

Gel Permeation Chromatography Using Controlled-Porosity Glass. I. Polyacrylamide-Formamide Solution

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Synopsis

The process of characterizing polyacrylamide and its partially hydrolyzed materials by gel permeation chromatography was examined. The use of controlled-porosity glass and formamide as the stationary phase and the eluent, respectively, resulted in chromatographic behavior in accord with the hydrodynamic volume concept for polyacrylamide fractions. The addition of a salt (KCl) to the eluent was found to retard the elution of the hydrolyzed polyacrylamide.

INTRODUCTION

Polyacrylamide (PAAm), in particular, partially hydrolyzed anionic polyacrylamide (PAAm-H), is an important material as a flocculant of sols or suspensions. PAAm-H with high molecular weight is more effective as a flocculant in primary clarification of waste water.^{1,2} According to the bridging mechanism of La Mer³ for the flocculation of sols and suspensions, it is expected that molecular parameters of PAAm or PAAm-H such as molecular weight, the radius of gyration, and the degree of hydrolysis have a large effect on flocculation activity. The evaluation of these parameters could be useful in the operation of waste water treatments.

Gel permeation chromatography (GPC) provides a useful technique for the characterization of polymers. The method is extremely rapid once a column set has been constructed; an analysis takes only a few hours of effective working time. However, the general application of GPC has often been limited because chromatographic resolution depends on the combination of eluent and column packings. In addition, the separation process has not been explained sufficiently for individual cases.

In this study, the efforts for seeking the optimum operation conditions of GPC for PAAm and PAAm-H have been established. Formamide (FAM), used as an eluent, gives a stable solution for PAAm and PAAm-H,^{4,5} thus minimizing any aging effects originating from the disaggregation or the disentanglement of polymer molecules.⁶ The controlled-porosity glass functions well as a defined

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stationary phase.⁷ In another phase of the research, special attention was paid to the separation mechanism of GPC, especially on the salt effects for the elution phenomena of PAAm-H.

EXPERIMENTAL

Materials

PAAm fractions used were obtained from the three commercial sources (average molecular weight of 3×10^6 , 3×10^5 , and 12×10^4) by the fractional precipitation method with use of water as solvent and methanol as precipitant. The commercial sources were obtained from Sanyo Chemical Industries Ltd. and Nitto Chemical Industry Co. Ltd., respectively. The molecular weight of PAAm samples was determined from the intrinsic viscosity-molecular weight relationship.⁴

PAAm-H samples were prepared from the commercial resins by hydrolysis, to the desired degree, with sodium hydroxide according to the procedure of Nagase.⁸ Precipitation-dissolution was repeated and a dialysis was also performed to remove any residual alkali. Then, the hydrolyzed polymers were passed through an ion exchange column, which changed COONa for COOH groups. The degree of hydrolysis was determined by colloid titration with methyl glycol chitosan, potassium polyvinyl sulfonate, and toluidone blue as indicator.⁹

The formamide used in this study was distilled twice under reduced pressure.

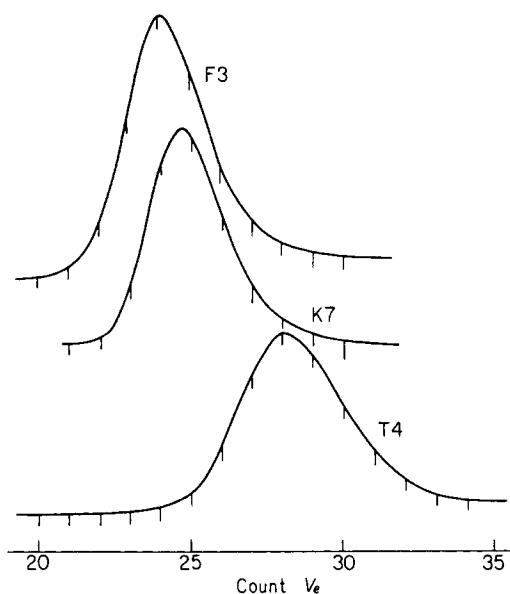


Fig. 1. Elution curves of polyacrylamide fractions (F3, K7, and T4) eluted with formamide under a flow rate of 1 ml/min. One count of the elution volume V_e corresponds to 5 ml.

