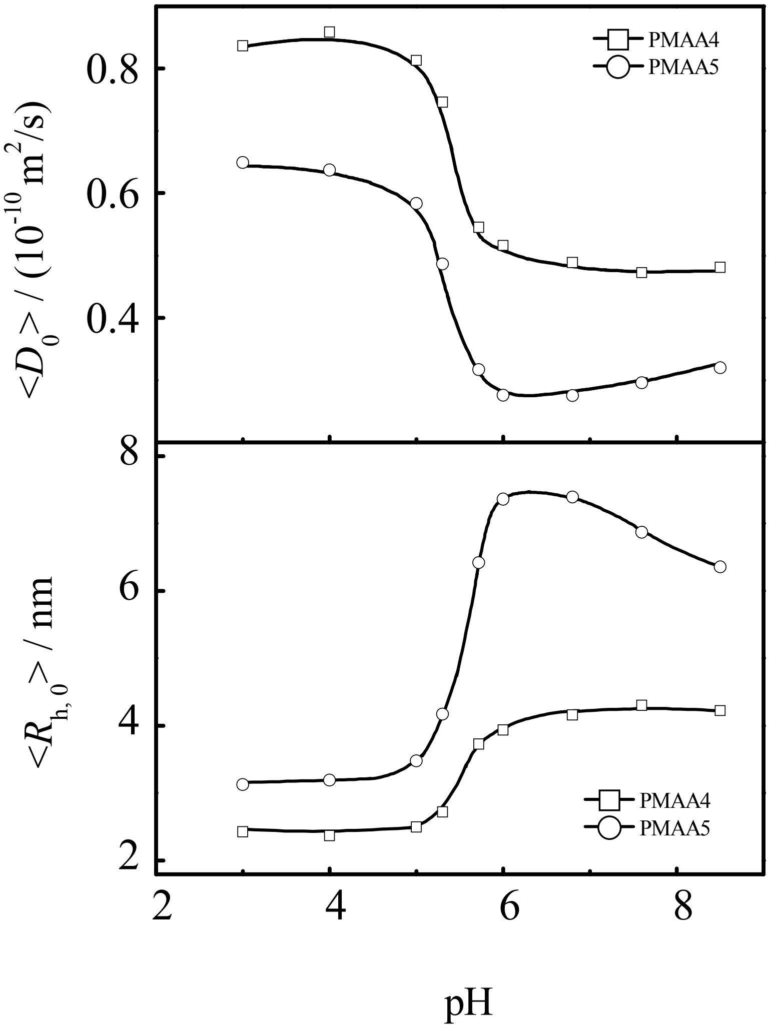

Figure 6. pH dependence of diffusion coefficients (<D_0>) and hydrodynamic radii (<$R_{h,0}$>) of PMAA4 and PMAA5.

The conformational change of PMAA in aqueous solutions has been investigated by viscometry measurements, fluorescence spectroscopy, laser-excited Raman spectroscopy and so on.[14, 31, 32, 56] Most of these techniques indirectly characterize the conformational changes. LLS is also difficult to observe the conformational change of short PMAA chains in aqueous solutions. Ruiz-Pérez et al[34]. reported that hydrodynamic radius of PMAA with the M_w of 80000 g/mol was ~ 8 nm at pH < 5.5, sharply increased to 15 nm at pH = 6.0, and then leveled off in the range of pH 6.0 – 10.0 by use of dynamic light scattering (DLS), where the ionic strength of the solution was 10 mM and the concentration of PMAA was 1.0 mg/mL. But they also stated that the quantity of the DLS data is not good enough to draw the conclusion that the transition is complete over a narrow range in pH. Figure 6 shows the pH dependence of $<D_0>$ and $<R_{h,0}>$ of two PMAA samples (PMAA4 and PMAA5). At pH < 5, $<R_{h,0}>$ is nearly a constant, indicating that the PMAA chain is collapsed with a lower $<R_{h,0}>$. The sudden increase in $<R_{h,0}>$ in the range of pH 5 – 6 reflects the conformation change from a collapsed conformation to a random coil. Our results show that the breadth of the transition range is larger than that reported by Ruiz-Pérez et al,[34] presumably due to the effect of the concentration of PMAA chains and/or the sensitivity of these two different methods (DLS and AUC). Then, for PMAA4 with a lower M_w, $<R_{h,0}>$ levels off at pH > 6. For PMAA5 with a higher M_w, we observed that $<R_{h,0}>$ slightly decreases at pH > 6. Chuang and Huang[49] studied the condensation behavior of monovalent and multivalent counterions surrounding the ionized PMAA chains in aqueous solutions by all-atom molecular dynamics simulations. They demonstrated that further ionization of the PMAA via increasing pH leads more sodium ions to condense on PMAA polymer chains which attract more than one charged monomers acting as a bridging agent of PMAA chains. Presumably due to this bridging effect, PMAA5 chain with a higher M_w shrinks a little bit, however PMAA4 chain might not be long enough to bend and form a bridging bond. Moreover, the possibility that water molecules can also play an important role in the formation of bridging bond cannot be ruled out.[57]

