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# Investigation of pH-induced conformational change and hydration of poly(methacrylic acid) by analytical ultracentrifugation†

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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 the sedimentation coefficient ( $s$ ) and the 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 stretch at pH higher than 5.2. Our results show that the sedimentation coefficient exhibits a minimum at pH  $\sim$  6.0, presumably due to the effect of the conformational change and the hydration state of PMAA chains. When pH increases from 6.0 to 8.5, PMAA chains with 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 by 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 values.

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## Introduction

Poly(methacrylic acid) (PMAA), one of the weak polyelectrolytes, has received increasing interest not only because of its promising applications in cosmetics,<sup>1,2</sup> pharmaceuticals,<sup>3,4</sup> drug testing<sup>5,6</sup> and biomedical technology,<sup>7,8</sup> but also due to its biophysical relevance to the folding and packing process of more complicated biomacromolecules such as proteins and DNA.<sup>9–11</sup> 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 increasing pH.<sup>12–15</sup>

The pH-induced conformational change of PMAA has been studied by different techniques such as viscometry,<sup>14,16,17</sup> fluorescence spectroscopy,<sup>18–27</sup> small angle X-ray scattering (SAXS),<sup>28–30</sup> Raman spectroscopy,<sup>31,32</sup> infrared spectroscopy,<sup>13</sup> laser light scattering<sup>33,34</sup> and analytical ultracentrifugation.<sup>35</sup>

However, it is hard to investigate the conformational changes 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 high resolution after the development of data analysis software like the SEDFIT program.<sup>36,37</sup> By use of SV, the conformational change of PMAA chains in aqueous solutions can also be obtained from the scales of the sedimentation coefficient ( $s$ ), the diffusion coefficient ( $D$ ) and the hydrodynamic radius ( $R_h$ ) to degree of polymerization ( $N$ ) in terms of Mark–Houwink–Kuhn–Sakurada (MHKS) equations,<sup>12,38</sup>

$$s_0 = K_s N^a \quad (1)$$

$$\langle D_0 \rangle = K_D N^{-b} \quad (2)$$

$$\langle R_{h,0} \rangle = K_R N^c \quad (3)$$

where  $s_0$ ,  $\langle D_0 \rangle$  and  $\langle R_{h,0} \rangle$  are the sedimentation coefficient, the diffusion coefficient and the 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 indices 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  $b = 1/3$ .<sup>38</sup> Moreover, Raman spectroscopy measurements indicate that PMAA chains exhibit a progressive conformational transition.<sup>39,40</sup> Yet, other studies reveal that it is cooperative.<sup>13,41</sup> So it is necessary to examine the pH-induced conformational changes of PMAA by the use of

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SV because of its high resolution size analysis of polymers.<sup>42</sup> 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.<sup>33,34</sup> Besides, the samples do not need labeling in SV measurements.<sup>39</sup> Howard and Jordan<sup>35</sup> studied the sedimentation and diffusion of PMAA by use of AUC, however due to the lack of powerful data analysis software like SEDFIT, the diffusion coefficient and the hydrodynamic radius of PMAA at different pH values with high resolution were not obtained at that time.<sup>16</sup>

It is well known that hydration is an important event in proteins.<sup>43–45</sup> The hydration of PMAA at different pH values can be considered as a prerequisite for understanding the protein hydration and is worthwhile for further exploration. However, the hydration of PMAA chains and the ion binding to carboxylic acid groups of PMAA have received little attention. Pleštil *et al.*<sup>46</sup> studied the binding of counterions and the hydration of PMAA chains 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<sup>47,48</sup> 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 toward 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 and Huang<sup>49</sup> studied the condensation of sodium ions surrounding the charged PMAA and the water molecules around PMAA using 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 solutions. 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 carboxylate groups. The hydrogen bond between the adsorbed water molecules enhances bridging behavior of monomers. Sulatha and Natarajan<sup>15</sup> also studied the conformation and hydration behavior of PAA and PMAA aqueous solutions using fully atomistic molecular dynamics simulations and found a 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  $1.2 \times 10^3$  to  $3.2 \times 10^5$  g mol<sup>-1</sup> as a function of pH by use of SV. Our aim is to understand the conformational change and the 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<sub>2</sub>HPO<sub>4</sub> and NaH<sub>2</sub>PO<sub>4</sub> 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<sup>-1</sup>, respectively.