TABLE I
Results of GPC and Viscometry of PAAM Samples at 45.0°C

Sample	Molecular weight	GPC, counts		[η], dl/g	
		FAM	FAM with KCl ^a	FAM	FAM with KCl ^a
F2	1.55×10^7	23.5	23.5	6.56	6.12
F3	5.15×10^6	24.1	24.4	3.91	3.36
K7	2.57×10^6	24.6	25.0		
Y2	1.33×10^6	25.1	25.4 (25.5 ^b)	2.18	1.70
T1	4.64×10^5	25.8	26.3		
Y3	4.69×10^5	26.0	26.4	1.38	1.19
T2	2.88×10^5	26.3	26.8	0.959 ^c	0.853
T4	1.22×10^5	28.0	28.9		
Y4	1.33×10^5	29.0			

^a $5 \times 10^{-3}M$.

^b $1 \times 10^{-1}M$.

^c Measured at 30.0°C.

Apparatus

A Waters Model 200 GPC was used equipped with four 4 ft \times $\frac{3}{8}$ in. stainless-steel columns which were packed (dry) with CPG-10 porous glass of mean pore diameter 3125, 486, 255, and 75 Å (Electro-Nucleonic Inc.). Two ml of the polymer solution with 0.05 to 0.1 g/dl was injected and eluted at 45°C under a flow rate of 1 ml/min.

The viscosity measurements were performed in FAM and in $5 \times 10^{-3}M$ KCl-FAM at 45°C using an Ubbelohde dilution viscometer.

RESULTS AND DISCUSSION

Typical GPC elution curves of PAAM fractions in FAM are given in Figure 1, where no abnormal (skewed) elution is observed. The GPC analyses of PAAM in FAM with KCl also gave similar curves. In Table I, the elution count at the appearance of the peak are given for the PAAM fractions. It is seen that the presence of KCl delays the elution slightly. Varying the salt concentration (e.g., $10^{-1}M$ and $5 \times 10^{-3}M$ of fraction Y2) does not cause any further shift of the peak elution position. It is of interest to examine the peak shifts in terms of the salt effect on the PAAM molecular dimension. Figure 2(a) illustrates an example of the solution viscosity behavior for a PAAM fraction, which is plotted according to the Kraemer equation. The solution containing potassium chloride gives a lower intercept, i.e., a smaller value of the intrinsic viscosity [η]. The intrinsic viscosities listed in Table I show that KCl in the solution produces a salting-out effect. A contraction of the solute molecules has been observed for water-soluble uncharged polymers¹⁰⁻¹² and for PAAM and FAM.¹³ Here, the decrease in intrinsic viscosity seems to be responsible for the shift of GPC chromatogram to higher elution volume. According to the polymer solution theory, the product of [η] and molecular weight M is the hydrodynamic volume, and if the separation mechanism of GPC can be attributed to the size of a solute alone, [η] M gives a universal curve for a given set of GPC operational variables, independent of the kind of solutes examined.¹⁴ This concept has been applied to both the elutions of PAAM fractions with and without KCl. As seen from Figure 3, all the hy-

