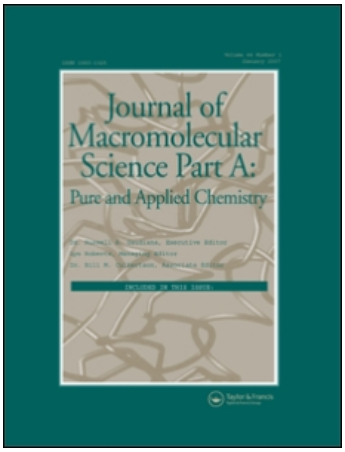

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Explanation of Ionic Sequences in Various Phenomena. II. Reversal of Colloid Charge and Ion Binding

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Explanation of Ionic Sequences in Various Phenomena. II. Reversal of Colloid Charge and Ion Binding

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SUMMARY

Previous proposed models for the structure of hydrated ions and the calculated values of the effective dielectric constant of such hydrated ions were used to explain the reversal of colloid charge and ion binding phenomena. In contrast to the conclusions made by Bungenberg de Jong, it is shown that the more soluble the counter-cation or counter-anion of a colloid charge, the greater is the ability of the counterion to reverse the electrical charge of the colloid. The reversal of charge phenomenon is therefore associated with the counterions's solubility, not its insolubility. The solubility sequence is determined by whether or not the carboxylate, sulfate, or phosphate ion is positively (A regions) or negatively hydrated. The phosphate group of DNA or RNA must be associated with a base by means of ion-ion bonds in order to produce the observed reversal of charge sequence. Just as in the reversal of charge phenomenon, the ion-binding phenomenon involves the electrostatic attraction of a counterion with the polyelectrolyte rather than a binding or insolubilization of the counterion. The reverse ion-binding sequence can be obtained if one dialyzes extensively in the presence of sufficient salt before physical measurements are made. This is because the solubility of a counterion determines the true electrostatic charge of the polymer. In other words, different concentrations of salt arise in the dialysis bag when different counterions are added because the activity coefficient of the counterion is determined by the solubility of the ion-ion complex between the counterion and the colloid's charged group.

INTRODUCTION

The reversal of charge and ion-binding phenomena have been known for some time and have been reviewed and studied by Bungenberg de Jong [1]. Ion binding studies have been done by numerous scientists [2], notably by Scatchard and his co-workers [3-6]. These two phenomena are the result of the interaction of added salt with the polyelectrolyte in aqueous solutions. Wall and his associates [7, 8] in 1950 proposed that counterions are rigidly attached to the polyelectrolytes and are therefore an integral part of the polyelectrolytes. Katchalsky et al. [9] present evidence to support this idea, but in contrast to the theory of Wall et al. [7, 8] contend that the associated counterions which form the atmosphere or "inner shell" around the polyelectrolyte are not rigidly held but rather are quite deformable and respond to external electrical fields. Consequently, in this inner shell there must be a continuous exchange of counterions with the surrounding medium just as there is a continuous exchange of hydrated water molecules of an ion with surrounding water molecules.

In the present study it will be shown that the presence of an inner shell of counterions can explain the reversal of charge and ion-binding phenomena. Furthermore, it will be seen that the greater the solubility of the counterion, the greater will be the ability of this counterion to reverse the electrostatic charge of the polyelectrolyte and to "bind" to the polyelectrolyte. As expanded below, this seemingly contradictory statement is due to the fact that once an ion-ion complex is produced, the other surrounding counterions are no longer attracted to the charged group on the polyelectrolyte and hence migrate away. That is, the ion atmosphere or inner shell is then temporarily destroyed until the ion-ion complex is destroyed by surrounding water molecules.

PHENOMENON OF REVERSAL OF CHARGE

Polarizability Theory and Its Flaws. An attempt to explain the reversal of charge phenomenon was made some time ago by Bungenberg de Jong [1]. He explains the various ionic sequences on the basis of the "polarizability" power of a cation or anion. According to him, the sequence for polarizability is phosphate > carboxylate > H_2O > sulfate. In Table 1 are listed some ionic sequences for the reversal of charge phenomenon. It should be noted that the carboxylate group can be associated in polarizability with either the phosphate or sulfate group. Thus, according to the theory stated by Bungenberg de Jong [1, p. 288], the phosphate colloids have the sequence $K^+ > Na^+ > Li^+$ as given in Table 1 because the phosphate ion is more polarizable than water. In addition, the sulfate col-

Table 1. Relative Concentrations of Cations Necessary to Obtain a Reversal of Colloid Charge[a]

Colloid	Sequence	
	Monovalent ions	Divalent ions
Na^+-pectinate ($-CO_3^-$) Na^+-agar ($-SO_4^-$) K^+-chondroitin sulfate ($-SO_4^-$) Na^+-arabinate ($-CO_3^-$)	$Li^+ > Na^+ > K^+ > Rb^+ > Cs^+$ (acidic sequence)	$Mg^{2+} > Ca^{2+} > Sr^{2+} > Ba^{2+}$ (acidic sequence)
Soya bean phosphatide ($-PO_4^-$)	$K^+ > Rb^+ > Na^+ > Cs^+ > Li^+$	$M^{2+} > Sr^{2+} > Ba^{2+} > Ca^{2+}$
Na^+-nucleate (DNA or RNA)	$K^+ > Na^+ > Li^+$	$Mg^{2+} > Sr^{2+} > Ca^{2+} > Ba^{2+}$
Egg lecithin ($-PO_4^-$)	$K^+ > Na^+ > Rb^+ > Cs^+ > Li^+$	$Sr^{2+} > Ba^{2+} > Mg^{2+} > Ca^{2+}$
Gelatin ($-CO_3^-$)	$K^+ > Na^+ > Li^+$	$Sr^{2+} > Ba^{2+} > Mg^{2+} > Ca^{2+}$
Casein ($-CO_3^-$)	$K^+ > Na^+ > Li^+$	$Ba^{2+} > Sr^{2+} > Mg^{2+} > Ca^{2+}$
Oleate ($-CO_3^-$)	$K^+ > Na^+ > Rb^+ > Cs^+ > Li^+$ (basic sequence)	$Ba^{2+} > Sr^{2+} > Ca^{2+} > Mg^{2+}$ (basic and nonpolar sequence)
Casein ($-NH_3^+$) (pH 3.4) Gelatin ($-NH_3^+$) (pH 2.7) Clupein ($-NH_3^+$) (pH 8.9)	$Cl^- > Br^- > I^- > SCN^-$	

