Journal of Macromolecular Science, Part A
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597274

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To cite this Article Erlander, Stig R. and Babcock, G. E.(1968) 'Explanation of Ionic Sequences in Various Phenomena. VII. The Concentration Dependence in Molecular Weight Determinations of Polyelectrolytes', Journal of Macromolecular Science, Part A, 2: 8, 1493 — 1520
To link to this Article: DOI: 10.1080/10601326808051912
URL: http://dx.doi.org/10.1080/10601326808051912

J. MACROMOL. SCI.—CHEM., A2(8), pp. 1493-1520, December, 1968

Explanation of Ionic Sequences in Various Phenomena. VII. The Concentration Dependence in Molecular Weight Determinations of Polyelectrolytes

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SUMMARY

The molecular weight of bovine plasma albumin (BPA) was examined by ultracentrifugal molecular weight determinations at low pH values in various concentrations of guanidinium thiocyanate (GSCN). The electrostatic charge on BPA under such conditions is approximately +100 for a molecular weight of about 70,000. It was shown that the value of B_w as obtained from the equation $(1/\bar{M}_w^{app}) = (1/\bar{M}_w^{ext}) + B_w(C_a + C_b)/2$ goes through a minimum at concentrations slightly less than 2.5 M GSCN for both dialyzed and undialyzed samples. The results illustrate that the value of the net electrostatic charge on BPA varies with the absolute concentration of salt and BPA. The minimum B_w is due to a reversal of charge phenomenon. At 2.5 M GSCN or higher the net charge on BPA is negative rather than positive because of the association of counterions. The greater the solubility of the counterion, the greater will be the reversal of charge effect. Addition of a more soluble salt therefore reduces the value of B_w faster than a less soluble salt. The extrapolated molecular weight values of BPA were the

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same whether dialysis was or was not carried out. This is because in most cases extrapolation of $1/\bar{M}_w^{app}$ to zero polyelectrolyte concentration is the same as extrapolation to zero net charge. The equations of Casassa and Eisenberg and of Scatchard and co-workers are therefore in error because they neglected to consider the variation in the net charge with a variation in the concentration of salt and polyelectrolyte. Plots of $\bar{M}_w^{app}/\bar{M}_z^{app}$ versus polyelectrolyte concentration can be used to detect erratic changes in the reversal of charge phenomenon. Such erratic changes are caused by greater association constants between counterion and polyelectrolyte because of clusters of charged groups on the polyelectrolyte. When $\bar{M}_w^{app}/\bar{M}_z^{app} > 1$ and increases with polymer concentration, extrapolation to $C_p = 0$ may not give the true molecular weight of the polymer because the net electrostatic charge may be unequal to zero at $C_p = 0$.

INTRODUCTION

In order to completely understand the effect of salt on the concentration dependence of polyelectrolytes in molecular weight determinations, one must also understand how ions interact with the electrostatically charged groups on polyelectrolytes. Such interactions are manifested in the reversal of charge phenomenon and ion binding of cations or anions to the polyelectrolyte. In a previous paper [1] these phenomena were discussed. Contrary to what has previously been believed, it was shown that the ability of an ion to reverse the electrostatic charge of a polyelectrolyte increases as the solubility of the counterion to the polyelectrolyte increases. For example, the negatively charged carboxylate ions on pectinate and arabinate have the following solubility sequence: $Li^+ < Na^+ < K^+ < Rb^+ < Cs^+$. Thus in this sequence the lithium ion has the least solubility, whereas the cesium ion has the greatest solubility for the carboxylate group. However, examination of the electrophoretic mobility shows that the lithium ion has the least capability of reversing the electrostatic charge on these polyelectrolytes. Thus the concentration of the cation necessary to reverse the negative electrostatic charge of the pectinate and arabinate goes according to the sequence $Li^+ > Na^+ > K^+ > Rb^+ > Cs^+$. A comparison of the solubility of the various cations with regard to the phosphate group on egg lecithin or with regard to the sulfate group on chondroitin sulfate and agar shows the same effect—that the greater the solubility of the counterion, the greater is the ability of this cation to reverse the electrostatic charge of the polyelectrolyte.

Examination of the effect of ion binding [1] shows that the same phenomenon is occurring with ion binding as in the case of the re-

versal of charge phenomenon. Thus the greater the solubility of the counterion to the polyelectrolyte, the greater will be the ability of the counterion to associate itself with the polyelectrolyte. This close association has been referred to as "ion binding." The ion-binding studies of Scatchard and co-workers [2-4] are therefore studies on the ability of the counterion to associate itself with the polyelectrolyte. This association only occurs for that ion which has an opposite charge to that of the polyelectrolyte. Wall and his associates [5, 6] came to the conclusion that a certain fraction of counterions is free to move in solution, whereas the remaining fraction is rigidly attached to the polyelectrolyte and move with it. Their conclusions were based on extensive studies on the conductance of self-diffusion of polyelectrolytes. This study is essentially the same as that for the reversal of charge phenomenon. However, as pointed out by Katchalsky et al. [7], the counterion is not rigidly attached to the polyelectrolyte but has a certain degree of freedom. Nevertheless, just as in the theory of Wall and co-workers [5, 6], even though the counterion atmosphere is by no means a fixed layer which does not respond to the external field as assumed by the theory of association, there exists an ionic atmosphere of counterions which can be considered as part of the polyelectrolyte. The results of Katchalsky et al. [7] again emphasize that ion binding or ion association is not the insolubilization of a counterion with the polyelectrolyte but rather must be associated with the solubility of the counterion with the charged groups on the polyelectrolyte. Thus, again as in the reversal of the charge phenomenon, the greater the solubility of the counterion, the greater will be its ability to "bind" to the polyelectrolyte.

In order to understand why such a relationship holds, one must also understand that more counterions can remain attracted to the charged groups of a polyelectrolyte if these groups remain charged. In other words, if an insoluble salt complex is formed with the monovalent counterion and the charged group of the polyelectrolyte, then the electrostatic charge of this group is canceled. Now other counterions which may have been in the vicinity of this charged group will diffuse away because there is no longer any attraction for other counterions. The greater the insolubility of the counterion with the charge group, the longer will be the time that it remains attached to the charge group. And the longer the time it remains attached to the charged group, the fewer will be the number of counterions which can be associated with the charge groups over a given time. Conversely, the more soluble the complex between the charged group and the counterion, the greater will be the number of counterions that can gather around the polyelectrolyte. And as seen above, the greater the number of counterions which can become thus associated with the polyelectrolyte the greater will be the reversal of charge phenomenon.

It should be pointed out that a reversal of charge of the polyelectrolyte can actually occur. In other words, if the association of the counterion is due mainly to the insolubilization of the counterion with the charged group, then no reversal of charge would occur. Rather, the charge on the polyelectrolyte would be reduced at most to zero. The fact that a reversal of charge can occur, such as changing a positively charged polyelectrolyte to a negatively charged one, shows that the phenomenon occurring is not due to the insolubilization of the counterion with the charged groups.

The above studies can now be applied to the concentration dependence of polyelectrolytes in the determination of their molecular weight. It is known that if salt is added to an aqueous solution of a polyelectrolyte, then the concentration dependence of the polyelectrolyte in the molecular weight determinations is decreased. It is also known that the concentration dependence of a polyelectrolyte is decreased if the molecular weight is obtained at or near the isoelectric point. Yet the charged groups of the polyelectrolyte at the isoelectric point still remain charged. Nevertheless, the net charge has been reduced to zero, and therefore the concentration dependence has also been reduced considerably. By applying the above discussion it can be concluded that the addition of salt reduces the concentration dependence of a polyelectrolyte because the counterions added reduce the electrostatic charge of the polyelectrolyte. In other words, a reversal of charge or "ion-binding" phenomenon is occurring. Consequently, the greater the amount of salt added, the greater will be reduction in the electrostatic charge. If, however, too much salt is added, then a reversal of charge phenomenon will occur. And now the concentration dependence of the polyelectrolyte will again increase.

