The solubility of cetylpyridinium complexes of biological polyanions in solution of salts

In a recent note the precipitation of acidic polysaccharides by long-chain quaternary ammonium salts was reported, and some qualitative and quantitative applications were described. The present communication deals with an extension of one of these aspects: the use of solutions of sodium sulphate to dissolve precipitated carboxylic polysaccharides selectively, leaving untouched precipitates containing sulphated polysaccharides. DUTTA, JONES AND STACEY[2] have described the different solubilities in saline of DNA and RNA complexes with "cetavlon". It seemed important to discover whether similar phenomena could be observed within each group of acidic polysaccharides, and to search for a principle underlying the solvent efficiency of any particular salt. The following experiments were performed with these ends in view.

A series of tubes each containing 0.4 mg polysaccharide and 1.0 mg (a small excess) cetylpyridinium chloride in 3.3 ml solution of varying salt concentration was kept overnight at 30° C to allow the precipitates to coagulate. After centrifugation the amount of cetylpyridinium remaining in the supernatant was estimated spectrophotometrically at 260 mμ, at which wavelength the molecular extinction is approximately 4,100. The quantity of precipitate formed can thus be followed as a function of salt concentration.

The concentration of calcium chloride required to prevent precipitation of four dextran sulphates varied from 2.8 *N* to 7.5 *N*, and seemed to depend both on degree of sulphation and molecular weight. The heparin (100 units/mg) complex did not precipitate from 3.0 *N* calcium chloride; nor did the complexes of two specimens of chondroitin sulphate from approximately 1.1 *N*.

The effect of a series of cations and anions on the solubility of the complex of one dextran sulphate was determined. The results are shown in Table I. Fig. 1 shows some of the curves which were obtained.

TABLE I

A COMPARISON OF THE SOLVENT POWER OF CATIONS AND ANIONS ON THE COMPLEX OF A DEXTRAN SULPHATE (16.6% S) WITH CETYLPYRIDINIUM

Cation (as chloride)	H^+	Li^+	Na^+	K^+	NH_4^+	$MeNH_3^+$	$Me_2NH_2^+$	Ca^{++}	Mg^{++}
Concentration (normality) required to maintain 70% of dextran sulphate in solution.	3.1	2.83	1.75	1.0	1.75	2.05	> 2.5	3.3	4.1

Anion	$(K)F^-$	$(Na)Cl^-$	$(Na)Br^-$	$(Na)NO_3^-$	$(NH_4)_2SO_4^{--}$
Concentration (normality) required to maintain 70% of dextran sulphate in solution.	> 4	1.75	1.1	1.8	3.8

Solubility curves of the precipitates of pectin, potassium hyaluronate, sodium alginate, sodium chondroitin sulphate (CSA), DNA, heparin and dextran sulphate (DS) were determined in solutions of potassium and magnesium chlorides and sodium sulphate. The results are summarised in Table II.

TABLE II

	POLYSACCHARIDE						
Salt	*Pectin*	*Hyaluronate*	*Alginate*	*D.N.A.*	*C.S.A.*	*Heparin (75 units/mg)*	*D.S.*
KCl	0.14	0.22	0.33	0.36	0.9	1.1	1.00
$MgCl_2$	0.04	0.19	0.30	0.35	1.00	1.6	4.1
Na_2SO_4	0.03	0.15	0.60	0.75	Not determined		

Normality of salt solution required to maintain 70% of polysaccharide in solution.

Certain generalisations can be made from these observations.

1. Complexes formed by polyanions with cationic detergents are soluble in salt solutions at concentrations which are characteristic and widely different, according to the structure of the polymer.

2. Complexes of carboxylic polysaccharides are soluble in much lower concentrations of all types of salt than those of the polysulphates, with DNA and RNA[2] in the intermediary range.

3. The solvent action of the anion on polysulphate complexes is inversely related to its energy of hydration; the same applies (up to a point) for the cation. Ionic strength seems to be of greater importance in the prevention of polycarboxylate and nucleic acid precipitation, especially when the density of charged groups is low, as in pectin.

4. The bond between the quaternary ammonium ion and ester sulphate groups differs considerably from that derived from carboxylate, and is probably stronger.

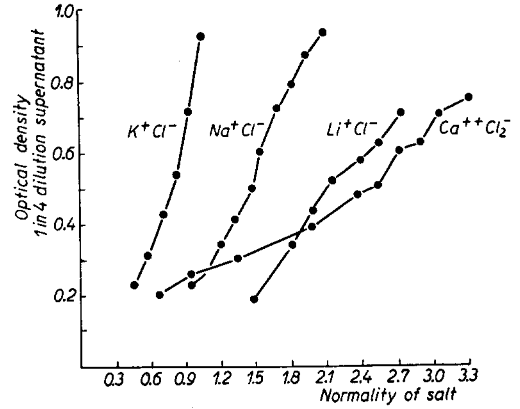

Fig. 1. The optical density was measured in 10 mm quartz cells at 260 mμ. A reading of 0.20 corresponds to complete precipitation, and 0.95 to no precipitation.

From the above and other experiments it is thought that two processes contribute to the formation of the precipitate.

1. Organic cations associate with the polyanion in solution displacing inorganic ions to some extent. When the energy of hydration of the polymer is insufficient to hold the long paraffin chains of the associated cetylpyridinium in solution, a new phase is formed.

2. Organic cations from solution exchange with inorganic ions remaining on the solid with release of energy, stabilising the precipitate.

Equilibrium in stage 1, between solid and solution phases depends on the ratio of the concentrations of quaternary ammonium and inorganic cations, their respective affinities for the anionic groups and on the hydration energy of the polymer, *i.e.* on its structure. The cation-anion affinity decreases with increasing solvation of both, *i.e.* is roughly proportional to the size, charge remaining constant (for review on ionic solvation see Ref. 3). This is probably because the strongest bond between cation and anion is an ion-pair, or a closely allied structure which can only be formed subsequent to the loss of water of solvation.

The affinity of COO^-, PO_4^-, SO_4^-, for a given cation seems to increase in that order; of cations for an anion, $Li < Na < K$; the order of increasing size. The hydrogen ion occupies a special place in such series because of its ability to form covalent bonds with anions.

The applications of these findings to biological materials, together with detailed interpretations and experimental data, will shortly be submitted for publication.

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Effect of salt concentration and pH on the rate of virus neutralization by purified fractions of specific antiserum

JERNE[1] and JERNE AND SKOVSTED[2] first reported that the rate of neutralization of bacteriophage by specific antiserum is considerably faster at low than at high salt concentration. This effect of salt concentration on the rate of virus neutralization has been confirmed by others[3,4]. The purpose of the present communication is to make a preliminary report of studies dealing