[a]Data obtained from Bungenberg de Jong [1]. All electrical mobility studies were done at approximately pH 10 unless designated otherwise.

loids have the reverse of this sequence ($Li^+ > Na^+ > K^+$) because the sulfate ion is less polarizable than the H_2O molecule. But consider now the carboxylate ion. As seen in Table 1, the $-CO_3^-$ ion behaves as if it has a polarizability greater than (as in the case of $-PO_4^-$) or less than (as in the case of $-SO_4^-$) that of water. This seemingly contradictory result was explained by Bungenberg de Jong [1, p. 293] as being due to the presence of the hydroxyl groups in the vicinity of the carboxyl ion. Consequently, when hydroxyl groups are present, the $-CO_3^-$ ion on the colloid behaves as the sulfate group and when they are absent it behaves as the phosphate ion.

But if this reasoning can be applied to the $-CO_3^-$ group, it should also be applied to the $-SO_4^-$ group. Examination of Table 1 shows that the only sulfate colloids studied were agar and chondroitin sulfate. But both of these polymers have hydroxyl groups in the vicinity of the sulfate ion. Consequently, by applying the above reasoning it can be concluded that both the sulfate and carboxylate groups actually have polarizabilities that are greater than that of H_2O and that the sequence $Li^+ > Na^+ > K^+$ is due to the presence of hydroxyl groups near the charged groups. But if such were the case, then the previous polarizability sequence phosphate > carboxylate > H_2O > sulfate which was observed in other experimental results is in error! Consequently, it can be concluded that the polarization theory is inadequate in explaining the experimental results.

More difficulty with the polarizability theory is encountered in the "irregular" sequences for phosphate colloids using divalent cations. Bungenberg de Jong [1, p. 290] admits that this difficulty cannot be explained using the polarization theory and that "still unknown factors must also play a role." Moreover, an extension of this polarizability theory by Morsi and Sterling [10] also seems to be inadequate in explaining the effect of cations on the retrogradation of amylose. Consequently, it is concluded that the "polarizability" of an ionic group does not determine the order of cations or anions in the reversal of charge phenomenon.

Similar postulates concerning polarizability of the added ion have been used to explain the binding of ions to various polyelectrolytes. Since the two phenomena are related, a theory will now be presented which will explain the reversal of charge and ion-binding phenomena. This theory will be based on my previous models for hydrated ions and the calculated dielectric constants for these ions [11, 12].

Significance of the Presence or Absence of A Regions on the Electrostatically Charged Groups of Hydrated Polyelectrolytes. To understand the reversal of charge phenomenon one must first examine how simple ions interact with each other. In a previous paper [12], it was shown how solubility sequences of various salts can be correlated with the effective dielectric constant and the presence of A regions. A summary of the explanations for solubil-

ity sequences of various salts is given in Table 2. It should be noted that if an anion has an A region, then the observed cationic solubility sequence is "acidic," whereas if the anion does not have an A region and if its effective dielectric constant is still less than that of water, then the cationic solubility sequence is "basic."

To apply the results of Table 2 to the reversal of charge phenomenon, one must first know whether the carboxylate, phosphorylate, and sulfate groups have A regions (positive hydration). The equilibrium constants and pK values for various carboxylic acids are given in Table 3.

The formate ion, as seen in Table 2, has the acidic solubility sequence, whereas the acetate ion has the basic solubility sequence. Consequently, it can be concluded that gluconate ion or any other type of sugar carboxylate ion will be positively hydrated and therefore will give the acidic solubility sequence. As the hydroxyl group is removed farther from the carboxyl group as in going from α-hydroxybutyric to γ-hydroxybutyric acid, then the positively hy-

Table 2. Cationic and Anionic Solubility Sequences for Various Anions and Cations[a] (Destruction of Salt Bonds)

Anions or cations	General sequence	Reason
ClO_4^-, NO_3^-, SCN^-, I^-, Br^-	Acidic: $Li^+ > Na^+ > K^+ > Rb^+ > Cs^+$	$D_- > D_1$
$(PO_3^-)_n$, PO_4^{-3}, $H_2PO_4^-$, CO_3^{2-}, F^-, formate	Acidic: $Li^+ < Na^+ < K^+ < Rb^+ < Cs^+$	A region on anion
Cl^-, SO_4^{2-}, HCO_3^-, acetate[b]	Basic: $K^+ < Na^+ < Rb^+ < Li^+ < Cs^+$	$D_- < D_1$
Cs^+, guanidinium	$SO_4^{2-} > I^- < Br^- < Cl^- < F^- < Ac^-$	$D_+ > D_1$
K^+, NH_4^+	$SO_4^{2-} < Cl^- < Br^- < I^- < F^- < Ac^-$	$D_+ < D_1$

[a]For further discussion and elaboration of these sequences, see the previous paper [12]. The insolubility sequence is comparable to the salting-out and reversal of charge sequences. The greater the interaction of the cation and anion (the greater the insolubility of the salt), the smaller will be the reversal of charge phenomena (see the text). The solubility data were obtained from Refs. [13] and [14]. The $(PO_3^-)_n$ sequence was obtained from the ion-binding studies of Strauss and Ross [15].