### Measurement of 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<sup>-1</sup>, respectively. The partial specific volume ( $v$ ) of PMAA was determined according to eqn (4),

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

where  $\rho_0$ ,  $\rho$  and  $C$  are the solvent density, the solution density and the concentration of the solute, respectively.<sup>50</sup> The value of  $v$  at different pH values was  $(0.683 \pm 0.014)$  ml g<sup>-1</sup>, which is close to the value ( $v = 0.68$  ml g<sup>-1</sup>) reported by Heitz *et al.*<sup>28</sup> Moreover, as the partial specific volume is almost independent of the molar mass, we used this value for all the PMAA samples.<sup>36</sup> The measurements of the  $v$  of PMAA samples at different pH values can be found in the ESI.†

### Sedimentation velocity (SV) measurement

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 was used as the reference. The rotational speed was 60 000 rpm for the three PMAA samples with the lowest  $M_w$  and 56 000 rpm for other PMAA samples. The wavelength was set to 220 nm during the experiments. Data were collected using the software provided with the instrument and analyzed using 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,<sup>36,37,51</sup>

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

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

$$s = \frac{u}{\omega^2 r} = \frac{d \ln r}{\omega^2 dt} \quad (6)$$

where  $u$  is the sedimentation velocity of the solute. The unit of  $s$  is Svedberg (S) or 10<sup>-13</sup> s.

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

$$D = \frac{k_B T}{6\pi\eta R_h} \quad (7)$$

$$M = \frac{k_B N_A T}{(1 - v\rho_0)D} s \quad (8)$$

with the Boltzmann constant  $k_B$ , the absolute temperature  $T$ , the hydrodynamic radius  $R_h$ , the solvent viscosity  $\eta$ , the molar mass  $M$ , the Avogadro number  $N_A$ , the solvent density  $\rho_0$  and the partial specific volume of solute  $v$ .

## Results and discussion

The 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 provided by Provencher. The continuous  $c(s)$  distribution model in the SEDFIT program could distinguish boundary spreading due to size heterogeneity from diffusion, so the diffusion coefficient and the molar mass of polymer samples can be evaluated using eqn (8). The typical sedimentation coefficient distributions of PMAA samples which are designated as PMAA1–PMAA7 are shown in Fig. 1, where the concentration of PMAA was  $0.6 \text{ mg ml}^{-1}$  and the pH value was 6.0. Fig. 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 the manufacturer as polydispersity indices are smaller than 1.2. The characterization data for 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 have a high

Table 1 Characterization data for PMAA samples

Sample	$M_w^a$ ( $\text{g mol}^{-1}$ )	$M_w^b$ ( $\text{g mol}^{-1}$ )	$M_w^c$ ( $\text{g mol}^{-1}$ )	$N^c$	$M_w^d$ ( $\text{g mol}^{-1}$ )
PMAA1	1250	990	1300	15	1720
PMAA2	3150	2510	2480	29	3590
PMAA3	8210	6540	4980	58	8130
PMAA4	34 700	27 600	18 200	211	36 400
PMAA5	76 800	61 200	38 000	442	82 600
PMAA6	163 000	129 800	95 600	1110	176 600
PMAA7	311 000	247 600	154 000	1790	274 500

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

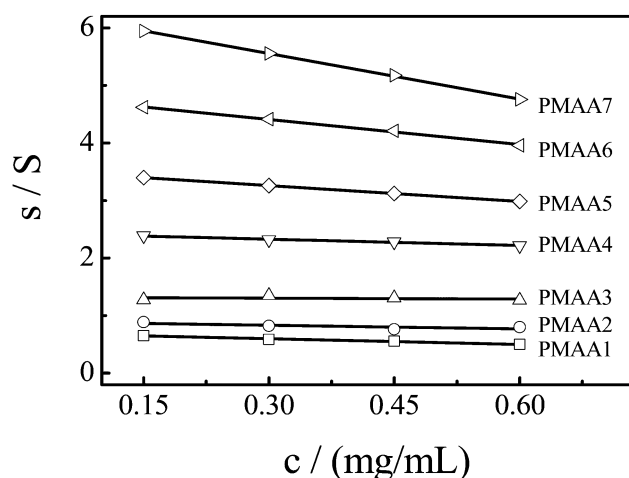


Fig. 2 Concentration dependence of sedimentation coefficients of PMAA at pH 8.5, where the concentrations are 0.15, 0.30, 0.45, and  $0.60 \text{ mg ml}^{-1}$ .

$M_w$  aggregate at pH = 3.0 and the  $M_w$  increases with increasing pH due to the hydration of PMAA chains, as we will discuss later.<sup>52</sup> Note that the  $M_w$  obtained by AUC is lower than that obtained by SEC, especially for those PMAA samples with the highest  $M_w$ . This is understandable because SEC gives a relative molecular weight due to the difference between the standards and polymer samples.

Fig. 2 shows the concentration dependence of  $s$ , which is the sedimentation coefficient of the PMAA sample at  $20^\circ\text{C}$  in an aqueous solution. When  $M_w$  is lower than  $18\,200 \text{ g mol}^{-1}$  (PMAA1–4),  $s$  is almost independent of the concentration. However, when  $M_w$  is higher than  $18\,200 \text{ g mol}^{-1}$  (PMAA5–7),  $s$  decreases with the concentration. These facts indicate that interactions between PMAA chains increase with  $M_w$ , which is a common phenomenon of hydrodynamic interactions for neutral polymers and polyelectrolytes.<sup>35,53–55</sup> Since the measurements were performed in very dilute solutions, the 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.