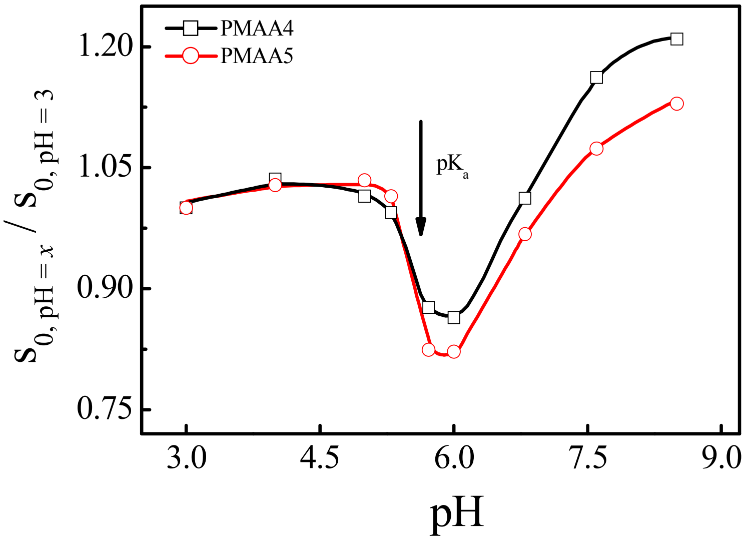

Figure 7. pH dependence of $s_{0,\ \mathrm{pH}\ =\ x}$ / $s_{0,\ \mathrm{pH}\ =\ 3}$, where $s_{0,\ \mathrm{pH}\ =\ x}$ and $s_{0,\ \mathrm{pH}\ =\ 3}$ are the sedimentation coefficients of PMAA4 and PMAA5 at infinite dilution at pH = x and pH = 3, respectively. The arrow indicates the $\mathrm{pK_a}$ of PMAA chains.

Figure 7 shows the pH dependence of the ratio of sedimentation coefficients of PMAA4 and PMAA5 at pH = x to those at pH = 3. At pH < 5, $s_{0,\ \mathrm{pH} = x}$ / $s_{0,\ \mathrm{pH} = 3}$ is nearly a constant, indicating that the conformation of PMAA remains unchanged at pH < 5. The ratio decreases in the range of pH 5−6 and exhibits a minimum at pH ~ 6. Then the ratio increases at pH > 6. Howard et al. studied the effect of degree of ionization on sedimentation coefficient of PMAA and their AUC measurements showed that sedimentation coefficient had a minimum when the degree of ionization was ~ 0.4, which is consistent with our results.[35] Note that the sedimentation coefficient is proportional to the product of the molar mass (M) and the diffusion coefficient (D), i.e. $s \propto M{\cdot}D$, which means that Figure 7 will be much clearer if we know the pH dependence of the molar mass of PMAA.

