J. Pharm. Pharmac., 1966, **18**, 409–416 Received December 23, 1965

Phase relationships in the simple coacervating system isoelectric gelatin : ethanol : water

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

The influence of molecular weight on coacervation in the system iso-electric gelatin : ethanol : water has been studied. The minimum concentration of ethanol required to produce coacervation decreased as the molecular weight of the gelatin increased. Analysis of the coacervate phase showed that the ethanol content was approximately constant and independent of both the molecular weight of the gelatin and the total concentration of ethanol in the system.

UNDER certain conditions macromolecular solutions may separate into two liquid layers. The term coacervation was introduced by Bungenberg de Jong & Kruyt (1929) to denote this kind of phase separation. If the opposition of charges between the colloidal components is the cause of this separation it is termed complex coacervation, whilst if it is due to "desolvation" it is said to be simple coacervation. In this latter instance the coacervate phase is rich in the colloidal component whilst the equilibrium liquid contains only negligible amounts of the colloid. Coacervation has been observed both in solutions of colloidal electrolytes (Bungenberg de Jong, 1937) and non-electrolytes (Dobry, 1938).

Because of its importance in plant and animal biology, complex coacervation has been extensively studied (Bungenberg de Jong, 1949), while few published data refer to simple coacervation. Many patents have been granted in recent years for various coacervating systems, both simple and complex, as coatings for pharmaceutical and other purposes (Green & Schleicher, 1956; Green, 1957). Phares & Sperandio (1964) have prepared samples of pharmaceuticals coated with coacervate layers.

We have examined the location of the coacervate phase obtained from the simple system isoelectric gelatin : ethanol : water in relation to the molecular weight of the gelatin and also the composition of the coacervate and equilibrium liquid.

Experimental

MATERIALS

Gelatin. The samples were alkali-processed hide gelatins having the characteristics given in Table 1. The gelatin samples were dried in thin layers at 110° for 12 hr and stored in air-tight containers. *Absolute ethanol* and *glass distilled water* were used.

METHODS

Determination of the number average molecular weight of gelatin. This was determined from the intrinsic viscosity by the method of Janus & Darlow (1962) using constants obtained by Pouradier & Venet (1950).

From The School of Pharmacy, Chelsea College of Science and Technology, Manresa Road, London, S.W.3.

* This work forms part of a thesis (S.A.H.K.) to be submitted for the degree of Ph.D. in the University of London.

TABLE 1. CHARACTERISTICS OF GELATIN SAMPLES

Sample	Number average M	Bloom number	Viscosity (cps, 6·67%)	Isoelectric point	Ash %
A	27,000	55	—	5·1	1·18
B	49,000	106	2·9 (60°)	5·1	1·21
C	60,000	160	4·1 (60°)	5·0	1·19
D	70,000	240	8·1 (40°)	5·2	1·20

Determination of the phase boundaries. A series of gelatin solutions containing from 5 to 30% w/w gelatin were prepared and adjusted to the isoelectric point by the dropwise addition of 2N hydrochloric acid. A weighed quantity of the coacervating agent (ethanol) was added to a weighed quantity of gelatin solution and the mixture equilibrated in a glass stoppered centrifuge tube at 40 ± 0·1°. Further additions of ethanol were made until a phase change was noted. The mixture was equilibrated for 24 hr at 40° and then centrifuged at 5000 rpm for 5 min to achieve separation.

The number and types of phase present at 40° were examined and the percentage composition at which a phase change occurred was calculated.

Measurement of the coacervate volume. The volume of the coacervate phase separated at 40° was measured directly in calibrated 10 ml centrifuge tubes.

Analysis of the coacervate phase and the equilibrium liquid. The method of Phares & Sperandio (1964) was modified to enable mixtures of gelatin: ethanol: water to be assayed. A total gelatin concentration of 5% w/w was used to allow comparison with the results of other workers. The gelatin solution was adjusted to the isoelectric point with 2N hydrochloric acid and the required weight of ethanol added. The stoppered tubes were then equilibrated for 5 days at 40 ± 0·1° to allow complete separation of the phases.

The coacervate phase was sampled and after suitable dilutions with water the refractive index and the specific gravity were determined at 40°. The percentage composition of the dilutions was calculated from the formulae

$$W = 1 - A - G \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (1)$$

$$G = \frac{\Delta RI - \alpha A}{\beta} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)$$

$$A = \frac{(\Delta \text{ sp.gr.})\,\beta - \Delta RI(\Delta \text{ sp.gr.} + \phi)}{(\beta\psi - \alpha\phi) + \Delta \text{ sp.gr.}\,(\beta - \alpha)} \quad .. \quad .. \quad (3)$$

Where A = g ethanol/g diluted coacervate phase G = g gelatin/g diluted coacervate phase, W = g water/g diluted coacervate phase, RI = refractive index, sp.gr. = specific gravity, α = slope of refractive index against concentration of ethanol (g/g of solution), β = slope of refractive index against concentration of gelatin (g/g of solution), ψ = slope of specific gravity against concentration of ethanol (g/g of solvent), ϕ = slope of specific gravity against concentration of gelatin (g/g of solvent).

