

Unusual Behavior of Atactic Poly(methacrylic acid) in Aqueous Solutions Monitored by Wide-Angle Light Scattering

Ksenija Kogej,^{*,†,‡,§} Hugo Berghmans,[‡] Harry Reynaers,[‡] and Sergio Paoletti[†]

Department of Chemistry, Catholic University of Leuven, Celestijnenlaan 200F, B-3001 Heverlee, Belgium, and Department of Biochemistry, Biophysics, and Macromolecular Chemistry, University of Trieste, I-34127 Trieste, Italy

Received: March 26, 2004; In Final Form: September 16, 2004

Wide-angle light scattering studies were performed on the atactic poly(methacrylic acid), HPMA, in 0.002 and 0.1 M HCl at 25 and 55 °C in a wide concentration range. The weight-average degree of polymerization of a single poly(methacrylate), PMA, chain \bar{N}_w (=1528) was estimated from scattering experiments on poly(sodium methacrylate) in 0.1 M NaCl. The intermolecular association between PMA chains in carefully prepared acidic aqueous solutions is evidenced in both angular and concentration dependencies of scattered light. The dependence of the reciprocal reduced scattering intensity on the square of the scattering vector is heavily curved at high angles, and it exhibits a minimum as a function of concentration. In 0.002 M HCl, the associated forms deaggregate on a time scale of several days. This process is accompanied by a decrease of the second virial coefficient, A_2 , and by chain expansion; the radius of gyration, R_g , increases from about 17 to about 28 nm after 0.17 and 576 h, respectively. The intermolecular association in 0.1 M HCl is stronger than in 0.002 M HCl and seems to be stable on a much longer time-scale. The time-average R_g value (about 18 nm) of the associated HPMA form in 0.1 M HCl indicates a more compact nature of entanglements; this fact decisively contributes to the overall stability of intermolecular aggregates. For HPMA dissolved in 0.1 M HCl, an increase in temperature from 25 to 55 °C brings about no increase in R_g . The changes in thermodynamic functions of association point to a process driven by hydrophobic interactions.

Introduction

Many biological systems involving water-soluble polymers have an ability to induce thickening or gelation of solutions that is driven by aggregation of some ordered forms of the polymer chain.^{1–7} The phenomenon can be induced by various physicochemical factors, i.e., by change in temperature, presence of suitable counterions, and/or sufficient ionic strength, and it usually involves a conformational transition of the macromolecular chain.^{1,4–7} The complicated structure of many biocolloids makes it difficult to determine the forces and mechanisms that are operative during the gelation process. Therefore, one tries to study simpler model systems.

Among synthetic polymers, poly(methacrylic acid), HPMA, is the simplest representative that is known to form thermoreversible gels in concentrated aqueous solutions.⁸ From viscometric and thermodynamic study of undissociated HPMA, Eliassaf and Silberberg⁸ have concluded that the molecule is intramolecularly bonded in dilute solutions. These intramolecular bonds in the dilute regime should eventually lead to agglomeration (e.g., gelation) in concentrated solutions caused by intermolecular hydrogen bonding between carboxyl groups of different chains. Closely related to gelation is the phenomenon of negative thixotropy or shear-induced viscosity change in

concentrated aqueous HPMA solutions.^{9–11} In negative thixotropic systems, the field of flow induces formation of intermolecular bonds⁹ and thus leads to the formation of a gel network. On standing, the gel state reverts spontaneously into the original solution state. There is little doubt that these peculiar properties of poly(methacrylate) (PMA) reflect the conformational transition of the PMA chain in aqueous solutions,^{12–14} which is induced by the ionization of carboxyl groups. Various forces are likely to be responsible for these phenomena, among them intra- and intermolecular hydrogen bonding,¹⁵ van der Waals interactions,¹⁴ and the so-called hydrophobic interactions associated with the methyl side groups.¹⁶

Despite a great number of works that have dealt with various solution properties of HPMA, there are rather few systematic light scattering studies. HPMA was studied by small-angle X-ray and neutron scattering techniques^{17–22} and by dynamic light scattering.²³ Wide-angle light scattering, WALS, on the other hand, was applied only in a limited number of cases^{15,17,24,25} although it is recognized as an attractive tool for the study of conformational changes and associative equilibria of polymers in solution.^{2–7} One has to stress that in most of the WALS studies on PMA^{15,17,24} the molar mass and the second virial coefficient were obtained from the intensity of the light scattered at an angle of 90°. Very few attempts deal with the evaluation of the angular dependence²⁵ of scattering that is the only one able to give valuable information on the size of the polymer in solution. The shape of the complete Zimm plot (linear or curved plots) could simultaneously point to association equilibria in solution.^{3–7,26,31,36}

From what was pointed out above, there seems to be a clear need for a more comprehensive wide-angle light scattering study

* To whom correspondence should be addressed. Ksenija Kogej, Faculty of Chemistry and Chemical Technology, University of Ljubljana, Aškerčeva 5, P.O. Box 537, SI-1001 Ljubljana, Slovenia. Tel: +(386-1)-2419-412. Fax: +(386-1)-2419-437. E-mail: ksenija.kogej@uni-lj.si.

[†] University of Trieste.

[‡] Catholic University of Leuven.

[§] On leave from the Faculty of Chemistry and Chemical Technology, University of Ljubljana, SI-1000 Ljubljana, Slovenia.

of HPMA. Therefore, the purpose of the present paper is to fill this gap and perform a systematic study of the solution properties of the atactic HPMA. Our main goal is to obtain the weight-average molar mass, the second virial coefficient, and the radius of gyration from light scattering measurements. Emphasis will be given to possible association processes in dilute HPMA solutions that could eventually explain the ability of HPMA to form gels in the concentrated regime and shed more light on the phenomenon of negative thixotropy. As the starting point for the achievement of this task, knowledge of the molecular parameters of the PMA chain under nonassociating conditions is crucial. This information can be safely achieved with the acid in the fully neutralized form. For this purpose, the WALS measurements will be first performed on the sodium salt of HPMA. The investigation will then deal with scattering of HPMA in aqueous solutions at zero degree of neutralization in dependence on the ionic strength of the solution (pH) and the temperature. A very broad concentration range of the polymer will be investigated in order to correlate the data at high and low concentrations. Special attention will be devoted to the treatment of the angular dependence of scattering and to the evaluation of the macromolecular dimensions. The present atactic chain will serve as the starting point for further understanding of the behavior of the isotactic polymer.

Materials and Methods

1. Materials. HPMA was prepared by polymerization of methacrylic acid using a standard procedure.²⁷ The absence of anhydride groups was checked by IR spectroscopy. The polymer was first characterized by the determination of chain tacticity and polydispersity. The tacticity of the polymer was determined from the corresponding ester form. For this purpose, the acid was dissolved in a methanol/water mixture (2:1) and ethereal diazomethane solution was added while stirring to yield the methyl ester of the polyacid, i.e., poly(methyl methacrylate), PMMA.²⁸ The tacticity of the resulting PMMA was determined from the signals of the α -methyl group in the ^1H NMR spectrum²⁹ and in the ^{13}C NMR spectrum³⁰ of a CDCl_3 solution. The polymer contains around 39% heterotactic, 49% syndiotactic, and 12% isotactic triads.

The polydispersity index, PI, was determined in the Polymer Characterization Laboratory of the POLYtech S.C.r.l., Trieste, by Dr. F. Z. on the sodium salt form of PMA in aqueous 0.1 M NaCl, in accordance with previously reported procedures,⁵ providing the value for the ratio of the relevant molar mass averages as $\bar{M}_z/\bar{M}_w/\bar{M}_n = 4.03:2.44:1$.

The stock solutions of HPMA for light scattering measurements were prepared by dissolving the dry acid polymer in Milli-Q water. For the preparation of poly(sodium methacrylate), NaPMA, stock solution, the dry HPMA was first dissolved in water and 1 M NaOH solution was slowly added under continuous stirring and blowing-through with N_2 until the pH of the solution was around 8.5. This value of pH corresponds to the equivalent point in the titration curve of HPMA with NaOH and was determined in a separate experiment.

