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The Precipitation of Polyanions by Long-Chain Aliphatic Ammonium Salts

5. THE INFLUENCE ON PRECIPITATION OF CHANGES IN THE AMINE STRUCTURE*

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Several authors have shown that many polyanions are precipitated from aqueous solution by organic cations containing a long chain of CH₂ units, e.g. cetylpyridinium and cetyltrimethylammonium (for a review see Scott, 1960). Polyanions that take part in this reaction include polyacrylic acid, negatively charged proteins, polyuronides, sulphated polysaccharides, polyphosphates, nucleic acids and borate complexes of neutral polysaccharides. It was concluded (Scott, 1956, 1960) that the reaction is probably general for all water-soluble polyanions.

Scott (1955a) demonstrated that the insoluble complexes were salts of the polyanion and the aliphatic ammonium cation. The stoichiometric combination of, e.g., cetylpyridinium with various polyanions was shown to be of use in the estimation of their acidic groups (Scott, 1955a). The complexes are very insoluble (less than 1 mg./100 ml.) and small amounts of polyanion may be precipitated quantitatively from dilute solutions: a useful step in the isolation of polyanions from biological

materials, since they can be easily recovered from the complex (Scott, 1960).

The mechanism of the precipitation reaction has been discussed (Scott, 1956) and it was suggested that there were two alternatives, depending on whether the quaternary ammonium ion was considered to react as micellar aggregates or as single ions. The available evidence was insufficient to allow a conclusion to be drawn. The emphasis in this paper is on the influence on the reaction of changes in the structure of the amine. As a result of the investigation it is possible to propose a mechanism of precipitation. Apart from the intrinsic interest of the reaction, it is desirable that it should be better understood so that improvements in the practical application may be made. A preliminary report of some of the results has been given (Scott, 1961).

MATERIALS AND METHODS

Abbreviations. R⁺, Organic cation; M⁺, inorganic cation; p^{z-}, polyanion with valency z.

Aliphatic ammonium salts

Unbranched primary amines with chain lengths C₈–C₁₈ were obtained from L. Light and Co. Ltd. They were converted into the hydrochloride without further purification.

* Part 4: Antonopoulos, Borelius, Gardell, Hamnström & Scott (1961).

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Table 1. *Precipitation of heparin and hyaluronate by primary amine hydrochlorides $C_nH_{2n+1}NH_3Cl$*

Heparin and hyaluronate in aqueous solution were titrated with solutions of primary ammonium salt to the flocculation end point (see Materials and Methods section). The values marked * are of low precision because of the small quantities of titrant added (0.02 ml.), and are upper limits.

Sodium heparinate	C_n					
	C_6		C_8		C_{10}	
Titration volume at the end point	0.68	3.3	0.52	2.58	0.52	2.53
Normality of heparin at the end point	0.0076	0.00158	0.01	0.002	0.0098	0.0025
Normality of amine at the end point	0.265	0.245	0.031	0.029	0.019	0.005
(Normality of amine) - (normality of heparin) = excess amine concentration	0.257	0.244	0.030*	0.027	0.009*	0.0025

Sodium hyaluronate	C_n							
	C_8		C_{10}		C_{12}		C_{14}	
Titration volume at the end point	0.6	2.8	0.52	2.58	0.54	2.58	0.56	2.56
Normality of hyaluronate at the end point	0.004	0.00071	0.0048	0.00097	0.0047	0.00097	0.0045	0.00098
Normality of amine at the end point	0.166	0.18	0.020	0.0165	0.0072	0.004	0.0050	0.00122
(Normality of amine) - (normality of hyaluronate) = excess amine concentration	0.162	0.18	0.015*	0.0155	0.0025	0.003	0.0005	0.0002

C_6 , C_8 , C_9 and C_{10} amines were titrated directly with 2N-HCl in water, by using a Cambridge pH meter, to a pH between 6.3 and 6.8. Amines with C_{12} , C_{14} and C_{16} chains were dissolved in ether, and HCl gas from a generator was bubbled through the solution. The solid hydrochloride separated out as a white deposit, which was washed with ether and recrystallized from water. Secondary amines, di-*n*-propylamine, di-*n*-amylamine and di-*n*-hexylamine were also obtained from L. Light and Co. Ltd. They were titrated in aqueous solution with 2N-HCl to pH 6.5.

