

Effect of electrolytes on coacervation of the systems gelatin-water-ethanol and gelatin-water-sodium sulphate

J. R. NIXON, SALEH A. H. KHALIL* AND J. E. CARLESS

In the ethanol system the electrolytes had a suppressive effect on the coacervation of isoionic gelatin, whilst at other pH values the phenomenon was favoured in the presence of polyvalent ions. In the sodium sulphate system the added electrolytes produced insignificant effects. The similarity between the systems studied and the complex coacervating system: gelatin⁽⁺⁾-water-acacia⁽⁻⁾ is discussed.

THE classification of the phenomenon of coacervation into simple and complex types by Bungenberg de Jong (1949) is based on the role played by the charges on the colloidal components. In simple coacervation it has been suggested that charge effects are negligible and phase separation occurs due to "desolvation," as in the systems: isoelectric gelatin-water-ethanol and isoelectric gelatin-water-sodium sulphate (Holleman, Bungenberg de Jong & Modderman, 1934). On the other hand complex coacervation occurs due to adequate charge opposition between the colloids involved, as in the systems gelatin⁽⁺⁾-water-acacia⁽⁻⁾ at pH 2.6-4.2. The extensive studies of Bungenberg de Jong and his co-workers (1949) have shown that complex coacervating systems are extremely sensitive to pH changes and electrolytic impurities. The effect of these factors on simple coacervating systems was not investigated in detail. Recently, Khalil, Nixon & Carless (1968) examined the role of pH on the coacervating systems: gelatin-water-ethanol and gelatin-water-sodium sulphate. For the ethanol system, coacervation was only possible at pH values in the vicinity of the isoionic point, whilst the sodium sulphate system showed a much wider pH coacervation range.

The present paper examines the effect of added electrolytes on the two systems previously studied by us and compares the results with data available on complex coacervating systems.

Experimental

MATERIALS

Gelatin. A lime-pretreated 240 Bloom sample as described by Khalil & others (1968) was used.

The gelatin was deionized by the mixed-bed ion-exchange technique of Janus, Kenchington & Ward (1951). *Coacervating agents:* Absolute ethanol and 20% w/w sodium sulphate solution. *Electrolytes:* All electrolytes, with the exception of aluminium chloride, were A.R. grade. The latter was a pure sample containing not less than 99% AlCl₃. *Glass distilled water* was used (pH 5.4, specific conductivity 4.8 μ mhos cm⁻¹).

From Department of Pharmacy, Chelsea College of Science and Technology, University of London, Manresa Road, London, S.W.3, England.

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METHODS

Determination of the onset of coacervation, measurement of the coacervate volume and analysis of the phases. These techniques have been described by Nixon, Khalil & Carless (1966) and Khalil & others (1968).

Measurement of turbidity. An Evans Electro-selenium Ltd. nephelometer was used. The coacervating agent was added to 3.0 ml portions of 0.6% w/v gelatin solution at $40^\circ \pm 0.05^\circ$. Readings were recorded after mixing and equilibrating the turbid mixture for 3 min at 40° . A gelatin solution of the same total concentration as in the test was used as a blank.

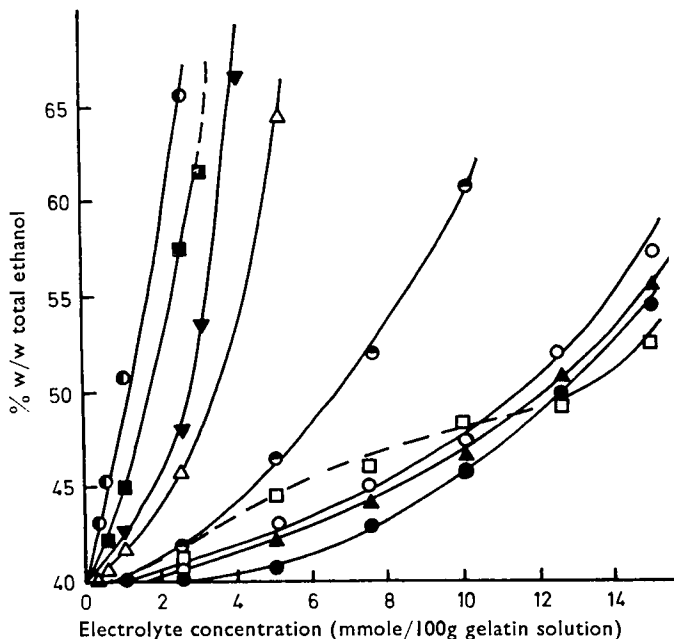
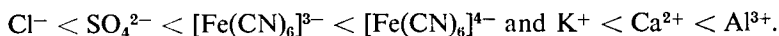


