CLXXXVII. EFFECT OF ADDITION OF SALTS ON THE ISOELECTRIC POINT OF PROTEINS. II.

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FRESH evidence will be given in this paper in support of the opinion that the free charge of protein particles in solution is dependent on the presence of neutral salts and on their concentration; in particular on the valency of their anti- and iso-ions.

It was shown in our previous communication [1931] that, as demonstrated by experiments on flocculation induced by heat or by addition of alcohol, proteins may be discharged at p_H other than that of the isoelectric point by the addition of a critical concentration of electrolyte, termed in our previous paper C_K . Experiments will be described in this paper, showing that at concentrations greater than the critical the protein particles may, at the same p_H , be supercharged, so that protein solutions are obtained in which, at p_H values smaller than that of the isoelectric point, the particles behave as anions, whilst at p_H higher than the isoelectric they behave as cations. The possibility of such supercharging was indicated by Kruyt's [1929] experiments on the action of sodium sulphate on the cataphoretic properties of gelatin at p_H 3·9, as well as by the work of Loeb [1924, 1925].

EXPERIMENTAL.

1. Gelatin.

Gelatin was purified by the method described in the preceding paper, and the flocculation of aqueous solutions of such gelatin, in the presence of 60–70 % of alcohol, was studied as before. Since it appeared probable that supercharging could most readily be achieved by the addition of electrolytes to solutions at a p_H differing little from that of the isoelectric point, hydrochloric acid was added to p_H 3·5–3·8, or sodium hydroxide to p_H 6·5–7·0.

1 % gelatin solutions at p_H 3·5 were treated with an excess (0·001–0·004 M) of potassium ferricyanide, the C_K of which is about 0·0001 M at this p_H . In the presence of alcohol (70 %) gelatin is quantitatively precipitated in the presence of 0·001 M $K_3Fe(CN)_6$, whilst when excess of this reagent is taken, 10–20 % of the gelatin remains in solution. Filtrates from such systems were taken for examination.

Various salts (sodium sulphate, potassium ferricyanide, calcium and magnesium chlorides) were added to these filtrates. The results obtained indicate that gelatin treated with an excess of ferricyanide at p_H 3.5 becomes practically insensitive to the action of multivalent anions, but is readily flocculated by salts giving rise to univalent anions and bivalent cations. Thus ordinarily the C_K of sodium sulphate is less than 0.001 M , and of magnesium chloride greater than 0.05 M , whilst supercharged gelatin is not flocculated by the addition of 0.02 M sodium sulphate, and is coagulated by 0.0005 M magnesium chloride.

Similar experiments were performed on alkaline gelatin solutions, at p_H 6.5–7.0, adding excess of neutralised aluminium chloride (0.002–0.005 M), when a precipitate formed, the bulk of which was increased by the addition of alcohol to 70 %. The filtrate contained gelatin which was sensitive to the action of multivalent anions, but not cations. Further addition of aluminium chloride did not lead to flocculation of the residual gelatin, and the same applies to magnesium chloride, the C_K of which is normally 0.0025 M , whilst in this case flocculation is not obtained using 0.02 M solutions. On the other hand, the C_K of sodium sulphate is now 0.0003–0.0008 M as compared with the normal value of over 0.01 M .

2. Serum-albumin.

A similar sensitisation to the action of cations and desensitisation to that of anions is obtained using serum-albumin solution at p_H 3.5 by the addition of excess ferricyanide (0.001–0.005 M), and, conversely, sensitisation to the action of anions and desensitisation to that of cations can be demonstrated in neutral solutions (p_H 6.5–7.0) by the addition of 0.01–0.02 M aluminium chloride. The results obtained are based on observations of coagulation after heating 10 min. at 100°.

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