[b]The acetate ion has the sequence $Rb^+ < Na^+ < K^+ < Li^+ < Cs^+$ and therefore can be considered as being intermediate between the acidic and basic sequences. However, the position of the Li^+ ion in this sequence shows that the acetate ion has more of a basic than an acidic type of solubility sequence.

Table 3. Equilibrium Constants for Various Carboxylic Acids

Acid	$K \times 10^5$	pK
Thioglycolic	29	3.54
Gluconic	25	3.60
Formic	21	3.68 (3.75) (A regions)
Glycolic	15	3.82
α-Hydroxybutyric	10.5	3.98
β-Hydroxybutyric	3.0	4.52
γ-Hydroxybutyric	1.9	4.72
Acetic	1.8	4.75 (B regions)
n-Butyric	1.5	4.82
Nonanoic	1.1	4.96

[a]Data obtained from Kendall [16]. Formate ions are positively hydrated (have A regions), while acetate ions are negatively hydrated (have B regions). The pK of formate is listed as 3.75 by Lange [14] ($K = 1.77 \times 10^{-4}$ instead of 2.1×10^{-4}). Therefore, the pK values listed in this table and Table 5 cannot be considered as the most accurate values.

drated carboxylate ion gradually becomes negatively hydrated (Table 3). Extension of the hydrocarbon chain increases the pK slightly. Thus the nine-carbon carboxylate nonanate must be negatively hydrated and must have a value of D_- greater than that of acetate.

The approximate pK values for various phosphates are listed in Table 4. Again, as in the case of the carboxylates, if a hydrocarbon is attached to the phosphate group as in monoethyl or monoisobutyl phosphate, then there is an increase in pK. Moreover, the pK is also increased if the phosphate is attached to a sugar by means of an oxygen atom. The tremendous increase in pK for inosinic acid must be due to the association of the negative charge of the phosphate ion with the positive charge of the base, inosine. That is, inosinic acid has the structure base-ribose-phosphate. Although the attachment of the $-CH_2-$ or sugar group to the phosphate will increase the pK of the phosphate ion, the greater than expected increase in pK must be due to a partial reduction of the electrostatic charge on the phosphate ion by an ion-ion association with the positive nitrogen atom of the base. Previous results on solubility data

and calculated D_+ values showed that such associations always increase the value of D_+ (decrease the electrostatic charge). Thus in nucleotides the phosphate is most likely negatively hydrated (absence of A regions), which is opposite to that of free phosphate. Table 4 shows, however, that for diphosphates ($H_4P_2O_7$) or disubstituted phosphates (dimethyl or diethyl phosphate) the pK is decreased. The decrease in pK for dimethyl phosphate must be due to the reduction in electron drawing power of the two oxygen atoms since monoethylphosphate has an increase in pK.

Table 4. Equilibrium Constants for Various Phosphoric Acids[a]

Type of side chain	Acid	K_1	pK_1
Free diacid	$H_4P_2O_7$	Complete	dissociation
	Dimethyl phosphoric	3.77	—0.58
Dihydrocarbon	Diethyl phosphoric	3.05	—0.48
Free acid	H_3PO_4	0.885	0.053
Monosugar	Erythritol phosphoric	0.686	0.16
	Mannide phosphoric	0.656	0.18
	Monoglycol phosphoric	0.500	0.30
	Monoglyceryl phosphoric	0.488	0.31
Monohydrocarbon	Monoethyl phosphoric	0.274	0.56
	Monoisobutyl phosphoric	0.261	0.58
Mono(sugar-base)	Inosinic	0.157	0.80

[a]Data obtained from Kendall [16]. The free acid (H_3PO_4) has A regions as shown in Table 3, while inosinic acid is negatively hydrated (no A regions).

The sulfate ion must undergo similar changes. Such an extrapolation can be made by examining the equivalent conductance of various sulfates. The equivalent conductances for monomethyl, monopropyl, and monoisobutyl sulfates are 368, 356, and 350 at a concentration of 0.03 mole/liter [16]. Hence the data show that the pK value of the sulfate increases with an increase in the chain length of the hydrocarbon just as in the case of substituted phosphates and carboxylates. Unfortunately, no data are given for sulfate sugars.

Relationship between the Solubility of Counterion and Its Ability to Reverse the Colloid or Polyelectrolyte Charge. A comparison of the solubility sequences of various anions with the corresponding

sequences for the reversal of charge phenomenon for the pectinate gives an acidic sequence. As shown in Table 3 the value of the pK for the carboxyl group for gluconic acid is smaller than that for the formic acid and hence should be positively hydrated (the presence of A regions). Consequently, it has the same solubility sequence as that of the formate and fluoride ions (Table 2). A close examination, therefore, shows the following relationship:

Relative concentration of cation required for reversal of charge of pectinate and arabinate:	$Li^+ > Na^+ > K^+ > Rb^+ > Cs^+$
Relative concentration of cation at the maximum solubility of a positively hydrated counteranion (A regions):	$Li^+ < Na^+ < K^+ < Rb^+ < Cs^+$

The same sequence in both cases is maintained. However, an important difference is observed. Namely, the cation which has the greatest solubility is the most effective in reversing the electrostatic charge of the pectinate polymer.

