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Role of pH in the coacervation of the systems: gelatin–water–ethanol and gelatin–water–sodium sulphate*

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Phase boundary determination, coacervate volume measurements and analysis of the phases have been made to assess the influence of pH on the coacervation of gelatin solutions by ethanol and sodium sulphate. Coacervation was found to be pH dependent. In the ethanol system coacervation was noticeable only within a pH range in the vicinity of the isoionic point; at other pH values either a viscous gel phase or floccules occurred. In the sodium sulphate system, coacervation occurred at all pH values examined. The effect of pH in changing the overall charge on the gelatin molecule is explained in relation to the formation of gelatin coacervates. Finally, the role of the coacervate phase in the microencapsulation of oil and solid particulates is discussed.

THE term coacervation was introduced by Bungenberg de Jong & Kruyt (1929) to describe the various cases of partial miscibility occurring in macromolecular systems where two isotropic liquid phases co-exist. Under optimum conditions the colloid-rich phase, the coacervate, contains the bulk of the colloidal components and the equilibrium liquid contains only a negligible amount of the colloid. Two main types of coacervation have been classified by Bungenberg de Jong (1949); simple coacervation occurs as the result of a decrease in the solubility of the colloidal components caused by the addition of non-solvents, as in the systems: isoelectric gelatin–water–ethanol and isoelectric gelatin–water–sodium sulphate, first investigated by Holleman, Bungenberg de Jong & Modderman (1934). Complex coacervation, on the other hand, results from the interaction of oppositely charged colloids and has been of academic interest in relation to some biological phenomena (Bungenberg de Jong & Booij, 1956). Recently a quantitative formulation to the theory of complex coacervation was attempted by Veis (1963).

Simple coacervation of gelatin systems has received little attention as it has been assumed that the phenomenon occurs as a result of dehydration and that the charge effects play no active role.

In the past few years, attention has focussed on the application of both types of gelatin coacervation to the microencapsulation of pharmaceuticals (Luzzi & Gerraughty, 1964; Phares & Sperandio, 1964) and as a superior method for the fractionation of heterodisperse polymers (Stainsby, 1954). The present paper examines the effects of pH on the simple coacervation of gelatin by ethanol and sodium sulphate.

Experimental

MATERIALS

Gelatin. Two samples were used having the characteristics given in Table 1. The gelatins were dried in thin layers at 110° for 12 hr and

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TABLE 1. CHARACTERISTICS OF GELATIN SAMPLES

Sample	Type	Source	Bloom number	pH	Viscosity (cps 6·67%)	Ash %	Isoelectric point	Isoionic point (PI)
A	Lime-pretreated	Hide	240	6·6	8·1 (40°)	1·20	5·2	4·9
B	Acid-processed	Pigskin	252	4·7	5·5 (60°)	1·11	9·2	8·9

stored in air-tight containers. *Absolute ethanol* and 20% *w/w sodium sulphate solution* were used as the coacervating agents. All *electrolytes* used were Analar and *water* was once distilled from an all-glass still (pH 5·1, specific conductivity 4·8 μ mhos cm^{-1}).

METHODS

Determination of isoionic point and preparation of ash-free gelatin. The isoionic point was determined by the mixed bed ion-exchange resin technique of Janus, Kenchington & Ward (1951) and large batches were deionized by the same technique. The maximum specific conductivity of the deionized product was 10–28 μ mhos cm^{-1} (40°) for 2–10% w/w gelatin solutions. The amount of ash estimated did not exceed 0·002%.

Adjustment of pH. Hydrochloric acid (2N) or sodium hydroxide (2N) was used to adjust the pH of the gelatin solutions. The use of buffers was avoided on account of the possible effect of electrolytes on gelatin coacervation. Measurements of pH values were made at 40° using glass/calomel electrodes.

Determination of the phase boundaries and analysis of the coacervate phase and equilibrium liquid. The methods described by Nixon, Khalil & Carless (1966) were used.

