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Commentary

The Hofmeister series and ionic strength

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With reference to the Hofmeister series there appear to be many misunderstandings about the observations made by Hofmeister; i.e. what is meant by salting-out (and in), concentration and ionic strength. A clear understanding of these terms is of utmost relevance to the understanding of the effects of salts on biological systems.

The original study by Hofmeister [1] was a milestone in protein chemistry. This was a study on the solubility of hen egg-white protein (ovalbumin?) in salt solutions and he determined the concentrations of various salts required to precipitate the protein. These results he presented (Table I) in terms of normality of the salt concentration. Since then many studies have added to the list of salts and extended lists of 'salting-out' anions and cations have been composed. What can be generally deduced from Hofmeister's results is that polyvalent anions are more effective in precipitating proteins than monovalent anions and that alkali metal cations are better than ammonium.

What we must consider is that the Hofmeister series is about the 'salting-out' of proteins and pertains to salts at high concentrations. This phenomenon of 'salting-out' of proteins is an important experimental means of isolating, fractionating and crystallizing proteins. Thus it is used today in hydrophobic-interaction chromotography besides classical ammonium sulphate fractionation, and precipitation with ammonium sulphate is still the primary means of obtaining protein crystals for X-ray diffraction studies [2].

'Salting-out' can also be effected by non-salts such as polyethylene glycols, glycerol and non-denaturing alcohols such as methane pentane diol [3,4]. The studies of these indicate that salting-out agents are excluded from the hydration shell of the protein whereas salting-in agents can penetrate this shell. Observations in line with this proposition were made by Chick and Martin [5] on the salting-out of egg albumin with ammonium sulphate. However salting-out may even be more complex than this, since high concentrations of salts probably change the structure of water and the partial molar volumes of salts change with concentration.

At low concentrations of salts, the solubility of proteins typically increases and we have the phenomenon of 'salting-in'. This was originally described by Mellanby [6]. His interpretation of the results obtained from the effects of various salts on the solubility of horse serum globulin is clearly an alternative definition of the concept of ionic strength. This concept was introduced later by Lewis and Randall [7], which they considered as a unifying principle applicable to *strong electrolytes in dilute solution*, i.e. to solutions of salts which were highly dissociated into their ionic components. The application of this concept to other solutions may be misleading. The sense of this concept is that the concentrations of the individual free-ionic species are considered and these approximate to the molal (molar) concentrations at infinite dilution, i.e. where ionic dissociation is complete or the activity coefficients approach unity. At lower degrees of dissociation the activity coefficients of electrolytes will be less than this; taking as an example from the paper of Lewis and Randall [7] that of KCl, at concentrations of 0.01, 0.1 and 1 molal, the activity coefficients are 0.922, 0.794 and 0.634, respectively.

An example of the sort of misunderstanding that can arise can be seen in a recent publication in which results are interpreted as correlating with the Hofmeister series [8]. Here the effects of various salts at high concentration on enzymatic activity were studied. These concentrations are presented as ionic strengths in molar units which has no physico-chemical meaning. Then it is claimed that the effects of the salts correspond to the Hofmeister series: sulphate > acetate > chloride > bromide > iodide. Now, if we look at Table I and the data for sodium and convert these values to 'ionic strength' using the Lewis and Randall relationship (μ =

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Table I

Data of Hofmeister [1] on the concentrations of salts required to precipitate egg-white protein, expressed as normalities

	Lithium	Sodium	Potassium	Ammonium	Magnesium
Sulphate	1.57	1.60	n.p.	2.03	2.65
Phosphate	n.d.	1.65	1.61	2.51	s.s.s.
Acetate	n.d.	1.69	1.67	n.p.	n.p.
Citrate	n.d.	1.68	1.67	2.71	n.d.
Tartrate	n.d.	1.56	1.51	2.73	n.d.
Bicarbonate	n.d.	n.p.	2.53	n.d.	n.d.
Chromate	n.d.	2.61	2.67	n.p.	n.d.
Chloride	c.p.	3.63	3.53	n.p.	n.p.
Nitrate	n.d.	5.42	n.p.	n.p.	n.p.
Chlorate	n.d.	5.52	n.p.	n.d.	n.d.

n.d., not determined; n.p., no precipitation; s.s.s., slightly soluble salt; c.p., changed protein appearance

$1/2\ \Sigma c_i z_i^2$), we find the series: acetate (1.69) > sulphate (2.4) >chloride (3.63). Obviously the correlation with the Hofmeister series is missing.

The origin of the use of 'ionic strength' in salting-out studies of proteins probably lies in the reports of Green [9] who attributes it to Cohn in his review of 1925 [10]. This reference is one that is frequently misquoted since in all his examples of salting-out and his use of the Setschenov equation to relate protein solubility to salt concentration, Cohn used salt molarity. A recent example which perpetuates this misquote and misconception of the Hofmeister series is a study on the crystallization of lysozyme [11]. Here the crystallization was studied at relatively low salt concentrations which for many proteins might be salting-in conditions, and the results provide us with the reflection that if Franz Hofmeister had worked with hen egg-white lysozyme instead of albumin, then the series that bears his name would be reversed.

In summary, the effects of salts on biological systems should only be considered in terms of the Hofmeister series at high salt concentrations, and these concentrations should be expressed as molarity (or normality); ionic strength should be reserved for low salt concentrations where the degree of dissociation is large.

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