The quaternary alkyltrimethylammonium salts were gifts from Imperial Chemical Industries Ltd. and Armour Hess Chemicals Ltd., 6 Arlington St., London, S.W. 1. C_8 , C_{10} , C_{12} and C_{14} were from the Imperial Chemical Industries Ltd. specimen collection, and were obtained as bromides. C_{16} and C_{18} were Arquad preparations from Armour Hess Chemicals Ltd. and were obtained as chlorides. All the compounds were used without further purification.

Didecyldimethylammonium bromide was obtained from the British Hydrological Corp., Colloidal Works, High Path, Merton, London, S.W. 19.

Stock solutions were prepared in water and were analysed for N content by the Kjeldahl method, performed according to Fawcett (1954) except that in general a digestion time of 1 hr. was used. Concentrations calculated from the N content were used as the basis for all calculations.

Polyanions

Hyaluronic acid. This was prepared from the exudate of a human myxoma according to the method of Scott (1960).

Heparin. This was commercial, 100 units/mg., obtained as an aqueous solution (50 mg./ml.) from Paines and Byrne Ltd., Pabyrn Laboratories, Greenford, Middlesex.

Keratosulphate. This was from nucleus pulposus, a gift of Dr Sven Gardell.

Sodium alginate (B.P.C.) and pectin (apple 200 grade). These were commercial samples from British Drug Houses Ltd., used without further purification. The pectin contained 8% methoxyl and 20% moisture.

All the above polyanions were sodium salts.

Titration-precipitation

Aqueous solutions of the polyanion containing 0.5–3 mg./ml. were incubated at 30° in a water bath in 5 ml. tubes. The aliphatic ammonium salt solution was added dropwise from a microburette, and mixed with the polyanion solution by gently rotating the tube. The 'end point' occurred when all the polyanion-organic cation complex had flocculated, leaving a clear solution between the particles (or, rarely, when there was no further increase in turbidity with addition of amine solution). Each titration was performed at least twice. The concentrations of the amine solutions varied from 1M for hexylammonium chloride to 0.01M for tetradecylammonium chloride, with the general aim of preventing too great a variation in titration volume throughout the series.

RESULTS

Titration-precipitation of polyanions

The precipitate of each polyanion forms in the presence of decreasing amounts of aliphatic ammonium salt as the chain length in the latter increases (Table 1). When a paraffin chain with more than 12 or 14 CH_2 units is involved, precipitation occurs when there is practically a one-to-one ratio of aliphatic ammonium cations with the anionic groups of the polymer. That is, the quantity of aliphatic ammonium salt required to produce a flocculent precipitate depends on the total quantity

of polyanion in the solution. The situation is quite different when the chain is of six CH_2 units. The formation of a flocculent precipitate then depends on the concentration of the amine cation, which is much higher (about 10^3 times) than that of the polyanion. There is no equivalence between polyanion and amine. If identical amounts of polyanion are titrated in different volumes of solution, the quantity of hexylammonium (C_6) ion needed to produce a flocculent precipitate is proportional to the total volume. The behaviour of amines with single chains of 8, 10 or 12 CH_2 units is intermediate between the two extremes, with a steady and marked trend towards stoichiometric combination as the carbon chain is lengthened. These points are illustrated in Table 1, which gives results of typical experiments involving heparin and hyaluronate.

If the amine cation is assumed to combine stoichiometrically with the polyanion in the precipitate at the end point, throughout the series C_6 – C_{16} (which is known to be the case, e.g. with cetylpyridinium), the quantity combined can be subtracted from the total amine in the solution to give the concentration of free amine, which will be called the excess amine concentration. It is expressed in normality and is nearly independent of polyanion concentration in these experiments. For the purpose of the calculation the polyanions were assumed to possess the structures generally attributed to them (e.g. as shown in Whistler & Smart, 1953).