Measurement of the coacervate volume. The volume of the separated coacervate phase was directly measured using calibrated 10 ml centrifuge tubes after equilibration at 40°. When the coacervate volume was too small for direct measurement the apparatus used was a glass vessel provided with a calibrated tube and a platinum stirrer. The coacervating agent was added from a microburette attached to one side of the apparatus.

Results

The phases separating at various pH values after the minimum ethanol or sodium sulphate concentration required to produce a phase change was added to gelatin solution, are shown in Tables 2 and 3. The 10% gelatin solutions require less coacervating agent because the gelatin molecules are in a state of greater initial aggregation; also, at the higher temperatures, they required less dehydrating agent to move the dispersion into the coacervate region. Whilst the initial gelatin concentration only affected the amount of coacervating agent required to produce a phase change, the behaviour of both systems differed considerably with pH changes. In the ethanol system separation into two isotropic liquid phases (coacervation) was noted only within a pH range of 4·4 to 6·9 for

TABLE 2. EFFECT OF pH ON THE TYPE OF PHASE SEPARATING IN THE SYSTEM GELATIN–WATER–ETHANOL. Minimum ethanol concentrations (% w/w) required to initiate a phase change. Temperature: 40° ± 0·1°, 5 g samples

	240 Bloom alkali-processed gelatin					250 Bloom acid-processed gelatin					
	Before deionization % w/w initial gelatin			After deionization % w/w initial gelatin			Before deionization % w/w initial gelatin			After deionization % w/w initial gelatin	
pH	5	10	pH	5	10	pH	5	10	pH	5	10
3·0	72·1 F	63·7 F	4·1	79·9 F	73·2 F	3·2	74·7 F	66·0 F	3·8	80·5 F	77·1 F
4·2	68·2 F	60·5 F	4·2	62·6 F	59·3 F	4·3	70·4 F	62·8 F	4·4	76·9 F	75·6 F
4·3	65·0 F	58·9 F	4·3	56·7 G	54·9 G	4·7*†	68·2 F	60·3 F	5·3	74·8 F	70·7 F
4·4	61·4 F	56·7 F	4·4	54·5 C	53·2 C	5·1	61·9 F	59·7 F	6·1	69·1 F	66·9 F
4·5	59·3 G	55·2 G	4·8	48·2 C	46·0 C	6·4	60·3 F	59·2 F	6·7	65·3 G	63·9 G
4·6	57·3 C	53·9 C	4·9‡	40·0 C	38·4 C	6·8	58·9 G	57·9 G	6·9	63·0 C	61·9 C
4·8	53·9 C	52·2 C	5·9	57·6 C	53·9 C	6·9	58·4 C	57·0 C	7·4	60·3 C	58·7 C
5·2†	42·1 C	41·6 C	6·3	60·3 C	57·3 C	7·1	57·6 C	56·3 C	8·1	57·0 C	55·2 C
5·9*	40·9 C	39·1 C	6·9	61·7 C	58·9 C	8·3	56·1 C	54·9 C	8·9‡	49·8 C	48·7 C
7·7	53·5 C	48·7 C	7·0	63·3 G	61·9 G	8·9	53·9 C	52·2 C	9·3	56·1 C	51·8 C
8·7	59·7 C	51·0 C	7·1	66·0 G	62·7 G	9·1†	51·0 C	50·2 C	10·2	59·7 C	55·5 C
9·0	65·6 C	53·2 C	8·1	69·9 F	66·6 F	9·7	53·2 C	51·8 C	10·8	64·9 C	61·9 C
9·2	66·9 C	53·8 C	10·6	79·0 F	70·4 F	10·1	57·6 C	54·9 C	11·0	69·1 G	65·4 G
9·3	67·4 G	54·5 G	—	—	—	10·9	59·7 C	56·7 C	11·3	72·6 G	68·2 G
9·8	68·4 F	56·4 F	—	—	—	11·3	61·9 G	60·8 G	11·7	77·7 F	73·3 F
11·1	73·9 F	61·9 F	—	—	—	11·4	66·7 G	63·0 G	—	—	—
—	—	—	—	—	—	11·8	75·8 F	71·3 F	—	—	—