The polyelectrolyte can therefore be considered as a variable species. Its electrostatic charge will depend on the concentration of both the counterion and the polyelectrolyte. Both of these concentrations change in the ultracentrifugal cell and, as seen in Eq. (11), the concentration coefficient depends on changes in the net charge as well as the value of the net charge itself. Consequently, the electrostatic charge of a polyelectrolyte will vary with the ultracentrifugal cell radius.

In 1960 Casassa and Eisenberg [8] put forth the theory that if a polyelectrolyte in a salt solution is dialyzed against the specific salt solution, then the polyelectrolyte could be treated as a two-component system. Their approach was similar to that of Johnson et al. [9], but in the latter case the polyelectrolyte was given a fixed composition. In other words, a certain amount of salt was subtracted from the polyelectrolyte to account for the Donnan effect of the polyelectrolyte. In the case of Casassa and Eisenberg [8], it was recognized that the activity coefficients of these ions may change. Consequently, they state that if each solution to be examined in the ultracentrifuge

is dialyzed against the salt solution, then the effect of the salt will cancel out when the extrapolation of the observed molecular weights is made to zero polyelectrolyte concentration.

These results have been used by a number of workers such as Woods et al. [10] in their study of mysosin and also by Jeffrey and Coates [11]. In the derivation of their equations, however, Casassa and Eisenberg [8] assume that the electrostatic charge of the polyelectrolyte is fixed. In other words, the electrostatic charge is assumed to be independent of the concentration of salt and polyelectrolyte. However, as seen above, this is not the case. As the concentration of salt or polymer is changed, the electrostatic charge of the polyelectrolyte will also be changed. Consequently, their equations are not valid because a new species of polyelectrolyte will be involved both at the different cell radii and at the different polymer concentrations. However, as shown below, if the extrapolation is made to zero concentration, then in most cases the electrostatic charge of the polyelectrolyte will be zero and the true molecular weight of the polyelectrolyte will be obtained. Hence the same molecular weight of the polyelectrolyte should be obtained whether the solution is dialyzed or not. Moreover, the concentration dependence for the dialyzed solutions will be different from those which are not dialyzed. In other words, the Donnan equilibrium effect will reduce the amount of salt in the dialyzed bag and, consequently, will change the concentration of the salt. The electrostatic charge of the dialyzed polyelectrolyte will therefore be different from that of the undialyzed. In both cases, however, the same molecular weight should be obtained. To examine this further, the molecular weight of bovine plasma albumin (BPA) will be determined at pH 1.5, where the electrostatic charge on BPA is approximately 100 for a molecular weight of 70,000.

EXPERIMENTAL

Armour's BPA, Lot No. T68204, was used in these studies. The salts were obtained from K & K Laboratories, Inc. The percentage of moisture in the BPA sample was obtained by dissolving the BPA in water and measuring the concentration with a Beckman Model D.U. spectrophotometer using $E^{1\%}_{1cm} = 6.67$ at 279 mu [12]. The pH was adjusted by adding 1.0 M HCl to the solution with stirring (magnetic) and measuring the change in pH with a pH meter. The molar concentration (M) of the guanidinium thiocyanate (GSCN) was obtained from the refractive index (n) of the aqueous solution according to [13]

$$N = (n - 1.3325)/A$$

The constant A equals 0.02521 for the GSCN up to its saturation point. The BPA was dialyzed with three or four changes of the salt solution. The dialysis was done in a sealed flask with gentle shaking. Equilibrium was reached in all samples after 72 hr. It is necessary to seal the flask because of the hydroscopic character of the salt solution. To remove the sample from the dialysis bag, it is necessary to maintain the bag in the salt solution. This is done by raising the dialysis bag partially out of the solution and then inserting a syringe into the bag to remove the solution. If this step is omitted, evaporation of water occurs and the concentration of the salt in the dialysis bag is not maintained at the same level. This procedure is extremely important for the concentrated solutions of GSCN. In all samples the BPA was centrifuged before dialysis in a swinging bucket rotor for 30 min at 25,000 rpm using a Spinco Model L preparatory centrifuge to remove the fat from the solution. The bottom 80% of each tube was retained.

A Spinco Model E ultracentrifuge, equipped with a Wolter phase-plate and a Spinco RTIC temperature control unit was used to determine the molecular weights of BPA [14]. The light source was positioned on the optical axis by using a mercury mirror in the cell as suggested by Trautman [15]. Pictures were taken by the schlieren optical system on Kodak spectroscopic I-D [2] plates. The temperature was maintained at 25°C in all equilibrium runs. The equilibrium was reached in all samples after 72 hr. Initial concentrations varied but were in general about 0.5, 0.3, 0.1, and 0.05%. In some cases samples were run using two instead of one double-sector 30-mm cell. One cell had a wedge window to differentiate it from the other. Also, different column heights in the two cells were obtained by using 0.30 ml of Kel-F polymer oil in one cell and 0.50 ml of Kel-F polymer in the other. Then 0.36 ml of the BPA was layered onto the Kel-F polymer oil to give approximately a 4.0-mm distance between the Kel-F polymer and the meniscus of the solution. The particular solvent used was added to the other side of the double-sector cell. The rotor was run at different speeds between 5227 and 16,200 rpm depending on the concentration of BPA and the molarity of salt. Higher speeds were necessary for the higher concentrations because concentration dependence reduced the slope of the pattern.

Initial concentrations of the BPA were obtained by using the synthetic boundary cell. The synthetic boundary cell was made from a double-sector, epoxy resin, carbon-filled center piece by cutting thin grooves at equal heights on both sides of the cell and two-thirds of the distance from the bottom of the cell to the top [16]. Lines were also cut at the top of the cell in order to equilibrate the pressure. It was observed in the more concentrated salt solutions that dialysis appreciably changed the concentration of the salt. To obtain this proper concentration of BPA the cell had to be run at low speed for approximately 2-3 hr to let the salt equilibrate by diffusion.

The density (ρ) of the salt solution was obtained by correlating the refractive index with experimentally determined values for ρ. The value of the partial specific volume for BPA was assumed to be equal to that in aqueous solutions in the absence of salt ($\bar{V} = 0.733$ [17]).

Because of aggregation problems, the 0.005 M GSCN solution was adjusted to pH 2.0 with HCl instead of to pH 1.5 as in the 2.5 and 5.0 M GSCN solutions. That is, addition of sufficient acid to make the pH equal to 1.5 increased the ionic strength and hence promoted more aggregation in the 0.005 M GSCN solutions. Possibly a few of the carboxylate groups may not be titrated at pH 2.0. However, even if the titration is incomplete, the results will not be effected because the BPA in 0.005 M GSCN behaves as if it has a large net charge (see Discussion).

Equations

The concentration dependence at a specific radius r for the determination of the weight-average ($\bar{M}_w$) and Z-average ($\bar{M}_z$) molecular weights by ultracentrifugal methods can be expressed by the equations [14, 18-22]

$$1/\bar{M}_{w,r}^{app} = (1/\bar{M}_{w,r}^{ext}) + B_r C_{p,r} \tag{1}$$

and

$$1/\bar{M}_{z,r}^{app} = (1/\bar{M}_{z,r}^{ext}) + B_{z,r} C_{p,r} \tag{2}$$

where the concentration coefficient $B_{z,r}$ equals $2B_r(\bar{M}_{w,r}^{ext}/\bar{M}_{z,r}^{ext})$. The subscript r refers to that value of the concentration of the polymer (Cp) or its molecular weight ($\bar{M}_w$ or $\bar{M}_z$) at the position r in the ultracentrifuge. Also the superscripts "app" and "ext" refer to apparent or extrapolated molecular weights. The apparent molecular weight is obtained at a finite value of Cp and the extrapolated molecular weight equals that value near Cp = 0, that is, as Cp approaches zero. For the value of $\bar{M}_w$ and $\bar{M}_z$ obtained on the entire sample and not at a specific value or r, we have

$$1/\bar{M}_w^{app} = (1/\bar{M}_w^{ext}) + B_w \left(\frac{hC_a + C_b}{2}\right) \tag{3}$$

and

$$1/\bar{M}_z^{app} = (1/M_w^{ext}) + B_z \left(\frac{hC_a + C_b}{2}\right) \tag{4}$$