FIG. 1. Effect of electrolytes on the occurrence of coacervation in the system: gelatin-water-ethanol. Minimum ethanol concentrations (% w/w) required to produce coacervation plotted against electrolyte concentration. Initial gelatin concentration 5% w/w. Temp. $40^\circ \pm 0.1^\circ$, pH 4.9 (pI). \bullet — \bullet , AlCl₃. \blacksquare — \blacksquare , K₄[Fe(CN)₆]. \blacktriangledown — \blacktriangledown , K₃[Fe(CN)₆]. \triangle — \triangle , CaCl₂. \circ — \circ , K₂SO₄. \circ — \circ , KI. \blacktriangle — \blacktriangle , KBr. \bullet — \bullet , KCl. \square — \square , KF.

Fig. 1 shows the effect of electrolytes on the minimum ethanol concentrations required to produce coacervation in isoionic gelatin. The suppressive effect of the electrolytes followed the valency rule for both the anions and cations:



In the concentrations used, the lyotropic effect was not clearly shown. Nevertheless the arrangement of the potassium halides, in the order of peptizing power, was in accordance with the Hofmeister series: $(\text{F}^-) < \text{Cl}^- < \text{Br}^- < \text{I}^-$. At low concentrations potassium fluoride showed anomalous results which can be attributed to pH changes caused by its ionization. Aluminium chloride produced similar effects.

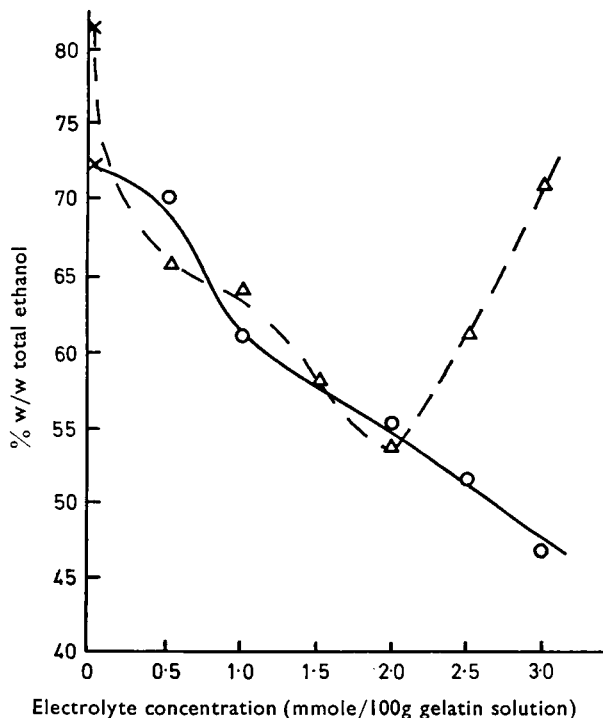


FIG. 2. Effect of calcium chloride (at pH 9.1) and potassium ferrocyanide (at pH 2.4) on coacervation in the system: gelatin-water-ethanol. Minimum ethanol concentrations required to produce a phase change plotted against electrolyte concentration. Initial gelatin concentration 5% w/w. Temp. $40^{\circ} \pm 0.05^{\circ}$. \times flocculation, O, Δ coacervation.

At pH values away from the isoionic point polyvalent ions favoured coacervation in mixtures which showed flocculation in the absence of these ions. Typical results are shown in Fig. 2 for Ca^{+2} at pH 9.1 and $[\text{Fe}(\text{CN})_6]^{4-}$ at pH 2.4.