For each set of measurements, the concentration of stock solutions was always the highest concentration measured in light scattering experiments. Special care was taken to ensure complete solubilization of the polymer. The stock solutions of the polymer in pure water were stirred for at least 24 h at room temperature prior to further treatment. Afterward, they were transferred quantitatively into a volumetric flask and a desired amount of 1 M HCl or 1 M NaCl was added to adjust the final concentration of the simple electrolyte in solution to the desired

value. HPMA was studied at two different HCl concentrations, i.e., in 0.002 M and in 0.1 M HCl, whereas NaPMA was studied in 0.1 M NaCl. The stock solutions were successively diluted with the corresponding solvent (i.e., with 0.002 M, 0.1 M HCl, or 0.1 M NaCl) to obtain the desired polymer concentration range. After dilution, solutions were left to equilibrate for another 24 h prior to filtering and light scattering measurements. Filtering was performed directly into measuring cells through Millipore filters (type GS) with a pore size of $0.22\ \mu\text{m}$ using a closed circuit involving a filter holder and a peristaltic pump. The filtering time was around 15 min. The first measurement was always performed approximately 10 min after filtration and thermal equilibration in the thermostatically controlled toluene bath. In all cases, the time dependence of scattering has been followed by performing additional experiments 3 h, 24 h, and in the case of HPMA in 0.002 M HCl also 576 h after filtration, keeping the solutions all the time in the measuring cells.

The concentration range for light scattering measurements was very broad. In 0.002 M HCl, two concentration regions were investigated; the higher one was 2–10 g/L and the lower one was 0.04–2 g/L, whereas in 0.1 M HCl and in 0.1 M NaCl only the lower concentration region was studied. Even at the highest concentration studied (i.e., at 10 g/L), these conditions correspond to a state where polymer chains do not overlap.²⁵ For example, the critical overlap concentration, c_p^* , for HPMA in 0.002 M HCl is approximately 40 g/L and for NaPMA in 0.1 M NaCl it is around 2.8 g/L. One can conclude that the condition $c_p < c_p^*$ is always satisfied.

2. Light Scattering Measurements. The static light scattering at 25 and 55 °C was measured at angles between 30° and 150° using a commercial Amtec MM 1000 photometer. A cylindrical cell, containing the solution, was immersed in a thermostatically controlled toluene bath. As the light source, a monochromatic and polarized beam of a He–Ne laser (Spectra Physics, model 124) operating at 632.8 nm was used. The refractive index increment, dn/dc , for PMA was taken from the literature. The following variation of dn/dc with the degree of neutralization, α_N , is reported:¹⁷

$$\frac{dn}{dc} = 0.171 + 0.092\alpha_N \text{ (mL/g)} \quad (1)$$

It gives $dn/dc = 0.171$ and $0.263\ \text{mL/g}$ for the acid ($\alpha_N = 0$) and for the salt ($\alpha_N = 1$), respectively. These values agree well with other dn/dc data in the literature³² and were used in elaborating the data obtained by the static light scattering method.

3. Analysis of the Light Scattering Data. In static light scattering experiments, one measures the excess Rayleigh ratio, R_θ , which permits the evaluation of the weight-average molecular weight, M_w , the second virial coefficient, A_2 , and the z -average root-mean-square radius of gyration, $(\langle R_g^2 \rangle_z)^{1/2}$, hereafter designated as R_g for simplicity. The experimental data were treated according to the classical Zimm procedure based on the Zimm formula.³¹ Extrapolations to zero angle ($\theta = 0^\circ$) and zero concentration ($c_p = 0$) were independently performed. In the former case, both linear and polynomial fitting of Kc_p/R_θ as a function of x were performed, where x denotes the square of the modulus of the wave vector q , given by the usual relationship

$$q = \frac{4\pi n_s \sin(\frac{\theta}{2})}{\lambda} \quad (2)$$

In eq 2, λ is the wavelength of light (i.e., 632.8 nm in this study, see the experimental section), and n_s is the refractive index

of the medium (i.e., $n_s = 1.33$ for water at 25 °C). In most cases, the second-order polynomial fitting gave the lowest values of the sum of the residuals.

3.1. Concentration Dependence. It will be demonstrated in Results and Discussion that the experimental scattering data on HPMa solutions at $\alpha_N = 0$ show a marked upward curvature that is usually referred to strong intermolecular interactions. The simplest interpretation of such behavior is the occurrence of association/aggregation.^{2,3} Various physical models can be applied for studying the association. A model which has gained rather general consensus is the so-called open association model (OAM) of Elias.²⁶ For a system having a polydispersity index, PI ($PI = M_w/M_n$, where the indexes w and n denote the weight and the number averages of the molar mass, respectively), the OAM introduces an apparent weight-average molar mass of an associated species, $(M_w)_{ass}$, as²⁶

$$(M_w)_{ass} = [(M_{w,0})^2 + 4000K_{ass} PI M_{w,0} c_p]^{1/2} \quad (3)$$

In eq 3, $M_{w,0}$ is the weight-average molar mass of the fundamental nonassociated species (unimer²⁶), K_{ass} is the association constant of the process, and c_p is the concentration of the polymer in g/L. By introducing eq 3 into the Zimm formula, the expression for the concentration dependence of scattering at $\theta \rightarrow 0^\circ$ takes the form

$$\frac{Kc_p}{R_0} = \frac{1}{[(M_{w,0})^2 + 4000K_{ass} PI M_{w,0} c_p]^{1/2}} + 2A_2c_p \quad (4)$$

Equation 4 is formally a three-parameter equation,^{4–7,26} namely, in $M_{w,0}$, K_{ass} , and A_2 . In fitting procedures used in this paper, the second virial coefficient was considered to be a constant for each series of experiments, irrespective of the extent of association.³¹ Although the model of Elias has been successfully applied to a very large number of associating polymer systems, still one should not stretch too much the interpretation of K_{ass} as a well-defined thermodynamic parameter. More generally, this constant should just be regarded as a phenomenological parameter describing a significant intermolecular interaction.

3.2. Angular Dependence. The experimental data were analyzed also for the angular dependence. The form factor, $P(\theta)$, depends on the shape and size of the scattering particle. $P(\theta)$ equals the ratio between the scattered intensity for large particles to the scattered intensity without interference. To a good approximation, $P(\theta)$ can be expressed as a polynomial expansion of the so-called concentration-dependent static correlation length,⁵ ξ :

$$P(\theta) = [1 - (q\xi)^2 + \text{const} \times (q\xi)^4 - \text{const}' \times (q\xi)^6 + \dots] \quad (5)$$

The radius of gyration is then obtained from ξ according to eq 6:

$$R_g = \sqrt{3}\xi \quad (6)$$

Moreover, the form factor of the macromolecule given by eq 5 can be looked upon as the approximated form of the Debye equation.³³ In the latter case, the angular-dependent part of the reciprocal intensity assumes the form

$$\left| \frac{Kc_p}{R_\theta} \right|_{c_p=0} = \frac{1}{(M_w)} \left[\frac{2}{(R_g^2 x)^2} (e^{-R_g^2 x} + (R_g^2 x - 1)) \right]^{-1} \quad (7)$$

where $x = q^2$.

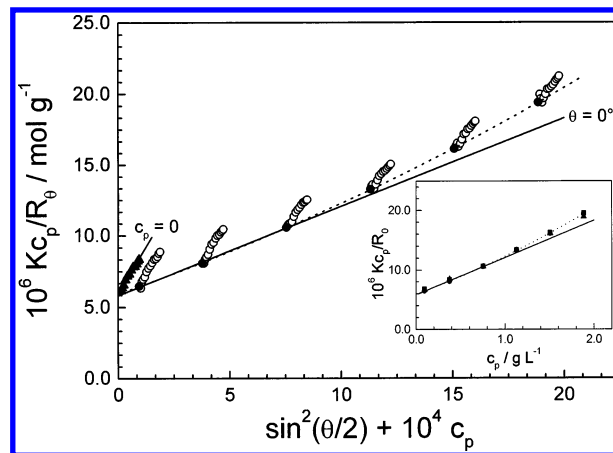


Figure 1. The Zimm plot for NaPMA in 0.1 M NaCl at 25 °C (1 measurement: $t = 0.17$ h). The inset shows the extrapolated values to zero angle for all measurements: $t = 0.17$ h (●), 3 h (■), 24 h (▲).

Linear, quadratic, and cubic polynomial fitting, as well as the Debye equation, was applied for the treatment of the angular dependence in the procedure of the evaluation of the radius of gyration of HPMa and NaPMA under different experimental conditions. For the evaluation of the molecular weight of the polymer, only linear and second-order polynomial q^2 -dependences were taken into account.