where $B_w = B_b(\bar{M}_{w,b}^{ext}/\bar{M}_w^{ext})$, $B_z = 2B_b(\bar{M}_{w,b}^{ext}/\bar{M}_z^{ext})$, and C_a or C_b refer to the polymer concentration at the meniscus or cell bottom, respectively. Moreover, the apparent weight-average molecular weight is

$$\bar{M}_w^{app} = \frac{C_b - C_a}{C^o(b^2 - a^2)} \left[\frac{RT}{(1 - \bar{V}_p\rho)\omega^2}\right] \tag{5}$$

where $\bar{V}_p$ equals the partial specific volume of the polymer, a and b equal the distance (in cm) from the center of the rotor to the meniscus and cell bottom, ρ equals the density of the solvent, and ω equals the rotor speed. The apparent Z-average molecular weight is

$$\bar{M}_z^{app} = \frac{\bar{M}_{w,b}^{app}C_b - \bar{M}_{w,a}^{app}C_a}{C_b - C_a}$$

$$= \left[\frac{\Delta\ (dC/r\ dr)}{\Delta C}\right]_{(a\ to\ b)} \left[\frac{RT}{(1 - \bar{V}_p\rho)\omega^2}\right] \tag{6}$$

where in general a refers to the meniscus and b to the solution and Kel-F interface ("cell bottom"). The other symbols are as designated previously [14, 18-22]. The value of h is $h = (B\bar{M}_w^{ext})_a/(B\bar{M}_w^{ext})_b$ and was neglected in obtaining the extrapolated molecular weights because the value of C_a is much smaller than C_b.

From the discussion given in the Introduction, it can be concluded that the electrostatic charge on a polyelectrolyte (PX_Z) varies according to the concentration of both salt and polyelectrolyte [22]:

$$PX_Z \longrightarrow PX_{(Z-Z\gamma_\pm)}^{+Z\gamma_\pm} + (Z\gamma_\pm)X^- \tag{7}$$

where Z equals the total possible charge on the polyelectrolyte and where $\gamma_\pm$ represents the fraction of charge resulting from ion association of the counterions. The value of $\gamma_\pm$ can be any number less than or equal to 1.0, which includes zero and negative numbers. Equation (7) therefore refers to any polyelectrolyte, even though complete dissociation of the ionic groups has occurred. That is, the ion $PX_{(Z-Z\gamma_\pm)}^{+Z\gamma_\pm}$ can be thought of as the completely dissociated polyelectrolyte ion P^{+Z} plus $(Z - Z\gamma_\pm)$ associated counterions. If a reversal of charge has occurred, then $-Z\gamma_\pm$ will be positive and $(Z - Z\gamma_\pm)$ will be greater than Z. Considering Eq. (7), values for the extrapolated molecular weights will be [21, 22]

$$\bar{M}_w^{ext} = \bar{M}_w\tau \tag{8}$$

and

$$\bar{M}_Z^{ext} = \bar{M}_Z \tau \tag{9}$$

where

$$\tau = 1 - M_{BX}\left(\frac{Z'\gamma'_{\pm}}{M'_{px_z}}\right)\left(\frac{1 - \bar{V}_{BX}\rho}{1 - \bar{V}_{px_z}\rho}\right) \tag{10}$$

The same value of τ is obtained for the molecular weights at a specific radius: $\bar{M}_{w,r}^{ext} = \bar{M}_{w,r}\tau$ and $\bar{M}_{z,r}^{ext} = \bar{M}_{z,r}\tau$. The primed values on $(Z'\gamma'_{\pm}/M'_{px_z})$ mean that this ratio is a value which represents the average polymer segment. The concentration coefficient B_b of Eqs. (3) and (4) is the same coefficient as obtained at the radius b and is equal to [22]

$$B_b = \ln\left[\frac{C_p}{M_p} + \frac{C_B}{M_B}\right]_b \left(\frac{d(Z'\gamma'_{\pm}/M'_{px_z})}{dC_p}\right)_b - \left(\frac{2Z'\gamma'_{\pm}}{M'_{px_z}}\right)_b \left(\frac{dC_B}{C_B\, dC_p}\right)_b + \left(\frac{\partial \ln \gamma_p}{M_{px_z}\, \partial C_p}\right)_{T,p,C_{Bxb}} \tag{11}$$

The same expression for the concentration coefficient is obtained for Eqs. (1) and (2) at a specific radius r except that $B_b = B_r$. The term $(\partial \ln \gamma_p/\partial C_p)$ in Eq. (11) should mainly be a function only of the nonelectrostatic interactions. Because such nonelectrostatic interactions for small globular proteins such as BPA should be small, the main terms in B_b are the $-2(Z'\gamma'_{\pm}/M'_n)d \ln C_B/dC_n$ and the $\ln(m_p + m_B)d(Z'\gamma'_{\pm}/M'_n)/dC_p$ terms. It is seen, therefore, that the concentration coefficient is a function of the variable charge $Z\gamma_{\pm}$.

Explanation of $Z\gamma_{\pm}$

In the interaction of hydrated counterions with the hydrated electrostatic groups of a polyelectrolyte, the water molecules of these ions encounter each other before ion-ion interactions can occur. Hence the ion-dipole-dipole-ion and the ion-dipole-ion interactions [23] determine whether the ion-ion interactions will take place. It would therefore seem reasonable to postulate that these water dipole interactions form the "inner shell" of Katchalsky et al. [7] and of Wall et al. [5, 6]. Such interactions between polyelectrolyte molecules and counterions should produce a net charge which is equivalent to the electrophoretic charge. Thus these interactions would be capable of producing the reversal of charge phenomenon, since electrophoresis is the method used to examine this phenomenon.

This charge will depend on the molarity of the added salt as well as the type of counterion in the added salt. At extremely high concentrations of polyelectrolyte or under circumstances where the counterion is being tightly bound to the polyelectrolyte as in the BPA plus 0.005 M GSCN example given below, the concentration of polyelectrolyte may also determine this net or electrophoretic charge. However, in other cases the concentration of the polyelectrolyte would most likely have only a slight effect on the value of the electrophoretic charge.

The extent of the above ion-dipole-dipole-ion, ion-dipole-ion, and ion-ion interactions determines the magnitude of intramolecular interactions. It should also be one of the main factors—but not the sole factor—in determining intermolecular interactions. These interactions are determined by the total number of counterions which are between two adjacent polyelectrolyte molecules even if some of these counterions are not included in the above inner shell. Consequently, as the solution is diluted at constant molarity of salt, the number of counterions between two adjacent molecules will increase. Hence the concentration of polyelectrolyte as well as that of the added salt will determine the extent of intermolecular interactions and consequently will also determine the average electrostatic charge that is involved in the interaction of adjacent polyelectrolyte molecules. It is this charge, therefore, which equals the value of $Z\gamma_{\pm}$ in the above equations. Moreover, as the concentration of the polyelectrolyte approaches zero, the value of this charge ($Z\gamma_{\pm}$) will also approach zero in the presence of added salt. That is, the domain of each polyelectrolyte molecule plus its associated and nonassociated counterions also increases as $C_p \rightarrow 0$, and at infinite dilution all these counterions in this extended domain will eliminate electrostatic interactions. Hence, according to Eq. (10), the value of τ will be one at $C_p = 0$, since then $Z\gamma_{\pm} = 0$. Thus the extrapolated molecular weight will equal the true molecular weight unless the extrapolation is erroneously made as in the example given below. This explains why Erlander and Senti [14] observed that the average extrapolated molecular weight of BPA was 74,000 ± 2000 under conditions where both the charge Z as determined by the pH and the added salt concentration varied dramatically. It should, however, be emphasized that for specific polyelectrolyte and salt concentrations, the electrophoretic charge will determine the magnitude of the value of $Z\gamma_{\pm}$ and hence will determine the magnitude of concentration coefficient B_r.

