CLXVI. EFFECT OF ADDITION OF SALTS ON THE ISOELECTRIC POINT OF PROTEINS. III.

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IN the present paper, experiments are described on the influence of electrolytes on the critical concentration, C_K , of alcohol necessary to flocculate gelatin solutions, and on the viscosity of aqueous and aqueous-alcoholic gelatin solutions.

EXPERIMENTAL.

Purification of gelatin. The gelatin taken, purchased from Coignet Père et Fils, had 1.49 % of ash which consisted almost exclusively of calcium phosphate, with traces of iron. After purification, the ash content fell to 1.1 %; for the concentrations of gelatin used in our experiments, viz. 0.15 and 0.9 %, this would correspond with 0.0165 and 0.099 g. per litre of solution, i.e. the concentrations of $\text{Ca}_3(\text{PO}_4)_2$ originally present were 0.00032 and 0.00192 *N*.

A filtered 4 % solution of gelatin in warm water was poured in a thin stream into 4 volumes of alcohol. The flocculent precipitate was left in contact with alcohol for 24 hours, when the solution was violently agitated, and allowed to settle. The supernatant clear solution was then decanted, and the precipitate was shaken with fresh alcohol and filtered. The residue after evaporation of alcohol was redissolved and the gelatin was reprecipitated as before, with the difference that the duration of contact with alcohol before shaking was increased to 48 hours. The residue after filtration was washed 4 times with 96 % alcohol, allowing the suspension to settle 3 hours before every decantation. The product so obtained was spread in a thin layer on sheets of glass, and dried *in vacuo* over calcium chloride, giving a white impalpable powder, the properties of which remained unchanged by further treatment with alcohol.

The action of electrolytes on gelatin solutions was examined by adding various concentrations of salts to the clear solution, and then adding sufficient 96 % alcohol to produce a milky opalescence. The volume of alcohol necessary (in future designated by *V*) is fairly constant for a given concentration of gelatin and electrolyte, and sufficiently reproducible results can, with a little experience, be obtained without any difficulty. The procedure adopted was as follows: 2 cc. of electrolyte solution were added to a series of 70 cc. test-tubes (pyrex) each of which contained 3 cc. of 0.5 % gelatin; the volume was made

up to 10 cc., and alcohol was added from a burette, in small portions, shaking after the addition of every portion.

Viscosity was determined using Ostwald's viscosimeter, and calculating the results from the simplified formula $\eta = T'/T$, where T' and T are the rates of flow of the gelatin solution examined, and of a solution of electrolyte of the same concentration as in the gelatin solution. Determinations were made at 40°, in view of Davis and Oakes's [1922] observation that gelation, with consequent augmentation of viscosity, does not take place at temperatures higher than 38.1°. 3 cc. of electrolyte solution were added to 27 cc. of 1 % gelatin buffered to the appropriate p_H or of a mixture of equal volumes of 2 % gelatin and 65 % alcohol in 50 cc. flasks, which were then stoppered, and allowed to stand for 1 hour at 40° before viscosity was measured. Determinations were made in a glass-walled thermostat at $40^\circ \pm 0.1^\circ$, and the values given are the mean of 4–6 determinations.

1. Influence of p_H and salts on V .

The results obtained are illustrated by the curve shown in Fig. 1. At the isoelectric point (p_H 4.85), V is at a minimum, viz. 6 cc. (30 %), whilst at

p_H 4.3, 14.5 cc. (56 %); at p_H 3.8, 22 cc. (66 %); at p_H 3.4, 34 cc. (74 %); and at p_H 2.6, 37 cc. (75.5 %) are necessary to produce opalescence. Similarly, at p_H above 4.85, V rises from 12 cc. at p_H 5.5 to 18 cc. at p_H 6.3, 22 cc. at p_H 7.5, 24 cc. at p_H 8.4 and 25.5 cc. at p_H 9.5.

Table I. *Action of $MgCl_2$.*

Conc. of $MgCl_2$ N	Least volume of alcohol required to produce turbidity at p_H						
	3.87	4.49	4.84	6.15	7.63	8.97	9.63
0	22	10	6.5	18.5	22	24	25.5
0.002	—	—	7.5	13	14.5	15.5	15.5
0.004	—	—	8.5	11.5	13	13.5	15.5
0.006	—	—	10	11	12.5	12.5	13.5
0.008	—	—	11.5	11.5	12	12	12.5
0.010	—	—	13	12	12	12	12
0.02	23	15	14.5	12.5	12.5	12	12.5
0.04	24	18	16	14.5	14.5	14	14
0.06	24.5	19	18	15	15.5	15.5	15
0.08	25	20	19.5	16.5	16	16.5	16.5
0.10	25	21	20	17	17	17.5	17

