Gel Permeation Chromatography using Controlled-Porosity Glass. II. Polyacrylamide Aqueous Solutions

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Synopsis

In a study of polyacrylamide in solutions we have required the rapid characterization on chromatographies. The application to the aqueous solution of the GPC using controlled-porosity glass has been examined from both the viewpoint of the effect of salt addition and the GPC mechanism. An adequate addition to neutral salt, $0.005M$ KCl to the eluent, gave rise to the elution behaviors being in accord with the hydrodynamic volume concept of the GPC separation.

INTRODUCTION

Polyacrylamide (PAAm) and its derivatives have been broadly employed as synthetic flocculants because of their high flocculation activities. This flocculation mechanism, however, has not been fully elucidated, as the object to be operated ranges widely in its properties.[1,2] For general comprehension of the flocculation process, well-characterized flocculant polymers, and suspensions are essential. The aqueous solutions of polymers with the activity do not give stable solutions, this being called the aging effect, and the proposed reasons for this phenomenon are disaggregation of entangled polymer molecules, and the chemical breakdowns of intermolecular peroxide and imide groups produced in the side reactions of polymerization.[3–6]

The rapidity of gel permeation chromatography, GPC, is very favorable to these polymers. However, the successful applications to water-soluble polymers are of limited number.[7,8] For the formamide solution of PAAm, the chromatographic behaviors on the stationary phase of controlled-porosity glass were understood by the concept of hydrodynamic volume in GPC.[9]

In the present work we have examined the applicability to PAAm aqueous solutions on the same stationary phase mentioned above, by employing well-characterized fractions, and the partially hydrolyzed materials were also put into the test. Furthermore, the molecular weight distribution of PAAm fractions is discussed from the resultant elution curves.

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EXPERIMENTAL

Materials

Acrylamide recrystallized from methanol was polymerized in an aqueous solution adjusted to pH of 10 by the addition of sodium carbonate at 40°C for 8 hr under a nitrogen atmosphere, with the use of the catalyst, sodium periodide. PAAm samples employed in this work were obtained by the fractional precipitation with water (solvent) and methanol (nonsolvent).[10] The refractionation was also performed to make a fraction designated P2. The molecular weight was determined from the light scattering measurement performed with FICA 50 photometer by the radiation of light of 436 nm in formamide at 30°C. The refractive index increment of 0.1033 ml/g was used for the data processing. Partially hydrolyzed material PAAm-H was prepared from a PAAm fraction, F3 by the alkaline hydrolysis with the changes of the alkaline concentration and the reaction period at 40°C.[11] After the dialysis against deionized water, the solution passed an ion exchanger column to produce a PAAm-H sample with the carboxylic side group. The degree of hydrolysis of PAAm-H samples of F3H2 and F3H3 was estimated as 3.2 and 21.9%, respectively, by the colloid titration at pH of 10.6 with standard methyl glycolchitosan and potassium poly(vinyl sulfate) solutions using toluidene blue as an indicator.[9] Neutral salts used were guaranteed grade, and fresh water twice distilled after deionization was immediately used.

METHODS

As the stationary materials for GPC, controlled-porosity glasses, CPG-10 of Electro-Nucleonic Inc. with pore sizes of 3125, 2000, 973, 493, 240, and 123 Å were packed as dry materials into the stainless steel columns of 4 ft $\times \frac{3}{8}$ in. The columns were connected to a Waters GPC model 200, and had the number of theoretical plates, 1400–1500/4 ft after the complete degas with the flow of a large quantity of water. The GPC measurement was performed with the injection volume of 2 ml and the flow rate of 1 ml/min at 30°C. The viscosity measurements were carried out with Ubbelohde dilution viscometers thermostatted within 0.01°C at 25.0, 40.0, and 50.0°C at the sixth day after mixing together the freeze-dried polymer sample and solvent. This process was made for gentle dissolution to avoid mechanical stirring.