RESULTS

The molecular weights obtained in 0.005 N GSCN at pH 2.0 are recorded in Table 1. For the dialyzed samples the values of the

Table 1. Apparent Molecular Weights of BPA in 0.005 M GSCN at pH 2.00[a]

Sample	Rotor speed, rpm	$\overline{M}_w^{app} \times 10^{-3}$	$\overline{M}_z^{app} \times 10^{-3}$	$\overline{M}_{w,a}^{app} \times 10^{-3}$	$\overline{M}_{w,b}^{app} \times 10^{-3}$	C^0	$\frac{C_a + C_b}{2}$	C_a	C_b
Dial.	9,945	89	56	157	61	0.147	0.29	0.027	0.547
Dial.	16,200	14	4	58	10	1.164	0.93	0.193	1.669
Undial.	5,227	109	219	104	181	0.174	0.20	0.099	0.300
Undial.	5,227	89	223	73	164	0.340	0.39	0.219	0.558
Undial.	11,272	27	10	48	19	0.577	0.61	0.221	0.993
Undial.	16,200	13	3	28	9	1.132	1.12	0.436	1.805

[a]The concentrations C^0, $(C_a + C_b)/2$, C_a, and C_b are in grams per 100 ml.

apparent weight-average molecular weight ($\bar{M}_w^{app}$) are always greater than those of the apparent Z-average molecular weight ($\bar{M}_z^{app}$). However, for the undialyzed samples the value of $\bar{M}_w^{app}$ does not become greater than the value of $\bar{M}_z^{app}$ until the concentration of BPA becomes greater than approximately $C_\beta^0 = 0.6$ g/100 ml. The dialyzed samples therefore behave as if they had a lower amount of salt. This would be expected on the basis of the Donnan equilibrium effect.

A plot of the reciprocal weight-average molecular weights versus the concentration is given in Fig. 1. From this figure it is seen that the dialyzed and undialyzed samples appeared to give the same type of curve. It is also seen that if the molecular weight were to be extrapolated from the higher concentrations, a negative molecular weight would be obtained. As will be shown below, this effect is caused by a variation in the strength of the association between counterion and polyelectrolyte. The molecular weights obtained at the cell bottom are in agreement with those obtained for the total weight-average molecular weight and give a similar type of curve. The extrapolated values indicate that the BPA molecules are aggregated. That is, previous results [14] show that the molecular weight of BPA at pH 1.8 in 0.1 M NaCl + HCl is 76.1×10^3.

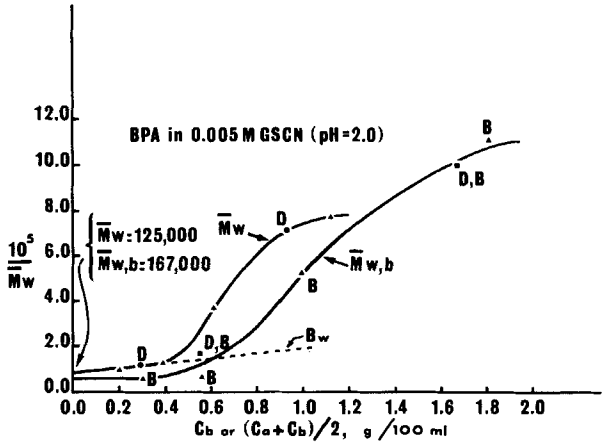

Fig. 1. Plot of $1/\bar{M}_w^{app}$ and $1/\bar{M}_{w,b}^{app}$ versus BPA concentration in 0.005 M GSCN at pH 2.0. The dialyzed samples are marked with a "D" for the $\bar{M}_w$ points (●) with a "D,B" for the $\bar{M}_{w,b}$ points (■). For the undialyzed samples the "B" designates those points (▲) obtained at the cell bottom, and the unmarked points (▲) designate the $\bar{M}_w$ values.

The higher molecular weights obtained with the GSCN salt or with greater amounts of salt and/or acid (see below) must therefore be due to a "salting out" of the BPA, i.e., the formation of aggregates. Thus the 0.005 M GSCN can cause the BPA to aggregate even though the 0.1 M NaCl did not, because the SCN^- ion forms a more soluble salt with the positive groups of BPA and hence has a greater ability to reduce (or reverse) the electrostatic charge on BPA. In Fig. 2 the reciprocal weight-average molecular weight obtained at the meniscus is plotted versus the meniscus concentration. In this figure it is seen that the concentration dependence is linear up to all concentrations, the maximum concentration being 0.436%. As seen in Fig. 1 the molecular weight plot for the weight-average molecular weight at the cell bottom and for the entire solution was also linear to approximately this maximum concentration.

The apparent molecular weights for BPA obtained in 2.5 M GSCN at pH 1.5 at various initial concentrations (C°) of BPA are listed in Table 2. A plot of the weight-average molecular weights for the entire solution is given in Fig. 3. Here it is seen that for those samples which have been dialyzed, the slope of the line is lower than that of the undialyzed samples. If we now examine the molecular weights obtained at the meniscus and cell bottom (Fig. 4), we also see that the apparent molecular weights obtained at the cell bottom give a lower slope than those obtained at the meniscus. In

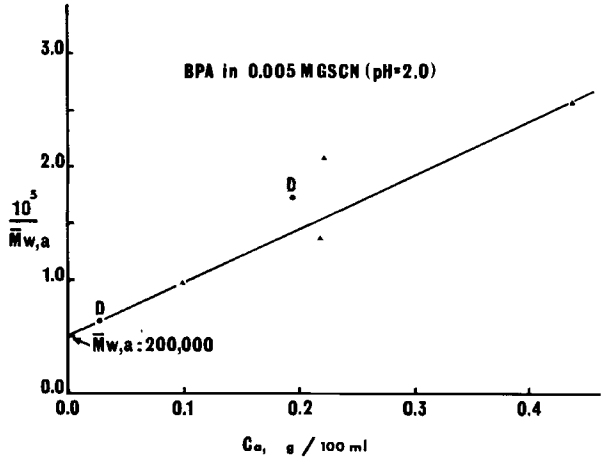

Fig. 2. Plot of $1/\bar{M}_{w,a}^{app}$ versus BPA concentration in 0.005 M GSCN at pH 2.0. The dialyzed samples (●) are designated "D" and the undialyzed (▲) are unmarked.

Table 2. Apparent Molecular Weights of BPA in 2.5 M GSCN at pH 1.5[a]

Sample	$\overline{M}_w^{app} \times 10^{-3}$	$\overline{M}_z^{app} \times 10^{-3}$	$\overline{M}_{w,a}^{app} \times 10^{-3}$	$\overline{M}_{w,b}^{app} \times 10^{-3}$	C^0	$\frac{C_a + C_b}{2}$	C_a	C_b
Dial.	107	120	105	116	0.484	0.572	0.246	0.899
Dial.	94	120	88	109	1.04	1.132	0.598	1.67
Undial.	106	110	103	108	0.310	0.352	0.161	0.543
Undial.	100	104	100	107	0.426	0.467	0.205	0.726
Undial	96	110	98	105	0.579	0.621	0.336	0.907

[a]All rotor speeds were 11,272 rpm. All concentrations are in grams per 100 ml.

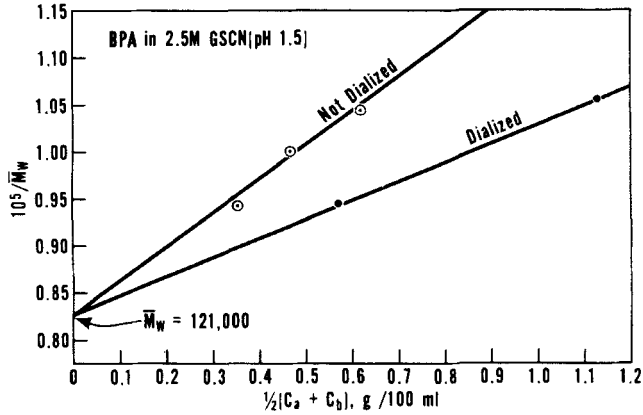

Fig. 3. Plot of $1/\bar{M}_w^{app}$ versus BPA concentration, $(C_a + C_b)/2$, in 2.5 M GSCN at pH 1.5, where C_a and C_b are the concentrations at the meniscus and cell bottom, respectively. The dialyzed samples (●) have less salt than the nondialyzed samples (⊙) because of the Donnan equilibrium effect.