3.3. Chain Stiffness. The persistence length, L_p , of a wormlike chain can be calculated from the radius of gyration using the equation of Benoit and Doty³⁴ as modified for polydisperse samples,³⁵

$$R_g^2 = L_p^2 \left(\frac{\bar{N}_z b}{3L_p} - 1 + \frac{2L_p}{\bar{N}_w b} \left(1 - \frac{L_p}{\bar{N}_n b} \right) \right) \quad (8)$$

where \bar{N}_z , \bar{N}_w , and \bar{N}_n are the average number of chain elements (degree of polymerization) with the index indicating the appropriate kind of the average and b is the length of the monomer unit. The persistence length of PMA was calculated from a numerical solution of eq 8 using the experimentally determined values of the molar mass averages.

Results and Discussion

1. Atactic NaPMA in 0.1 M NaCl. The main purpose of the scattering experiments with NaPMA was to achieve information on the molar mass of the isolated PMA chain. Figure 1 shows the Zimm plot obtained for NaPMA in aqueous 0.1 M NaCl 0.17 h after filtration. The investigated polymer concentration range was 0.094–2 g/L. Figure 2 reports the plot of Kc_p/R_θ versus q^2 for each NaPMA concentration: the angular dependence of the scattered light is slightly curved. The curves through the experimental points correspond to a second-order polynomial fitting in q^2 , which produces the lowest values of the sum of the squares of the residuals. All the data for the evaluation of M_w in NaPMA solutions were, therefore, treated in this way. The inset of Figure 1 shows the concentration dependence of the zero-angle values, Kc_p/R_0 , for all three sets of measurements (performed at various times, i.e., 0.17, 3, and 24 h after filtration). It can be seen that the Kc_p/R_0 points do not depend on time. They show a positive deviation from the linearity only for concentrations higher than approximately 1 g/L. This fact points to the presence of a positive third virial coefficient in eq 4.³¹ Still, the behavior in the lower c_p range reasonably allows determining a consistent value of the molar mass of the NaPMA chain. The weight-average molar mass of

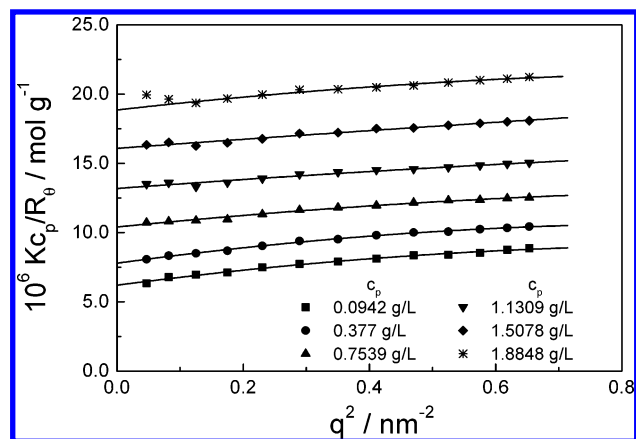


Figure 2. The dependence of the inverse of the reduced scattering intensity, Kc_p/R_θ , on the square of the scattering vector, q^2 , for NaPMA in 0.1 M NaCl at 25 °C (1 measurement: $t = 0.17$ h).

TABLE 1: Data Obtained for the Atactic NaPMA in 0.1 M NaCl at 25 °C from the Analysis of Zimm Plots

t/h	linear Kc_p/R_0 vs c_p		second-order polynomial Kc_p/R_0 vs c_p	
	M_w (kg mol ⁻¹)	$10^3 A_2$ (mol mL g ⁻²)	M_w (kg mol ⁻¹)	$10^3 A_2$ (mol mL g ⁻²)
0.17	172	3.2	169	2.71
3	165	2.93	162	2.42
24	163	2.95	163	2.79
average	167	3.03	165	2.64

TABLE 2: Data Obtained for the Atactic HPMA in Aqueous 0.002 M HCl at 25 °C from the Analysis of Zimm Plots

set	t/h	$M_{w,0}$ (kg mol ⁻¹)	$10^3 A_2$ (mol mL g ⁻²)	$10^{-4} K_{ass}$
1	0.17	204 ^a	0.41 ^a	0.027 ^a
2	0.17	172 ^a	1.23 ^b	9.5 ^b
3a	0.17	184 ^a	1.32 ^b	7.5 ^b
3b	3	170 ^a	1.21 ^b	6.1 ^b
4a	0.17	208 ^a	1.03 ^b	9.0 ^b
4b	3	172 ^a	0.9 ^b	6.1 ^b
4c	24	149 ^a	0.8 ^b	4.9 ^b
4d	576	131 ^a	0.5 ^b	0.8 ^b

^a Open association model: variable $M_{w,0}$, A_2 , and K_{ass} . ^b Open association model: fixed $M_{w,0} \equiv 131$ kg/mol, variable A_2 and K_{ass} .

NaPMA in 0.1 M NaCl was calculated using both a linear fitting in c_p of the three lowest concentrations and a second-order polynomial fitting of all concentrations. The latter procedure gives a better fit of the experimental points than the former one, as can be appreciated from the comparison between the dotted and solid curves in Figure 1. However, the agreement between the values of M_w and A_2 obtained by the two fitting procedures is rather good. This can be seen from the results that are reported in Table 1 for each set of measurements. As pointed out above, no time dependence of M_w or A_2 is indicated, at least for the period of 1 day. The last line in Table 1 gives the average of all values. The second-order-polynomial average of M_w ($=165$ kg/mol) was accepted as the weight-average molar mass of the isolated NaPMA chain. From this, one then obtains a weight-average degree of polymerization equal to 1528 ($=\bar{N}_w$). For the acid form, the latter value corresponds to the weight-average relative molecular mass of 131 000 ($=M_{w,0}$; cf. eq 3).

2.1. Atactic HPMA in 0.002 M HCl at 25 °C. The scattering experiments with HPMA in 0.002 M HCl at 25 °C were first performed in a concentration range from 2 to 10 g/L (cf. Table 2, set 1). The resulting Zimm plot is shown in Figure 3. Different fitting procedures were used, according to Materials and

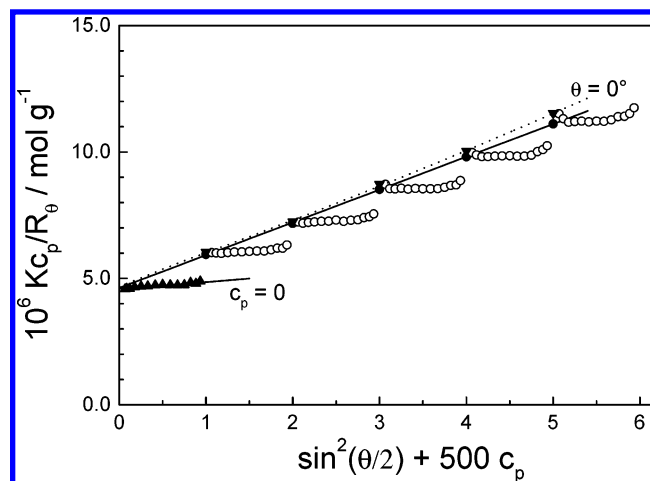


Figure 3. The Zimm plot of HPMA in 0.002 M HCl at 25 °C in the high c_p range (set 1).

Methods. The average value of M_w therefrom was equal to 214 ± 6 kg/mol, corresponding to an apparent value of N_w equal to 2488 ± 70 . The second virial coefficient was positive with an average value of $A_2 = (3.2 \pm 0.2) \times 10^{-4}$ mol mL/g². These values are not reported in Table 2. The table gives only the results obtained by OAM (see Materials and Methods). The positive value of A_2 suggests that under these experimental conditions HPMA is still far from being in theta-conditions, in agreement with recent reports in the literature.¹⁷

Clearly, there is a disagreement between the values of the apparent degrees of polymerization for NaPMA in 0.1 M NaCl ($N_w = 1528$) on one hand and for HPMA in 0.002 M HCl on the other. The rather high discrepancy is certainly beyond the accuracy limits of the technique and could point to a possibility that HPMA molecules in aqueous solutions are subjected to significant intermolecular interactions (association/aggregation processes).