Table 2 shows values of the excess amine concentrations, for a series of aliphatic ammonium ions with one or two paraffin chains, in the presence of several polyanions. With heparin and alginic acid

the values for amines with more than 10 or 12 CH_2 units became so small as to be not measurable.

Fig. 1 shows that the plot of the logarithm of the excess amine concentration against chain length (C_n) of the amine is a straight line, for all the polyanions tested in this way.

In a similar series of experiments with unneutralized polyacrylic acid as the sole polyanion, Saito (1955) showed that the plot of the log of the total amine concentration against n was a straight line, in the region of shorter chain lengths, but that the slope became very much less as the chain length increased to C_{12} and above. This is mainly because the total amine concentration at flocculation point is partly determined by the polyanion concentration and is much less a property of the amine itself. When Saito's results are recalculated to give excess amine concentrations, the line is straight at all chain lengths, as shown in Fig. 1.

The difference between the excess amine concentrations of primary and quaternary amines with the same paraffin chain is very considerable. It was observed that the precipitates obtained with the primary amines were almost always clean and flocculent, with a clear end point, whereas the quaternary amines more frequently gave a slightly diffuse end point, especially when the chain length was C_{12} or less. Keratosulphate, which is not easily titrated by quaternary amines (e.g. cetylpyridinium) because it gives a diffuse end point, and also because the precipitate may redissolve in the presence of a large excess of quaternary amine, was easily titratable with the longer-chain primary amines, such as tetradecylammonium chloride, to give a very good flocculent precipitate. There was

Table 2. *Excess amine concentrations of aliphatic ammonium salts in the presence of several polyanions*

The excess amine concentrations, expressed in normalities, of amine salts with aliphatic chains R, are in each case averages of several experiments of the type presented in Table 1. Where the appropriate value is not entered, it was not measured. Excess amine concentrations are included for keratosulphate, although it is not a well-characterized polysaccharide, because of its interesting behaviour in these systems (see the Results section).

		R							
	Precipitant	C_3H_7	C_5H_{11}	C_6H_{13}	C_8H_{17}	C_9H_{19}	$\text{C}_{10}\text{H}_{21}$	$\text{C}_{12}\text{H}_{25}$	$\text{C}_{14}\text{H}_{29}$
Sodium pectinate	RNH_3^+	—	—	—	0.25	—	0.036	0.006	—
Sodium hyaluronate	RNH_3^+	—	—	1.0	0.15	—	0.015	0.002	0.0002
	RNMe_3^+	—	—	—	—	—	0.3	0.016	0.005
	R_2NH_2^+	> 0.75	> 0.2	> 0.1	—	—	—	—	—
Sodium alginate	RNH_3^+	—	—	—	0.036	—	0.004	—	—
Sodium heparinate	RNH_3^+	—	—	0.25	0.028	0.005	0.003	—	—
	R_2NH_2^+	> 0.8	> 0.2	Turbidity at 0.125	—	—	—	—	—
	RNMe_3^+	—	—	—	0.27	—	0.04	0.0055	—
Sodium kerato-sulphate	RNH Me_2^+	—	—	—	—	—	0.022	—	—
	RNH_3^+	—	—	0.5	0.149	0.07	0.04	—	—
	Didecyl dimethyl-ammonium bromide	—	—	—	—	—	0.001	—	—

Fig. 1. Excess amine concentrations (EAC) and critical micellar concentrations (CMC) of normal aliphatic amines plotted logarithmically against the number of CH_2 units in the aliphatic paraffin chain. —, Excess amine concentrations, which are of primary amines only; ---, critical micellar concentrations; $\blacktriangle$, sodium pectinate; +, sodium hyaluronate; $\bullet$, sodium heparinate; $\circ$, polyacrylic acid; $\square$, primary aliphatic ammonium chlorides in water; Δ , quaternary trimethylammonium bromides in concentrated sodium chloride solution. The points on the polyacrylic acid line are calculated from the data of Saito (1955). The CMCs in sodium chloride solution are based on data of Corrin & Harkins (1947), and of Phillips (1955). The CMCs in water are according to Kleven (1948).

no apparent tendency for the precipitate to redissolve in the presence of excess of tetradecylammonium chloride. Keratosulphate is also precipitated cleanly by didecyldimethylammonium bromide.