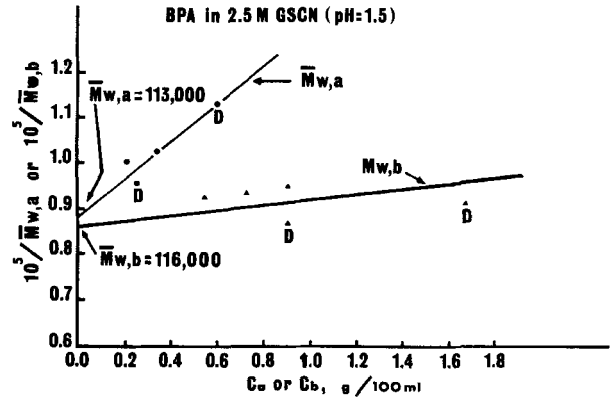

Fig. 4. Plot of $1/\bar{M}_{w,a}^{app}$ or $1/\bar{M}_{w,b}^{app}$ versus the BPA concentration in 2.5 M GSCN at pH 1.5. The $\bar{M}_{w,b}$ values (▲) are obtained at lower relative concentration of salt than the $\bar{M}_{w,a}$ values (●). Hence the BPA has less concentration dependence at the cell bottom ($\bar{M}_{w,b}$ values) because of a lower negative electrostatic charge (reversal of charge phenomenon). The dialyzed samples are marked with a "D".

the dialyzed $\bar{M}_w$ samples, the BPA solutions have smaller molarities of salt (GSCN) than in the undialyzed solutions. Hence the lower concentration coefficient for its apparent molecular weights is opposite of what one would normally expect. In other words, normally one would expect an increase in the concentration coefficient as the molarity of salt is decreased.

In Figs. 5 and 6 the reciprocal weight-average molecular weight is plotted against the respective concentration using the data given in Table 3. In contrast to the data for the 2.5 M GSCN solutions, the BPA in the 5.0 M GSCN solutions has a greater concentration dependence for the dialyzed samples than for the undialyzed samples. Yet just as in the 2.5 M GSCN solutions, the concentration of salt in the dialyzed samples is still less than that in the nondialyzed samples. The curvature for the plots of the molecular weights obtained for the dialyzed samples is a manifestation of this larger concentration dependence. It should be noted that the molecular weight obtained for the nondialyzed samples is linear to a much greater concentration of BPA. The results indicate that the electrostatic charge on the BPA in the dialyzed samples is greater than that of the nondialyzed samples for the 5.0 M GSCN solutions. And as noted, this is reverse of the effect obtained in the 2.5 M GSCN solutions.

A comparison of the values of B_w and $B_{w,D}$ obtained from Figs. 1, 3, and 5 is given in Table 4. The added subscript "D" on $B_{w,D}$ refers to those values obtained on dialyzed samples of BPA. These values show that the values of B_w go through a minimum at 2.5 M GSCN for both dialyzed and undialyzed samples. Moreover, Table 4 as well as the figures show that within experimental error the same molecular weight is obtained for the dialyzed and undialyzed samples.

DISCUSSION

Reversal of Charge on BPA at 2.5 M GSCN

Previous experimental data of Erlander and Senti [14] illustrate that the more insoluble the counterion, the less will be its ability to lower the electrostatic charge of the polyelectrolyte and the greater will be the resulting value of B_w. The solubility sequence for NH_4^+ and consequently for $R{-}NH_3^+$ is $Cl^- < Br^- < I^- < SCN^-$ in solubility [23]. Yet it was shown [14] that at pH 3.1 the values of B_w for BPA are 0.7 in 0.01 M NaSCN ($\bar{M}_w$ = 73,000) and 1.3 in 0.01 M NaCl ($\bar{M}_w$ = 73,000) or B_w (NaCl) > B_w (NaSCN). Consequently, the greater the solubility of the counterion, the greater will be the reduction in B_w. In other words, these data indicate that the concentration coefficient is dependent on a variable charge ($Z\gamma_\pm$) as given in

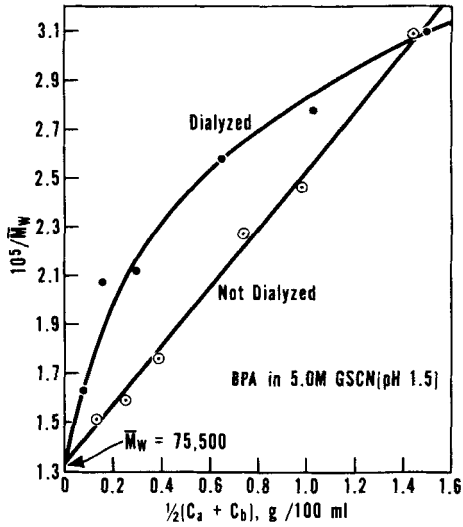

Fig. 5. Plot of $1/\bar{M}_W^{app}$ versus BPA concentration in 5.0 M GSCN at pH 1.5 for dialyzed (●) and undialyzed (⊙) samples. The value of B_W for the undialyzed samples is smaller because a reduction in the negative charge on BPA is occurring (greater association of G^+ ions with the SCN^- + BPA association).

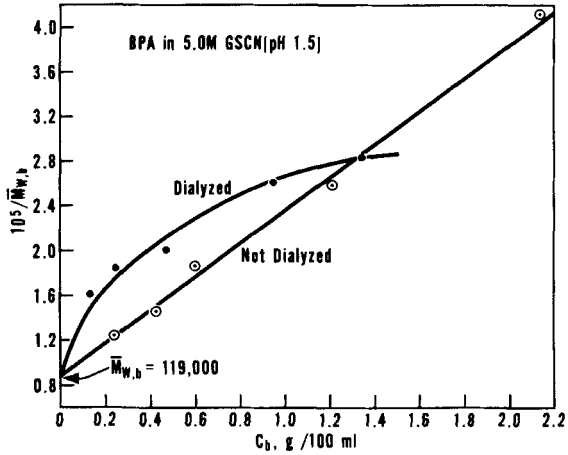

Fig. 6. Plot of $1/\bar{M}_{W,b}^{app}$ versus BPA concentration in 5.0 M GSCN at pH 1.5 for dialyzed (●) and undialyzed (⊙) samples. The undialyzed samples have a greater concentration of salt in comparison to the dialyzed samples.

Table 3. Apparent Molecular Weights of BPA in 5.0 M GSCN at pH 1.5[a]

Sample	$\bar{M}_w^{app} \times 10^{-3}$	$\bar{M}_z^{app} \times 10^{-3}$	$\bar{M}_{w,a}^{app} \times 10^{-3}$	$\bar{M}_{w,b}^{app} \times 10^{-3}$	C^0	$\frac{C_a + C_b}{2}$	C_a	C_b
Dial.	61	60	63	61	0.063	0.083	0.028	0.129
Dial	48	56	48	53	0.138	0.158	0.064	0.250
Dial.	50	51	47	50	0.267	0.297	0.133	0.476
Dial.	39	41	34	38	0.653	0.653	0.358	0.948
Dial.	36	35	34	35	0.995	1.032	0.739	1.340
Dial.	32	20	36	27	1.497	1.500	0.943	2.051
Undial.	66	79	112	82	0.093	0.132	0.022	0.242
Undial.	63	80	31	69	0.187	0.252	0.075	0.429
Undial	57	61	31	54	0.312	0.390	0.135	0.645
Undial.	44	37	44	39	0.642	0.741	0.269	1.290
Undial.	32	13	47	24	1.380	1.43	0.716	2 14

[a] All rotor speeds were 11,272 rpm. All concentrations are in grams per 100 ml.

Table 4. Comparison of Ultracentrifuge Molecular Weights and Their Concentration Coefficients for BPA for Various Molarities of GSCN at pH 1.5[a]

Molarity of GSCN	$\bar{M}_W \times 10^{-3}$ ($\bar{M}_W = \bar{M}_{w,D}$)	$B_W \times 10^3$ (undialyzed)	$B_{w,D} \times 10^3$ (dialyzed)	Expected charge on BPA
0.005	125	1.20	1.20	(+)
2.5	121	0.29	0.21	(−)
5.0	75.5	1.20	4.20	(−)

[a]Units for B_W and $B_{W,D}$ as obtained from $\Delta(1/\bar{M}_W)/\Delta C$ are (ml/g) (mole/g) or ml-mole/g^2; pH = 2.0 for the 0.005 M GSCN solution.