Type of phase separated: F = flocculate; G = gel; C = coacervate.
‡ = *isoionic point*; † = *isoelectric point*; * = pH of gelatin solution without adjustment.

the deionized alkali-processed gelatin (pI 4·9) and pH 6·9 to 10·8 for the deionized acid-processed sample (pI 8·9). Outside these pH ranges a highly viscous gel phase separated and at extreme pH values flocculation occurred, Fig. 1. Both the gel phase and the floccules produced showed no coacervate droplets, characteristic of coacervate phase. Commercial lime-pretreated gelatin samples are less sensitive to pH changes because of the electrolytic impurities, mainly calcium salts which are bonded to the carboxyl groupings of the gelatin on the alkaline side of the isoelectric

TABLE 3. EFFECT OF pH ON THE TYPE OF PHASE SEPARATING IN THE SYSTEM: GELATIN–WATER–SODIUM SULPHATE. Minimum sodium sulphate concentrations (% w/w) required to initiate a phase change. Temperature: 40° ± 0·1°, 5 g samples

	240 Bloom alkali-processed gelatin					250 Bloom acid-processed gelatin					
	Before deionization % w/w initial gelatin			After deionization % w/w initial gelatin			Before demineralization % w/w initial gelatin			After demineralization % w/w initial gelatin	
pH	5	10	pH	5	10	pH	5	10	pH	5	10
2·1	6·35 C	6·13 C	2·4	6·38 C	6·22 C	3·2	6·80 C	6·34 C	2·4	6·70 C	6·48 C
3·8	6·61 C	6·40 C	3·7	6·51 C	6·36 C	4·1	6·93 C	6·77 C	3·3	6·93 C	6·60 C
4·3	6·92 C	6·84 C	4·4	7·11 C	6·92 C	4·7*	7·42 C	6·98 C	4·6	7·08 C	6·77 C
4·9	7·95 C	7·47 C	4·6	7·36 C	7·26 C	5·6	7·68 C	7·18 C	5·2	7·36 C	6·92 C
5·2†	8·47 C	8·11 C	4·9‡	7·89 C	7·81 C	6·4	7·85 C	7·60 C	6·7	7·48 C	7·13 C
5·9*	8·49 C	8·32 C	5·5	8·31 C	8·03 C	8·0	8·16 C	8·01 C	8·4	7·63 C	7·35 C
6·7	8·55 C	8·47 C	5·9	8·66 C	8·31 C	9·1†	8·47 C	8·24 C	8·9‡	7·80 C	7·74 C
7·7	8·91 C	8·71 C	6·6	8·90 C	8·45 C	9·7	8·68 C	8·40 C	9·7	7·86 C	7·78 C
8·0	9·46 C	9·02 C	8·3	9·27 C	9·10 C	10·4	9·11 C	8·79 C	10·8	8·40 C	8·10 C
9·1	9·48 C	9·17 C	8·9	9·45 C	9·22 C	10·7	9·24 C	8·90 C	11·3	8·78 C	8·36 C
9·3	9·51 C	9·26 C	9·1	9·63 C	9·26 C	—	—	—	—	—	—
10·2	9·54 C	9·33 C	9·6	9·70 C	9·37 C	—	—	—	—	—	—
—	—	—	10·5	9·78 C	9·48 C	—	—	—	—	—	—

* pH of the gelatin solution without adjustment; † isoelectric point; ‡ isoionic point.
C = Coacervate.