DISCUSSION

Precipitation by micelles versus precipitation by single ions

The consideration of the mechanism of the precipitation of polyanions by organic cations in the absence of added electrolyte is complicated by the fact that amphipathic organic cations can react in two different states, as single ions or as micelles, and both are often present in solution together. It is possible to explain many of the experimental observations by postulating that either single ions or micelles are involved, to the exclusion of the other, and there is a third possibility, which cannot be totally excluded, that both may be involved simultaneously.

It was suggested by Teryama (1955) that the precipitate that he obtained on mixing equivalent amounts of cellulose sulphate and cetyltrimethylammonium bromide was an example of combination between oppositely charged colloidal particles, such as he had observed between the polycation chitosan and several polyanions. The positively charged micelle of cetyltrimethylammonium bromide was thought to be analogous to the positively charged polymer of chitosan. Scott (1955*b*) suggested that single amphipathic ions might be the precipitant, and later concluded (Scott, 1956) that polyanions could be precipitated by quaternary ammonium ions at concentrations so small that micelles were unlikely to be present.

In the present paper it is shown that polyanions are precipitated by organic cations that are not normally regarded as forming micelles, e.g. hexylammonium, and by octylammonium and decylammonium at concentrations far below those in which micelles are known to be present.

If the precipitation of polyanions depends on the existence of micelles, a given organic cation should not be capable of precipitating polyanions until it is above the concentration at which micelles are formed (the 'critical micellar concentration'). This implies that the excess amine concentration of an organic cation should be constant, irrespective of the nature of the polyanion. Table 1 shows that this is not so: there is a very marked dependence of this value on the structure of the polyanion.

Further, if micelles of organic cation are the effective precipitants, the graph of the log of the excess amine concentration against n , the number of carbon atoms in the organic cation aliphatic chain, should be parallel to, if not coincident with, the graph of log of critical micellar concentration against n . Fig. 1 shows that the two lines are widely separated and of quite different slope.

It is therefore concluded that micelles do not participate in the precipitation reaction in the absence of added electrolyte. It remains to be shown that the experimental observations can be explained on the basis of precipitation by single ions.

Flocculation end point

The occurrence of a flocculation end point may be accounted for by applying the Law of Mass Action to the equation

which gives

$$\frac{[P^z - zR^+]}{[P^z - zM^+]} = K \frac{[R^+]^z}{[M^+]^z}. \quad (2)$$

The left-hand expression will be equated with the degree of precipitation, D . If arbitrary values of K are chosen (the precise values are immaterial) and

the ratio $[R^+]/[M^+]$ is calculated for 1, 50 and 99 % precipitation, for even quite small values of z (≥ 10 –20), D increases from very small values to almost 100 % for a very small change in the value of $[R^+]/[M^+]$. On either side of a very small range of values of $[R^+]/[M^+]$, the polyanion exists almost entirely as $P^{z-}zR^+$ or $P^{z-}zR^+$. The flocculation end point, which is sharp, is the visible manifestation of the sudden appearance of $P^{z-}zR^+$ in large quantity.

Equation (2) predicts an influence of molecular weight on precipitation by organic cations, since z is directly proportional to the molecular weight, if P^{z-} is a homogeneous polymer.

Free energy of formation of $P^{z-}zR^+$

The relationship

$$\Delta F = -RT \ln K \quad (3)$$

may be applied to equation (1) where K is the equilibrium constant and F is the free-energy change involved. Certain assumptions are made, which are mentioned in this discussion.