The action of $MgCl_2$ is different according to its concentration and to the p_H of the medium. Thus at and below the isoelectric point (i.p.) $MgCl_2$ increases V to an extent diminishing with diminishing p_H , V rising from 6.5 to 20 cc. at p_H 4.85, from 10 to 21 cc. at p_H 4.49 and from 22 to 25 cc. at p_H 3.87. Above the i.p., V is smaller in the presence of $MgCl_2$ than in its absence; for every p_H , however, a minimum V is obtained for a certain concentration of $MgCl_2$, above and below which V is greater and may, at p_H close to the i.p., even exceed the value found in the absence of electrolytes. The smallest value of V observed at p_H 6.15 is 11 cc. in the presence of 0.006–0.01 N $MgCl_2$, as compared with the value of 6 cc. found in the absence of salts at p_H 4.8, showing that the charge on the particles cannot be reduced by addition of $MgCl_2$ to that found at the isoelectric point.

Table II. *Action of Na_2SO_4 .*

Conc. of Na_2SO_4 N	Least volume of alcohol required to produce turbidity at p_H						
	3.20	4.43	4.73	5.4	6.1	6.7	7.1
0	35	14.5	6	13	18.5	20	21.5
0.002	17	10	19.5	23	31.5	34	36.5
0.004	15	9.5	22	25	33	36.5	38
0.006	13	8.0	23	25	33	36	37
0.008	12.5	8.5	24	25.5	31.5	34	36
0.01	12	9.0	24	25.5	30	33	33.5

0.006 N Na_2SO_4 increases V from 6 to 24 cc. at the i.p., and decreases it below the i.p. from 35 to 12 cc., at p_H 3.2. Above the i.p., Na_2SO_4 greatly increases V , the values of which are considerably higher at all values of $p_H > 4.85$ than in the absence of electrolytes. It is not possible on the basis of the experimental material available to decide whether this effect is due to formation of $CaSO_4$ by interaction between Na_2SO_4 and Ca combined with gelatin, or to augmentation of the charge on the colloidal particles. As the concentration of Na_2SO_4 is increased, V rises to a maximum value, and then falls; thus, at p_H 7.1 V rises from 21.5 cc. in the absence of Na_2SO_4 to 38 cc. in the presence of 0.004 N Na_2SO_4 , and falls to 33.5 cc. for 0.01 N Na_2SO_4 .

The action of CaCl_2 was studied only at p_H 5.6 and was found to be analogous to that of MgCl_2 . Thus V falls from 14 to 7 cc. when CaCl_2 is added to 0.003 N , rising to 19.5 cc. when the concentration is increased to 0.1 N .

Fig. 2. Influence of $\text{La}(\text{NO}_3)_3$ on the volume of alcohol required to produce turbidity of gelatin solutions at p_H 5.5.

In the case of $\text{La}(\text{NO}_3)_3$ (studied only at p_H 5.5), V falls sharply from 14 to 3.5 cc. as the concentration of $\text{La}(\text{NO}_3)_3$ is increased from 0 to 0.0001 N , and again rises sharply to 26 cc. in 0.0004 N $\text{La}(\text{NO}_3)_3$. KCl , NaCl and MgSO_4 have only a very slight effect on the value of V (Table III).

Table III. Action of KCl , NaCl and MgSO_4 at p_H 5.3.

Conc. of salt N	Least volume of alcohol required to produce turbidity in the presence of		
	KCl	NaCl	MgSO_4
0	14	14	14
0.00075	14	14	14
0.0015	14	14	14
0.0031	14	14	14
0.0062	14	14	13.5
0.0125	14	14	13.5
0.025	15	14	13.6
0.05	16	15	13.0
0.1	17	16	13.0

A comparison of the actions of MgCl_2 , Na_2SO_4 and MgSO_4 on portions of the same gelatin solution at p_H 5.6 showed (Fig. 3) that as the concentrations of salts giving rise to bivalent anions and univalent cations are increased,

V at first increases and then diminishes; in the case of those giving univalent anions and bivalent cations, V at first falls and then rises, whilst bi-bivalent electrolytes have practically no action.

Fig. 3. No. of cc. of alcohol necessary to produce turbidity in the presence of Na_2SO_4 , MgCl_2 and MgSO_4 .