The above observation is opposite to the conclusions given by Bungenberg de Jong [1, p. 288]. In examining phosphates and sulfates, he stated that the cation or anion that produces the least soluble salt is the most effective in reversing the colloid charge. His prediction did not consider the entire sequence (including Rb^+ and Cs^+ ions) and did not consider a reversal of the solubility sequence of $Li^+ < Na^+ < K^+$ to $Li^+ > Na^+ > K^+$ when the anion changes from positive hydration to negative hydration. In his example of the phosphate group [1, p. 288], he cites the solubility sequence of the phosphate ion. But the phosphate ion is positively hydrated, whereas that of inosinic acid is negatively hydrated. Moreover, the phospholipids have a base in close proximity to their phosphate group just as in inosinic acid. Consequently, the pK of the phosphate group on lecithins and phosphatides must be much greater than that of inorganic phosphate just as in the case of ionosinic acid. Thus Bungenberg de Jong [1] compared a negatively hydrated ion with a positively hydrated inorganic salt. In other words, the solubility sequence for the phosphate group on egg lecithin and soya bean phosphatide should be basic (absence of A regions) and not acidic as in the case of inorganic phosphate. Therefore, a correct comparison of the solubility sequence for negatively hydrated phosphate ions with the reversal of charge phenomena for such groups gives

Relative concentration of cation required for reversal of charge on egg lecithin:	$K^+ > Na^+ > Rb^+ > Cs^+ > Li^+$
Relative concentration of cation for maximum solubility of negatively hydrated anion (no A regions):	$K^+ < Na^+ < Rb^+ < Li^+ < Cs^+$

The above comparison shows that both sequences are basic and are thus in agreement. Consequently, the results on phospholipids are the same as those for carboxylate polysaccharides: The more soluble the salt, the greater is its effectiveness in reversing the colloid charge. The results of Table 1 also show that in nucleic acids the phosphate group must be associated with the base. That is, DNA or RNA give the same type of reversal of charge sequence as the phospholipids. Consequently, the bases in RNA or DNA must be reducing the charge per unit surface area of the phosphate ion just as in the case of inosinic acid or the phospholipids.

The same type of study can be applied to sulfate polysaccharides. Examination of Tables 1 and 2 yields the relationship:

Relative concentration of cation required for reversal of charge on chondroitin sulfate and agar:	$Li^+ > Na^+ > K^+ > Rb^+ > Cs^+$
Maximum solubility of cation for positively hydrated anion (A regions):	$Li^+ < Na^+ < K^+ < Rb^+ < Cs^+$

Thus upon examination of the entire sequence (including the Rb^+ and Cs^+ cations), it is seen that in both solubility and reversal of charge phenomena, the acidic sequence is obtained. If only the ions Li^+, Na^+, and K^+ are examined, then the sequence could be acidic or basic and no definite conclusions could be made. However, the above comparison shows definitely that sulfate groups on polysaccharides are positively hydrated just as in the case of carboxylate groups.

The effect that introducing hydroxyl groups on the aliphatic chain of carboxylate ions has on the reversal of charge phenomena can be seen in Table 5. As the number of hydroxyl groups increases, the pK of the carboxylic acid will decrease and the degree of positive hydration will increase. Hence the sequence changes from basic to acidic as hydroxyl groups are introduced. If part of the carboxyl groups are titrated (pH 10 to 6), then the resulting colloid will produce a more acidic sequence. That is, it becomes more difficult for the hydrated Li^+ ion to associate with the polymer because of repulsion between the positively hydrated water (A region) on the Li^+ ion and the polar –COOH group. The lower the number of cations which can associate with a colloid, the greater will be the required concentration of the cation to reverse the negative charge of the colloid. Consequently, if Li^+ ions are repelled by –COOH groups, then a greater concentration of Li^+ ions will be required to reverse the charge. Two effects are therefore shown in Table 5: the effect of changing from negatively to positively hydrated carboxylate ions and the effect of titrating part of the carboxylate ions.

The results given in Table 1 for gelatin and casein at about pH 10 show that the carboxylate groups on these two proteins are

Table 5. Relative Concentrations of Cations Necessary to Obtain a Reversal of Colloid Charge[a]

Colloid	pH	Sequence		Type of sequence
		Monovalent ions	Divalent ions	
Arabinate	6-10	$Li^+ > Na^+ > K^+$	$Mg^{2+} > Ca^{2+} > Sr^{2+} > Ba^{2+}$	Acidic
Hexaoxystearate	6	$Li^+, Na^+ > K^+$	$Mg^{2+} > Sr^{2+} > Ca^{2+} > Ba^{2+}$	Acidic
Trioxystearate	6	$Li^+, Na^+ > K^+$	$Ba^{2+} > Sr^{2+} > Mg^{2+} > Ca^{2+}$	Acidic
Hexaoxystearate	10	$K^+ > Na^+, Li^+$	$Sr^{2+} > Ba^{2+}, Ca^{2+} > Mg^{2+}$	Basic
Trioxystearate	10	$K^+ > Na^+, Li^+$	$Ba^{2+} > Sr^{2+}, Ca^{2+} > Mg^{2+}$	Basic
Oleate	7-10	$K^+ > Na^+ > Li^+$	$Ba^{2+} > Sr^{2+} > Ca^{2+} > Mg^{2+}$	Basic