The degree of polymerization dependence of  $s_0$  of PMAA in a double logarithmic plot is shown in Fig. 3. It is clear that there

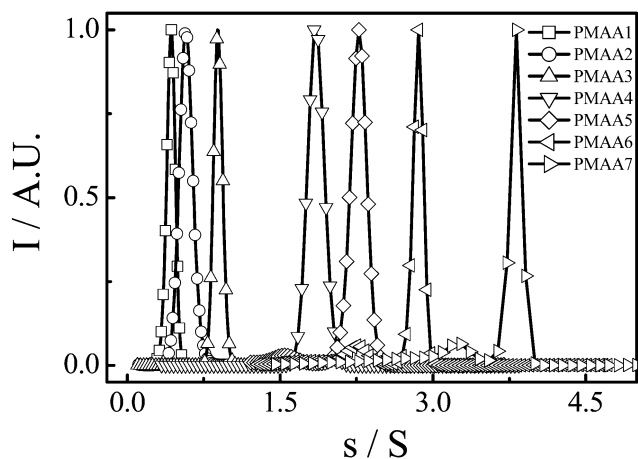


Fig. 1 Sedimentation coefficient ( $s$ ) distribution of PMAA samples in phosphate buffer solution at pH 6.0, where the concentration of each PMAA sample is  $0.60 \text{ mg ml}^{-1}$  and the ionic strength of the phosphate buffer is  $100 \text{ mM}$ .

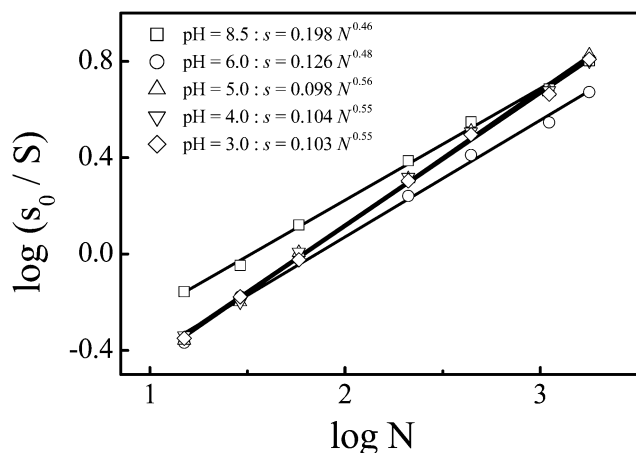


Fig. 3 Degree of polymerization ( $N$ ) dependence of the PMAA sedimentation coefficient at infinite dilution ( $s_0$ ) at different pH values.

Table 2 Scale prefactors and the corresponding scale indices of MHKS equations

pH	$s_0 = K_s N^a$		$\langle D_0 \rangle = K_D N^{-b}$		$\langle R_{h,0} \rangle = K_R N^c$	
	$K_s$ (S)	$a$	$K_D$ ( $\times 10^{10}$ m <sup>2</sup> s <sup>-1</sup> )	$b$	$K_R$ (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

is a scaling relationship between  $s_0$  and the degree of polymerization, *i.e.*  $s_0 = K_s N^a$ . 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,  $a$  is  $\sim 2/3$  and for a linear flexible random coil chain,  $a$  is  $\sim 0.4-0.5$ .<sup>38</sup> The value of  $a$  is 0.46 at pH = 8.5, and  $\sim 0.56$  when pH is lower than 5.0, indicating that the conformation of the polymer chain is a random coil at high pH values and is collapsed but not a compact one at low pH values since  $a$  is smaller than  $2/3$ .

It is known that not only the sedimentation coefficient but also the diffusion coefficient ( $\langle D \rangle$ ) and the hydrodynamic radius ( $\langle R_h \rangle$ ) can be obtained in SV experiments with the help of the SEDFIT program. Fig. 4 shows that  $\langle R_h \rangle$  increases with the concentration when the weight average molar masses of PMAA samples are higher than 18 200 g mol<sup>-1</sup> (PMAA5-7) at high pH values such as 8.5. Note that Ruiz-Pérez *et al.*<sup>34</sup> reported that the hydrodynamic radius of PMAA with the  $M_w$  of 80 000 g mol<sup>-1</sup> 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<sup>-1</sup>. From Fig. 4, we can say that there are still non-negligible interactions between PMAA chains with  $M_w$  higher than 18 200 g mol<sup>-1</sup> when the concentration is higher than 0.30 mg mL<sup>-1</sup>. But the hydrodynamic radii of PMAA samples do not change when the concentration of PMAA is lower than 0.30 mg mL<sup>-1</sup>. Note that the interaction between PMAA chains is the largest at pH = 8.5 as PMAA chains are fully ionized. Thus, in this study, the  $\langle R_h \rangle$ s of PMAA with a