2. Influence of electrolytes on viscosity.

The viscosity- p_H curve has a minimum at the I.P., η being 1.575 at p_H 2.5, 1.480 at p_H 3.3, 1.340 at p_H 3.8, 1.275 at p_H 4.5, 1.260 at p_H 4.84, 1.315 at p_H 5.7, 1.388 at p_H 6.5, 1.400 at p_H 7.3, 1.416 at p_H 8.0 and 1.424 at p_H 9.8 (Fig. 1).

Table IV. Influence of electrolytes on viscosity.

Conc. of salt N	Viscosity at p_H 4.0 in the presence of			Viscosity at p_H 6.4 in the presence of		
	NaCl	KCl	LiCl	NaCl	KCl	LiCl
0	1.370	1.370	1.370	1.367	1.367	1.367
0.0006	1.368	1.370	1.370	1.360	1.352	1.307
0.0012	1.355	1.366	1.364	1.352	1.325	1.310
0.0025	1.337	1.345	1.345	1.337	1.310	1.313
0.005	1.327	1.329	1.329	1.323	1.305	1.308
0.01	1.313	1.313	1.310	1.311	1.308	1.311
0.02	1.292	1.292	1.292	1.308	1.316	1.308
0.04	1.283	1.290	1.291	1.314	1.320	1.314

At p_H below the I.P. the action of uni-univalent electrolytes is of the same type for the three salts studied (KCl, NaCl, LiCl); these salts all lower η to about the same extent, and the same applies to MgCl_2 , whilst the action of $\text{La}(\text{NO}_3)_3$ is smaller than is that of alkali or alkaline earth salts. $\text{K}_3\text{Fe}(\text{CN})_6$

Table V. *Influence of KCl, MgCl₂, La(NO₃)₃, Na₂SO₄ and K₃Fe(CN)₆ on viscosity at p_H 3.4.*

Conc. of salt (N)	KCl	MgCl ₂	La(NO ₃) ₃	Na ₂ SO ₄	K ₃ Fe(CN) ₆
0	1.324	—	—	—	—
0.0006	1.322	1.316	1.323	1.310	1.252
0.0012	1.314	1.306	1.313	1.308	1.186
0.0025	1.292	1.288	1.303	1.270	1.150
0.005	1.270	1.264	1.292	1.227	} Partial coagulation
0.01	1.262	1.254	1.283	1.216	
0.02	1.252	1.246	1.270	1.210	
0.04	1.254	1.246	1.257	1.210	

Table VI. *Influence of KCl, MgCl₂, La(NO₃)₃, Na₂SO₄ and K₃Fe(CN)₆ on viscosity at p_H > isoelectric point.*

Conc. of salt N	p _H 6.5			p _H 7.4		p _H 8.5
	KCl	MgCl ₂	K ₃ Fe(CN) ₆	MgCl ₂	Na ₂ SO ₄	La(NO ₃) ₃
0	1.378	1.378	1.378	1.400	1.400	1.420
0.0006	1.373	1.357	1.367	1.384	1.094	1.408
0.0012	1.355	1.334	1.348	1.360	1.090	1.366
0.0025	1.334	1.322	1.342	1.334	1.378	1.302
0.05	1.314	1.303	1.340	1.284	1.356	1.246
0.01	1.306	1.289	1.340	1.240	1.334	1.236
0.02	1.308	1.279	1.336	1.218	1.318	1.244
0.04	1.310	1.276	1.338	1.218	1.310	1.254

Table VII. *Influence of salts on viscosity in aqueous-alcoholic systems.*

Conc. of salt N	p _H 3.8		p _H 4	p _H 4.5			p _H 6.01	
	Na ₂ SO ₄	K ₃ Fe(CN) ₆	La(NO ₃) ₃	KCl	MgCl ₂	K ₃ Fe(CN) ₆	Na ₂ SO ₄	MgCl ₂
0	2.794	2.794	2.753	2.698	—	—	2.623	2.623
0.0003	2.742	2.671	—	—	—	—	2.616	2.580
0.0006	—	—	2.770	2.686	2.694	2.650	—	—
0.0007	2.686	2.600	—	—	—	—	2.613	2.531
0.0012	—	—	2.792	2.672	2.686	2.564	—	—
0.0015	2.638	2.522	—	—	—	—	2.606	2.516
0.0025	—	—	2.800	2.664	2.660	2.476	—	—
0.003	2.620	Turbidity	—	—	—	—	2.611	2.508
0.005	—	—	2.815	2.650	2.640	Turbidity	—	—
0.006	2.618	2.514	—	—	—	—	2.613	2.504
0.01	—	—	2.822	2.642	2.632	—	—	—
0.0125	2.622	2.518	—	—	—	—	2.617	2.504
0.02	—	—	2.823	2.640	2.626	2.436	—	—
0.025	2.628	2.526	—	—	—	—	2.620	2.509
0.04	—	—	2.826	2.640	2.622	2.444	—	—