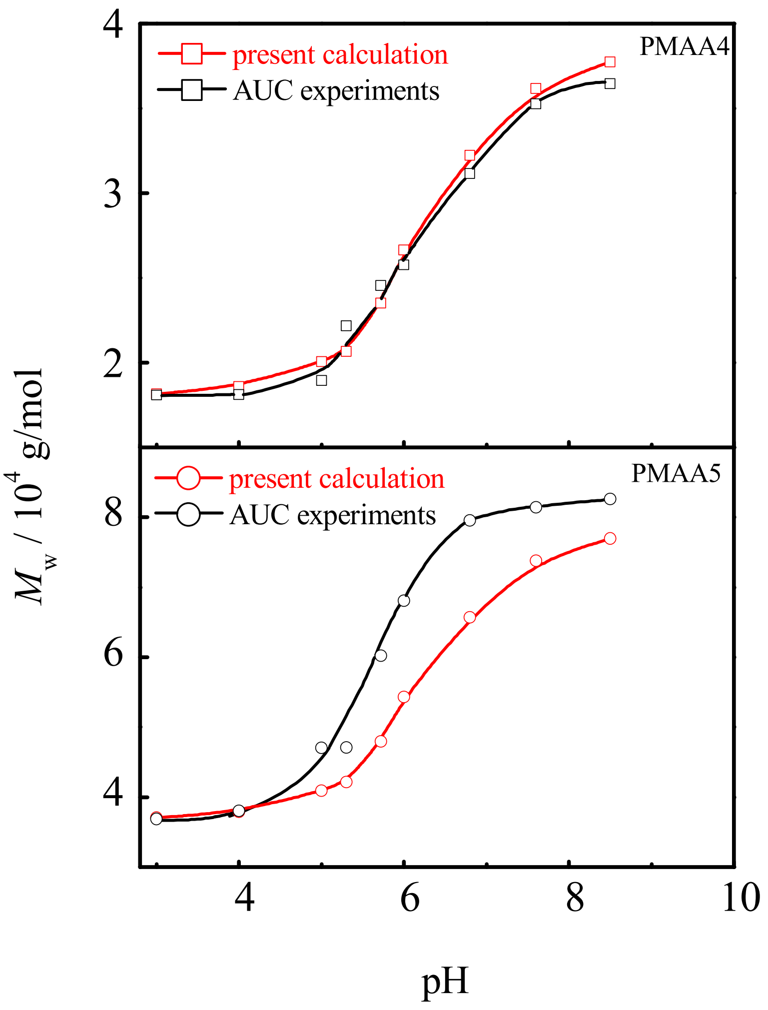

Figure 8. pH dependence of M_w of PMAA4 and PMAA5, which were obtained by AUC experiments and calculation.

Figure 8 shows the pH dependence of M_w of PMAA4 and PMAA5. At pH < 4, M_w is nearly a constant. M_w increases in the range of pH 4 – 7 and then levels off at pH > 7, presumably due to the binding of water molecules and ions to the carboxylate groups by the hydrogen bonds and electrostatic interactions.[15, 49, 57, 58] From Figure 6 and Figure 8, we know that at pH < 4, both molar mass and diffusion coefficient remain unchanged, so sedimentation coefficient is nearly a constant. In the range of pH 4 – 6, the sedimentation coefficient decreases because the effect of diffusion coefficient dominates over that of molar mass. At pH > 6, the effect of molar mass on the sedimentation coefficient dominates over that of diffusion coefficient. Gustavsson et al.[59] studied the binding of sodium ions to carboxylate groups of PMAA at different pH values by nuclear magnetic resonance. They stated that sodium ions do not bind to carboxylate groups when the degree of ionization (α) ≤ 0.3 and the portion of carboxylate groups that bind sodium ions is ($\alpha - 0.3$) when α is in the range of $0.3 - 1.0$, where α is related to the pH of the solutions.[60, 61] Assuming that each carboxylate group binds three water molecules and each sodium ion binds four water molecules,[57, 62] and the number of water molecules binding to the carboxylate group and sodium ion decrease by one when carboxylate group and sodium ion binds to each other, the molar mass of PMAA at different degree of ionizations can be calculated, as shown in Figure 8. Clearly, the calculated molar mass is consistent with our AUC experimental data, especially for PMAA4. Note that even if considering the water molecules binding to the condensed sodium ions, the total number of water molecules per repeating unit of PMAA is only ~ 5, which is smaller than the number of water molecules 18 reported by Pleštil et al.,[46] further indicating that other water molecules may not strongly bind to COO^- groups and only a small portion of water molecules move together with COO^- groups. For PMAA5, the molar masses from the AUC experiments are larger than the calculated data, the reason is presumably that some water molecules bind to the hydration shells of carboxylate groups and sodium ions and move with the PMAA chains, as shown in Figure 9.