RESULTS AND DISCUSSION

Salt Influence on the Characterization

PAAm has been investigated in water containing neutral salts at comparatively high salt concentration such as $1M$ NaNO$_3$ and 10% NaCl.[12–16] The most probable reason for this seems to suppress the polyelectrolytic behaviors of solutions caused by the contaminating hydrolyzed materials in the samples, which arose from the lack of purification. Then, before GPC experiments, the solutions of the PAAm fractions of interest were examined viscometrically. The extrapolation to zero concentration to obtain the intrinsic viscosity [η] was performed easily even for the salt-free system with the use of the Kraemer and the Huggins

equations, without encountering any difficulty to salt-free systems of polyelec-trolytes. The influence of the salt presence on $[\eta]$ is shown in Figure 1(A) for typical neutral salts such as potassium chloride and calcium chloride. Within the range of salt concentration examined up to $1M$, any significant change in $[\eta]$ is not observed, and, moreover, there exists no appreciable difference for these two kinds of salt. These evidences undoubtedly account for the nonionic character of the PAAm fractions employed in this study and also support the above presumption for the addition of salt. Furthermore, the temperature de-pendences of a fraction P4 shown in Figure 1(B) explain that aqueous media are better to PAAm than formamide used in the previous work.[9] It can be said that $[\eta]$ of well-fractionated PAAm in aqueous media is hardly affected by the pres-ence of neutral salt and temperature. The intrinsic viscosity obtained in a salt-free system and the weight-average molecular weight M_w from the light scattering method summarized in Table I and the others[17] give the following relation:

$$[\eta] = 2.67 \times 10^{-4} M_w^{0.66} \qquad \text{(water, 25.0°C)} \qquad (1)$$

The value of the exponent is in good agreement with that found in $1M$ NaNO$_3$.[13] The wide applicability of eq. (1) over temperature and salt concentration, re-vealed above, will be later incorporated with the subsequent chromatographic behaviors of PAAm.

As for addition of neutral salts to the eluent of GPC, there have been found many examples, even for the elutions of nonionic water-soluble polymers: poly(vinyl alcohol) and dextran on the stationary phase of the deactivated Porasil of porous silica,[18] and polyvinyl alcohol on that of glyceryl CPG.[19] We employed
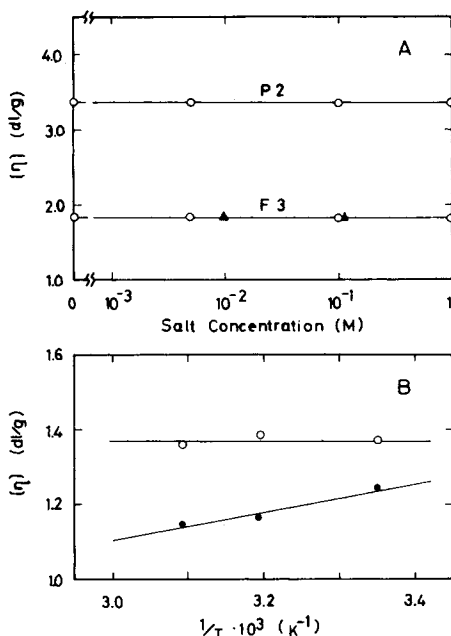

Fig. 1. Environmental influence on the solution viscosity of polyacrylamide: (A) effect of neutral salt addition of potassium chloride (O) and calcium chloride (▲), (B) temperature effect on poly-acrylamide sample of P4-water (O) and P4-formamide (●) systems.

TABLE I
GPC and Viscometric Results for Polyacrylamide Samples

| Sample | $M_w \times 10^{-4}$ | $[\eta](dl/g)$[a] | GPC elution volume (counts)[b] | | | |
			Water	0.005M KCl	0.05M KCl	0.5M KCl
P2	159.	3.310		28.1		
F2	107.	2.460	24.7	29.5	30.1	30.1
F3	57.3	1.820	25.0	31.5	31.5	31.5
P4	41.3	1.373		33.1	33.5	
H3	13.9	0.687		36.5	36.6	

[a] Measured in water at 25.0°C.
[b] 1 count = 5 ml.