From (2), ignoring activity coefficients:

$$K = \frac{[P^{z-}zR^+][M^+]^z}{[P^{z-}zM^+][R^+]^z} = D \frac{[M^+]^z}{[R^+]^z} \quad (4)$$

If two organic cations, R_1^+ and R_2^+ , are chosen:

$$\frac{K_{R_1^+}}{D_1} = \frac{[M^+]_1^z}{[R_1^+]^z} \quad (5)$$

and

$$\frac{K_{R_2^+}}{D_2} = \frac{[M^+]_2^z}{[R_2^+]^z} \quad (6)$$

From (5) and (6), if $D_1 = D_2$, the degree of precipitation at the flocculation point:

$$\frac{K_{R_1^+}}{K_{R_2^+}} = \frac{[M^+]_1^z [R_1^+]^z}{[M^+]_2^z [R_2^+]^z} \quad (7)$$

Substituting from (3), and if $[M^+]_1 = [M^+]_2$:

$$\frac{\Delta F_{R_1^+} - \Delta F_{R_2^+}}{RT} = z \ln \frac{[R_2^+]}{[R_1^+]}. \quad (8)$$

$[R_1^+]$ and $[R_2^+]$ are equivalent to the two excess amine concentrations (EAC_1 and EAC_2) at flocculation point, therefore:

$$\Delta F_{R_1^+} - \Delta F_{R_2^+} = zRT \ln \frac{EAC_1}{EAC_2} \quad (9)$$

The ratio of the two excess amine concentrations is approximately 10 (Table 1). $\Delta F_{R_1^+} - \Delta F_{R_2^+}$ is the free-energy change associated with the transfer of $2z$ CH_2 units from water to the complex, and therefore the average value of the free-energy change associated with two CH_2 units is:

$$\frac{\Delta F_{R_1^+} - \Delta F_{R_2^+}}{z} = 2.3 RT.$$

This is in approximate agreement with the value of $2.7 RT$ obtained from the transfer of two CH_2

units from water to oil and in good agreement with $2.4 RT$, the value obtained by Phillips (1955) for the transfer of two CH_2 units from water to a detergent micelle.

It is an approximate value since, although the volumes of titration at flocculation point were generally not very different from R_1^+ to R_2^+ (by taking more concentrated solutions of organic cation as the excess amine concentration increased), $[M^+]_1$ was seldom exactly the same as $[M^+]_2$. It also assumes that D is the same at the flocculation point for different organic cations.

Conversely, if the 'oily' nature of the polyanion-organic cation complex is assumed, then

$$\Delta F_{R_1^+} - \Delta F_{R_2^+}$$

would be expected to be constant, whereupon (9) becomes

$$\ln \frac{EAC_1}{EAC_2} = \text{a constant},$$

which is equivalent to the straight-line relationship shown in Fig. 1.

This argument predicts the constancy of the excess amine concentration of a polyanion if $[M^+]$ is constant, at any given ratio of $[P^{z-}zR^+]/[P^{z-}zM^+]$, i.e. at a given percentage precipitation. In these circumstances the excess amine concentration is a constant of the polyanion, and could in theory be used in the characterization or isolation of the polyanion.

Electrostatic factor in the formation of $P^{z-}zR^+$

The formation of detergent micelles in solution depends on a balance between two opposing factors: (1) the alignment together of hydrocarbon chains, in order to minimize their area of contact with water; (2) the repulsion between cationic groups at the end of the hydrocarbon chains. The repulsive force is considerably lessened if the cationic groups are screened from each other by a high electrolyte concentration, and it has been experimentally observed (Corrin & Harkins, 1947) that the critical micellar concentration is much lower in salt solutions than in water. The slope of log critical micellar concentration against n , the number of CH_2 units in the hydrocarbon chain, has been given as 0.5 in concentrated electrolyte solution by Phillips (1955) based on the data of Taylor, as compared with 0.3 in water. A line has been plotted in Fig. 1, with the value for decyltrimethylammonium bromide (Corrin & Harkins, 1947) in concentrated sodium chloride solution as the fixed point for a line of slope 0.5. A second line based on the findings of Kleven (1948) for primary amine hydrochlorides in water is also plotted, showing a much smaller slope. The line of log excess amine concentration has about the same slope as the line of log critical micellar concentration in concen-