In view of these findings, it was decided to undertake further investigations with HPMA in aqueous HCl solutions by performing light scattering experiments in a more dilute regime. Two sets of measurements with HPMA in 0.002 M HCl at 25 °C were performed covering the concentration range from around 0.1 to 2 g/L (sets 2 and 3). We have carefully analyzed the resulting Zimm plots in the first place according to the q^2 -dependence. The Kc_p/R_θ versus q^2 plots of set 3b are shown in Figure 4 as an example. Other cases resulted in a similar angular dependence. It is clearly seen from Figure 4 that Kc_p/R_θ versus q^2 plots at low polymer concentrations are heavily curved at high q^2 values, which is often attributed to intermolecular association.^{3,5,31,37,38}

All the angular dependence data for HPMA in 0.002 M HCl solutions were fitted with a polynomial function of the second order. Figure 5 gives the zero-angle values for HPMA in 0.002 M HCl including the high concentration range (set 1) and the low concentration range (sets 2, 3a, and 3b). One can see that the Kc_p/R_0 values in the high c_p range fit well with those obtained at lower c_p values. Moreover, inspection of the plot in the dilute regime (below 2 g/L, for details see the inset in Figure 5) reveals a pronounced and asymmetric upward curvature, which is diagnostic for association processes.^{4–7,26} Figure 6 gives the zero-angle points for sets 4a–d, which were obtained in the dilute regime only (c_p below 2 g/L), for a more extensive analysis of the time dependence (see Materials and Methods). Again, a decreasing trend of Kc_p/R_0 with increasing c_p at low concentrations is seen, as one expects for associating systems. One can conclude that the choice of the concentration range of

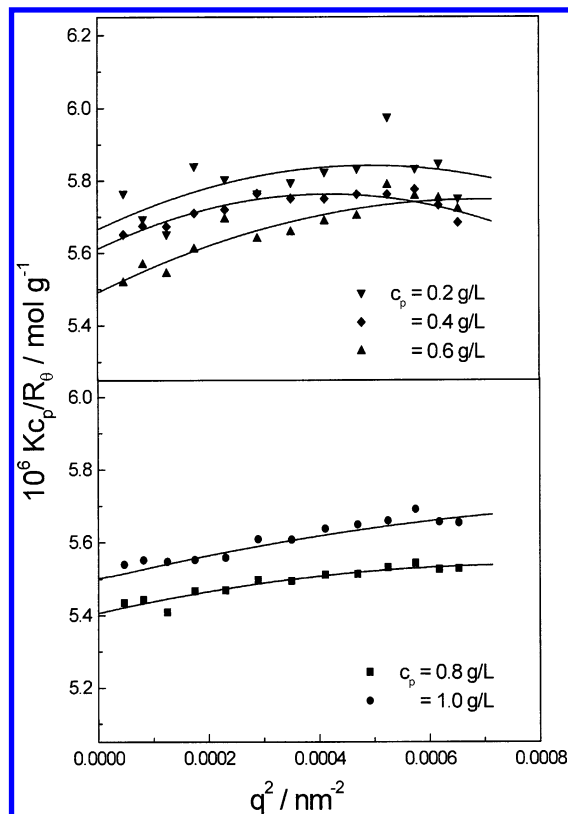


Figure 4. The dependence of the inverse of the reduced scattering intensity, Kc_p/R_0 , on the square of the scattering vector, q^2 , for HPMA in 0.002 M HCl at 25 °C (set 3b).

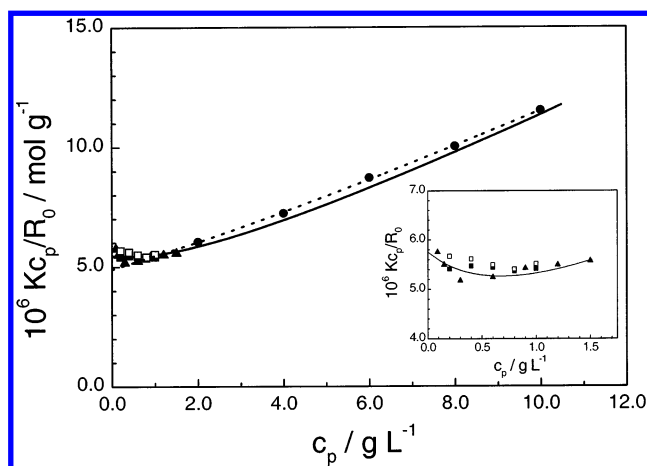


Figure 5. The dependence of the inverse of the reduced scattering intensity at zero angle, Kc_p/R_0 , on polymer concentration, c_p , for HPMA in 0.002 M HCl at 25 °C (set 1, ● (polynomial q^2 -dependence); set 2, ▲; set 3a, ■; set 3b, □). The dashed line corresponds to the OAM treatment of the data points designated by ● (set 1, high c_p range); the solid line is the result of the OAM treatment for all the data points measured at $t = 0.17$ h (sets 1, 2, and 3a). The inset shows the enlargement of the plot for c_p below 1.7 g/L. The solid line in the inset is the result of the OAM treatment for data set 2 (▲).

the polymer is crucial in making any conclusions on the presence or absence of intermolecular association from light scattering data of HPMA. Studying the polymer only in the region of high concentrations and neglecting its behavior in the dilute regime can easily overlook the presence of associative equilibria.¹⁷

For associating systems, the data are often treated by the approach of Elias.²⁶ Therein, any process (or processes) that leads to the formation of particles of higher molecular weight from molecules of lower molecular weight is treated as a

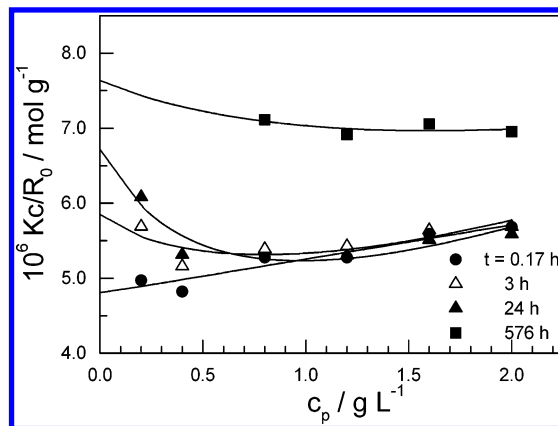


Figure 6. The dependence of the zero-angle values, Kc_p/R_0 , on polymer concentration, c_p , and time for HPMA in 0.002 M HCl at 25 °C (set 4).

multimerization process. Two extreme types of association can take place. The first one is the model of *open association*. In this one, a continuous distribution of the associated forms of the fundamental nonassociated form into dimers, trimers, and so forth is assumed. The other extreme is the *closed type of association* by which the “unimer” (i.e., the fundamental nonassociated form) is in equilibrium with an n -mer of molecular weight M_n only. Because the possible mechanism for associative processes in HPMA solutions is at present completely unknown, we have decided to keep to the most general and best documented approach,^{4–7,26} which is the OAM.

2.2. Treatment of Association of HPMA Chains in 0.002 M HCl. Application of the OAM model to the various sets of data, using $M_{w,0}$, K_{ass} , and A_2 as fitting parameters, revealed a markedly *decreasing* trend of $M_{w,0}$ upon decreasing concentration and increasing time from filtration (cf. column 3 in Table 2). Interestingly enough, the value of $M_{w,0}$ from the low concentration range after 24 days exactly corresponds to the \bar{N}_w value obtained for the sodium salt form of HPMA. This result points to the success of the OAM procedure, at least as a phenomenological approach to describe the interchain interactions of HPMA acid. Therefore, we decided to use the constant value of 131 kg/mol for $M_{w,0}$ as the accepted “true” molar mass value of the unassociated polymer (corresponding to $\bar{N}_w = 1528$) in a new set of fitting procedures of the data, using K_{ass} and A_2 as the only fitting parameters. The results of this calculation are reported in the last two columns of Table 2.

The decrease of the molar mass, which was ascertained by the previous procedure, is accompanied by a *decrease of the apparent association constant* and by a *simultaneous decrease of the second virial coefficient*. For example, for the particular case of set number 4, K_{ass} decreases by more than 10 times, i.e., from 9.0×10^4 to 0.8×10^4 , whereas A_2 decreases from 1.03×10^{-3} to 0.5×10^{-3} mol mL g⁻² at $t = 0.17$ and 576 h, respectively. It is worthwhile to stress that the values of A_2 are here taken into account merely as a relative measure of how polymer–solvent interactions change in the course of time.