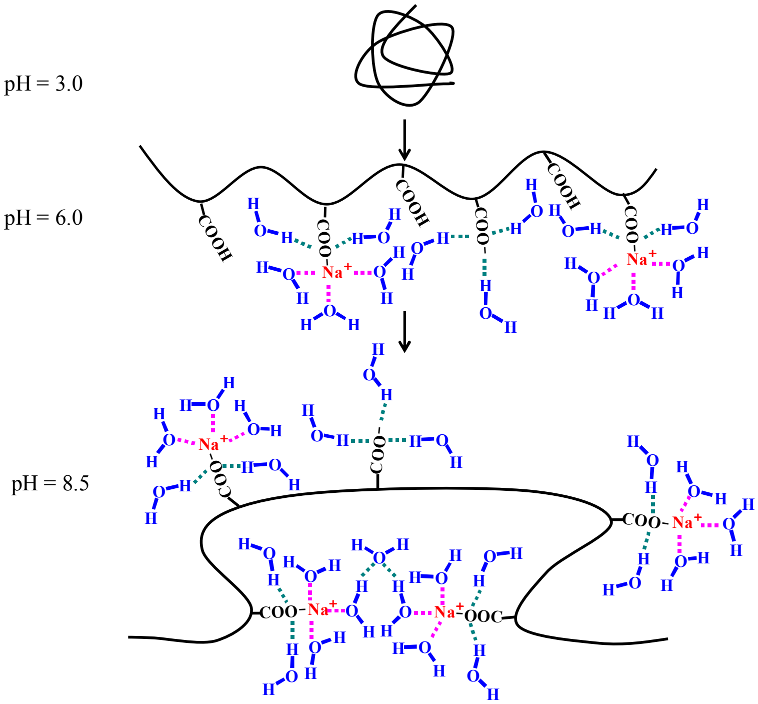

Figure 9. Schematic diagram of pH-induced conformational change of PMAA and the interactions between carboxylate groups, sodium ions and water molecules at different pH values.

Conclusion

We have investigated the sedimentation coefficient, diffusion coefficient and hydrodynamic radius of a series of PMAA with different molar masses at different pH values in dilute aqueous solutions by use of analytical ultracentrifuge (AUC). The scale prefactors and corresponding scale indexes obtained from AUC experiments indicate that the PMAA chain is collapsed at pH < 4, and it stretches at pH > 5.0. Our results show that $\langle R_{h,0} \rangle$ is nearly a constant at pH < 4, indicating that the PMAA chain is collapsed with a lower $\langle R_{h,0} \rangle$. The sudden increase in $\langle R_{h,0} \rangle$ in the range of pH 4 – 6 reflects the conformation change from a collapsed conformation to a random coil. For PMAA with a lower M_w, $\langle R_{h,0} \rangle$ levels off at pH > 6 and for PMAA with a higher M_w, $\langle R_{h,0} \rangle$ slightly decreases at pH > 6. Moreover, the molar mass of PMAA chain is nearly a constant at pH < 4, increases in the range of pH 4 – 7 and then levels off, presumably due to the binding of the water molecules and sodium ions to the ionized carboxylate groups.

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Xiaoyan Wang, Xiaodong Ye, Guangzhao Zhang