From the value obtained, the composition of the undiluted coacervate phase was calculated.

Samples of the equilibrium liquid were similarly diluted and the refractive index determined. The concentrations of ethanol and gelatin were calculated using the equation of the tie line.

$$A = \gamma + \sigma G \quad .. \quad .. \quad .. \quad .. \quad (4)$$

where γ and σ are constants for each tie line.

By simple mathematical manipulation the concentration of gelatin (G_E) and ethanol (A_E) in the diluted equilibrium liquid can be shown to be given by equations 5 and 6.

$$G_E = \frac{A_E - \gamma}{\sigma} \quad .. \quad .. \quad .. \quad .. \quad (5)$$

$$A_E = \frac{\sigma \Delta RI + \gamma\beta}{\sigma\alpha + \beta} \quad .. \quad .. \quad .. \quad (6)$$

Results

The ternary diagrams for the gelatins examined are shown in Fig. 1. The positions of the various phases produced on transition from a clear

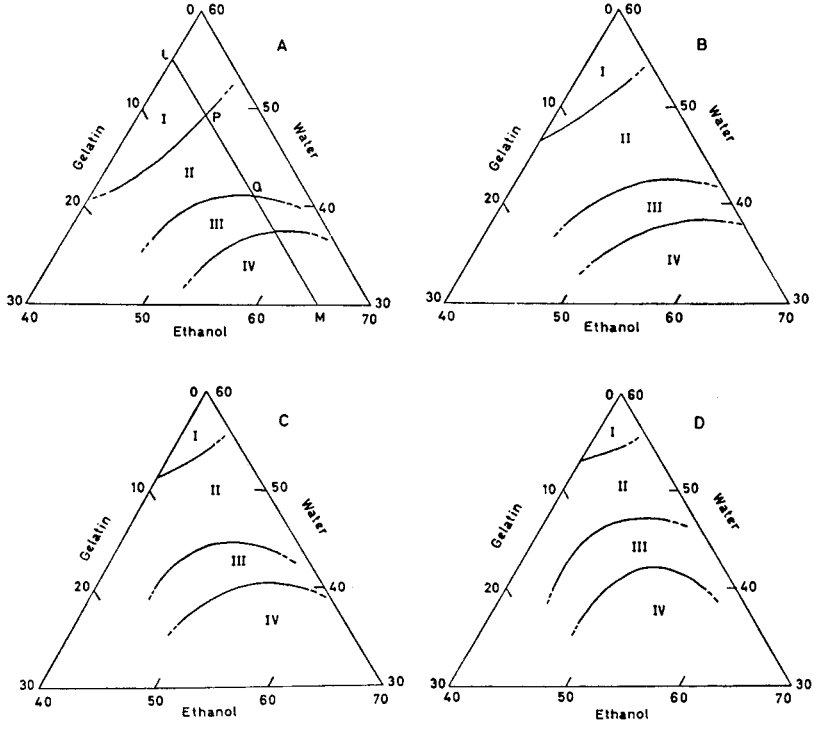

FIG. 1A-D. Ternary diagrams of the phase boundaries in the system iso-electric gelatin : ethanol : water. Gelatin Number Average Molecular Weight : A, 27,000 ; B 49,000 ; C, 60,000 : D, 70,000. I Clear isotropic liquid ; II Coacervate and equilibrium liquid ; III Coacervate, precipitated gelatin and liquid ; IV Precipitated gelatin and liquid. Temperature, 40° ± 0·1°. pH = isoelectric point.

solution to complete flocculation of the gelatin component are shown. In Fig. 1A, the line LPQM represents mixtures containing 5% w/w gelatin. As the concentration of ethanol was increased above 40% w/w, transition into a turbid region (II) occurred at point P. On microscopic examination this was found to consist of coacervate droplets (20–30 μ diameter) in a clear equilibrium liquid. The coacervate phase was viscous and showed no birefringence under polarised light.

Increasing concentrations of ethanol, from 48 to 52%, produced a decrease in coacervate volume (Table 2) and an increase in viscosity. At constant ethanol concentrations the volume of the coacervate phase was proportional both to the amount of gelatin present in the system and to the molecular weight of the gelatin (Tables 2 and 3).