Eq. (11). Also the decrease in B_W in going from the 0.005 M GSCN solution to the 2.5 M GSCN can be explained on the basis of a reduction in the electrostatic charge $Z\gamma_\pm$. Thus the data given under Results will be examined in this light.

As seen in Table 4, the minimum in B_W cannot be correlated with a change in molecular weight. Consequently, this minimum must be due to a minimum in the value of the electrostatic charge. In other words, the rate of change in the value of the electrostatic charge ($Z\gamma_\pm$) on BPA governs the concentration coefficient B_W [see Eq. (11)]. And if the value of $Z\gamma_\pm$ is reduced or increased for a specific value of C_p, then the value of B_W will also be reduced or increased. The value $Z\gamma_\pm$ is, however, the net charge and not the total number of charges. Thus at the isoelectric point of BPA, the net charge will be zero ($Z\gamma_\pm = 0$) but the total number of charges on BPA will be approximately 200. Yet B_W is equivalent to zero at the isoelectric point, which illustrates that B_W is a function of the net charge $Z\gamma_\pm$. The electrostatic charges on BPA therefore do not have to be canceled in order to reduce B_W. Consequently, the association of counterions to BPA (reversal of charge phenomenon) can cause a lowering of the value of B_W just as the elimination of the net charge at the isoelectric point results in $B_W = 0$. But as seen in Table 4 the net charge on BPA as deduced from the B_W values is much smaller in 2.5 M GSCN than that in 0.005 M or 5.0 M GSCN at a specific value of C_p. Consequently, the minimum in B_W at 2.5 M GSCN illustrates that the net charge on BPA is being reversed; otherwise B_W would not again increase at 5.0 M GSCN.

The dialyzed samples should always have less salt than the undialyzed BPA samples, owing to the Donnan equilibrium effect.

This fact is important when a comparison of the B_W coefficients is made, because a change in the initial concentration of salt will change the value of B_W by altering the number of counterions in the domain. In Fig. 3 and Table 4 it is seen that the value of B_W for the 2.5 M GSCN solutions is greater than that of $B_{w,D}$. Yet B_W is obtained from a higher salt concentration than $B_{w,D}$. If the positive charge on BPA is being reduced by the added salt as indicated in the above comparison of the 0.005 M GSCN and the 2.5 M GSCN solutions, then the greater the salt concentration, the greater will be the reduction in B_W. Consequently, if the net charge on BPA is being reduced, then B_W should be less than $B_{w,D}$ because the salt concentration is greater for the undialyzed (B_W) samples. But the reverse of this is true. Therefore, for the 2.5 M GSCN equilibrium runs, the addition of salt increases the electrostatic charge on the BPA. In other words, the net charge on BPA in 2.5 M GSCN must be negative and an increase in salt concentration increases this negative charge because of an increase in the number of SCN^- ions associated with the BPA. Examination of Fig. 4 indicates, however, that in the comparison of B_a and B_b, factors other than salt molarity must be considered, since $B_{w,a} > B_{w,b}$ even though the molarity of the salt may be greater at the cell bottom than at the meniscus (see below).

In the 5.0 M GSCN solutions the reverse of the above is seen. That is, the value of B_W is less than that of $B_{w,D}$. Considering the effect of 2.5 M GSCN, the only plausible conclusion is that the coion to the counterion on the BPA is reducing the net negative charge. In other words, the positive guanidinium ions (G^+) as well as the SCN^- ions are now becoming associated with the BPA polyelectrolyte. Thus the results in 5.0 M GSCN show that there can be a reduction in the reversed charge at high concentrations of salt.

Variations in the Concentration Dependence of BPA in 0.005 M GSCN and Negative Extrapolated Molecular Weights

The results obtained in the 0.005 M GSCN indicate that B_W and $B_{w,D}$ are approximately equal. This result is most likely due to the low concentration of salt and hence the inability to have large variations in the values of B_W and $B_{w,D}$. Nevertheless, it is seen from Fig. 1 that a drastic change in the electrostatic charge occurs when the concentration of BPA is increased above approximately 0.5% BPA. Ordinarily, as the concentration of polymer is increased, the concentration dependence will decrease, not increase, as seen in Fig. 1. Consequently, the effect shown in Fig. 1 is an unusual effect not seen in uncharged polymers. At this concentration there are 70 moles of GSCN for every mole of BPA (70 molecules of GSCN for every +100 charges), assuming a molecular weight of 70,000 for

BPA*. However, some SCN^- ions must be free in solution because there must exist a dynamic equilibrium between the BPA molecules and the associated counterions. Thus the number of counter-ions associated with the BPA is most likely less than 0.7 counterions per charge at this drastic change in B_w ($B_{w,b}$). Nevertheless, the drastic increase in the value of B_w at about 0.5% BPA indicates that the association constant between SCN^- ions and the BPA polyelectrolyte has suddenly increased.

The concentration dependence will be a function of both the molarity of the salt and the polyelectrolyte at high concentrations of polyelectrolyte. Hence, for the 0.005 M GSCN solution, the reduction in this large B_w value at polyelectrolyte concentrations greater than about 1.0% for $\overline{M}_w$ or about 1.4% for $\overline{M}_{w,b}$ is due to the C^2 term (or higher terms) in the concentration dependence. In other words, in the association of counterions to the BPA, one must consider that a certain fraction of the salt will not be associated with the polyelectrolyte. Thus both the total free volume that a counterion can migrate into and the ratio of salt to polymer are important. That is, both the concentration of polymer and the ratio of salt to polymer are significant.

Another important point can be realized from Fig. 1. If only the data at concentrations which are greater than 0.5% BPA had been considered, then a negative molecular weight would have been obtained. In other words, one must extrapolate to zero charge as well as to zero concentration of polyelectrolyte. In the case of BPA, the reduction in charge is more important than the reduction in nonelectrostatic interactions. Consequently, extrapolation to zero charge is more important than extrapolation to zero polymer concentration. When the strength of the association of counterions with the BPA is weak, then the change in the value of $Z\gamma_\pm$ with a change in C_p (or a change in C_{Bx}) will be different than if such an association were strong. Thus to extrapolate to the correct molecular weight, one must use relatively large variations in concentrations in comparison to the difference between the lowest concentration and the extrapolated value ($C_p = 0$). In this way one can be assured that the

*As noted in the section, Equations, the ratio of charge to molecular weight ($Z'\gamma'_\pm/M'$) rather than the molecular weight alone is the important factor in determining the value of B_w. Hence, even though some aggregation has occurred by means of either salting out or by formation of intermolecular disulfide bonds, this aggregation or increase in $\overline{M}_w$ should not effect the electrostatic factors in the concentration coefficient as long as the ratio of charge to molecular weight remains constant. Thus the approximate molecular weight of the monomer ($\overline{M}_w = 70,000$) can be used in discussing the value of $Z'\gamma'_\pm/M'$.

extrapolated value is essentially at the point where $Z\gamma_{\pm} = 0$ and hence $\bar{M}_w^{ext} = \bar{M}_w$. Consequently, one must consider that the association constant between counterion and polymer may vary with the concentration of polymer and that this variation may or may not be linear from $C_p = 0$ to the experimental points.