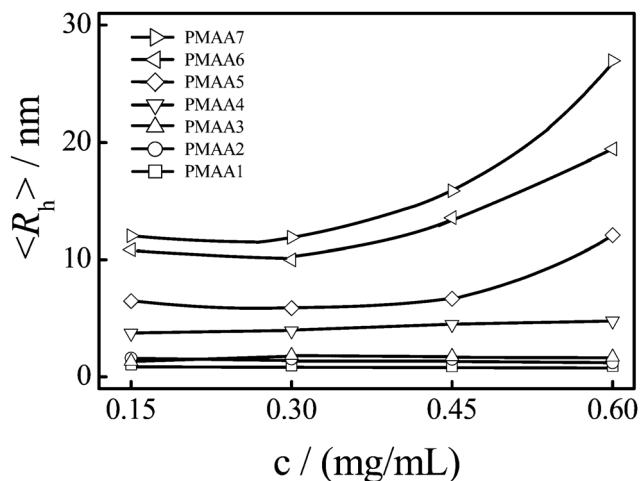


Fig. 4 Concentration dependence of the hydrodynamic radius ( $\langle R_h \rangle$ ) of PMAA at pH = 8.5.

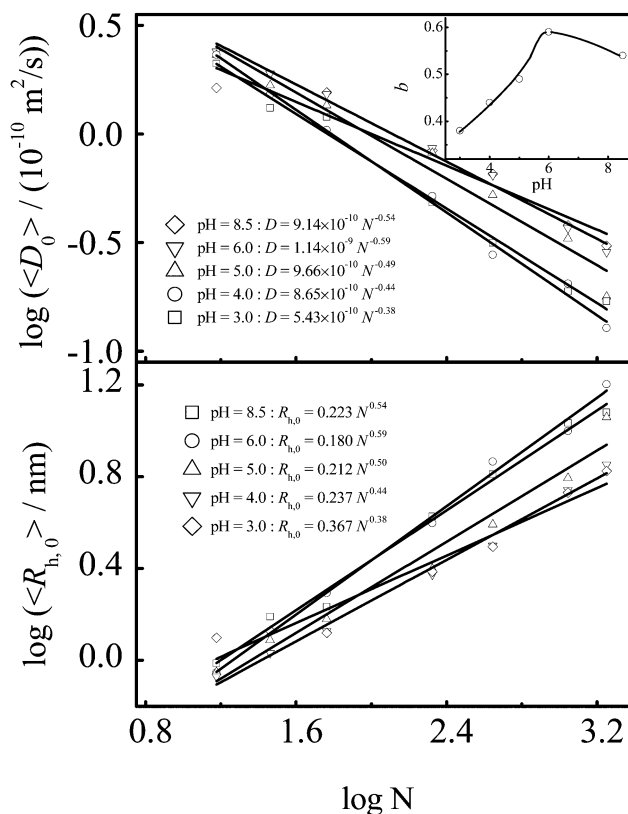


Fig. 5 Degree of polymerization ( $N$ ) dependence of diffusion coefficients ( $\langle D_0 \rangle$ ) and hydrodynamic radii ( $\langle R_{h,0} \rangle$ ) of PMAA samples at different pH values, where the  $\langle D \rangle$  and  $\langle R_h \rangle$  of PMAA with a concentration of 0.15 mg mL<sup>-1</sup> are used as  $\langle D_0 \rangle$  and  $\langle R_{h,0} \rangle$ . The inset shows the pH dependence of  $b$ .

concentration of 0.15 mg mL<sup>-1</sup> at different pH values are used to eliminate the effect of concentration on the hydrodynamic radius at all pH values.

Fig. 5 shows the  $N$  dependence of  $\langle D_0 \rangle$  and  $\langle R_{h,0} \rangle$  in a double logarithmic plot, where  $\langle D_0 \rangle$  and  $\langle R_{h,0} \rangle$  are the values of  $\langle D \rangle$  and  $\langle R_h \rangle$  of PMAA with a concentration of 0.15 mg mL<sup>-1</sup>, respectively.

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

The conformational change of PMAA in aqueous solutions has been investigated by viscometry measurements, fluorescence spectroscopy, laser-excited Raman spectroscopy, and so on.<sup>14,31,32,56</sup> Most of these techniques indirectly characterize the conformational changes. It is also difficult to observe the conformational change of short PMAA chains in aqueous solutions using LLS. Ruiz-Pérez *et al.*<sup>34</sup> reported that the hydrodynamic radius of PMAA with the  $M_w$  of  $80\,000\text{ g mol}^{-1}$  was  $\sim 8\text{ nm}$  at  $\text{pH} < 5.5$ , sharply increased to  $15\text{ nm}$  at  $\text{pH} = 6.0$ , and then leveled off in the range of  $\text{pH} 6.0$ – $10.0$  by use of dynamic light scattering (DLS), where the ionic strength of the solution was  $10\text{ mM}$  and the concentration of PMAA was  $1.0\text{ mg mL}^{-1}$ . 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 of pH. Fig. 6 shows the pH dependence of  $\langle D_0 \rangle$  and  $\langle R_{h,0} \rangle$  of two PMAA samples (PMAA4 and PMAA5). At  $\text{pH} < 5$ ,  $\langle R_{h,0} \rangle$  is nearly constant, 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 pH range of 5–6 reflects the conformational 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.*,<sup>34</sup> 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  $M_w$ ,  $\langle R_{h,0} \rangle$  levels off at  $\text{pH} > 6$ . For PMAA5 with a higher  $M_w$ , we observed that  $\langle R_{h,0} \rangle$  slightly decreases at  $\text{pH} > 6$ . Chung and Huang<sup>49</sup> 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 PMAA by increasing the pH leads to condensation of more sodium ions on PMAA polymer chains which attract more than one charged monomer acting as a bridging agent of PMAA chains. Presumably due to this bridging effect, the PMAA5 chain with a higher  $M_w$  shrinks a little bit, however the 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 bonds cannot be ruled out.<sup>57</sup>