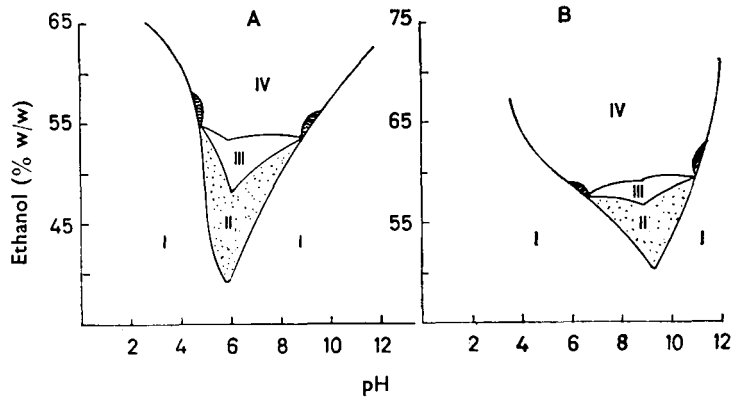

FIG. 1. Phase relationship at various pH values in the system gelatin–water–ethanol. A. Alkali-processed gelatin (I.E.P. 5·2). B. Acid-processed gelatin (I.E.P. 9.2). Initial gelatin concentration: 10% w/w Temperature: 40° ± 0.1°. I = clear isotropic phase, II = coacervation, III = Three phases, IV = Flocculation. Hatched area = gel phase.

point. For the commercial alkali-processed sample the pH range for coacervation was extended to 4·6–9·2. The addition of equivalent amounts of calcium chloride to demineralized gelatin produced a similar insensitivity to pH changes on the alkaline side of the isoelectric point. High concentrations of sodium chloride (1–2M) produced similar results.

Coacervation by sodium sulphate occurred at all pH values examined and lower electrolyte concentrations were required on the acid side of the isoionic point (Table 3).

The isothermal triangular diagrams of phase boundaries, Fig. 2 A–F, show the location of the coacervation region (II) relative to the other phases present at various pH values. At values away from the isoelectric point the boundaries move towards higher ethanol concentrations. The bandwidth of both the coacervation region (II) and the three-phase zone (III) is relatively narrow and, outside the effective pH range for coacervation, the system passes directly from the clear isotropic liquid region (I) to the flocculate region (IV). In sodium sulphate systems, all the phase boundaries are displaced away from the sodium sulphate corner at pH values on the acid side of the isoelectric point (Fig. 2 F) and the reverse occurs on the alkaline side.

Changes in coacervate volume at various pH values are shown in Figs 3 and 4. In the ethanol system, variations in the pH farther from the isoionic point not only increased the percentages of ethanol required for maximum coacervate volume, but also the volume of the separated coacervate phase at the maxima (Fig. 3A,B). In the sodium sulphate system pH values on the acid side of the isoionic point produced a displacement of the maxima in the coacervate volume plots towards the ordinate axis and also a decrease in the coacervate volume (Fig. 4A). On the alkaline side of the isoionic point the coacervate produced showed a marked increase in volume and a lower viscosity. Plots of the maximum