Proposed Method for Detecting Whether the Net Charge Extrapolates to Zero as C_p Approaches Zero

It is desirable to have some method for detecting whether extrapolation to $C_p = 0$ yields a value at $Z\gamma_{\pm} = 0$. Such a method may be obtained by examining the ratio of B_a/B_b. That is, if B_a/B_b suddenly changes from a decreasing function of C_p to an increasing function, one would expect that $d(Z\gamma_{\pm})/dC_p$ has also been changed. As seen in Eq. (11), the value of $\bar{M}_{w,a}{}^{app}$ will be a function of B_a. Consequently, the ratio $\bar{M}_{w,a}{}^{app}/\bar{M}_{w,b}{}^{app}$ when plotted against the concentration should give some insight into how the ratio B_a/B_b changes with polymer concentration. Because of the relationship $\bar{M}_{w,r}{}^{app} = \bar{M}_{w,r}^{ext}/(1 + B_r\bar{M}_{w,r}^{ext}C_r)$, the ratio $\bar{M}_{w,a}{}^{app}/\bar{M}_{w,b}{}^{app}$ becomes

$$\bar{M}_{w,a}{}^{app}/\bar{M}_{w,b}{}^{app} = (K_{w,b}/K_{w,a})\,(\bar{M}_{w,a}^{ext}/\bar{M}_{w,b}{}^{ext}) \tag{12}$$

where

$$K_{w,b} = 1 + B_b\bar{M}_{w,b}{}^{ext}\,C_b \tag{13}$$

$$K_{w,a} = 1 + B_a\bar{M}_{w,a}^{ext}\,C_a \tag{14}$$

In other words, $\bar{M}_{w,a}{}^{app}/\bar{M}_{w,b}{}^{app}$ is proportional to B_bC_b/B_aC_a. Therefore, if $\bar{M}_{w,a}{}^{app}/\bar{M}_{w,b}{}^{app} < 1$ or $\bar{M}_{w,a}{}^{app} < \bar{M}_{w,b}{}^{app}$ then $B_a > B_b$ unless, of course, drastic changes occur in $\bar{M}_{w,b}{}^{ext}/\bar{M}_{w,a}^{ext}$ or C_b/C_a. Consequently, the value of $\bar{M}_{w,b}{}^{app}$ can be greater than $\bar{M}_{w,a}{}^{app}$ because of heterogeneity [16] and also because of a greater concentration dependence (greater value of $Z\gamma_+$) at the meniscus ($B_a > B_b$). Conversely, $\bar{M}_{w,a}{}^{app}$ can be greater than the apparent molecular weight at the cell bottom, $\bar{M}_{w,b}{}^{app}$ when $Z\gamma_{\pm}$ is greater at the cell bottom (when $B_a < B_b$) and when the value of C_b is much greater than C_a.

In Fig. 7 a plot of $\bar{M}_{w,a}{}^{app}/\bar{M}_{w,b}{}^{app}$ versus concentration shows that B_b/B_a changes erratically. That is, $B_bC_b/B_aC_a < 1$ at low concentrations and $B_bC_b/B_aC_a > 1$ at high concentrations of BPA. At the low concentrations of BPA, the value of $\bar{M}_{w,a}{}^{app}/\bar{M}_{w,b}{}^{app}$ decreases slightly with an increase in C_p. In other words, the fact that $C_b > C_a$ may produce the lower apparent molecular weight at the

meniscus. In addition, the value of $Z\gamma_{\pm}$ may be greater at the meniscus than at the cell bottom. However, for normal solutions $Z\gamma_{\pm}$ should be less at the meniscus because of a larger extended domain on the protein at r = a. That is, the value of $Z\gamma_{\pm}$ is governed by both the concentration of added salt and the polyelectrolyte concentration (see discussion above on $Z\gamma_{\pm}$). Furthermore, a decrease in $\overline{M}_{w,a}{}^{app}/\overline{M}_{w,b}{}^{app}$ implies that the value of B_bC_b/B_aC_a is changing. But experimental evidence in Fig. 1 indicates that both B_b and B_a remain constant for the low concentration range (< 0.4 g/100 ml). Consequently, the initially slight decrease in the ratio $\overline{M}_{w,a}{}^{app}/\overline{M}_{w,b}{}^{app}$ in Fig. 7 is most likely due to a greater percentage change in the concentration of C_b as compared to C_a as the initial concentration C^0 increases.

In cases where the association of the counterion is strong, the competition between polyelectrolyte molecules for the counterions is more important than the ability of the counterion to escape into free regions. Now, as the concentration of polyelectrolyte increases with cell radius, there will be a decrease in the ratio of counterion to polyelectrolyte as well as a decrease in free space. The decrease in this ratio with r means that more counterions will be associated with the polyelectrolyte at the meniscus, where the ratio of GSCN/BPA is greatest, than at the cell bottom, where the ratio GSCN/BPA is least. Consequently, the value of $(Z\gamma_{\pm})_a$ will be less than $(Z\gamma_{\pm})_b$ and it follows that $B_a < B_b$ and $\overline{M}_{w,b}{}^{app} < \overline{M}_{w,a}{}^{app}$. Thus the ratio $\overline{M}_{w,a}{}^{app}/\overline{M}_{w,b}{}^{app}$ becomes greater than 1 and increases dramatically with C_p. Consequently, there is a sudden

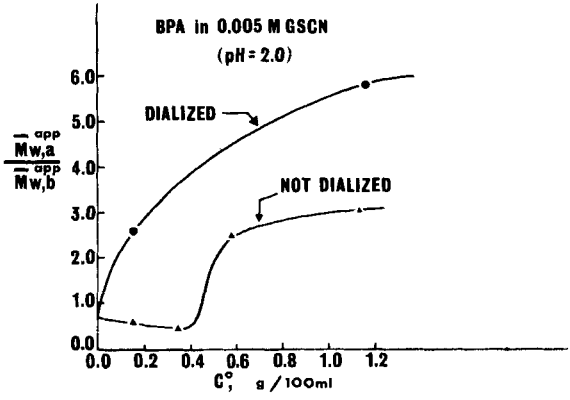

Fig. 7. Plot of $\overline{M}_{w,a}{}^{app}/\overline{M}_{w,b}{}^{app}$ versus BPA concentration in 0.005 M GSCN at pH 2.0 for dialyzed (●) and undialyzed (▲) samples. The dialyzed samples have less salt concentration and therefore the erratic change in $\overline{M}_{w,a}/\overline{M}_{w,b}$ occurs at a lower BPA concentration.

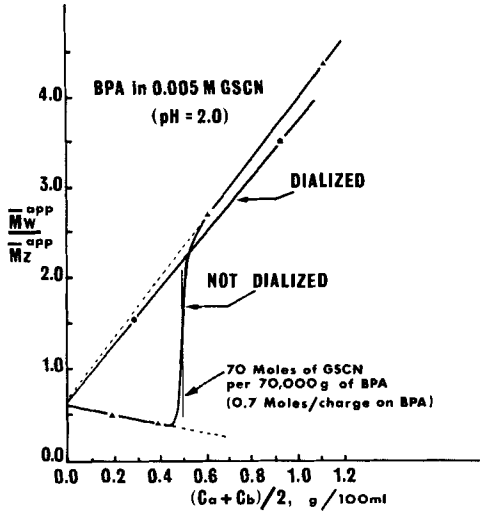

Fig. 8. Plot of $\bar{M}_w^{app}/\bar{M}_z^{app}$ versus BPA concentration in 0.005 M GSCN at pH 2.0 for dialyzed (●) and undialyzed (▲) samples. The dramatic change in the association constant between SCN^- ions and BPA is manifested more in this plot than in Fig. 7 because of the definition of $\bar{M}_z^{app}$ [Eqs. (6) and (17)].

change in the value of this ratio when the association constant increases. Such increases in the association of counterions with BPA have been noted in "ion-binding" studies in the work of Scatchard and co-workers [2-4].

Such strong associations are possible if one considers that groups of positive charges rather than statistically located positive charges exist on BPA. These groups would be able to maintain an attraction for SCN^- ions more than single ions because when the electrostatic charge on one of these ions on the group is canceled by the formation of an insoluble salt bond with the counterion ($R-NH_3^+SCN^-$), the neighboring positive charges will still attract the SCN^- ions which remain in this domain. Thus at some critical concentrations ($C_p > 0.5\%$ and $C_{BX} = 0.005$ M GSCN in this experiment) the BPA begins to lose these tightly associated SCN^- ions and the value of $Z\gamma_\pm$ begins to change at a different rate with a change in C_p.