The decrease of K_{ass} for HPMA in 0.002 M HCl implies that intermolecular association between HPMA chains that is present at the beginning becomes increasingly more unfavorable as time from preparation increases. Consequently, HPMA molecules undergo a change from some associated/aggregated state toward the molecularly dispersed one, as shown by the approach of the M_w to the value of the molar mass for a single chain. One of the reasons for these observations could be that the initial state of HPMA in 0.002 M HCl after filtration of the solutions just prior to light scattering measurements is in fact not an

equilibrium state. It is very probable that a field of shear, to which solutions are exposed during the filtering procedure, induces detectable interchain connections in the case of HPMA in aqueous HCl. This is in accordance with reports in the literature over the years on the shear-induced gelation (*negative thixotropy*) of concentrated aqueous HPMA solutions.^{9–11} In those authors' opinion,⁹ negative thixotropy in HPMA is probably due to the fact that more intermolecular bonds are likely to be formed in a field of flow than in a system at rest. Our measurements clearly show, in accordance with the above reports,^{9–11} that the physical cross-links between HPMA molecules in 0.002 M HCl are indeed rather strong, as the chains return to their nonassociated equilibrium form only after a rather long time after mechanical agitation has ceased. At the atomic level, driving forces for such shear-induced association could be hydrogen bonds between carboxyl groups on neighboring chains, the tendency of the methyl side groups to avoid unfavorable contact with water molecules, or a combination of these. Based on the fact that solutions of poly(acrylic acid) show no tendency toward shear-induced association, we believe that the sole hydrogen bonding ability of the polymer is not a sufficient factor to induce aggregation of chains. Another appealing explanation can be traced to the relative stiffness of the chain, the possibility of giving rise, under shear, to transient anisotropic structures with an increased tendency to lateral alignment/association.

The simultaneous decrease of A_2 indicates that polymer/solvent interactions become disadvantageous in the course of time. From the thermodynamic point of view, the observed decrease of A_2 with time could be explained by invoking an increase of the number of unfavorable contacts between the methyl side groups on HPMA and the solvent water molecules, after the cessation of shear. Clusters of aggregated chains offer effective, albeit temporary, protection of these contacts. At the same time, the cohesive forces inside those clusters when the solvent is 0.002 M HCl are too weak; therefore, the clusters are progressively disintegrated. The exposure of CH_3 -groups to the solvent leads to stronger hydrophobic interactions and consequently to the lowering of A_2 . Still, the overall balance of the interactions in 0.002 M HCl favors the isolated, molecularly dispersed form as the true equilibrium form. A schematic representation of the initial ($t = 0.17$ h) and final ($t = 576$ h) states in the disintegration of chain clusters in 0.002 M HCl is shown in Scheme 1.

3. Atactic HPMA in 0.1 M HCl at 25 and 55 °C. Further scattering studies of HPMA were undertaken at higher H^+ concentrations. Aqueous 0.1 M HCl was used as the solvent at two temperatures, i.e., at 25 and 55 °C. The analysis of the time dependence of scattering was undertaken only at 25 °C. The higher temperature, 55 °C, was chosen because of its importance in the phase diagram of HPMA.⁸ The measurements with HPMA in 0.1 M HCl were performed only in the low concentration range of the polymer, from around 0.06 to 1.4 g/L.

The analysis of the light scattering data for HPMA in 0.1 M HCl was carried out in the same way as outlined above; i.e., a polynomial function of the second order was used for fitting the angular dependence data. Again, these plots were curved and the curvature was more pronounced at lower concentrations³⁹ (cf. Figure 2 in ref 39). Figure 7 shows the Kc_p/R_0 values obtained at 25 °C (0.17, 3, and 24 h after the filtering procedure) and at 55 °C. Much like before, the OAM method was applied for the treatment of the zero-angle points, using $M_{w,0}$ both as a variable and as a fixed parameter. The results are reported in

SCHEME 1: Schematic Representation of the Disintegration of Chain Clusters between HPMA Molecules in 0.002 M HCl

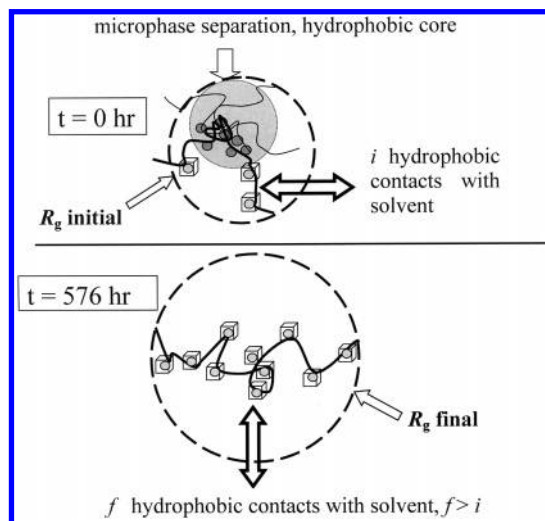


TABLE 3: Data Obtained for the Atactic HPMA in Aqueous 0.1 M HCl at 25 °C and at 55 °C from the Analysis of Zimm Plots

$T/^\circ\text{C}$	t/h	$M_{w,0}$ (kg mol ⁻¹)	$10^3 A_2$ (mol mL g ⁻²)	$10^{-4} K_{\text{ass}}$
25	0.17	192 ^a	1.37 ^b	13.9 ^b
25	3	180 ^a	1.28 ^b	12.3 ^b
25	24	187 ^a	1.32 ^b	13.5 ^b
25 (average)		186^a	1.32^b	13.1^b
55	0.17	170 ^a	1.24 ^b	8.2 ^b

^a Open association model: variable $M_{w,0}$, A_2 , and K_{ass} . ^b Open association model: fixed $M_{w,0} \equiv 131$ kg/mol, variable A_2 and K_{ass} .

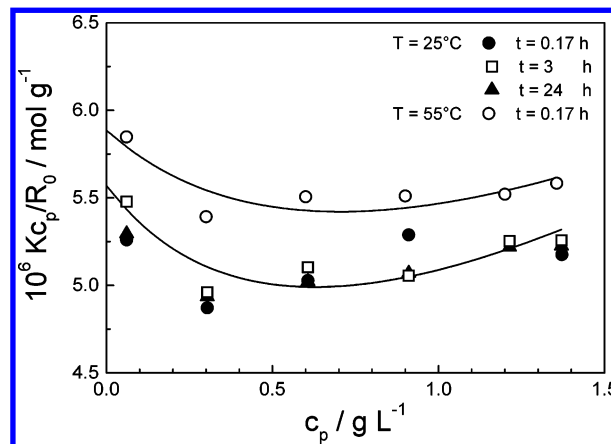


Figure 7. The dependence of the zero-angle values, Kc_p/R_0 , on polymer concentration, c_p , for HPMA in 0.1 M HCl at 25 and 55 °C. The time dependence is given only for 25 °C.

Table 3. The resulting $M_{w,0}$ values are constant within the limits of the accuracy of the WALS method. Therefore, the average values are also included in the table. By comparing the latter values as given by the OAM (186 kg/mol at 25 °C and 170 kg/mol at 55 °C) with the $M_{w,0}$ value proposed for the truly nonassociated form (131 kg/mol), one concludes that HPMA is largely associated in 0.1 M HCl at both temperatures. This is confirmed by the K_{ass} values: the average values are 13.1×10^4 and 8.2×10^4 at 25 and 55 °C, respectively (see the last column in Table 3). Moreover, K_{ass} in 0.1 M HCl is higher than the corresponding values for HPMA in 0.002 M HCl (compare with values in Table 2). All these observations suggest that 0.1

TABLE 4: Radius of Gyration, R_g (in nm), for Atactic NaPMA in Aqueous 0.1 M NaCl at 25 °C

t/h	linear q^2 -dependence	quadratic q^2 -dependence	cubic q^2 -dependence	Debye function	average
0.17	44.5 ± 1.2	53.1 ± 3.4	53.4 ± 2.3	42.4 ± 1.5	48.4 ± 5.7
3	41.3 ± 1.4	48.3 ± 5.7	48.0 ± 2.5	39.4 ± 1.3	44.3 ± 4.6
24	40.3 ± 2.9	54.9 ± 8.4	49.9 ± 5.0	39.2 ± 1.9	46.1 ± 7.6
average	42.0 ± 1.8	52.1 ± 5.8	50.4 ± 3.3	40.3 ± 1.6	46.2 ± 5.9

M HCl is a worse solvent for the isolated HPMA chain than 0.002 M HCl and a better one for the associated form. As a consequence, the cross-links that are likely to be induced by shearing of solutions during filtering seem to be permanent or at least remain operative during a much longer period of time as suggested also by Ohoya et al.^{10,11} The negative charge fraction on the PMA chain in 0.002 M HCl is extremely low. From titration curves of our HPMA sample in water,³⁶ it has been estimated that the degree of ionization is around 1.6% for $c_p = 0.8$ g/L. In the presence of 0.002 M HCl, the ionization of HPMA is further suppressed. Moreover, the 50-fold increase of H^+ concentration on passing from 0.002 M HCl to 0.1 M HCl brings this value practically to zero, thus canceling even the weakest possibility of interchain repulsion and favoring aggregation.