TABLE 1. EFFECT OF ELECTROLYTES ON THE OCCURRENCE OF COACERVATION IN THE SYSTEM: GELATIN-WATER-SODIUM SULPHATE. Minimum sodium sulphate concentrations (% w/w) required to produce coacervation at various electrolyte concentrations

Salt concentration (mmol/100 g gelatin solution)	AlCl_3	CaCl_2	KCl	KF	KBr	KI	K_2SO_4	$\text{K}_3[\text{Fe}(\text{CN})_6]$	$\text{K}_4[\text{Fe}(\text{CN})_6]$
0	7.81 (Blank)								
0.25	7.78	7.81	7.81	7.83	7.82	7.82	7.81	7.81	7.80
0.5	7.62	7.83	7.80	7.86	7.82	7.84	7.80	7.82	7.79
1.0	7.55	7.80	7.83	7.88	7.82	7.83	7.80	7.80	7.78
2.5	7.31	7.81	7.80	7.91	7.84	7.85	7.78	7.77	7.76
5.0	7.21	7.84	7.82	7.93	7.84	7.86	7.80	7.77	7.78
7.5	—	—	7.81	7.90	7.86	7.86	7.80	7.79	7.76
10.0	—	—	7.81	7.91	7.85	7.88	—	—	—

Temperature: $40^{\circ} \pm 0.1^{\circ}$, pH = 4.9 (pI).
Initial gelatin concentration: 10% w/w (5 g used).

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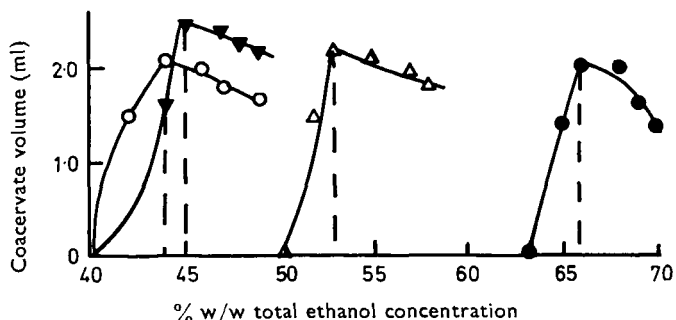


FIG. 3. Effect of electrolytes on changes in coacervate volume at various ethanol concentrations. Total gelatin concentration 4% w/w. Electrolyte concentration 2 mmole/100 g gelatin solution. Temp. $40^{\circ} \pm 0.05^{\circ}$, pH 4.9 (pI). \circ — \circ , blank. \blacktriangledown — \blacktriangledown , CaCl_2 . \triangle — \triangle , $\text{K}_4[\text{Fe}(\text{CN})_6]$. \bullet — \bullet , AlCl_3 .

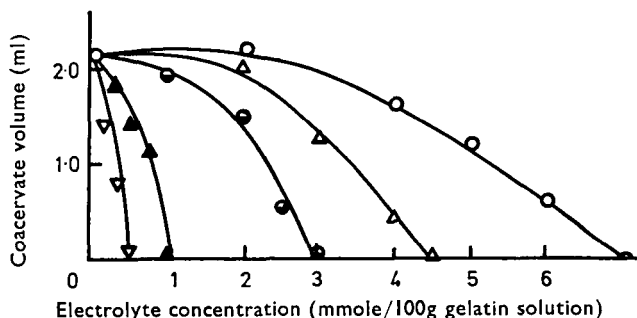


FIG. 4. Effect of electrolytes on changes in coacervate volume at a constant composition. Gelatin 4% w/w ethanol 44% w/w, water 52% w/w. Temp. $40^{\circ} \pm 0.05^{\circ}$, pH 4.9 (pI). ∇ — ∇ , AlCl_3 . \blacktriangle — \blacktriangle , $\text{K}_4[\text{Fe}(\text{CN})_6]$. \bullet — \bullet , CaCl_2 . \triangle — \triangle , K_2SO_4 . \circ — \circ , KI .

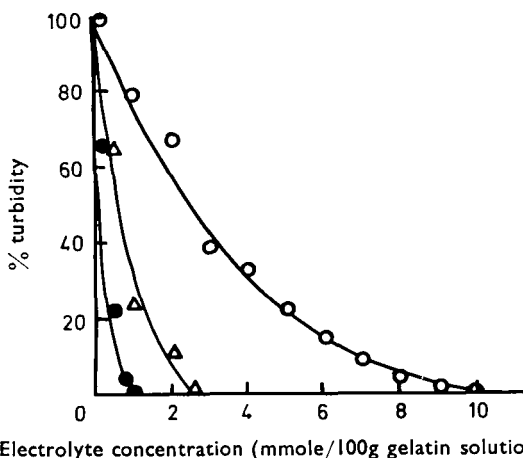


FIG. 5. Percentage turbidity plotted against electrolyte concentration (the turbidity in the absence of electrolytes was taken as 100%). Temp. $40^{\circ} \pm 0.05^{\circ}$, pH 4.9 (pI). \bullet — \bullet , AlCl_3 . \triangle — \triangle , CaCl_2 . \circ — \circ , KCl .