Further insight into this change in B_b/B_a can be seen in Fig. 8, where $\bar{M}_w^{app}/\bar{M}_z^{app}$ is plotted against concentration. This plot is similar to that given in Fig. 7 but magnifies the changes because of the definitions of $\bar{M}_w$ and $\bar{M}_z$. In other words, from Eqs. (5) and (6) one can derive

$$\frac{\bar{M}_w^{app}}{\bar{M}_Z^{app}} = \frac{K_{w,b}K_{w,a}\bar{M}_w^{ext}(C_b - C_a)}{K_w\bar{M}_{w,b}^{ext}C_bK_{w,a} - \bar{M}_{w,a}^{ext}C_aK_{w,b}} \quad (15)$$

where $K_{w,a}$ and $K_{w,b}$ are defined by Eqs. (13) and (14) and where

$$K_w = 1 + B_b\bar{M}_{w,b}^{ext}(hC_a + C_b)/2 \quad (16)$$

Or Eq. (15) can be rearranged to give

$$\frac{\bar{M}_w^{app}}{\bar{M}_Z^{app}} = \frac{(C_b/C_a)K' - K'}{(C_b/C_a)K_{w,a} - K_{w,b}} \quad (17)$$

where $K' = K_{w,b}K_{w,a}/K_w$ and when it is assumed that $\bar{M}_{w,b}^{ext} = \bar{M}_{w,a}^{ext} = \bar{M}_w^{ext}$. Thus if $K_{w,b}$ is greater than $K_{w,a}$, the value of $(C_b/C_a)K_{w,a} - K_{w,b}$ will become small and $\bar{M}_w^{app}/\bar{M}_Z^{app}$ will become large. That is, a corresponding difference will not exist in the numerator because the same value (K') is used rather than two different values ($K_{w,a}$ and $K_{w,b}$). Thus if $B_b/B_a > 1$, then $\bar{M}_w^{app}/\bar{M}_Z^{app} > 1$ just as $\bar{M}_{w,a}^{app}/\bar{M}_{w,b}^{app} > 1$. Consequently, both plots (Figs. 7 and 8) give the same information concerning the change in the ratio of the concentration coefficients (B_b/B_a).

For the dialyzed samples of Fig. 8 the drastic increase in $\bar{M}_w^{app}/\bar{M}_Z^{app}$ occurs at a lower polymer concentration, because the dialyzed samples have less salt present with the BPA. The drastic change in $\bar{M}_w^{app}/\bar{M}_Z^{app}$ or in $\bar{M}_{w,a}^{app}/\bar{M}_{w,b}^{app}$ occurs at the same concentration that a corresponding change occurs in the $1/\bar{M}_w$ versus $(C_a + C_b)/2$ plot (Fig. 1).

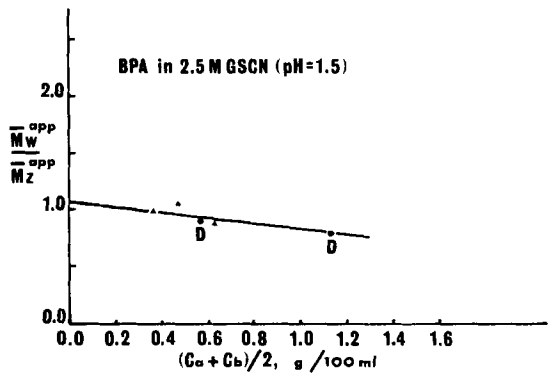

Fig. 9. Plot of $\bar{M}_w^{app}/\bar{M}_Z^{app}$ versus BPA concentration in 2.5 M GSCN at pH 1.5. The dialyzed samples (●) are represented by a "D," whereas the undialyzed (▲) are unmarked.

In Fig. 9 for 2.5 M GSCN the ratio $\bar{M}_w^{app}/\bar{M}_z^{app}$ does not change drastically because now the tightly associated SCN^- is not involved. Just as at the low concentrations of BPA in the 0.005 M GSCN solutions, the value of $\bar{M}_w^{app}/\bar{M}_z^{app}$ decreases with an increase in C_p because the ratio of GSCN/BPA is less at the cell bottom.

In Fig. 10 for 5.0 M GSCN we see a change in the ratio $\bar{M}_w^{app}/\bar{M}_z^{app}$ at the higher concentrations of BPA. The change occurs first in the solutions containing the larger amount of salt (the undialyzed samples). The BPA in these solutions will have a lower negative electrostatic charge because of a reduction in the reversed electrostatic charge (see above). Thus the BPA at the meniscus will have more GSCN associated with it than the BPA at the cell bottom. And the greater the amount of GSCN associated, the lower will be the value of $Z\gamma_{\pm}$. Consequently, the drastic change in $\bar{M}_w^{app}/\bar{M}_z^{app}$ for the higher concentrations of BPA in 5.0 M GSCN may be due to a greater charge on BPA at the cell bottom because of its relatively lower concentrations of GSCN.

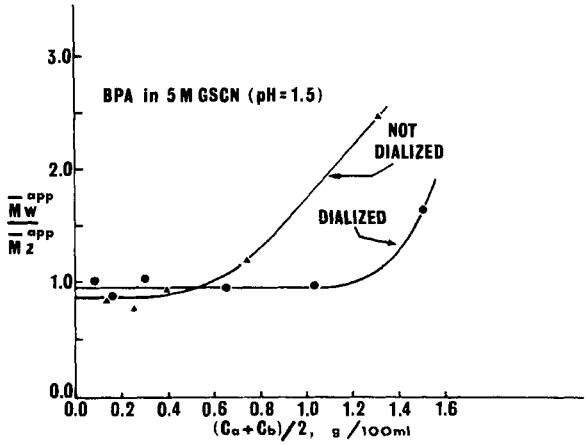

Fig. 10. Plot of $\bar{M}_w^{app}/\bar{M}_z^{app}$ versus BPA concentration in 5.0 M GSCN at pH 1.5 for dialyzed (●) and undialyzed (▲) samples.

CONCLUSIONS

The results on the molecular weight concentration coefficient of BPA in the various solutions of GSCN show that the value of this concentration coefficient can be increased or decreased with an increase in concentration of the GSCN salt. The addition of salt therefore does more than just reduce electrostatic interacts. The increase in B_w with an increase in salt concentration can only be

explained on the basis that the added salt is increasing the electrostatic charge of the polyelectrolyte. On the basis of previous data [14] and comparisons [1,23], it can be concluded that this ability to reverse the electrostatic charge on a polyelectrolyte will increase with an increase in the solubility of the counterion. Consequently, for proteins having more ϵ—NH_3^+ groups than guanidinium groups, the sequence will be $Cl^- < Br^- < I^- < SCN^-$ in ability to reverse the electrostatic charge. For negatively charged groups having positively hydrated water, the ability of the counterion to reverse or reduce the electrostatic charge on the polyelectrolyte will be according to the acidic sequence $Li^+ < Na^+ < K^+ < Cs^+$. If the negatively charged groups are negatively hydrated, the sequence $K^+ < Na^+ < Li^+ < Cs^+$ will result.

The results therefore show that the correct molecular weight can be obtained either with or without prior dialysis. It is recommended that dialysis be not carried out, because as noted under Experimental, the resulting change is salt concentration upon dialysis interferes with the synthetic boundary determination of the initial concentration. Dialysis is not required because, contrary to the theory of Casassa and Eisenberg [8] and Johnson et al. [9], the electrostatic charge of the polyelectrolyte varies with the absolute concentrations of both the salt and the polyelectrolyte. Exact molecular weights of polyelectrolytes are therefore obtained, because in extrapolating to zero polyelectrolyte concentration at constant molarity of salt, the value of the net electrostatic charge on the polyelectrolyte ($Z\gamma_\pm$) is extrapolated to zero.

In certain cases such extrapolations will give erroneous results because the association constant between the counterion and the polyelectrolyte may change. Plots of $\bar{M}_w^{app}/\bar{M}_Z^{app}$ are useful in detecting such changes. If $\bar{M}_w^{app}/\bar{M}_Z^{app}$ is greater than 1 and is increasing with an increase in polyelectrolyte concentration, the salt concentration is too low for reliable extrapolation to zero value of $Z\gamma_\pm$ at $C_p = 0$. In other words, under such conditions, the counterions which are being exchanged with the medium are associated strongly to the polyelectrolyte because of local groupings of charges on the polyelectrolyte and because of insufficient counterions for such groups. The extrapolation to $C_p = 0$ is unsafe under these conditions because it is not known if or at what value of C_p the value of the association constant between the counterion and polyelectrolyte will change.

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Accepted by editor August 5, 1968
Received for publication August 29, 1968