TABLE 2. CHANGE IN COACERVATE VOLUME WITH INCREASE IN ETHANOL CONCENTRATION

Gelatin % w/w *M* 27,000	Ethanol % w/w	Water % w/w	Coacervate volume ml
5	48	47	0·6
	50	45	0·4
	52	43	0·28
10	46	44	1·3
	48·5	41·5	0·9
15	44	41	1·9
	46	39	1·5

TABLE 3. THE EFFECT OF MOLECULAR WEIGHT ON COACERVATE VOLUME AT 40° IN THE SYSTEM GELATIN 5·32% w/w; ETHANOL 46·81 w/w; WATER 47·87% w/w

Number average *M*	27,000	49,000	60,000	70,000
Coacervate volume (ml)	0·65	1·20	1·65	2·20

When the ethanol concentration was further increased, a region (III) was reached in which coacervate and equilibrium liquid existed in equilibrium with precipitated gelatin. Finally, at sufficiently high ethanol concentrations, all the gelatin was precipitated (IV).

The most obvious effect of an increase in the molecular weight of the gelatin was that a lower concentration of ethanol was required to produce any phase change at a given gelatin concentration (Fig. 2, which is based on the positions of the phase boundaries in Fig. 1). These phase boundary lines were parallel, which indicated that the increase in concentration of ethanol required to produce any phase change was independent of the molecular weight of the gelatin. A linear relationship existed between the number average molecular weight and the total ethanol concentration required to produce a phase change.

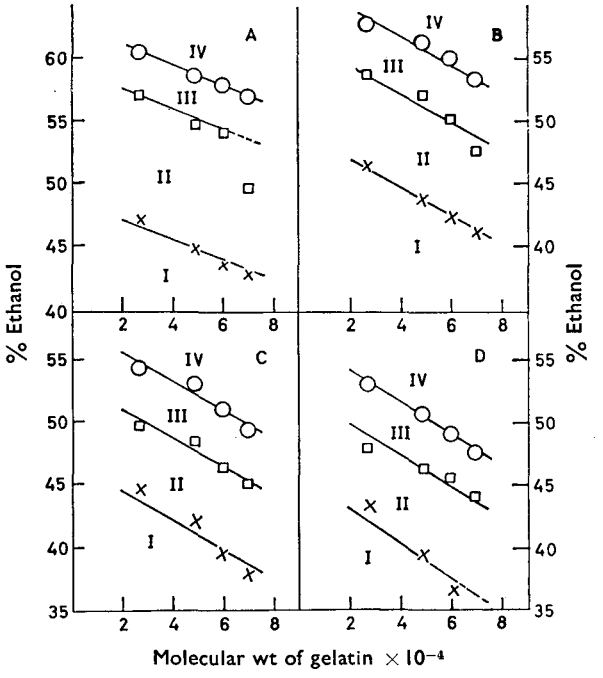

FIG. 2. The effect of molecular weight of gelatin on coacervation. Gelatin concentration % w/w: A, 2·5; B, 5; C, 10; D, 15. I Clear isotropic liquid; II Coacervate and equilibrium liquid; III Coacervate, precipitated gelatin and liquid; IV Precipitated gelatin and liquid. Temperature, 40° ± 0·1. pH = isoelectric point.

Analysis of the coacervate and corresponding equilibrium liquid (Table 4) showed that the ethanol content of the coacervate was approximately constant irrespective of the total ethanol concentration in the mixture and of the number average molecular weight of the gelatin.

TABLE 4. THE EFFECT OF GELATIN MOLECULAR WEIGHT ON THE COMPOSITION OF COACERVATE AND EQUILIBRIUM LIQUIDS

Gelatin Average number M	Percentage w/w compositions								
	Total mixture			Coacervate			Equilibrium liquid		
	Gelatin	Ethanol	Water	Gelatin	Ethanol	Water	Gelatin	Ethanol	Water
27,000	5	48	47	19·3	37·0	43·7	3·1	49·4	47·5
	5	50	45	23·0	38·0	39·0	2·0	52·2	45·8
	5	52	43	25·4	37·4	37·2	1·3	54·7	44·0
	5	54	41	28·0	37·0	35·0	0·7	57·2	42·1
49,000	5	45	50	12·3	36·8	50·9	1·9	48·5	49·6
	5	47	48	15·9	36·3	47·8	1·0	50·6	48·4
	5	49	46	19·3	37·7	43·0	0·7	52·6	46·7
	5	51	44	22·2	37·5	40·3	0·6	54·6	44·8
70,000	5	43	52	15·3	36·3	48·4	0·5	45·9	53·6
	5	44·5	50·5	16·4	36·7	46·9	0·2	47·9	51·9
	5	46	49	17·3	36·9	45·8	0·1	49·6	50·3
	5	47·5	47·5	18·7	36·2	45·1	0·1	51·5	48·4