potassium chloride as the salt, and performed GPC measurement with the column combination of 3125-2000-493-240 Å. In Figure 2 typical chromatograms eluted with water and saline water are shown for both PAAm and PAAm-H. For the PAAm sample, the presence of salt lets the elution volume increase appreciably, but the increase in the salt concentration over 0.001M does not cause further remarkable elution volume change. Taking into account the properties of PAAm in solutions mentioned above, the volume increase can be assigned to the change of the stationary phase that would be induced by the presence of salt. The total volume increase of the GPC columns, ~2 counts (= 10 ml) for the addition of the salt to water which was observed in the elutions of methanol and acetone, is in favor of the above insight. On the other hand, the elution curves
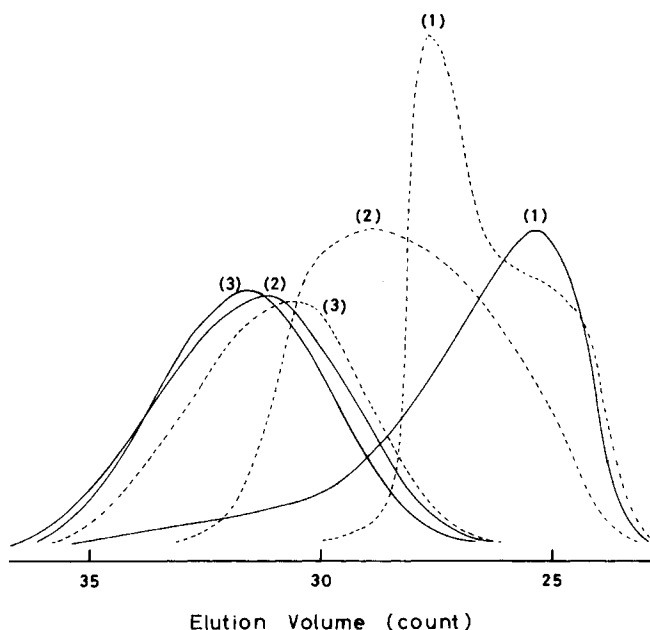

Fig. 2. Salt-dependent chromatograms of a polyacrylamide sample of F3 (solid curves) and the partially hydrolyzed materials of F3H2 (broken curves), as eluents: (1) pure water, (2) $10^{-3}M$ KCl, and (3) $5 \times 10^{-3}M$ KCl. The column set of 3125-2000-493-240 Å was used.

of the PAAm-H sample is more complicated than those of PAAm, depending on the salt concentration. As expected, PAAm-H behaves exactly as an anionic polyelectrolyte, and the expanded molecule in water seems to be excluded from the slightly negatively charged pores of CPG.[19] This trend should be reduced with the increase in the salt concentration, as seen in Figure 2. Judging from the elution behaviors shown in Figure 2, the favorable elutions to characterize PAAm and PAAm-H samples on this chromatography would be expected for the systems containing an appropriate amount of neutral salt.

GPC of PAAm in 0.005M Potassium Chloride

The molecular weight dependence on the peak elution volume performed with the analytical column set of 3125-973-493-123 Å is given in Table I, and it is seen that the resolution for the molecular weight is recognized for 0.005M and the elution volume becomes steady if the salt concentration exceeds 0.05M. The chromatographic behavior was examined in detail in 0.005M KCl aqueous solution. Figure 3 shows an example for the proportionality between the concentration of the injected sample and the area on the chromatogram, and no adsorption of PAAm onto the stationary phase is found for this system. The elution volume is also examined for the PAAm concentration, as shown in Figure 4. In addition to an overall view of the molecular-weight-dependent elution volume, the higher the M_w the more the concentration dependence of the volume. This elution volume increase with the increase in the sample size is similar to the elution behavior with organic eluent on the same packing materials,[20] and is in good agreement with the reported studies on the operational variables using Styragel[21] and Porasil.[22] It seems that the overloading does not occur within the range examined. Figure 5 demonstrates the standard calibration curve constructed from the elution experiments made on the solution of 0.1 g/dl.