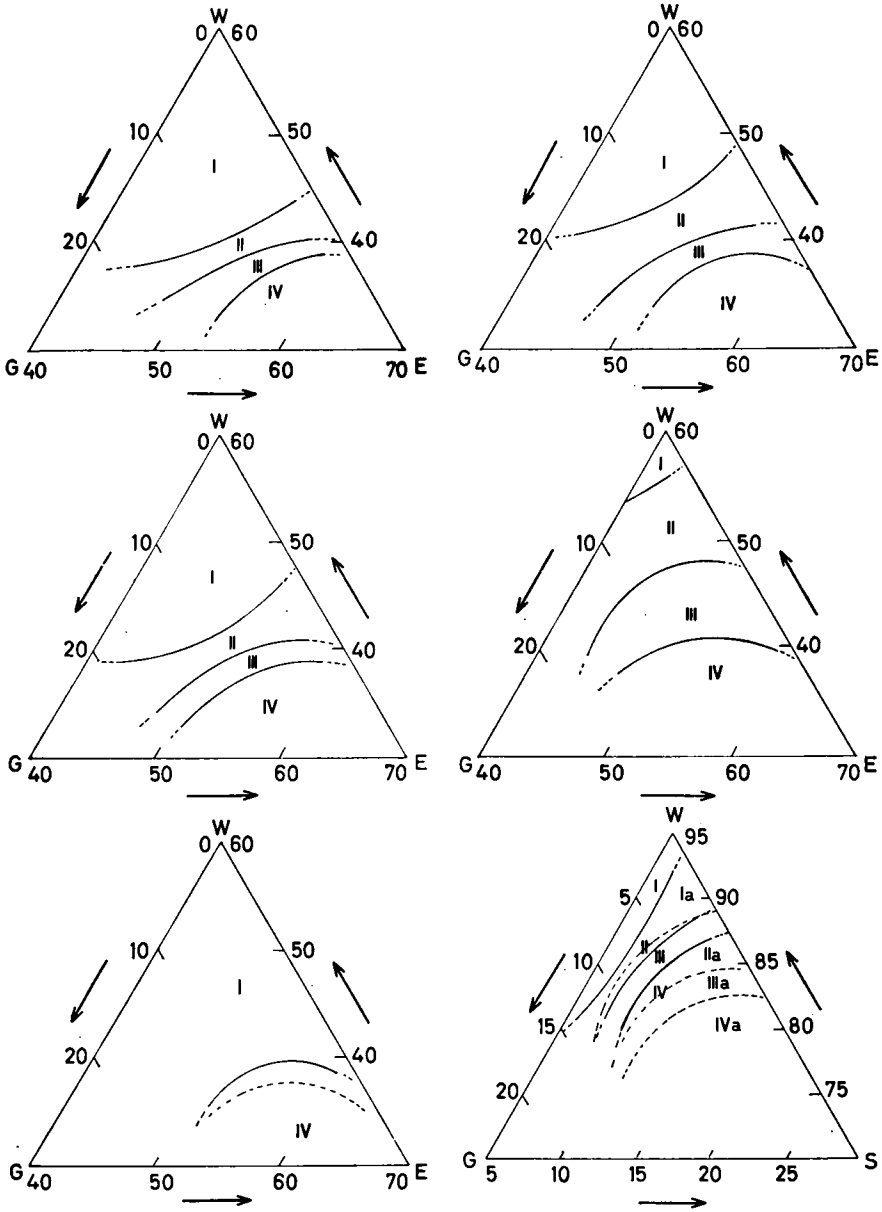

FIG. 2. A–F. Triangular diagrams of phase regions in the systems (GWE) gelatin–water–ethanol (A–E) and (GWS) gelatin–water–sodium sulphate (F) at various pH values. A (top left) pH 4·6. B (top right) pH 4·8. C (middle left) pH 5.2 (I.E.P.). D (middle right) pH 8.6. E (bottom left) solid line pH 4.4, broken line pH 9·8. F (bottom right) solid line pH 3·1 (regions I-IVa), broken line pH 8·1 (regions I–IV). I. Clear isotropic region. II. Coacervation (coacervate + equilibrium liquid). III. Three phase region (precipitated gelatin + coacervate + equilibrium liquid. IV. Flocculate region. W→G=gelatin. G→E=ethanol. E→W=water.