All figures are the mean of three experiments

Fig. 3 and Table 4 clearly indicate this point, but it should be noted that in the region of the initial formation of coacervate, the composition of coacervate and equilibrium liquid would be approximately the same. This means that the proportion of ethanol in the coacervate would be higher than the equilibrium concentration reached on passing further into the coacervate region, whilst the corresponding equilibrium liquid would contain a proportionately higher colloid concentration.

Discussion

Because of inadequate physico-chemical criteria for defining coacervates and coacervation the terms have been misused in the past and Lawrence (1954) suggested that this nomenclature is not necessary. However, coacervates do have unique characteristics in that they separate out as optically active isotropic liquids in equilibrium with a liquid containing only negligible amounts of the colloid, and the continued use of the term to distinguish them from other colloid-rich phases such as liquid crystals has been supported by Dervichian (1949).

Because of the polydisperse nature of the gelatin it is not strictly accurate to construct a three component phase diagram, but Fergusson & Richardson (1932) have shown that heterogeneous colloids (commercial soaps) behave effectively as a single component, and a triangular diagram showing the regions where one, two or three phases exist in equilibrium is valid (Dervichian, 1954).

The order of occurrence of the different phases in the system studied in this work resembled closely the phase equilibria in the system gelatin: water : ammonium sulphate (Dervichian & Van den Berg, 1948) where in each instance the coacervate was separated from the flocculate region by a zone containing coacervate, solid gelatin and equilibrium liquid.

The influence of gelatin average molecular weight on the position of the phases is shown in Fig. 1. The relative position of all the phases moved towards the ethanol corner as the molecular weight decreased. There was no direct relationship between the band width of a phase and the concentration of gelatin in the system. A linear decrease in band width of the coacervate phase was found up to approximately 12% w/w gelatin after which the range of ethanol concentration over which this phase was present sharply increased. The corresponding changes in the width of the three phase system (Fig. 2) were much smaller but whereas the coacervate band showed a minimum width the three phase system had a maximum in the region of 12% w/w gelatin. Fig. 2 also shows that the molecular weight of the gelatin used had no effect on the band width of a particular phase, but only on its relative position within the triangular diagram because the boundaries of the phases were essentially parallel.

Once coacervation had occurred, the further addition of ethanol produced a decrease in coacervate volume (Table 2). It is also possible, from the intercept of the line XA (Fig. 3) with the tie lines, to deduce the relative weights of each of the two phases in equilibrium as the ethanol concentration is progressively increased. The relative weight of the coacervate as a

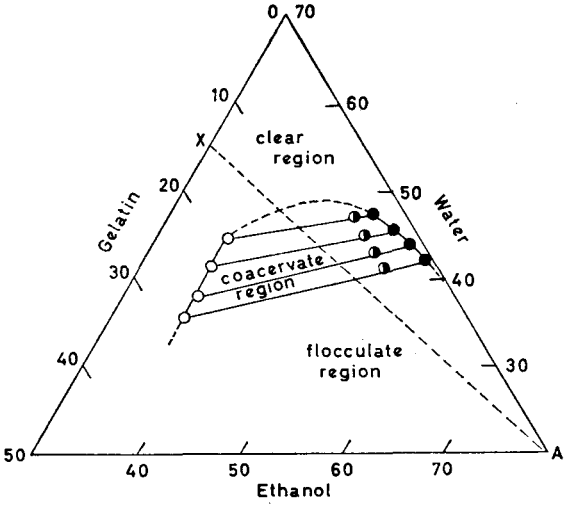

FIG. 3. The composition of coacervate and corresponding equilibrium liquid. Gelatin Number Average Molecular Weight 27,000. ○, Coacervate; ●, Equilibrium liquid; ◑, Total Mixture. Temperature, 40° ± 0·1°. pH = isoelectric point.