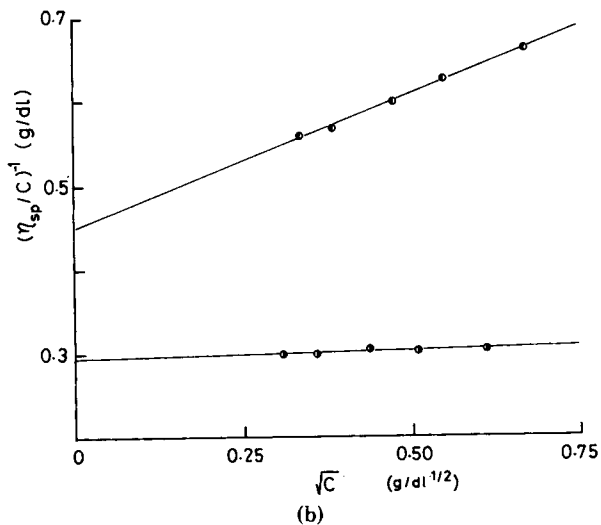
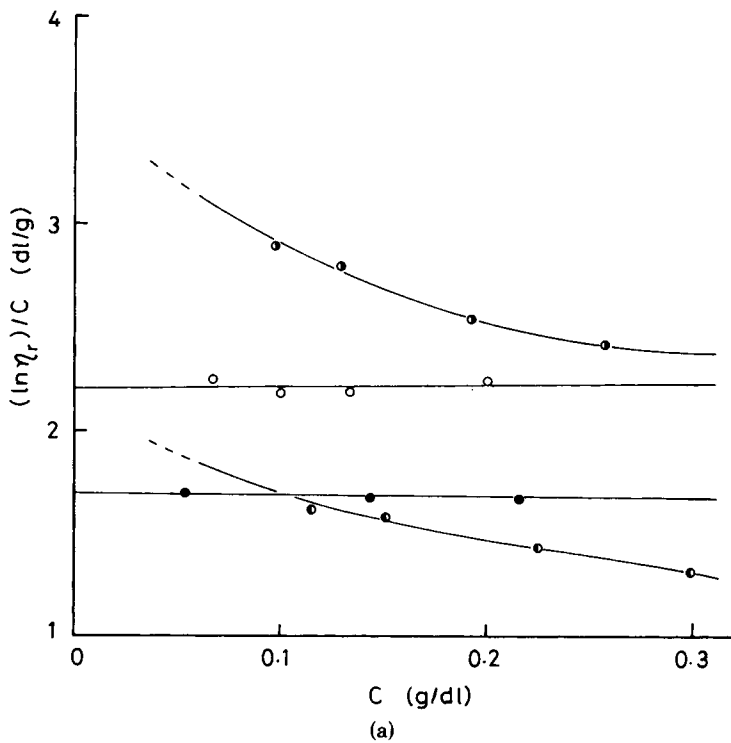


Fig. 2. (a) Solution viscosity behavior of a polyacrylamide (Y2) and a partially hydrolyzed polymer (30-30) at 45.0°C: Y2 in formamide (○) and in $5 \times 10^{-3}M$ KCl-formamide (●), and 30-30 in formamide (○) and in $5 \times 10^{-3}M$ KCl-formamide (●). (b) Replotted concentration dependence for the ionic polymer (30-30) shown in Fig. 2(a) according to the Fuoss equation¹⁵ $\eta_{sp}/c = A/(1 + B\sqrt{c}) + D$ where A , B , and D are constants and $A + D$ corresponds to the intrinsic viscosity. Solvents are formamide (○) and $5 \times 10^{-3}M$ KCl-formamide (●).

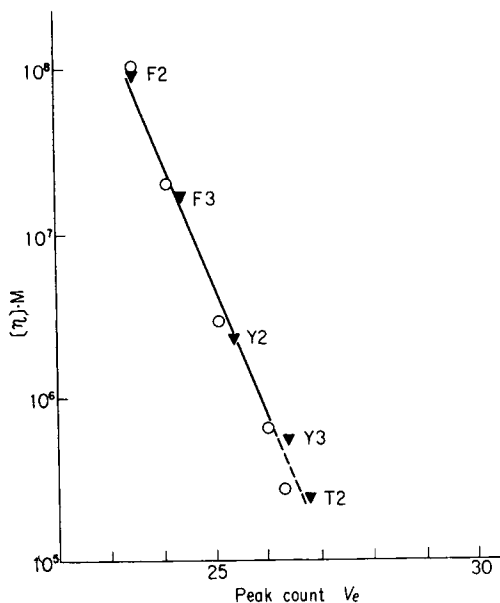


Fig. 3. Universal curve of polyacrylamide in formamide (—O—) and $5 \times 10^{-3}M$ KCl-formamide (—▲—). The elution volume V_e on the abscissa is the value at the peak of the chromatograms.