TABLE 2. EFFECT OF CALCIUM CHLORIDE ON THE COMPOSITION OF THE COACERVATES AND EQUILIBRIUM LIQUIDS IN THE SYSTEM GELATIN-WATER-ETHANOL

Calcium chloride concentration (mmole/100 g gelatin solution)	Percentage w/w compositions					
	Coacervate			Equilibrium liquid		
	Gelatin	Ethanol	Water	Gelatin	Ethanol	Water
0	19.7	36.9	43.4	0.6	47.4	52.0
0.6	17.0	38.2	44.8	0.9	47.4	51.7
1.2	14.2	39.5	46.3	1.2	46.9	51.9
1.8	11.4	41.6	47.0	1.4	47.1	51.5
2.4	8.6	43.1	48.3	1.8	46.7	50.5
3.0	One phase					

Temperature: $40^{\circ} \pm 0.1^{\circ}$, pH = 4.9 (pI).

Percentage w/w compositions of the total mixtures: 4 (gelatin) 46, (ethanol) and 50 (water).

In the sodium sulphate system all electrolytes added, with the exception of potassium fluoride and aluminium chloride, had a negligible effect on the minimum sulphate concentrations necessary for coacervation (Table 1).

Coacervate volume plots, Fig. 3, show also the suppressive effect of the electrolytes in the ethanol system, where higher ethanol concentrations were required to produce the same coacervate volume. At a fixed ethanol concentration in the system the presence of electrolytes produced a gradual decrease in the coacervate volume. The effect again followed the valency rule (Fig. 4). Turbidity determinations, Fig. 5, show complementary results with the coacervate volume measurements.

The effect of calcium chloride on the composition of both the coacervate phase and equilibrium liquid is shown in Tables 2 and 3. The results for the ethanol system are plotted in Fig. 6. At constant compositions of gelatin, water and ethanol an increase in the calcium chloride concentration resulted in the composition of both phases approaching one another. At a sufficiently high calcium chloride concentration (point P, Fig. 6) coacervation was completely suppressed and the system remained in the one-phase region.

TABLE 3. EFFECT OF CALCIUM CHLORIDE ON THE COMPOSITION OF THE COACERVATES AND EQUILIBRIUM LIQUIDS IN THE SYSTEM: GELATIN-WATER-SODIUM SULPHATE

Calcium chloride concentrations (mmole/100 g gelatin solution)	Percentages w/w compositions					
	Coacervate			Equilibrium liquid		
	Gelatin	Sod. sulph.	Water	Gelatin	Sod. sulph.	Water
0	14.4	7.0	78.6	0.6	11.5	87.9
0.6	14.1	7.1	78.8	0.6	11.8	87.6
1.2	14.2	6.9	78.9	0.6	11.4	88.0
1.8	14.0	6.9	79.1	0.6	11.0	88.4
2.4	14.3	6.9	78.8	0.8	11.0	88.2
3.0	14.4	7.2	78.4	0.8	10.9	88.3

Temperature: $40^{\circ} \pm 0.1^{\circ}$, pH = 4.9 (pI).

Percentage w/w compositions of the total mixtures: 4 (gelatin), 10.2 (sodium sulphate) and 85.8 (water).

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In the sodium sulphate system the presence of calcium chloride produced no noticeable effect on the composition of both phases, Table 3. This may be attributed to the formation of the slightly soluble salt, calcium sulphate, coupled with insufficient Cl^- concentration to produce any peptizing effect in the presence of SO_4^{2-} ions.