[a]For $Li^+ > K^+$ in one of the above sequences, the concentration of Li^+ ion required to reverse the charge will be greater than that of K^+. Therefore, in this case, the Li^+-colloid salt is more insoluble than the K^+-colloid salt (see the text). Data obtained from Bungenberg de Jong [1].

negatively hydrated (no A regions) just as in the case of oleate. The values of the pK_2 of the carboxylate group on the side chain of glutamate and aspartate ions are pK_2 4.3 and pK_2 3.9, respectively [17]. Consequently, from these pK values one would expect that the carboxylate group should have A regions. However, according to Tanford and Havenstein [18], the normal value for pK_2 for proteins is about pK_2 4.6. This value suggests that the $-CO_3^-$ groups are negatively hydrated (no A regions). Consequently, if the pK_2 value given by Tanford and Havenstein is accepted, then the sequences given in Table 1 agree with the conclusions concerning the pK of carboxylate groups (Table 2). That is, both cases point to the fact that the carboxylate groups of gelatin and casein do not have A regions.

The above results on carboxylate, phosphate, and sulfate groups can be applied to study the effect of anions rather than cations on reversing a colloidal charge. Thus the greater the solubility of the anion (the coion of the positively charged colloid), the greater will be the ability of the anion to reverse the electrostatic charge of the colloid or protein. A comparison of Tables 1 and 2 shows that the positively charged groups on the casein, gelatin, and clupein should have dielectric constants less than that of water ($D_+ < D_1$), as in the case of the $-NH_3^+$ group. The guanidinium group is therefore excluded as a major participator in the reversal of charge phenomena because its sequence is opposite to that of the $-NH_3^+$ group. The pK of the guanidinium group is greater than 12 for both the amino acid arginine [17] and protein ribonuclease [18]. However, the values [17] for the pK of the $-NH_3^+$ (lysine) groups on ribonuclease are 9.6 and 10.2, respectively, which are quite close to the pK value of about 9.3 for the NH_4^+ ion (NH_4OH). Consequently, the amino groups on proteins must have values of D_+ less than that of water, just as in the case of the NH_4^+ ions. The anionic sequence for the reversal of charge phenomena for the proteins listed in Table 1 must therefore pertain to the amino and possibly the imidazole groups and not the guanidinium group.

PROPOSED THEORY FOR REVERSAL OF CHARGE PHENOMENON

The question is now asked: Why are those ions which have the greatest solubility with respect to the colloid ions more effective in reversing the colloid charge? First, it must be remembered that the colloid charge as observed by physical measurements depends on the total charge at the surface of the colloid. Because of electrostatic attractions, ions of opposite charge will seek each other. Consequently, the counterions will exist in the immediate vicinity of a polyelectrolyte. If salt is added to the aqueous solution, then more ions can act as counterions. The association of a counterion

and ion can be compared to the association of water of hydration with its ion. The water is not permanently hydrated but rather is associated for a specific average length of time and then is cast off or repelled. Another water molecule then takes its place. If the exchange is rapid, the ion will associate itself with many water molecules.

In the same manner, if the length of time for an ion-ion complex between a counterion and a charged group on the polyelectrolyte is relatively short because of large repulsive forces, then many counterions can become associated with the charged group because the charge of the colloid group will statistically exist for longer periods of time. But associated counterions exist in the "inner shell" of the colloid and thus are capable of reducing or reversing the colloid's net electrostatic charge. And as shown [12], the stability of the ion-ion complex will depend on the value of $D_{\pm}$ for the charged groups and on the presence or absence of A regions. For example, if the value of $D_{\pm}$ for both the counterion and the colloid charge are similar, then the stability of an ion-ion complex will be relatively large. If both charged groups have A regions, then again the relative stability will even be greater [12]. And the greater this stability (or the insolubility), the greater will be the statistical length of time for the ion-ion complex. Consequently, the reversal of charge phenomenon is related to the solubility (or stability) of the ion-ion complex between the counterion and the colloid's charged group. Conversely, if an ion-ion complex is formed for a long period of time, the charge on this colloid group will be destroyed for longer periods of time and counterions will not seek the colloid's group during the ion-ion complex. Consequently, the more soluble or less stable is the ion-ion complex between colloid group and counterion, the greater will be the counterion's effectiveness in reversing the charge of the colloid, i.e., the greater will be the number of counterions that can gather around a specific colloid charge.

An important point is that the electrostatic charge is actually reversed. If the reversal of charge phenomena were actually due to the insolubilization of a salt as proposed by Bungenberg de Jong [1], there would be no reversal of charge. Rather, the electrostatic charge would at most be reduced to zero. The experimental fact that the charge can be reversed is therefore in agreement with the explanation of the above ionic sequences.

The above conclusions can also be applied to the divalent cations as shown in Table 1. Thus the sequence is changed from basic to acidic in going from negatively to positively hydrated carboxylate ions. (Compare with the solubility sequences of inorganic salts given previously [12].) In general, divalent cations are more effective in reversing the colloid charge than the monovalent cations. This is not because of the presence of A regions on divalent cations, but it is because the electrostatic (ion-ion) attraction of a divalent

cation is greater than that of a monovalent cation. Hence a divalent cation cannot escape from the vicinity of the colloid charge as readily as a monovalent cation.