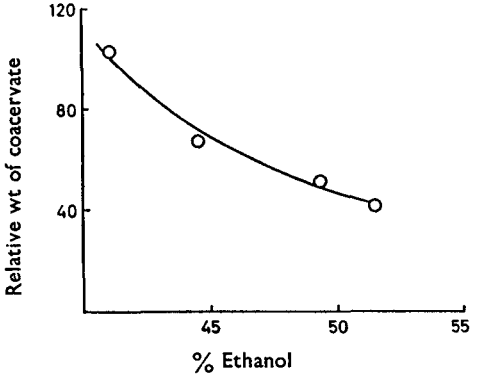

FIG. 4. Variation of the relative weight of the coacervate as a function of total ethanol concentration in the system. Ethanol gradually added to a system containing 15% w/w gelatin: 30% ethanol; 55% w/w water (line XA in Fig. 3).

function of ethanol concentration is shown in Fig. 4. Neither the decrease in relative weight nor volume was directly proportional to the ethanol concentration; an increase in the viscosity of the coacervate was also noticed. As the bulk of the gelatin was contained in this phase and the ethanol content remained constant, the changes must be due to the gradual dehydration of the coacervate as the overall percentage of ethanol in the system was increased.

Analyses of the two phases in equilibrium indicated that the coacervate contained almost a constant percentage of coacervating agent irrespective of the gelatin used or of the total ethanol concentration (Table 4). The

ethanol range was found to be between 36·3 and 38·0% w/w. This was similar to results (36·4–39·9%) obtained by Holleman, Bungenburg de Jong & Modderman (1934) using a less precise method of assay and an alkali processed gelatin of unknown molecular weight. In systems using sodium sulphate as coacervating agent a similar condition can be deduced from results obtained by Holleman & others (1934) and by Phares & Sperandio (1964), but the figures differ depending on the type of gelatin used. With alkali processed gelatin Holleman & others found an average value of 6·7% whilst with acid pretreated gelatin, Phares & Sperandio obtained a value of 9·5%.

With the data available at present it is difficult to be precise about the liquid nature of the coacervate phase or to speculate how the solvent-precipitant liquid is held by the gelatin. The modified theory of coacervation (Bungenberg de Jong, 1949; Basu & Bhattacharya, 1952) suggest that the inclusion liquid is immobilised within the spiral of the flexible colloid molecule. If this is so then the higher molecular weight gelatins, because of their greater flexibility, would be easier to coacervate than the less flexible shorter chain length material, as is shown in Fig. 1. The modified theory of coacervation suggests that the ethanol-water mixture held within the loops of the gelatin molecule can be divided into two parts: an occlusion liquid of the same composition as the equilibrium liquid and an excess of water which is present as water of hydration. Although this type of calculation is obviously imprecise, the amount of water of hydration g per g of gelatin was found to be: 0·2 (*M* 27,000), 0·4 (*M* 49,000), 0·3(*M* 70,000). This is similar to the value of 0·3 reported by Holleman & others (1934).

Acknowledgements. The authors wish to thank Mr. H. McGurk of Richard Hodgson & Sons Ltd, Beverley, Yorkshire, for gelatin samples of known viscosity, Bloom number and isoelectric point.

References

Basu, S. & Bhattacharya, G. (1952). *Science, N.Y.*, **115**, 544–546.
Bungenberg de Jong, H. G. & Kruyt, H. R. (1929). *Proc. Sect. Sci. K. ned. Akad. Wet.*, **32**, 849–856.
Bungenberg de Jong, H. G. (1937). *Kolloidzeitschrift*, **79**, 223–32; **80**, 221–231, 350–360.
Bungenberg de Jong, H. G. (1949). *Colloid Science*, Vol. 2, pp. 232–480, Editor, Kruyt, H. R. Amsterdam: Elsevier.
Dervichian, D. G. (1949). *Research, Lond.*, **2**, 210–217.
Dervichian, D. G. (1954). *Discuss. Faraday Soc.*, **18**, 231–239.
Dervichian, D. G. & Van den Berg, H. J. (1948). *Bull. Soc. Chim. biol.*, **30**, 559.
Dobry, A. (1938). *J. chim. Phys.*, **35**, 559.
Fergusson, R. H. and Richardson, A. S. (1932). *Ind. Engng Chem.*, **24**, 1329–1336.
Green, B. K. & Schleicher, L. (1956). U.S. patent 2,730,456.
Green, B. K. (1957). U.S. patent 2,800,457.
Holleman, L. W. J., Bungenberg de Jong, H. G. & Modderman, R. S. T. (1934). *Kolloidbeihefte*, **39**, 356.
Janus, J. W. & Darlow, R. L. R. (1962). *Nature, Lond.*, **194**, 1075–1076.
Lawrence, A. S. C. (1954). *Discuss. Faraday Soc.*, **18**, 229–230.
Phares, R. E. & Sperandio, G. J. (1964). *J. pharm. Sci.*, **53**, 515–521.
Pouradier, J. & Venet, A. (1950). *J. Chim. phys.*, **47**, 391.