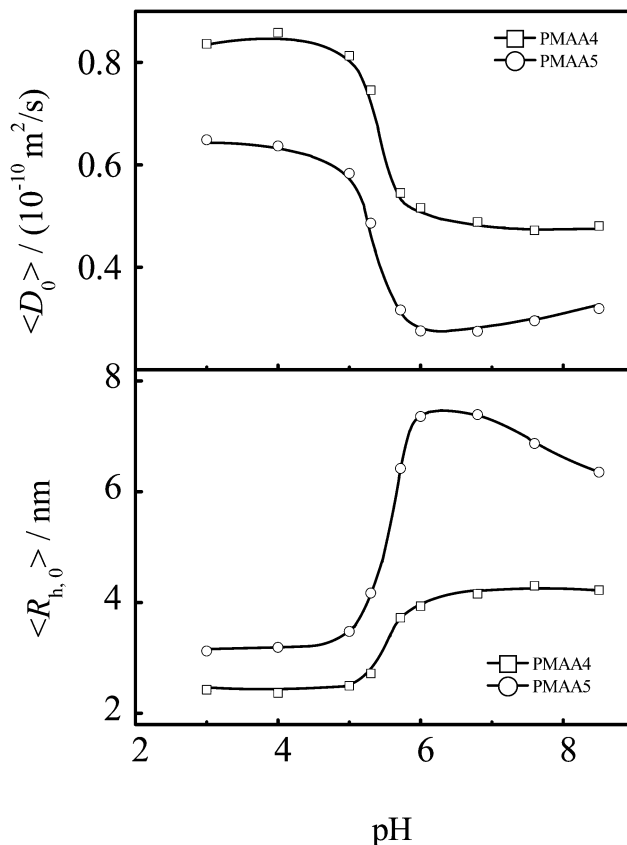


Fig. 6 pH dependence of diffusion coefficients ( $\langle D_0 \rangle$ ) and hydrodynamic radii ( $\langle R_{h,0} \rangle$ ) of PMAA4 and PMAA5.

Fig. 7 shows the pH dependence of the ratio of sedimentation coefficients of PMAA4 and PMAA5 at  $\text{pH} = x$  to those at  $\text{pH} = 3$ . At  $\text{pH} < 5$ ,  $s_{0,\text{pH}=x}/s_{0,\text{pH}=3}$  is nearly constant, indicating that the conformation of PMAA remains unchanged at  $\text{pH} < 5$ . The ratio decreases in the pH range of 5–6 and exhibits a minimum at  $\text{pH} \sim 6$ . Then the ratio increases at  $\text{pH} > 6$ . Howard and Jordan studied the effect of degree of ionization on the sedimentation coefficient of PMAA and their AUC measurements showed that the sedimentation coefficient had a minimum when the degree of ionization was  $\sim 0.4$ , which is consistent with our results.<sup>35</sup> 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 Fig. 7 will be much clearer if we know the pH dependence of the molar mass of PMAA.

Fig. 8 shows the pH dependence of  $M_w$  of PMAA4 and PMAA5. At  $\text{pH} < 4$ ,  $M_w$  is nearly constant.  $M_w$  increases in the pH range of 4–7 and then levels off at  $\text{pH} > 7$ , presumably due to the binding of water molecules and ions to the carboxylate groups by the hydrogen bonds and electrostatic interactions.<sup>15,49,57,58</sup> From Fig. 6 and 8, we can say that at  $\text{pH} < 4$ , both the molar mass and the diffusion coefficient remain unchanged, so the sedimentation coefficient is nearly constant. In the pH range of 4–6, the sedimentation coefficient decreases because the effect of the diffusion coefficient dominates over that of the molar mass. At  $\text{pH} > 6$ , the effect of the molar mass on the sedimentation