According to the concept of hydrodynamic volume, the product of $[\eta]$ and M, the molecular weight, gives a universal calibration curve, irrespective of the kind
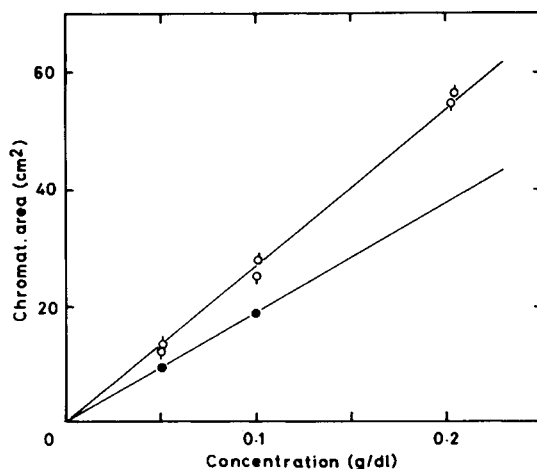

Fig. 3. Area on a chromatogram and the concentration of injected solutions of polyacrylamide samples of F3 (◯) and P4 (◌), and the partially hydrolyzed materials of F3H3 (●). Eluted with $5 \times 10^{-3}M$ KCl.
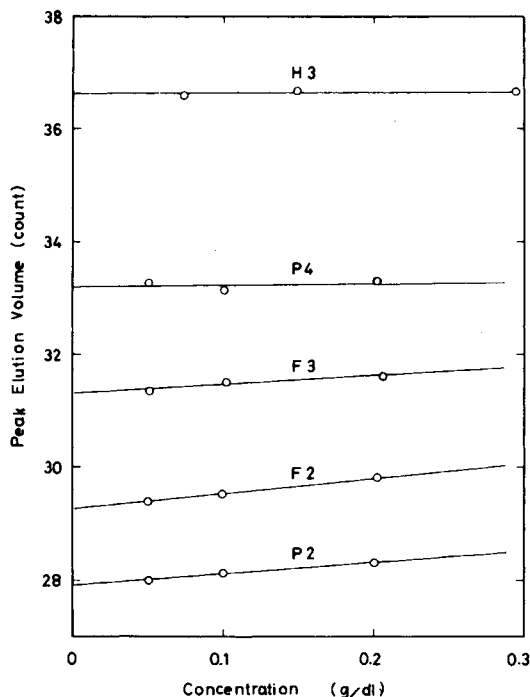
Fig. 4. Injected sample size and the retention volume (in 5 ml/count) of polyacrylamide samples under the elution with $5 \times 10^{-3}M$ KCl.

of solutes, under the concerned condition.[23] Since the constancy of $[\eta]$ over the wide range of both salt concentration and temperature was established for PAAm in the previous section, the $[\eta]$ value depicted in Table I can be applied to $[\eta]$ for this saline system, without any correction. By substituting M_w for M, a universal curve is constructed as shown in Figure 6, and the linear relationship on the semilogarithmic plots is found in such wide range of the hydrodynamic volume. This might be explained by considering the sizes of solute and pore of the stationary phase as follows: even for the highest molecular weight sample of PAAm employed, the root mean-squared end-to-end distance calculated from Flory–Fox equation[24] with the universal constant, $\Phi = 2.5 \times 10^{23}$ (in cg) is about 1300 Å, which is sufficiently less than the pore diameter of 3125 Å of the controlled-porosity glass with the largest pore. If the resolution is limited by the radius of gyration of the solute,[25] then the combination of eq. (1) and the pore size leads to the limiting molecular weight of 10^7, whose elution volume would correspond to the void volume of the operational variables. The hydrodynamic volume and its concentration dependence were examined further, based on Rudin's semiempirical scheme.[26] The hydrodynamic volume of solvated molecule V_h is expressed with δ times the unswollen volume at the amorphous state and can be evaluated at the respective concentration through the relation between δ and the volume fraction of solute in solution. By applying eq. (1), the trends appearing in Figure 4 were reproduced, and in Figure 7 the estimated V_h of PAAm fractions is plotted against the experimental elution volume. Some scatter would probably be due to the different molecular weight distributions of PAAm samples.
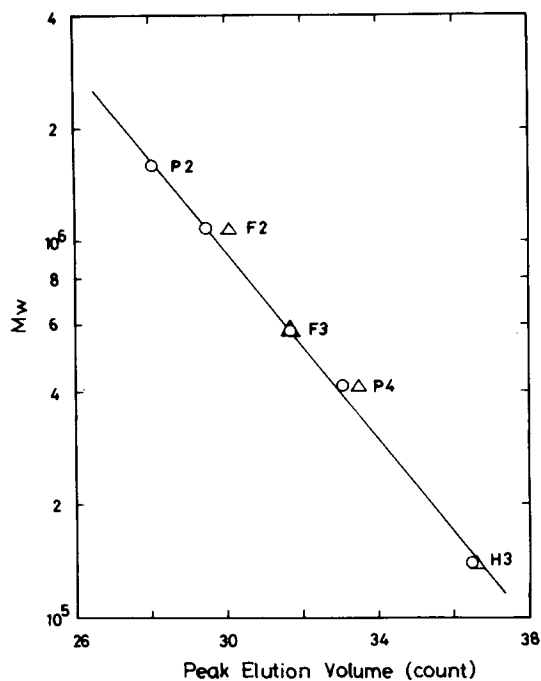
Fig. 5. Standard calibration curve for the elution with $5 \times 10^{-3}M$ KCl (O) and $5 \times 10^{-2}M$ KCl (△) of polyacrylamide samples made on the sample size of 0.1 g/dl.