Reversal of Charge Phenomenon on Proteins. In Fig. 22 of Bungenberg de Jong's review [1, p. 299], it is seen that the ability of an anion to change the electrostatic charge of a protein varies with the type of protein. Thus casein binds I^-, Br^-, and Cl^- more strongly than gelatin, and gelatin binds anions more strongly than clupein. The entire sequence ($I^- > Br^- > Cl^-$) is shifted. Moreover, the ratio between the concentrations of the ions has changed slightly. For gelatin, the ratio of the chloride concentration divided by the iodide concentration necessary for reversal of charge is $[Cl^-]/[I^-] = 2.3$, whereas the corresponding ratio for casein is $[Cl^-]/[I^-] = 2.9$. This slight change in ratio is most likely due to the more guanidinium groups in gelatin. Casein [19] has 25 arginine, 19 histidine, and 61 lysine groups, whereas gelatin [19] has 49 arginine, 5 histidine, and 32 lysine groups per 10^5 g.

Despite the larger number of argine groups, gelatin still maintains the same ionic sequence as that of casein, indicating that its guanidinium groups are possibly involved in salt bonds or are hidden in some manner. Because the larger affinity of anions for casein is for all anions despite their value of D_-, this greater retention of anions near the surface of casein must be due to localized concentrations of lysine (ϵ-amino) or histidine groups. In other words, it cannot be due to the larger number of such groups on the casein. Such an increase in localized concentration of positive charges does not allow an anion to escape as readily. A large number of positive charges located in certain specific areas rather than located statistically on the surface would serve as chelating agents for any approaching or nearby anion. Therefore, casein would appear to have better anionic chelating properties than gelatin and gelatin more than clupein.

ION BINDING TO POLYMERS

The reversal of charge phenomenon can be considered an "ion-binding" phenomenon, and consequently the conclusions given above are applicable to more general ion-binding studies. Therefore, ion-binding studies involve two different phenomena: The counterions may be associated with the charged groups on the polyelectrolyte or they may have become insolubilized (formation of ion-ion complexes) with the charged groups. As we have seen above, the greater the solubility of a counterion, the greater will be its ability to associate with a charged group. By "ability" is meant that there will be a greater number of counterions participating and hence there

will be a greater reversal of charge effect. Insolubilization of a counterion with a charged group on a polyelectrolyte is not permanent. Rather, as stated above, it is only a time factor, since the insoluble salt is still in dynamic equilibrium with the medium.

The above conclusion that ion binding can be an association of a counterion with a polyelectrolyte is in agreement with the conclusions made by Nagasawa and Holtzer [20] as well as with the above concept of an "inner sphere [9]". They state that the ion-binding studies are caused by a change in the activity coefficient of the ion. More recently Manning and Zimm [21] have stated that the byion, the ion whose charge has the same sign as that of the polyelectrolyte, has an acitivity coefficient equal to about unity. The byion is strongly repelled by the polyelectrolyte and is thus far out in the solution. On the other hand, the counterion is closely associated with the polyelectrolyte and its activity coefficient is therefore quite small. Consequently, a counterion may appear to be bound to a polyelectrolyte by ion-ion complexes, but in reality it is only associated with its charged groups. These interactions between the counterion and the polyelectrolyte charge involve the effective dielectric constant of the B region of an ion. Consequently, the interactions are dipole-dipole interactions [12] in most cases rather than ion-ion pair formations as postulated by Szwarc [22].

The results on ion binding depend on whether the sample has or has not been dialyzed prior to physical measurement. This point will be discussed more fully below. But first, let us examine all studies where dialysis was not used prior to physical measurements. Scatchard and co-workers [3, 4] have examined the binding of ions to bovine serum mercaptalbumin (BMA) or bovine serum albumin (BSA) by measuring the conductivity of a salt solution before and after addition of the protein. Since BMA or BSA contains more $-NH_3^+$ groups than guanidinium groups [19], it can be concluded that any "binding" studies are the result of an interacting with $-NH_3^+$ groups. Now the "binding" sequence $Cl^- < Br^- < I^- < SCN^-$ is obtained for BSA and BMA [3, 4]. That is, the SCN^- ion "binds" more strongly than any of the other anions. But according to Table 2 and the above discussion, this sequence for the $-NH_3^+$ group shows that the "binding" studies are not a result of insolubilization of the counterion with the $-NH_3^+$ group. Rather, the I^- and SCN^- ions, which "bind" the strongest, have the greatest solubility. Consequently, these "binding" studies examined by Scatchard et al. [3, 4] involve a reversal of charge phenomenon, i.e., an ion association rather than an ion-insolubilization phenomenon.

This conclusion can be made clear by examining the experimental method used by them in determining the degree of ion "binding". The experiments of Scatchard et al. [3, 4] were carried out in the following manner. Two compartments were separated by an anion-exchange membrane. This membrane only allowed the pas-

sage of anions. Consequently, in such experiments if one side of the membrane is deficient in anions, there will be a difference in electrical potential. In the experiment, salt-free BMA was added to one side. Both sides initially had the same amount of salt. The addition of BMA now produces a potential because it "binds" or immobilizes some of the anions.

What actually has happened is that the anions have become associated with the BMA in the same manner as the reversal of charge phenomenon. The greater the solubility of the anion with the colloid groups, the greater will be its degree of association as explained above. In terms of activity coefficients, the activity coefficient of the more soluble counterion will be lowest because more of its ions can become associated with the charged groups. Consequently, the "binding" sequence $Cl^- < Br^- < I^- < SCN^-$ is obtained [3-6].