trated electrolyte solution. The screening action of the concentration of negative charges on the polyanion is analogous to the screening effect of a high electrolyte concentration. The concentration of negative charge of a polyanion is, however, much higher than that of almost any attainable electrolyte solution, and the screening action of a polyanion such as heparin is probably equivalent to that of a 'swamping excess' of electrolyte, i.e. the maximal effect is being exerted. The slope of log excess amine concentration should be dependent on charge density, which determines the screening power. There is a suggestion that the slope of the pectin line is less than that of the heparin line (Fig. 1), corresponding to a decrease in the screening action. The slope of log excess amine concentration for polyacrylic acid is only slightly greater than that for log critical micellar concentration in water, presumably reflecting the very low charge density, and therefore low screening capacity, of unneutralized polyacrylic acid. The generally low values of the excess amine concentrations with polyacrylic acid, as compared with carbohydrate polyanions, are probably due to the fact that organic cations can cohere to the main polymer chain of polyacrylic acid, which is of a hydrocarbon nature, as well as to each other. The hydrophilic carbohydrate polymers do not offer comparable absorption sites.

Qualitative hypothesis of the precipitation process

The implications of the single-ion mechanism can be qualitatively visualized by considering the sequence of events during the titration of a polyanion in aqueous solution with an organic cation (Scott, 1960). In the initial stages the organic cation is distributed throughout the solution, some of it being associated with the anionic groups of the polymer. The percentage of organic cation bound to the polyanion is determined by the affinity of the cation for the anionic groups. With increasing concentrations of organic cation, more is associated with the polyanion until 'bound' organic cation is likely to find itself close to another organic cation, similarly bound. The paraffin chains of both ions can then lie together or 'coalesce' with the liberation of free energy, which results in a big apparent increase in affinity of the organic cation for the polyanion. The apparent affinity of organic cation for the polyanion added at this point is greater than the affinity of organic cation at the outset, when there was no possibility that this type of co-operative binding could occur. At this stage the percentage of organic cation associated with the polyanion begins to increase. The amount of overlap of the paraffin chains with each other increases as more organic cation is bound, until organic cation must be bound at sites that are surrounded

by previously bound organic cation. At this stage there is a maximum value of the affinity of organic cation for the polyanion. When all anionic sites are occupied by organic cations, the charge of the polyanion is neutralized. The solution contains polyanion-organic cation complexes, which are outwardly similar to oil droplets, with an inner core of polyanion that may be almost completely hidden from the water. It is then possible for these neutral hydrophobic particles to coalesce to form a separate phase. The structure of the neutral polyanion-organic cation complex is analogous to that of the micelle of organic cation that is present in aqueous solution. In the micelle the ionized groups are turned outwards to the water, with the hydrocarbon chains turned inwards away from the water. The polyanion-organic cation complex is a 'reversed' micelle.

The main driving force leading to the formation of the polyanion-organic cation 'reversed' micelle and the ordinary micelle is the same. The presence of a hydrocarbon chain in water breaks up the water structure, and with it many hydrogen bonds. There is therefore a tendency for the water to expel the hydrocarbon chain, or, failing this, to reduce the area of contact to a minimum. This is done if the hydrocarbon chains can be made to lie together, as in micellar structures.

The following points arise from a consideration of this mechanism:

(a) The degree of overlap between adjacent organic cations depends on the length of the paraffin chain in the cation. A very long chain will be able to 'coalesce' to a very large extent with a neighbouring, similar chain. The free energy liberated is considerable, and, therefore, the apparent affinity for the polyanion is greater than if the chain length was shorter. Thus the partition of single organic cations between the solution and polyanion is in favour of the polyanion, i.e. there is a low concentration of free (unbound) organic cation. If a shorter chain is involved, the degree of overlap is less, the affinity is therefore less, and the partition of organic cation between solution and polyanion is more in favour of the solution. This is illustrated in Table 1. The excess amine concentrations (= free organic cation) of the long-chain organic cations are much less than those of short-chain organic cations.