The comparison of the values of the second virial coefficient in 0.002 M and in 0.1 M aqueous HCl leads to the apparent contradiction that a higher value of A_2 is noticed in the presence of a “worse” solvent. Again, this discrepancy can be resolved if one assumes that A_2 actually corresponds only to the *solvated* part of the associated form of HPMA and does not reflect interactions of the “hidden” hydrophobic parts of the HPMA chain with the solvent.

An estimate for the free enthalpy change, ΔG° , accompanying the association process is obtained from the K_{ass} values; $\Delta G_{328}^\circ (= -30.9$ kJ/mol) is more negative than $\Delta G_{298}^\circ (= -29.2$ kJ/mol), showing that the increase in temperature promotes intermolecular association of HPMA chains. From the temperature dependence of ΔG° , one can estimate the change in entropy as $\Delta S^\circ = 31$ J/(K mol) and the change in enthalpy as $\Delta H_{298}^\circ = -20$ kJ/mol. These values, albeit very crude ones, are comparable to the ones reported for the phase separation of, e.g., hydrocarbons in water, driven by hydrophobic interactions.⁴⁰ Moreover, the signs of the enthalpy and entropy changes are the same as those reported by Crescenzi et al.^{41,42} for the atactic HPMA in aqueous NaCl on the basis of potentiometric and direct calorimetric measurements. The difference in magnitude stems from the different normalization. In the case of refs 41 and 42, it is per mole of repeating unit, whereas in the present case it is per mole of “interpolymer contact”, which likely involves many repeating units.

The values for the radius of gyration should confirm the above assumption on stronger association and/or a more compact form of HPMA in 0.1 M HCl than in 0.002 M HCl. In the following,

we give a detailed treatment for the evaluation of the size of poly(methacrylic) chains under different experimental conditions.

4. Radius of Gyration and Persistence Length. The radius of gyration was evaluated by fitting the angular dependence of the scattering data according to the different formalisms outlined in Materials and Methods. One first obtains for each polymer concentration a value of the apparent radius of gyration, R_g , eq 6. The R_g value at zero concentration is then obtained from a plot of R_g versus c_p . Extrapolation of R_g to $c_p = 0$ has been in all cases performed by using a polynomial function of the second order (quadratic) in c_p . The results are reported in Tables 4 and 5, and they clearly show that R_g values depend on the fitting procedure as a function of q^2 . In general, the lowest values are obtained by using the Debye expression (eq 7), which gives results very close to those from the linear fitting of the q^2 -dependence. The polynomial function of the second order (quadratic) produces the highest R_g values. However, the quadratic function shows the lowest values of the sum of the squares of the residuals, whereas the Debye function shows the highest ones. Taking all that into consideration and in the absence of any clear-cut criterion, it was decided to take the average of the different estimates (see the last columns in Tables 4 and 5), at the cost of overestimating the standard deviations (SDs).

In the case of HPMA in 0.002 M HCl, these results showed a trend upon increasing time that was beyond the SD values. On the other side, the average values of R_g neither for the Na^+ salt form nor for the acid form in 0.1 M HCl allow for claiming a neat time dependence. In the case of NaPMA, the constancy is certainly due to the achievement of conditions of true molecular dispersion. On the contrary, in the case of HPMA in 0.1 M HCl, the K_{ass} values clearly show that association remains substantial and practically constant over a period of 24 h, thus explaining the essentially constant values of R_g .

In the extensive discussion on $M_{w,0}$ (see above), it has been pointed out that HPMA chains are intermolecularly associated in acidic solutions. The molecular dimensions for the acidic form reported in the following should be looked at as values at variable extent influenced by the association of HPMA. Only in the case of NaPMA (and, very likely, for the acidic form after 24 days in 0.002 M HCl) do they describe the dimensions of a single polyion chain.

4.1. NaPMA in NaCl. The agreement between the R_g values for NaPMA obtained with the different fitting procedures is excellent. The average value (46 ± 6 nm) points to a comparatively expanded coil, mainly as the result of electrostatic repulsions. No straightforward literature comparison can be made for the value of R_g . In their recent paper, Muroga et al.⁴³ use small-angle X-ray scattering (SAXS) to investigate two samples of PMA, both in acid and in salt form. Unfortunately, for NaPMA in 0.1 M NaF they do not give any value of R_g . A useful comparison can be drawn from the careful WALLS

TABLE 5: Radius of Gyration, R_g (in nm), for Atactic HPMA in Aqueous HCl

[HCl]	$T/^\circ\text{C}$	t/h	linear q^2 -dependence	quadratic q^2 -dependence	cubic q^2 -dependence	Debye function	average
0.002	25	0.17	8.74 ± 3.2	28.0 ± 4.3	21.9 ± 3.9	8.7 ± 3.2	16.8 ± 9.7
	25	3	11.0 ± 2.3	28.3 ± 3.3	22.3 ± 2.5	11.0 ± 2.2	18.2 ± 8.6
	25	24	15.6 ± 2.6	29.7 ± 3.2	24.5 ± 3.8	15.5 ± 2.6	21.3 ± 7.0
	25	576	20.7 ± 5.8	37.2 ± 12.9	31.5 ± 10.3	20.4 ± 5.6	27.5 ± 8.3
0.1	25	0.17	14.3 ± 1.9	26.6 ± 3.7	21.2 ± 2.7	14.2 ± 1.9	19.1 ± 6.0
	25	3	13.9 ± 2.3	19.4 ± 4.4	16.7 ± 4.5	13.8 ± 2.4	16.0 ± 2.7
	25	24	17.0 ± 2.7	18.0 ± 3.4	20.2 ± 2.9	15.6 ± 2.0	17.7 ± 1.9
	25	average	15.1 ± 2.3	21.3 ± 3.8	19.9 ± 3.4	14.5 ± 2.1	17.7 ± 3.4
	55	0.17	17.0 ± 0.9	16.8 ± 2.9	13.0 ± 2.4	17.1 ± 0.6	16.0 ± 2.0

analysis that Schweins et al.⁴⁴ performed on sodium poly-(acrylate), NaPA. They found that 0.1 M NaCl is a good solvent for NaPA: a power law is observed for the M_w dependence of R_g . By use of the latter equation, one calculates that for a polymer having the same M_w as our NaPMA sample the value of R_g is 29 nm. Considering that one might reasonably expect a substantial chain expansion for a sterically more crowded polymer like NaPMA with respect to NaPA, the former moreover presenting a substantial fraction of stereoregularity (i.e., around 50% syndio and 10% iso), the comparison of the results for the two companion molecules seems encouraging indeed.

The persistence length, L_p , was obtained from eq 8. Taking into account the overall average of R_g (46.2 nm; cf. last column in Table 4), the resulting value of L_p is 11 ± 3 nm, and that of the Kuhn segment, l_K , is 22 ± 6 nm. As first pointed out by Reed,⁴⁵ the values of l_K in good solvents (particularly so for polyelectrolytes at moderate to low values of the ionic strength) incorporate excluded volume effects, so they should be looked upon as “apparent” values, l_K' . Still, this observation does not hamper the applicability of the “wormlike chain” model to our NaPMA sample, for which the number of Kuhn segments in a chain is about 18, considering that its contour length is around 385 nm. From Figure 5 of the quoted paper by Schweins et al.,⁴⁴ one can calculate that the apparent Kuhn segment length for NaPA with $M_w \approx 165$ kg/mol in 0.1 M NaCl is about 10 nm, in agreement with the expected larger stiffness of NaPMA with respect to NaPA. From the analysis in theta conditions (i.e., in 1.5 M NaCl), those authors give for NaPA the value of “intrinsic” l_K equal to 4.2 nm. This value is in remarkable contrast with the value of the “intrinsic” persistence length claimed by Muroga et al. for NaPMA in 0.1 M NaF, i.e., 0.40 ± 0.05 nm.⁴³