Conc. of salt N	p _H 6.5		p _H 7.2	p _H 7.5		
	Na ₂ SO ₄	K ₃ Fe(CN) ₆	La(NO ₃) ₃	KCl	MgCl ₂	K ₃ Fe(CN) ₆
0	2.698	2.698	2.730	—	2.750	—
0.0003	2.698	2.705	—	—	—	—
0.0006	—	—	2.580	2.746	2.720	2.754
0.0007	2.690	2.706	—	—	—	—
0.0012	—	—	2.514	2.742	2.670	2.754
0.0015	2.692	2.708	—	—	—	—
0.0025	—	—	2.468	2.732	2.630	2.758
0.003	2.693	2.712	—	—	—	—
0.005	—	—	Turbidity	2.724	2.610	2.760
0.006	2.697	2.711	—	—	—	—
0.01	—	—	Turbidity	2.718	2.594	2.758
0.0125	2.699	2.713	—	—	—	—
0.02	—	—	2.474	2.718	2.598	2.760
0.025	2.701	2.719	—	—	—	—
0.04	—	—	2.486	2.710	2.602	2.758

lowers η more than do the previously mentioned electrolytes; this salt, however, produces partial flocculation at concentrations of 0.005 and 0.01 *N*.

Above the i.p., salts possessing univalent cations and multivalent anions (Na_2SO_4 , $\text{K}_3\text{Fe}(\text{CN})_6$) have a feeble action than have uni-univalent salts (KCl), which, again, are less active than are salts of multivalent metals ($\text{La}(\text{NO}_3)_3$). In the case of $\text{La}(\text{NO}_3)_3$ η falls with concentration to a minimum at 0.01 *N*, again rising at higher concentrations of salt (Table VI).

In aqueous-alcoholic media uni-univalent electrolytes lower η on both sides of the i.p. Salts giving rise to multivalent ions of opposite charge from the protein have a more marked action, which augments with increase in the valency. Thus, below the i.p., Na_2SO_4 has a more powerful action than has KCl, whilst that of $\text{K}_3\text{Fe}(\text{CN})_6$ is, up to a certain concentration, still more marked, partial flocculation taking place. At higher concentrations of $\text{K}_3\text{Fe}(\text{CN})_6$ its flocculating action ceases, and η again rises slightly. $\text{La}(\text{NO}_3)_3$ causes increase in η below the i.p. Above the i.p. depression of η increases with the valency of the cation of multi-univalent salts (KCl, MgCl_2 , $\text{La}(\text{NO}_3)_3$), Na_2SO_4 is practically without action, and $\text{K}_3\text{Fe}(\text{CN})_6$ very slightly increases η .

SUMMARY AND DISCUSSION.

1. The previous finding that the concentration (C_K) of alcohol necessary for the flocculation of gelatin sols is smallest at the isoelectric point (p_H 4.8) has been confirmed for purified preparations of gelatin. The flocculation-alcohol concentration curve (Fig. 1) has a greater slope at $p_H < 4.8$ than at $p_H > 4.8$. Negatively charged micelles are more readily coagulated by alcohol than are positively charged ones.

2. The value of C_K at the isoelectric point is increased in the presence of MgCl_2 or Na_2SO_4 , whilst MgSO_4 is without effect. The action of uni-multivalent salts at the isoelectric point can, according to Pauli [1930], be ascribed to combination between the salt and zwitterions, yielding products of the type $\text{XH}_3\text{N.R.CO}_2\text{M}$, with the production of double salts of different activities, to which the charge of the particles is due.

3. At all p_H values salts giving rise to ions of the same valency do not affect the value of C_K .

4. At $p_H < 4.8$ salts yielding multivalent anions and univalent cations lower C_K at small concentrations, and slightly raise it at $p_H > 4.8$. This effect is due to the charge of the colloidal particles; the greater the original charge the greater the percentage lowering of C_K . Thus *V*, the volume of alcohol necessary to produce turbidity, is 14 cc. at p_H 4.25, whilst in the presence of 0.06 *N* Na_2SO_4 *V* is 8 cc., and at p_H 3.2 *V* falls from 35 to 13 cc.