This binding sequence is the same as that obtained for the reversal of charge phenomenon for casein, gelatin, and clupein (Table 1). In the reversal of charge phenomenon, a greater concentration of Cl^- ions are required to reverse the charge because not as many Cl^- ions can become associated with the protein. That is, the Cl^- ion is more insoluble. In the binding studies of Scatchard et al [3-6], the Cl^- ion is again less soluble. Hence less Cl^- ions than SCN^- ions are associated with the BMA. Consequently, in both binding and reversal of charge studies, the greater the solubility of an anion with the charged group, the greater will be the association of the anion.

The anionic sequence obtained by Kronman and Foster [23] for changing the specific rotation or sedimentation value of BSA at low pH values is the same as the anion-binding sequence. The SCN^- ion decreases the rotation more than the Cl^- ion because of a decrease in electrostatic repulsion between charged groups. That is, the SCN^- will be associated more with BSA than the Cl^- ion. Thus with SCN^- ions there will be a greater increase in counterion concentration in the vicinity of the charged groups. This increase in counterion concentration will reduce electrostatic repulsion and thus reduce the swelling of the BSA. The change in sedimentation values is also in accord with this mechanism. Thus, contrary to the viewpoint of Kronman and Foster [23], the anionic effect is not one of "binding" or the formation of an insoluble ion-ion complex between the polymer charge and its counterion, but rather the anionic effect is the reversal of charge or ion association effect.

Similar reasoning can be applied to other anionic sequences such as that observed by Pedersen [24]. However, the cationic sequence obtained by Pedersen at pH 2.0 cannot be a reversal of charge effect because essentially all negative charges (carboxylate ions) had been neutralized. At pH 2.0 Pedersen [24] observed that the sedimentation constants decreased according to the sequence

$Li^+ > Na^+ > K^+ > Rb^+ > Cs^+$, which is the acidic sequence. In other words, the $s_{20,w}$ value of BSA is greater in 0.2 M LiBr than in 0.2 M CsBr at pH 2.0. Because all the carboxylate groups have been titrated, the acidic sequence must be due to the salting-out of the neutral —COOH group [25]. Examination of Table I of Ref. [25] shows that the same sequence observed by Pedersen [24] is observed in the salting-out of o-phthalic acid. Thus by salting-out the —COOH group, the BSA is forced to contract upon itself. Consequently, for LiBr solutions there is an increase in $s_{20,w}$ over that observed in CsBr solutions.

EFFECT OF DIALYSIS

If a polymer solution is first dialyzed before physical measurements are made, the reverse ionic sequence may be obtained. In the reversal of charge phenomena, the colloid was not dialyzed against the medium. Consequently, for the carboxylate polymer pectinate (Table 1) the observed acidic sequence means that the mobility of the polyelectrolyte is greater in the presence of Li^+ ions than in the presence of Cs^+ ions. On the other hand, Strauss et al. [26, 27] dialyzed extensively before measuring the mobility of polyphosphate in various salt solutions. In their acidic sequence, the mobility of $(PO_3^-)_n$ is slower in the presence of Li^+ ions than in the presence of Cs^+ ions. This is exactly the reverse of the mobilities obtained without dialysis.

The explanation of the above results can be made by employing the Donnan equilibrium effect. Because the Li^+ ion has the least solubility, the smallest electrostatic charge on $(PO_3^-)_n$ will be obtained in Li^+ solutions. The average electrostatic charge of the polyelectrolyte will therefore increase according to the sequence $Li^+ < Na^+ < K^+ < Rb^+ < Cs^+$, because this is the solubility sequence of the unaltered phosphate ion (Table 3). The Donnan equilibrium effect will therefore be least with the Li^+ solution, because this charge involves ion-ion interactions rather than the reversal of charge phenomenon. Consequently, after extensive dialysis the concentration of salt in all samples will be lower inside than outside the dialysis bag. However, the concentration of salt in the dialysis bag will be greatest in the Li^+ solution with respect to all the other solutions because of the lower electrostatic charge of the polymer. Thus in the studies of Strauss and Bluestone [27], the mobility of the $(PO_3^-)_n$ was being compared in solutions having different ionic strengths. The solution with the greatest ionic strength (the Li^+ solution) will have the lowest mobility because the greater the concentration of the Li^+ ion, the greater will be the reversal of charge phenomena. In other words, the greater the concentration of salt, the greater will be the number of Li^+ ions associated with the poly-

electrolyte. Consequently, the mobility sequence obtained after dialysis of the $(PO_3^-)_n$ is in agreement with the solubility sequence of the phosphate group because of the Donnan equilibrium effect.

The above conclusions can be expressed mathematically in the following manner. Consider the extensive dialysis of an aqueous polymer solution containing m moles/liter of M_ZP against a salt solution containing n moles/liter of LiBr or n moles/liter of CsBr. Here M_ZP for complete dissociation gives $M_ZP \rightarrow ZM^+ + P^{-z}$. After extensive dialysis has been completed, the molarity of the dialysate (solution with polymer) is the same (n) as the original salt solution before dialysis. However, during the extensive dialysis, the polymer solution has picked up a certain amount of salt, so that the chemical potentials on both sides of the dialysis bag are equal. Thus

$$\mu_{LiBr(1)} = \mu_{LiBr(2)} \qquad \text{and} \qquad \mu_{CsBr(1')} = \mu_{CsBr(2')}$$

where (1) and (1′) refer to the polymer-free salt solution and (2) and (2′) refer to the solution inside the dialysis bag. In terms of the chemical potential of the ions;

$$\mu^0_{Li} + RT \ln a_{Li(1)} + \mu^0_{Br} + RT \ln a_{Br(1)}$$
$$= \mu^0_{Li} + RT \ln a_{Li(2)} + \mu^0_{Br} + RT \ln a_{Br(2)} \qquad (1)$$