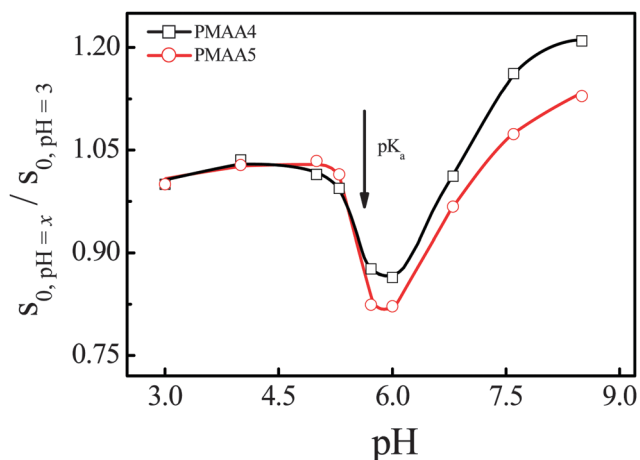


Fig. 7 pH dependence of  $s_{0,pH=x}/s_{0,pH=3}$ , where  $s_{0,pH=x}$  and  $s_{0,pH=3}$  are the sedimentation coefficients of PMAA4 and PMAA5 at infinite dilution at pH =  $x$  and pH = 3, respectively. The arrow indicates the  $pK_a$  of PMAA chains.

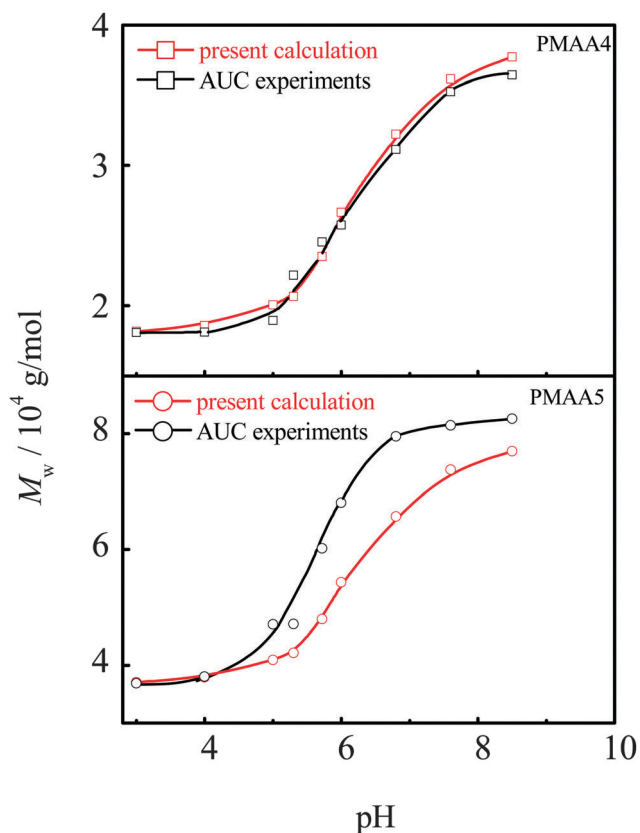


Fig. 8 pH dependence of  $M_w$  of PMAA4 and PMAA5, which were obtained by AUC experiments and calculation.

coefficient dominates over that of the diffusion coefficient. Gustavsson *et al.*<sup>59</sup> 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 ( $\alpha$ )  $\leq 0.3$  and the portion of carboxylate groups that bind sodium ions is ( $\alpha - 0.3$ ) when  $\alpha$  is in the range of 0.3–1.0, where  $\alpha$  is related to

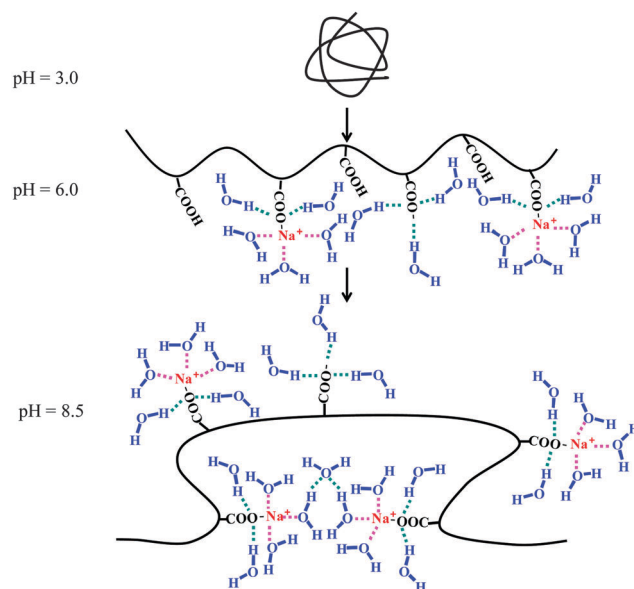


Fig. 9 Schematic diagram of pH-induced conformational change of PMAA and the interactions between carboxylate groups, sodium ions and water molecules at different pH values.

the pH of the solutions.<sup>60,61</sup> Assuming that each carboxylate group binds three water molecules and each sodium ion binds four water molecules,<sup>57,62</sup> and the number of water molecules binding to the carboxylate groups and sodium ions decrease by one when carboxylate groups and sodium ions bind to each other, the molar mass of PMAA at different degrees of ionization can be calculated, as shown in Fig. 8. Clearly, the calculated molar mass is consistent with our AUC experimental data, especially for PMAA4. Note that even if we consider the water molecules binding to the condensed sodium ions, the total number of water molecules per repeating unit of PMAA is only  $\sim 5$ , which is smaller than the number of water molecules of 18 reported by Pleštil *et al.*,<sup>46</sup> further indicating that other water molecules may not strongly bind to  $\text{COO}^-$  groups and only a small portion of water molecules move together with  $\text{COO}^-$  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 Fig. 9.