4.2. HPMA in HCl. All R_g values for HPMA in 0.002 M HCl increase with increasing time, in agreement with the previously found decrease of $M_{w,0}$, A_2 , and K_{ass} . This implies that the deaggregation of HPMA molecules (presumably accompanied by the penetration of solvent inside the clusters) leads to some expansion of the chain segments involved in the contacts. The process is a rather slow one. The ratio between the lowest average value for the radius of gyration of HPMA in 0.002 M HCl at $t = 0.17$ h ($R_g = 17$ nm) and that of its sodium salt form ($R_g = 46$ nm) is about 1:2.7, whereas for the corresponding one at $t = 576$ h ($R_g = 28$ nm) it is only 1:1.6. The latter increase in dimensions caused by neutralization of the acid groups is comparable to the one reported previously by Pleštil et al.²¹ They studied salt-free HPMA solutions by small-angle neutron scattering at various degrees of neutralization. Owing to the difference in molar mass (and distribution) between the two samples and, even more important, due to the large difference in the ionic strength, no direct comparison of absolute R_g values can be made. Still, a similar relative increase in dimensions of the chain (1.8-fold) was found in their case from the pure acid to the fully neutralized form. At variance with this, Moan et al.²⁵ have found a 2.6-fold increase for the radii of gyration of PMA in water from $\alpha_N = 0.27$ to $\alpha_N = 1.0$. One is led to believe that in their case the process of chain solubilization at the lowest value of α_N had not yet been fully achieved.

The radius of gyration shows its lowest value in 0.1 M HCl: the average value over 24 h is about 18 ± 3 nm at 25 °C. This confirms our previous assumption that stronger interchain association would result in a more compact form of HPMA. The apparent similarity between initial parameters in both HCl

concentrations (compare R_g values in Table 5 and also M_w and A_2 values in Tables 2 and 3 at $t = 0.17$ h for 0.002 M HCl and the time-average values for 0.1 M HCl) can be most simply traced back to the highly nonequilibrium conditions, which largely reflect the state of the polymer in the solid phase.

When the temperature is raised to 55 °C, R_g for HPMA in 0.1 M HCl slightly reduces to 16 ± 2 nm. The uncertainty of the measurement does not allow drawing a clear conclusion, although a slight decrease of R_g upon heating would be in expected agreement with the increased tendency toward association as revealed by the values of ΔG° . Comparison with other values of R_g is not straightforward because of the difference in molar masses and also in measuring conditions. Muroga et al.⁴³ studied by SAXS over a scattering vector range from 0.2 to 2.5 nm⁻¹ two samples of HPMA in acidic aqueous solution (pH = 2.3–2.8), having M_w values of 26.8 and 40.6 kg/mol, respectively. They report for R_g the values of 3.5 ± 0.4 and 3.6 ± 0.4 nm, respectively. Experimental data on poly-(acrylic acid), HPA, may be useful also in this case. Recently, Taylor and Stivala⁴⁶ reported data, obtained from SAXS over a scattering vector range from 0.12 to 1.6 nm⁻¹, on HPA in water in the acid form. For their sample, having $M_w = 590$ kg/mol, they found R_g equal to 60 nm.

We calculated the persistence length for HPMA only in 0.002 M HCl at 576 h, a condition which can reasonably be assimilated to equilibrium, or very close to it: L_p is 3.6 ± 2.2 nm. This value is comparable with, but larger than, the literature data. For the persistence length of HPMA in water, Pleštil et al.²² obtained for $\alpha_N \leq 0.2$ $L_p = 0.8$ nm and $L_p = 1.2$ nm for $0.2 < \alpha_N < 0.5$. Muroga et al.¹⁹ report a value in the range from 0.66 to 1 nm for the stereoregular counterpart of HPMA in its ester form, i.e., for the isotactic PMMA in acetone. In their more recent paper, Muroga et al.⁴³ claim that “the persistence length for PMA in aqueous solution containing added salt (0.4 ± 0.05 nm) is somewhat smaller than for PMA in salt-free acidic aqueous solution (0.5 ± 0.1 nm)”. Furthermore, they conclude that the local conformation of the polymer is not changed by the presence of charges¹⁹ and that “the contracted form of PMA changes to an expanded random coil in the higher pH region without a significant change in the chain flexibility, that is, predominantly by the excluded volume effect.”⁴³ This statement further justifies inclusion of poly(acrylic acid) in the comparative discussion. Taylor and Stivala⁴⁶ give for the persistence length of HPA in water the value of 5.85 nm, which is even slightly larger than ours. A possible explanation for the very low values of L_p for the data of Pleštil et al.²² and of Muroga et al.^{19,43} is that a very careful consideration of possible time effects and the push for low polymer concentrations has been used only in the present investigation at variance with the previous ones. Therefore, we would conclude that those values of R_g and of L_p are underestimated due to the noncomplete dissolution of chain clusters. However, it should be pointed out that R_g is certainly a less sensitive parameter to detect time dependence effects related to deaggregation than the weight-average molar mass and the association constant. The present conclusions referring to association have been at first more firmly deduced from the study of the concentration dependence in the low concentration regime, followed by that of the angular dependence.

Conclusions

A careful light scattering analysis of HPMA in aqueous HCl solutions under various experimental conditions, e.g., different ionic strength, degree of ionization, and temperature, has been

performed. The results of the study point to a complex behavior, which is at present not yet fully understood. Still, our results clearly demonstrate a high tendency toward intermolecular association of the acid form of PMA, on the basis of the following observations:

(1) The weight-average degree of polymerization of HPMA in acidic conditions is up to 63% higher than the corresponding value for a single chain, as determined for the completely neutralized sodium salt form, NaPMA, in 0.1 NaCl.

(2) The reciprocal reduced intensity of scattered light for HPMA in 0.002 and in 0.1 M HCl is not a linear function of the square of the wave vector; a pronounced downward curvature is found in the high q^2 range, in particular for low polymer concentrations.

(3) The zero-angle values of the reduced light scattering intensity, obtained in a very broad region of concentrations (from around 0.01 to 10 g/L), show a distinctive upward curvature in the dilute regime, which is also prognostic for associative systems. Such curvature can be easily overlooked by performing the experiments only in the high concentration region.

The overall association behavior seems to be a complicated result of both equilibrium and kinetic phenomena. In general, many polymers in the solid state are well-known to give well-disperse solutions only with much difficulty, mostly due to the combination of energetic and entropic terms giving rise to metastable states trapping them in the solid form. More specifically, one might suggest that hydrophobic interactions play an important role, involving methyl groups of different chains. This hypothesis is indirectly supported by the well-known lower critical solution temperature (LCST) behavior of HPMA. The formation of interchain hydrogen bonding is an additional very likely possibility to take into account. Also, previous findings^{9–11} suggested the presence of shear-induced association (or negative thixotropy) of poly(methacrylic) acid solutions; this could happen either during mechanical stirring or/and during the high shear process of filtration prior to light scattering measurement. However, no conclusive statement can be made so far on the cause of such strong tendency to associate. What is clear is that after preparation of solutions, HPMA in 0.002 M HCl progressively gives rise to less associated forms. The tiny residual fraction of charge that is left on the chain under those conditions of pH (about 1.6%) and the low value of the ionic strength of the system are likely responsible for a small but significant residual electrostatic contribution eventually leading to complete chain solubilization. At variance, aqueous 0.1 M HCl seems to annihilate almost completely the repulsive electrostatic interactions, at least on the time scale of 24 h. For the latter solvent, nothing can be said on the ultimate equilibrium form of HPMA on the basis of our data. In all cases, however, the rate of deassociation is so slow as to enable one to work under quasi-equilibrium conditions. The time required for a light scattering experiment is very small if compared with the overall time of polymer dissolution. Hence, it is justified to apply to the investigated systems the formalism pertaining to equilibrium association (OAM), keeping in mind the “apparent” meaning of the derived “association constants”.