TABLE II
Results of GPC and Viscometry of PAAm-H Samples at 45.0°C

Sample	Degree of hydrolysis, %	GPC, counts		[η], dl/g	
		FAm	FAm with KCl ^a	FAm	FAm with KCl ^a
300-8	8.2	23.6	39.0		
300-24	24.7	24.1	39.0		
30-6	6.9	24.6	(39.2 ^b)		
30-30	30.2	25.9	38.5 (37.0 ^c)	3.39	2.22
12-12	12.4	25.5	39.5		
12-35	35.6	26.5	39.6		

^a $5 \times 10^{-3}M$.

^b $5 \times 10^{-4}M$.

^c $10^{-1}M$.

hydrodynamic volumes evaluated for FAm and FAm with $5 \times 10^{-3}M$ KCl almost give one line in the plot. This result shows that the hydrodynamic volume of the PAAm fractions plays a dominant role in the separation mechanism as has been the case in conventional GPC. Therefore, the present GPC system is applicable in obtaining the molecular characteristics of PAAm samples. Of course, the range of the application has limitations, as can be seen from considering the magnitudes of the molecular dimension and the pore size in the glasses used as a stationary phase.

The chromatogram of partially hydrolyzed PAAm samples in FAm was nearly normal, compared with that of PAAm fractions with and without the salt. Table II shows the results of the chromatographic and viscometric analyses for PAAm-H samples. For PAAm-H samples from the same source, the peak appearance depends on the degree of hydrolysis. This is a consequence of unfa-

avorable side reactions such as scission of the main chain in the course of hydrolysis. The higher degree of hydrolysis for some of the samples required a longer time period for reaction and the use of a more concentrated alkaline solution. The addition of KCl produced a tremendous effect on the retention volume—over 10 counts (50 ml) of the delay of an elution—up to just before the appearance of impurity peaks. This was observed to be independent not only of molecular weight but also of the degree of hydrolysis. Furthermore, increasing the salt concentration does not substantially affect these chromatographic results. The magnitude of this retardation is so great that it cannot be explained solely on the basis of scission of the polymer chain.

We have tried to explain the large chromatographic shift with use of the viscosity data for sample 30-30 as shown in Figure 2(a). A clear polyelectrolytic dependence on the concentration can be seen, i.e., an upward turn in the low polymer concentration and a partial suppression with increasing ionic strength by the addition of neutral salt. The difficulty of the extrapolation to zero concentration, particularly of a no-salt-containing system, was overcome by replotting based on the Fuoss equation of polyelectrolyte solutions, as shown in Figure 2(b). According to the hydrodynamic volume concept mentioned before,

$$\log([\eta]M) = I - KC \quad (1)$$

where K is the slope of the universal curve as shown in Figure 3, I is a constant, and C is the count of the peak appearance. Then the amount of shift in count, ΔC , due to the volume change can be written as follows:

$$\Delta C = -\Delta[\eta]/2.3K[\eta] \quad (2)$$

Substituting the relevant data ($K = 0.78$) gives $\Delta C = 0.24$ counts as the shift, far less than the observed change. Thus, we must consider some special inter-