## Conclusion

We have investigated the sedimentation coefficient, the diffusion coefficient and the hydrodynamic radius of a series of PMAA polymers with different molar masses at different pH values in dilute aqueous solutions by use of analytical ultracentrifugation (AUC). The scale prefactors and the corresponding scale indices obtained from AUC experiments indicate that the PMAA chain is collapsed at pH  $< 4$ , and stretches at pH  $> 5$ . Our results show that  $\langle R_{h,0} \rangle$  is nearly 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 conformational 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 chains is nearly 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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## Notes and references

- 1 S. Moldoveanu, *Analytical Pyrolysis of Synthetic Organic Polymers*, Elsevier Science, 2005, p. 333.
- 2 Y. L. Robert, the Role of Polymers in Cosmetics: Recent Trends, *Cosmetic Nanotechnology*, American Chemical Society, 2007, pp. 3–56.
- 3 C. Donini, D. N. Robinson, P. Colombo, F. Giordano and N. A. Peppas, *Int. J. Pharm.*, 2002, **245**, 83.
- 4 A. N. Zelikin, J. F. Quinn and F. Caruso, *Biomacromolecules*, 2006, **7**, 27.
- 5 F. Wei, M. Zhang and Y. Q. Feng, *Electrophoresis*, 2006, **27**, 1939.
- 6 M. Zhang, F. Wei, Y. F. Zhang, J. Nie and Y. Q. Feng, *J. Chromatogr. A*, 2006, **1102**, 294.
- 7 W. Tjandra, J. Yao, P. Ravi, K. C. Tam and A. Alamsjah, *Chem. Mater.*, 2005, **17**, 4865.
- 8 H. T. R. Wiogo, M. Lim, V. Bulmus, L. Gutiérrez, R. C. Woodward and R. Amal, *Langmuir*, 2012, **28**, 4346.
- 9 T. G. Wensel, C. F. Meares, V. Vlachy and J. B. Matthew, *Proc. Natl. Acad. Sci. U. S. A.*, 1986, **83**, 3267.
- 10 M. Takahashi, K. Yoshikawa, V. V. Vasilevskaya and A. R. Khokhlov, *J. Phys. Chem. B*, 1997, **101**, 9396.
- 11 S. K. Tripathy, J. Kumar and H. S. Nalwa, *Handbook of Polyelectrolytes and Their Applications*, Scientific, Stevenson Ranch, CA, 2002.
- 12 N. M. Wiederhorn and A. R. Brown, *J. Polym. Sci.*, 1952, **8**, 651.
- 13 M. Mandel, J. C. Leyte and M. G. Stadhouder, *J. Phys. Chem.*, 1967, **71**, 603.
- 14 H. Okamoto and Y. Wada, *J. Polym. Sci., Polym. Phys. Ed.*, 1974, **12**, 2413.
- 15 M. S. Sulatha and U. Natarajan, *Ind. Eng. Chem. Res.*, 2011, **50**, 11785.
- 16 A. Katchalsky, *J. Polym. Sci.*, 1951, **7**, 393.
- 17 A. Katchalsky and H. Eisenberg, *J. Polym. Sci.*, 1951, **6**, 145.
- 18 T. S. Chen and J. K. Thomas, *J. Polym. Sci.*, 1979, **17**, 1103.
- 19 B. Bednář and H. Morawetz, *Macromolecules*, 1985, **18**, 1940.
- 20 D. Y. Chu and J. K. Thomas, *J. Am. Chem. Soc.*, 1986, **108**, 6270.
- 21 J. Horský and H. Morawetz, *Makromol. Chem.*, 1988, **189**, 2475.
- 22 B. Bednář, J. Trněná, P. Svoboda, S. Vajda, V. Fidler and K. Procházka, *Macromolecules*, 1991, **24**, 2054.
- 23 G. J. Liu, J. E. Guillet, E. T. B. Al-Takrity, A. D. Jenkins and D. R. M. Walton, *Macromolecules*, 1991, **24**, 68.
- 24 I. Soutar and L. Swanson, *Macromolecules*, 1994, **27**, 4304.
- 25 I. Soutar and L. Swanson, *Polymer*, 1994, **35**, 1942.
- 26 R. V. Pereira and M. H. Gehlen, *Macromolecules*, 2007, **40**, 2219.
- 27 D. Pristinski, V. Kozlovskaya and S. A. Sukhishvilia, *J. Chem. Phys.*, 2005, **122**, 014907.
- 28 C. Heitz, M. Rawiso and J. François, *Polymer*, 1999, **40**, 1637.
- 29 Y. Muroga, T. Yoshida and S. Kawaguchi, *Biophys. Chem.*, 1999, **81**, 45.
- 30 K. Kogej, E. Theunissen and H. Reynaers, *Langmuir*, 2002, **18**, 8799.
- 31 J. L. Koenig, A. C. Angood, J. Semen and J. B. Lando, *J. Am. Chem. Soc.*, 1969, 7250.
- 32 J. B. Lando, J. L. Koenig and J. Semen, *J. Macromol. Sci. Phys. B*, 1973, **7**, 319.
- 33 M. Sedláč, Č. Koňákt, P. Štěpánek and J. Jakeš, *Polymer*, 1987, **28**, 873.
- 34 L. Ruiz-Pérez, A. Pryke, M. Sommer, G. Battaglia, I. Soutar, L. Swanson and M. Geoghegan, *Macromolecules*, 2008, **41**, 2203.
- 35 G. J. Howard and D. O. Jordan, *J. Polym. Sci.*, 1954, **12**, 209.
- 36 P. Schuck, *Biophys. J.*, 2000, **78**, 1606.
- 37 J. Lebowitz, M. S. Lewis and P. Schuck, *Protein Sci.*, 2002, **11**, 2067.
- 38 S. E. Harding, *Biophys. Chem.*, 1995, **55**, 69.
- 39 H. Morawetz, *Macromolecules*, 1996, **29**, 2689.
- 40 K. Kogej, J. Cerkovnik, H. Berghmans and S. Paoletti, *Acta Chim. Slovaca*, 2001, **48**, 395.
- 41 E. V. Anufrieva, T. M. Birshtein, T. N. Nekrasova, O. B. Ptitsyn and T. V. Sheveleva, *J. Polym. Sci., Part C: Polym. Symp.*, 1967, **16**, 3519.
- 42 S. Wu, X. Y. Wang, X. D. Ye and G. Z. Zhang, *J. Phys. Chem. B*, 2013, **117**, 11541.
- 43 L. Y. Zhang, L. J. Wang, Y.-T. Kao, W. H. Qiu, Y. Yang, O. Okobiah and D. P. Zhong, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 18461.
- 44 Y. Maruyama and Y. Harano, *Chem. Phys. Lett.*, 2013, **581**, 85.
- 45 K. Tompa, M. Bokor, T. Verebelyi and P. Tompa, *Chem. Phys.*, 2015, **448**, 15.
- 46 J. Pleštil, Y. M. Ostanevich, V. Y. Bezzabotnov and D. Hlavatá, *Polymer*, 1986, **27**, 1241.
- 47 A. Ikegami, *Biopolymers*, 1968, **6**, 431.
- 48 A. Ikegami, *J. Polym. Sci., Part A: Gen. Pap.*, 1964, **2**, 907.
- 49 Y. T. Chung and C. I. Huang, *J. Chem. Phys.*, 2012, **136**, 1.