Thus we have for either experiment,

$$a_{Li^+(1)}\, a_{Br-(1)} = a_{Li^+(2)}\, a_{Br-(2)} \qquad (2a)$$

and

$$a_{Cs^+(1')} a_{Br-(1')} = a_{Cs^+(2')} a_{Br-(2')} \qquad (2b)$$

The activity coefficients of the anions and cations in the dialysate and of the byion can be considered as being equal. Thus $\gamma_{Li^+(1)} = \gamma_{Br-(1)} = \gamma_{Br-(2)}$ and $\gamma_{Cs^+(1')} = \gamma_{Br-(1')} = \gamma_{Br-(2')}$. Equations (2a) and (2b) therefore become, in terms of molar concentrations (C),

$$C_{Li^+(1)}\, C_{Br-(1)} = C_{Li^+(2)}\, C_{Br-(2)}\, (\gamma_{Li^+(2)}/\gamma_{Br-(1)}) \qquad (3a)$$

and

$$C_{Cs^+(1')}\, C_{Br-(1')} = C_{Cs^+(2')} C_{Br-(2')} (\gamma_{Cs^+(2')}/\gamma_{Br-(1')}) \qquad (3b)$$

But because of extensive dialysis the concentration of LiBr in (1) is the same as the concentration of CsBr in (1′) or $C_{Li^+(1)}\, C_{Br-(1)} =$

$C_{Cs^+(1')}\ C_{Br(1')}$. Furthermore, $\gamma_{Br-(1)}$ should be equal $\gamma_{Br-(1')}$. Equating Eqs. (3a) and (3b) gives

$$C_{Li^+(2)}\gamma_{Li^+(2)}C_{Br(2)} = C_{Cs^+(2')}\gamma_{Cs+(2')}C_{Br-(2')} \tag{4}$$

Consider now the ionization of the polyelectrolyte M_ZP. Because the charged groups are relatively close together, the solution in the domain of the polyelectrolyte will be the same as a highly concentrated salt solution. Therefore, all the groups will not be ionized. For the LiBr solution we have

$$(Li)_Z P \rightleftharpoons (Z\alpha)\,Li^+ + (Li)_{(Z-Z\alpha)}P^{-Z\alpha} \tag{5a}$$

and for the CsBr solution we have

$$(Cs)_Z P \rightleftharpoons (Z\alpha')Cs^+ + (Cs)_{(Z-Z\alpha')}\,P^{-Z\alpha'} \tag{5b}$$

where α and α' represent different degrees of ionization. The concentration of Li^+ or Cs^+ ions in (2) or (2') is equal to the concentration of cations from the ionized polymer plus the concentration of salt which has dialyzed into the bag. Thus $C_{Li^+(2)} = (Z\alpha)C_p + C_{Br^-(2)}$ and $C_{Cs+(2)} = (Z\alpha')C_p + C_{Br^-(2)}$, where C_p is the molar concentration of polymer. Substitution of these values into Eq. (4) gives

$$\gamma_{Li^+(2)}[(Z\alpha)\,Cp + C_{Br-(2')}]\,C_{Br-(2)}$$

$$= \gamma_{Cs^+(2')}[(Z\alpha')\,Cp + C_{Br-(2')}]\,C_{Br-(2')} \tag{6}$$

For dilute salt solutions $(Z\alpha)C_pC_{Br-(2)} \gg C^2_{Br-(2)}$ and $(Z\alpha')C_pC_{Br-(2')} \gg C^2_{Br-(2')}$. Consequently, Eq. (6) can be approximated as

$$C_{Br-(2)}/C_{Br-(2')} = [\gamma_{Cs^+(2')}/\gamma_{Li^+(2)}]\,(\alpha'/\alpha) \tag{7}$$

If $\gamma_{Cs^+(2')} = \gamma_{Li^+(2)}$, then Eq. (7) becomes

$$C_{Br-(2)}/C_{Br-(2')} = \alpha'/\alpha \tag{8}$$

The degree of ionization of the polymer in the LiBr solution will be less than that in the CsBr solution. Thus $\alpha < \alpha'$ or $\alpha'/\alpha > 1$. Consequently, the concentration of LiBr in the presence of polymer will be greater than the respective concentration of CsBr because of the Donnan equilibrium effect. The activity coefficient of the Cs^+ ion may be less than that of the Li^+ ion because more Cs^+ ions can associate with the polyelectrolyte ($\gamma_{Cs^+(2')} < \gamma_{Li^+(2)}$ or

$[\gamma_{Cs^+(2')}/\gamma_{Li^+(2)}] < 1$). Consequently, the activity coefficients will destroy part of the Donnan equilibrium effect [Eq. (7)]. However, from the experimental results it is apparent that this effect is not great. Consequently, the salt concentration will be greater in the LiBr solution than in the CsBr solution. Hence the agreement between solubility and binding as observed by Strauss and Bluestone [27] can be explained by the Donnan effect.

In conclusion it can be stated that the ion-binding and reversal of charge phenomena are merely effects which can be attributed to electrostatic attraction of the polyelectrolyte to its counterion. The greater the solubility of the counterion and the charged group, the greater will be the number of counterions which can become associated with that particular group. Thus the greater will be the reduction in the apparent charge of the polyelectrolyte. The association will be stronger if there are a number of charged groups on the polymer which are positioned relatively close together. Such groups can act as chelating agents for the counterion as described above. Consequently, the "strong-binding" sites of Scatchard et al. [3-6] and others are therefore only such chelation effects.

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