Salts yielding univalent anti-ions and multivalent iso-ions, *e.g.* MgCl_2 at p_H 4.8, considerably raise the value of C_K to an extent which is greater the closer the p_H is to 4.8. This effect may be ascribed to increase in the free charge of gelatin particles. The opinion that this phenomenon is analogous to

that of the formation of double salts of diminished activity, as with protein (*e.g.* $\text{NH}_3\text{Cl} \cdot \text{R} \cdot \text{CO}_2 \frac{1}{2}\text{Mg}$), with consequent diminution in the negative charge, lacks experimental proof, although it is in agreement with the zwitterion theory, and with Kruyt's [1929] observation of augmented cataphoresis of gelatin in the presence of Na_2SO_4 at p_{H} 4.4.

5. The action of salts at $p_{\text{H}} > 4.8$ is analogous to that at $p_{\text{H}} < 4.8$, with the difference that anions are anti-ions, and cations iso-ions. Augmentation of C_{K} by Na_2SO_4 can be observed at p_{H} as high as 7.1. The further the p_{H} is from 4.8, the more marked is the action of univalent anti-ions. A certain concentration of salt can be found giving maximum increase in C_{K} ; the value of this optimum concentration diminishes with increasing p_{H} .

6. The dual action of a salt is the more marked the greater is the difference between the valencies of its ions. Thus $\text{La}(\text{NO}_3)_3$ at p_{H} 5.3 in low concentrations lowers C_{K} , and hence the charge of the particles, and at higher concentrations augments it to values even higher than are found in the absence of $\text{La}(\text{NO}_3)_3$. According to the interpretation of Przyłęcki [1931] and of Przyłęcki and Giedroyć [1931] this effect is due to supercharging. Since the discharging of the colloidal particles takes place at very low concentrations of salt (0.0004 %), the supercharging of the particles by the anti-ions similarly takes place at low concentrations of salt, with consequent absence of discharging action by higher concentrations. The action of CaCl_2 is similar to that of $\text{La}(\text{NO}_3)_3$ but much feebler, supercharging being observed at a concentration of 0.006 *N*. Were this phenomenon to be ascribed to reduction in the charge due to ionised carboxylic groups, it would have, according to the zwitterion theory, to be assumed that only positively charged $-\text{NH}_3^+$ groups remain, to which the charge of the particles is due.

The reason for the increase in C_{K} at p_{H} 7.1 in the presence of Na_2SO_4 is not obvious. This effect may be due to the action of SO_4 on the remaining dissociated $-\text{NH}_3^+$ groups, or to the reaction:

7. The p_{H} -viscosity curves of aqueous systems are very similar to the p_{H} - V curves, minimum viscosity being at p_{H} 4.85, with a rapid rise in viscosity on the acid side of this point, and a gentle rise on the alkaline side (Fig. 1). This similarity is due to the identity of the factors leading to change in the charge of the particles, both increase in C_{K} and in viscosity being ascribable to increase in the charge.

8. The action of salts on the viscosity of gelatin sols is not in agreement with their effect on the value of C_{K} . Salts yielding univalent iso-ions and anti-ions diminish viscosity at all values of p_{H} . The actions of salts yielding the same anti-ions and different iso-ions are very nearly the same irrespective of the salt taken (KCl , LiCl , NaCl at p_{H} 4.0), whilst where the anti-ions are different but the iso-ion identical (KCl , LiCl , NaCl at p_{H} 6.4), the magnitude of the effect obtained varies with the different salts.

9. Salts yielding multivalent anti-ions and univalent iso-ions depress viscosity to an extent increasing with the valency of the anti-ion. As the concentration of salts of this type is increased, viscosity falls to a minimum value, again rising with higher concentrations.

10. Salts yielding multivalent iso-ions and univalent anti-ions ($\text{K}_3\text{Fe}(\text{CN})_6$) at p_{H} 6.5 diminish viscosity to a smaller extent than do uni-univalent salts (KCl).

11. The action of salts on the viscosity of aqueous-alcoholic solutions of gelatin is different from that in the absence of alcohol. Under certain conditions viscosity is increased by addition of salts. The Schulze-Hardy rule is followed in such systems, although in certain cases the viscosity at first falls and then rises as the concentration of salt is increased. Salts yielding univalent anti-ions and multivalent iso-ions increase viscosity at all concentrations ($\text{K}_3\text{Fe}(\text{CN})_6$ and Na_2SO_4 at $p_{\text{H}} > 4.8$, and $\text{La}(\text{NO}_3)_3$ at $p_{\text{H}} < 4.8$).

12. No explanation of the difference in the action of salts on viscosity in aqueous and in aqueous-alcoholic media is, in the present state of knowledge of this subject, apparent.

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