Thus the concerned elution behaviors, that is, observed in the system of PAAm–CPG–0.005M KCl seems to be understood in the framework of current GPC studies of the separation.
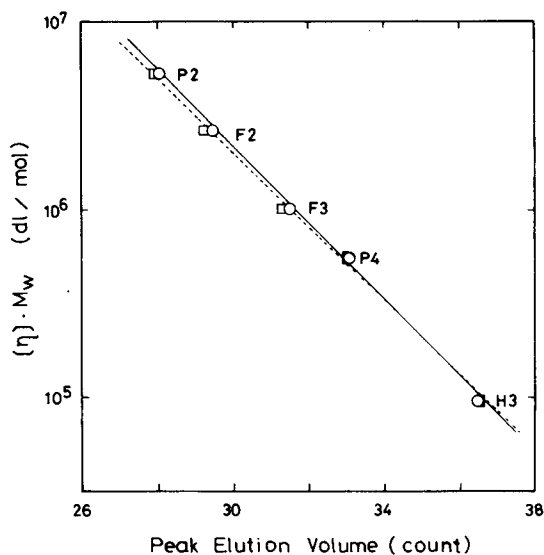

Fig. 6. Hydrodynamic volume and the retention volume of polyacrylamide samples under the elution with $5 \times 10^{-3}M$ KCl. The solid line connects the open circles made on the sample size of 0.1 g/dl, and the broken line connects the squares made on the extrapolated zero sample size.
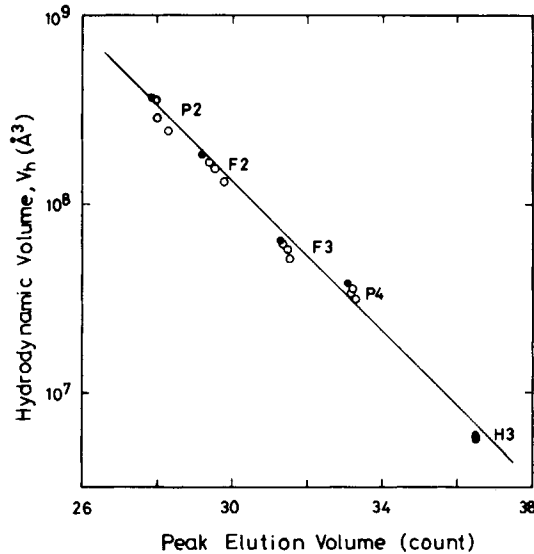
Fig. 7. Estimated hydrodynamic volume V_h (O) of a polyacrylamide sample in finite polymer concentration and the experimental retention volume; (●) V_h's extrapolated to the infinite dilution.