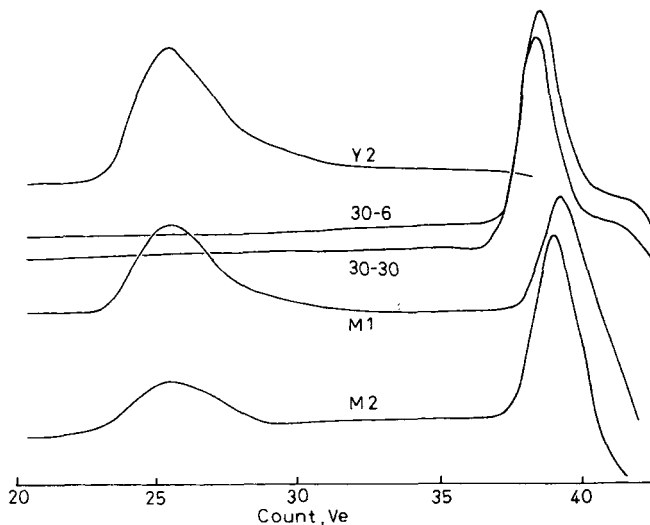


Fig. 4. Elution behavior of polyacrylamide (Y2), the partially hydrolyzed samples (30-6 and 30-30), and the mixtures (M1 and M2) of Y2 and 30-30; M1(2:1) and M2(1:2). Eluted with $5 \times 10^{-3}M$ KCl-formamide.

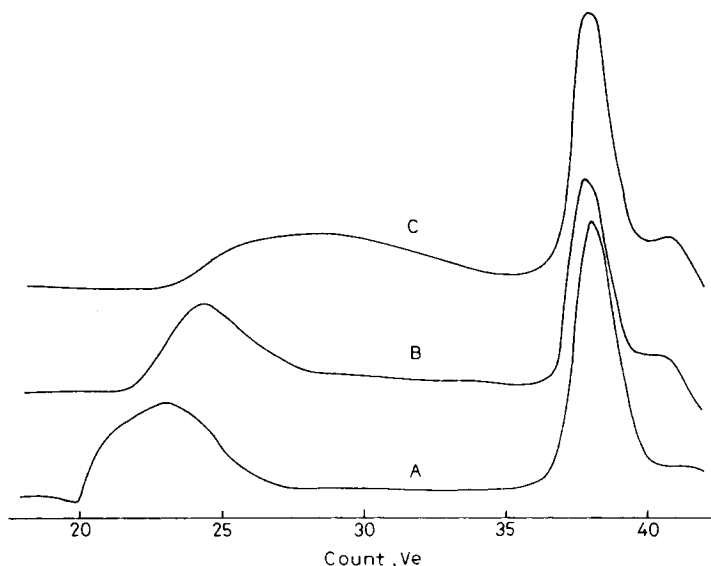


Fig. 5. Elution curves of technical polyacrylamide (average molecular weight of A, 3×10^6 ; of B, 3×10^5 ; and of C, 12×10^4) eluted with $5 \times 10^{-3}M$ KCl-formamide.

actions between the solute and the stationary phase induced by the existence of the salt. A physical absorption of PAAm-H onto the stationary phase, similar to the enhanced flocculation activity under the coexistence of simple salts, is most plausible.¹

Solute-substrate interactions prevent quantitative measurement of the molecular weight distribution of PAAm-H samples by GPC. Nevertheless, the abnormal elution behavior observed for PAAm-H can be employed qualitatively to detect "anionic polyacrylamide." Figure 4 shows the chromatograms of the mixed samples M1 and M2 having different ratios of Y2 and 30-30. It can be seen that the mixtures are well resolved into each component and the ratio of their areas on the chromatogram is consistent with the weight ratio before mixing. Subsequently, the technical polyacrylamides were eluted under the same conditions, namely, with $5 \times 10^{-3}M$ KCl-formamide. As shown in Figure 5, all chromatograms are composed of two distinctly separated peaks. Under elution with pure formamide, these products gave only a single peak situated in the range of the first one. From their positions, the first peak would correspond to PAAm and the second to PAAm-H, being anxious that it would also appear at the position of the second peak if the commercial resins contained low molecular weight impurities. Such an existence of hydrolyzed groups can be expected to some extent from the usual polymerization processes,¹⁶ and the presence of such anionic acrylamide may contribute largely to the actual flocculation activity.

In conclusion, the GPC undertaken in this work gives useful information about molecular characteristics such as molecular weight and molecular weight distribution of polyacrylamide and detects the existence of partially hydrolyzed polyacrylamide.

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