(b) There should be considerable overlap of adjacent bound organic cations when the anionic sites of a polymer are close together. By the reasoning of the previous paragraph, the affinity of organic cations for a polyanion of high charge density should be higher than for one of low charge density, i.e. there should be a low excess amine concentration for polyanions of high charge density. Table 1 shows that this is true. In pectin

there is approximately one anionic group per four sugar units, in hyaluronic acid one per two sugar units, in alginic acid one per one sugar unit, and in heparin one and a half (or more) anionic groups per sugar unit. The excess amine concentrations decrease in the order pectin > hyaluronate > alginate > heparin, for any particular organic cation. The result obtained by this reasoning is the same as that arrived at earlier, on the basis of the electrostatic screening action of the anionic groups of the polymer. It is because the screening power and the degree of overlap of adjacent bound organic cation are functions of the same variable (i.e. the distance between adjacent anionic groups).

(c) If two short chains, each containing n CH_2 units, are present in an organic cation, there is a smaller possibility of overlap with similar organic cations bound on adjacent sites of a polymer than if one chain of $2n$ CH_2 units is involved. It is probable that the two chains of the dialkylammonium cation lie together, producing a more globular shape, without the necessary length to overlap with adjacent organic cations. Thus the dipropylammonium has a much higher excess amine concentration than hexylammonium, and even diamylammonium is not as good a precipitant as hexylammonium (Table 2). However, when each chain in the dialkylamines is considerably increased, the total bulk of CH_2 units presumably offsets to some extent the lack of length in the paraffin chain, and overlap of the more rigid globule is possible on two sides.

(d) The organic cation–polyanion affinity is affected by the nature of the cationic group of the organic cation. It seems from the experiments in Table 1 that the primary ammonium cation has a greater affinity for anionic groups than has the quaternary ammonium ion. The increased binding of primary ammonium ions, as compared with quaternary ammonium ions, is approximately equivalent to the addition of two CH_2 units to the quaternary ammonium ion paraffin chain. The greater affinity of primary ammonium ions for anionic groups is presumably responsible for the sharper and clearer end points observed with, for example, tetradecylammonium chloride as compared with cetylpyridinium chloride.

An examination of the hypothetical structures of the precipitates produced according to either the micellar or single-ion mechanisms suggests that they are probably identical. In both cases polyanion is embedded in a matrix of paraffin chains. By the micellar mechanism the paraffin chains align together before combination with the polyanion, whereas by the single-ion mechanism alignment takes place partly during combination, and then by aggregation of the polyanion–organic cation particles. However, the process by which the end

is reached may be important in practice, and it seems unnecessary to postulate that micelles are required for the precipitation of polyanions from aqueous solution. This does not mean that precipitation by micelles is not possible, but it is suggested that precipitation by single ions is the more effective, and will usually occur before conditions for micellar precipitation are ripe. This is especially true of precipitation by primary ammonium salts.

SUMMARY

1. The precipitation of alginate, heparin, hyaluronate, keratosulphate and pectinate from aqueous solution by aliphatic ammonium ions containing paraffin chains of from $[\text{CH}_2]_6$ to $[\text{CH}_2]_{18}$, and with several types of cationic group, has been studied.
2. The efficiency of precipitation increases in a regular and marked manner with increase of chain length. This effect can be accounted for if the insoluble complexes are assumed to be 'oily' particles, and a free-energy change of about $1.2 RT$ is associated with the transfer of CH_2 from the aqueous phase to the particle.
3. The concentration of organic cation required to precipitate a polyanion depends on the structure of the polyanion. Polyanions with high charge densities are precipitated more readily than less-densely charged polyanions.
4. It is concluded that precipitation of polyanions by primary ammonium salts is unlikely to be a micellar phenomenon. The occurrence of a flocculation end point can be accounted for by applying the Law of Mass Action to the reaction equation, involving single ions.
5. A qualitative hypothesis of the reaction mechanism is proposed.

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