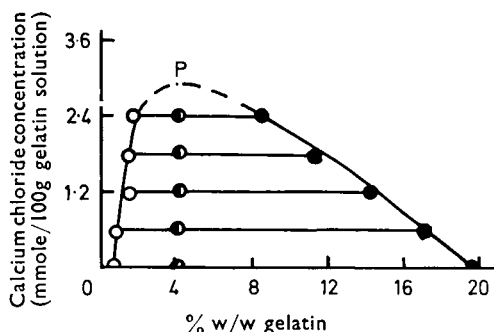


FIG. 6. Effect of calcium chloride on changes in the gelatin content of the coacervates, ●, and equilibrium liquids, ○. Composition of the total mixtures, ●; gelatin 4% w/w, ethanol 46% w/w, water 50% w/w. Temp. $40^\circ \pm 0.1^\circ$. pH 4.9 (pI).

Discussion

The addition of low concentrations of electrolytes to isoionic gelatin results in a decrease of the mutual attractive forces due to the screening of the functional groups on gelatin. This produces the unfolding of the coiled structure and an increase in viscosity occurs (Overbeek & Bungenberg de Jong, 1949). The effect of electrolytes on coacervation may be explained on the same basis. The shielding of the functional groups by the ions of the added electrolyte decreases the inter- and intra-molecular attractive forces. The peptizing property of the ions may exert a secondary influence. Salts of the type 1-1 (e.g., KCl) would suppress the interaction by both the positive and negative groupings to the same extent. With salts of the type 1-4, 1-3 or 1-2 the polyvalent anions would, in the given order, screen off the positively charged basic groups to an increasingly greater extent than the effect of the monovalent cations on the negatively charged carboxyl groups of the aspartic and glutamic residues. This results in an imbalance of the original charge distribution, characteristic for the isoionic state and the mutual attractive forces diminish according to the electrolyte-sequence previously mentioned (Fig. 1).

At pH values away from the isoionic point, charge opposition occurs between the added polyvalent ions and the ionized groups on gelatin. Any repulsive force resulting from a pH variation would be overcome by the selective fixation of the polyvalent ions at the oppositely charged sites on the gelatin. Under such conditions coacervation occurs. The coacervates produced at pH 2.4 in the presence of ferrocyanide or ferricyanide ions showed a unique property. Whilst a clear single liquid phase was not restored on dilution with water, as occurred with other simple coacervating systems, a change in pH or the addition of an excess

of the above electrolyte solutions restored complete clarity. In this respect these coacervates are analogous to the known complex coacervates of the type: gelatin⁽⁺⁾-water-acacia⁽⁻⁾ where the ferrocyanide or ferricyanide ions replace the arabinic anions.

In the present work both the coacervate volume and turbidity measurements gave complementary results which showed visually the suppressive effect of electrolytes in the ethanol system (Figs 4 and 5). Analyses showed the changes produced in the phase composition by the presence of calcium chloride (Tables 2 and 3). In the ethanol system the coacervate phase showed more pronounced changes than its corresponding equilibrium liquid. The calcium chloride concentration was paralleled by a decrease in the gelatin and an increase in the liquid (ethanol + water) contents of the coacervate phase. This was due to the larger space occupied by the coiled structure as a result of unfolding under the influence of calcium chloride. At a sufficiently high concentration of calcium chloride the system remained clear in the one-phase region where the composition throughout would be the same.

The results reported here, together with the data presented in a previous paper on the effect of pH on gelatin coacervation (Khalil & others, 1968) clearly indicate the role played by the ionogenic groups on gelatin in the coacervation phenomenon. The results also showed that gelatin coacervation by non-electrolytes, e.g., ethanol was of different type from coacervation by electrolytes, e.g., sodium sulphate. The former system was extremely sensitive to pH changes and the addition of electrolytes whilst the latter resisted these factors due to the protection offered by the electrolyte used in coacervation. It is suggested that the coacervation of systems such as isoionic gelatin-water-non-electrolyte is comparable with complex coacervating systems such as gelatin⁽⁺⁾-water-acacia⁽⁻⁾. In both cases phase separation results from charge opposition. In the so-called complex coacervation this charge opposition is between the two colloids whilst with the simple coacervation it is between oppositely charged sites on the isoionic gelatin. The presence of ethanol, as a coacervating agent, was necessary to enhance this latter effect sufficiently to produce phase separation.

This suggestion is supported by the fact that both systems are affected, in a qualitatively similar fashion, by changes in pH and electrolytic impurities. It thus appears that the classification of coacervation phenomenon into "simple" and "complex" types is no longer valid.

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