- 50 K. Nishinari, K. Kohyama, P. A. Williams, G. O. Phillips, W. Burchard and K. Ogino, *Macromolecules*, 1991, **24**, 5590.
- 51 W. Mächtle and L. Börger, *Analytical Ultracentrifugation of Polymers and Nanoparticles*, Springer, Berlin, Heidelberg, New York, 2006.
- 52 K. Kogej, H. Berghmans, H. Reynaers and S. Paoletti, *J. Phys. Chem. B*, 2004, **108**, 18164.
- 53 A. J. Rowe, *Biopolymers*, 1977, **16**, 2595.
- 54 W. Brown, *Macromolecules*, 1984, **17**, 66.
- 55 Z. L. Luo and G. Z. Zhang, *J. Phys. Chem. B*, 2009, **113**, 12462.
- 56 A. F. Olea and J. K. Thomas, *Macromolecules*, 1989, **22**, 1165.
- 57 S. P. Ju, W. J. Lee, C. I. Huang, W. Z. Cheng and Y. T. Chung, *J. Chem. Phys.*, 2007, **126**, 224901.
- 58 M. S. Sulatha and U. Natarajan, *Ind. Eng. Chem. Res.*, 2012, **51**, 10833.
- 59 H. Gustavsson, B. Lindman and T. Bull, *J. Am. Chem. Soc.*, 1978, **100**, 4655.
- 60 J. C. Leyte and M. Mandel, *J. Polym. Sci., Part A: Gen. Pap.*, 1964, **2**, 1879.
- 61 A. Pohlmeier and S. Haber-Pohlmeier, *J. Colloid Interface Sci.*, 2004, **273**, 369.
- 62 J. N. Israelachvili, *Intermolecular and Surface Forces*, Academic Press, 2011.