The molecular dimensions as obtained by R_g values show that HPMA is in its most compact form in solutions at very low pH (in our case this is in aqueous 0.1 M HCl), at elevated temperatures (herein at 55 °C), and at high polymer concentrations. Lowering of the H^+ concentration and the ionic strength leads to the formation of increasingly more loose clusters (higher R_g), which allow for a greater penetration of the solvent inside the associated form. With a very slow kinetics, the system

eventually reaches a state of true molecular dispersion. In our opinion, the described behavior is strongly dependent on the stereochemical structure of the polymer. Unfortunately, most of the published works on the atactic HPMA do not report the precise stereochemical composition of the samples. The polymer chain of our HPMA was a “typical” atactic one with approximately equal amounts of heterotactic and syndiotactic triads and very few isotactic ones. Highly isotactic HPMA (i-HPMA) displays different solution behavior than the syndiotactic or the atactic ones.^{13,19,47–49} One of the important differences is the insolubility of i-HPMA in water at zero degree of neutralization.¹³ Even in the case of the fully neutralized form, a stereoregular polyanion could possibly manifest a much higher chain stiffness with respect to its atactic counterpart, resembling, e.g., the case of stereoregular biological polyelectrolytes. It is, therefore, reasonable to expect that a polymer with distinctively different tacticity, for example, a highly uniform isotactic chain, would lead to a completely different pattern of behavior. Consequently, it is of great importance to conduct the experiments on polymer samples with well-defined stereoregularity in order to be able to understand these complicated phenomena. Work is in progress along this line in our laboratories.

Acknowledgment. The helpful support of Dr. Janez Cerkovnik, University of Ljubljana, and of Dr. Flavio Zanetti, POLYtech S.C.r.l., Trieste, for performing the NMR and the SEC-LALLS determinations, respectively, is gratefully acknowledged. The Flemish Institute for Science and Technology (IWT), the Fund for Scientific Research-Flanders (FWO), the Belgian Government (IUAP-IV/11), and the University of Trieste supported this work (the stay of K.K.).

References and Notes

- (1) Tanaka, F. *Macromolecules* **2003**, *36*, 5392–5405.
- (2) Wissenburg, P.; Odijk, T.; Kuil, M.; Mandel, M. *Polymer* **1992**, *33*, 5328–5330.
- (3) Hossain, K. S.; Ohya, E.; Ochi, A.; Magoshi, J.; Nemoto, N. *J. Phys. Chem.* **2003**, *107*, 8066–8073.
- (4) Vanneste, K.; Mandel, M.; Paoletti, S.; Reynaers, H. *Macromolecules* **1994**, *27*, 7496–7498.
- (5) Bongaerts, K.; Reynaers, H.; Zanetti, F.; Paoletti, S. *Macromolecules* **1999**, *32*, 675–682.
- (6) Bongaerts, K.; Reynaers, H.; Zanetti, F.; Paoletti, S. *Macromolecules* **1999**, *32*, 683–689.
- (7) Bongaerts, K.; Paoletti, S.; Denef, B.; Vanneste, K.; Cuppo, F.; Reynaers, H. *Macromolecules* **2000**, *33*, 8709–8719.
- (8) Eliassaf, J.; Silberberg, A. *Polymer* **1962**, *3*, 555–564.
- (9) Eliassaf, J.; Silberberg, A.; Katchalsky, A. *Nature* **1955**, *176*, 1119.
- (10) Ohoya, S.; Matsuo, T. *J. Colloid Interface Sci.* **1979**, *68*, 593–595.
- (11) Ohoya, S.; Hashiya, S.; Tsubakiyama, K.; Matsuo, T. *Polym. J.* **2000**, *32*, 133–139.
- (12) Leyte, J. C.; Mandel, M. *J. Polym. Sci., Part A* **1964**, *2*, 1879–1891.
- (13) Crescenzi, V. *Adv. Polym. Sci.* **1968**, *5*, 358–386 and references cited therein.
- (14) Mandel, M.; Leyte, J. C.; Stadhouder, M. G. *J. Phys. Chem.* **1967**, *71*, 640–649.
- (15) Silberberg, A.; Eliassaf, J.; Katchalsky, A. *J. Polym. Sci.* **1957**, *23*, 259–284.
- (16) Liquori, A. M.; Barone, G.; Crescenzi, V.; Quadrioglio, F.; Vitagliano, V. *J. Macromol. Chem.* **1966**, *1*, 291–305.
- (17) Heitz, C.; Rawiso, M.; François, J. *Polymer* **1999**, *40*, 1637–1650.
- (18) Moan, M. *J. Appl. Crystallogr.* **1978**, *11*, 519–523.
- (19) Muroga, Y.; Noda, I.; Nagasawa, M. *Macromolecules* **1985**, *18*, 1580–1582.
- (20) Pleštil, J.; Mikeš, J.; Dušek, K.; Ostanevich, Ju. M.; Kunchenko, A. B. *Polym. Bull.* **1981**, *4*, 225–231.
- (21) Pleštil, J.; Ostanevich, Ju. M.; Bezzapotonov, V. Yu.; Hlavata, D.; Labsky, J. *Polymer* **1986**, *27*, 839–842.

- (22) Pleštil, J.; Ostanovich, Ju. M.; Bezzabotnov, V. Yu.; Hlavata, D. *Polymer* **1986**, 27, 1241–1246.
- (23) Sedlak, M.; Konak, Č.; Štepanek, P.; Jakeš, J. *Polymer* **1987**, 28, 873–880.
- (24) Katchalsky, A.; Eisenberg, H. *J. Polym. Sci.* **1951**, 6, 145–154.
- (25) Moan, M.; Wolff, C.; Ober, R. *Polymer* **1975**, 16, 781–784.
- (26) Elias, H. G. Association and Aggregation as Studied via Light Scattering. In *Light Scattering from Polymer Solutions*; Huglin, M. B., Ed.; Academic Press: London, 1972; Chapter 9, pp 397–457.
- (27) Davenport, J. N.; Wright, P. V. *Polymer* **1980**, 21, 287–292.
- (28) Klesper, E.; Strasilla, D.; Regel, W. *Makromol. Chem.* **1974**, 175, 523–534.
- (29) Nagasawa, M.; Murase, T.; Kondo, K. *J. Phys. Chem.* **1965**, 69, 4005–4012.
- (30) Chapman, A. J.; Billingham, N. C. *Eur. Polym. J.* **1980**, 16, 21–24.
- (31) Kratochvil, P. *Classical Light Scattering from Polymer Solutions*; Elsevier Science Publishers B. V., Amsterdam, 1987.
- (32) Vorreux, G.; Morcellet, M.; Loucheux C. *Macromol. Chem.* **1982**, 183, 711–720.
- (33) Flory, P. J. *Statistical Mechanics of Chain Molecules*; Interscience: New York, 1969; pp 340–350.
- (34) Benoit, H.; Doty, P. *J. Phys. Chem.* **1953**, 57, 958–963.
- (35) Hunt, M. L.; Newman, S.; Scheraga, H. A.; Flory, P. J. *J. Phys. Chem.* **1956**, 60, 1278–1290.
- (36) Unpublished results from this laboratory.
- (37) Kratochvil, P. Particle Scattering Functions. In *Light Scattering from Polymer Solutions*; Huglin, M. B., Ed.; Academic Press: London, 1972; Chapter 7, pp 333–379.
- (38) Hyde, A. J.; Wippler, C. *J. Polym. Sci.* **1962**, 58, 1083.
- (39) Kogej, K.; Cerkovnik, J.; Berghmans, H.; Paoletti, S. *Acta Chim. Slov.* **2001**, 48, 395–406.
- (40) Tanford, C. *The Hydrophobic Effect: Formation of Micelles and Biological Membranes*; Wiley-Interscience: New York, 1980.
- (41) Delben, F.; Crescenzi, V.; Quadrioglio, F. *Eur. Polym. J.* **1972**, 8, 933–935.
- (42) Crescenzi, V.; Quadrioglio, F.; Delben, F. *J. Polym. Sci., Part A-2* **1972**, 10, 357–368.
- (43) Muroga, Y.; Yoshida, T.; Kawaguchi, S. *Biophys. Chem.* **1999**, 81, 45–57.
- (44) Schweins, R.; Hollmann, J.; Huber, K. *Polymer* **2003**, 44, 7131–7141.
- (45) Reed, W. F.; Ghosh, S.; Medjahdi, G.; François, J. *Macromolecules* **1991**, 24, 6189.
- (46) Taylor, T. J.; Stivala, S. S. *J. Polym. Sci., Part B: Polym. Phys.* **2003**, 41, 1263–1272.
- (47) Hatada, K. *J. Polym. Sci., Part A: Polym. Chem.* **1999**, 37, 245–260.
- (48) Costantino, L.; Crescenzi, V.; Quadrioglio, F.; Vitagliano, V. *J. Polym. Sci., Part A-2* **1967**, 5, 771–780.
- (49) Leyte, J. C.; Arbouw-van der Veen, H. M. R.; Zuiderweg, L. H. J. *Phys. Chem.* **1972**, 76, 2559–2561.