Estimate of the Molecular Weight Distribution of PAAm

As the goal of this work, we have tried to gain the molecular weight characterization from the elution envelopes of PAAm discussed in the previous section. It is well known that the first step required is the establishment of an expression linking the elution volume and the molecular weight, called the calibration curve. When the monodisperse or the narrow polymers are not available for the concerned GPC operations, the alternative ways to approach the expression must be applied.[27] In the present case, the relation obtained from Figure 5 is found to be an approximate expression by considering the process of the preparation and the fractionation performed on the PAAm samples. Therefore, we have sought the appropriate relation of the elution volume and the molecular size, within the limitations of available data such as the elution volume envelope and the weight-average molecular weight for the respective sample. The following functionality was primary assumed for M molecular weight and V elution volume

$$\log M = a - bV \qquad (2)$$

where a and b are constants. This relation seems to be accepted widely and has become conventional. The elution volumes V_i's on a GPC envelope, which were read out along with the height H_i's at about 150 points by an on-line chart reader having the resolution of 0.1 mm, were converted to the molecular weights M_i's for the presumed values of a and b. In practice, the values of a and b from Figure 5 were used, and the value of M_w^{GPC} was evaluated through the relationship

$$M_w^{\mathrm{GPC}} = \sum_i M_i H_i / \sum_i H_i$$

These processings were applied to the GPC envelopes of all PAAm samples with the aid of a medium size computer TOSBAC 3400. Then the sum S defined by

TABLE II
Molecular Weight Characterization of Polyacrylamide Samples by GPC with the eluent of
0.005M KCl

Sample	$M_w^{LS} \times 10^{-4}$ [a]	$M_n^{GPC} \times 10^{-4}$	$M_w^{GPC} \times 10^{-4}$	$M_z^{GPC} \times 10^{-4}$	M_w^{GPC}/M_n^{GPC}
P2	159.	94.1	133.8	167.9	1.42
F2	107.	62.2	106.0	152.3	1.71
F3	57.3	41.3	75.7	133.0	1.83
P4	41.3	25.0	42.1	60.1	1.68
H3	13.9	7.48	13.9	20.5	1.85

[a] LS denotes light scattering measurement.

eq. (3), of the difference between M_w^{GPC} and M_w^{LS} from the GPC and the light scattering (LS) method, respectively, was calculated for assessing the set of values of a and b:

$$S = \sum_{\text{samples}} \frac{|M_w^{GPC} - M_w^{LS}|}{M_w^{LS}} \tag{3}$$

To minimize the magnitude of S, the values of a and b were successively altered and all the above processes were iterated. Thus the obtained values of a^* and b^* were applied to eq. (2), and the number-average and z-average molecular weights, respectively, M_n and M_z were evaluated through the relations of

$$M_n^{GPC} = \sum_i H_i / \sum_i \frac{H_i}{M_i}$$

and

$$M_z^{GPC} = \sum_i M_i^2 H_i / \sum_i M_i H_i$$

The results are shown in Table II. As for M_w, the agreement is reasonable except a fraction F3, and the dispersity for a fraction P2 seems to reflect the refractionation process applied to this fraction. The values of M_w^{GPC}/M_n^{GPC} in Table II can be said to be reasonable when taking into account the difficulty for the fractionation to be experienced commonly in aqueous systems.[28]

The analytical method applied here is generally feasible in most cases, and the replacement of M_n for M_w is possible if the values of M_n are known. In spite of the assumption of eq. (2), the method would be employed till the monodisperse standards of molecular weight become available for aqueous systems.

It might be concluded that an adequate addition of neutral salt gives rise to the rapid characterization of PAAm with GPC employing the controlled-porosity glass in aqueous systems, and the elution behavior of an aqueous PAAm solution containing more than 0.005M potassium chloride is well in accord with the hydrodynamic volume concept on which the separation mechanism can be assigned to the volume difference of solutes. Thus, the characterization of PAAm by this chromatography is assessed by the well-established GPC experiments. For partially hydrolyzed materials, the elution behaviors are complicated, depending on the amount of salt presence. Regarding this point, GPC with the use of formamide as a mobile phase[9,29] serves an alternative way to the GPC performance.

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