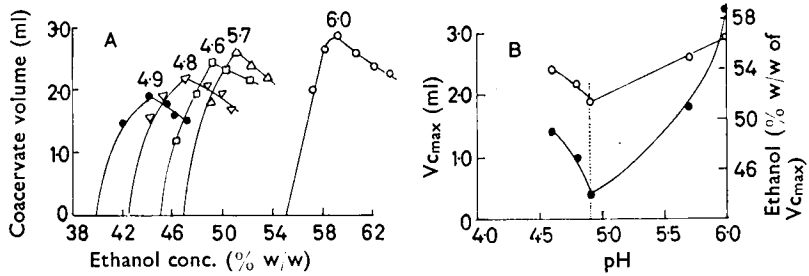

FIG. 3A. Change in coacervate volume with increasing ethanol concentrations at the pH values shown on the curves. Total gelatin concentration 4% w/v. Gelatin used: 240 Bloom pI 4.9. B. Maxima of coacervate volume (Vc $_{max}$) and their ethanol concentration plotted against pH from A. ○ Coacervate volume. ● Ethanol concentration.

coacervate volume, or the concentrations of coacervating agent which produced these maxima, against pH show minimal values at pH 4·9 in the ethanol system (the isoionic point of the alkali-processed gelatin) and pH 3·1 in the sodium sulphate system (Figs 3B, 4B). At these values the coacervates were highly viscous.

Analyses of the coacervates and their corresponding equilibrium liquids are given in Tables 4 and 5, and the values are plotted in Fig. 5 for the ethanol system. The position of the partial miscibility curves within the phase diagram, Fig. 5, depended on the pH in a manner analogous to the location of the coacervation region at a similar pH. At a fixed concentration of gelatin in the total mixture, the coacervates produced from ethanol systems showed a maximum percentage of colloid at the isoionic point which decreased at other pH values (Table 4). The corresponding equilibrium liquids contained low percentages of gelatin (<3% w/w), the minimum occurring at the isoionic point. Because of the increased concentration of ethanol required to produce coacervation at pH values away from the isoionic point, the equilibrium liquids at these pH values were relatively richer in ethanol content. Variation in the pH also produced a noticeable effect on the ethanol content of the coacervates.

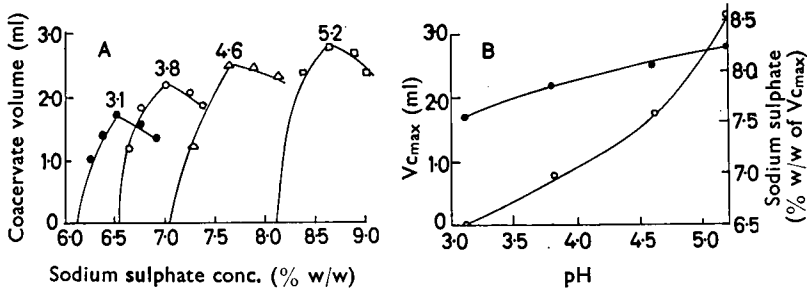

FIG. 4. A. Change in coacervate volume with increasing sodium sulphate concentrations at the pH values shown on the curves. B. Maxima of coacervate volume (Vc $_{max}$) and their sodium sulphate concentrations plotted against pH. ● Coacervate volume. ○ Sodium sulphate concentration.

TABLE 4. COMPOSITIONS OF THE COACERVATES AND EQUILIBRIUM LIQUIDS AS A FUNCTION OF pH, IN THE SYSTEM: GELATIN–WATER–ETHANOL. Gelatin used: demineralized 240 Bloom alkali-processed. Temperature: 40° ± 0·1°

	Percentage w/w compositions								
	Total mixture			Coacervate			Equilibrium liquid		
pH	Gelatin	Ethanol	Water	Gelatin	Ethanol	Water	Gelatin	Ethanol	Water
4·7	4	45	51	11·1	41·8	47·1	2·8	46·1	51·5
	4	47	49	13·8	42·3	42·9	1·6	47·9	50·5
4·8	4	44	52	11·6	39·4	49·0	2·8	45·2	52·0
	4	46	50	14·3	39·8	45·9	2·2	47·3	50·5
4·9*	4	42	54	14·1	37·1	48·8	2·2	42·6	55·2
	4	44	52	16·9	36·4	46·7	1·4	45·4	53·2
	4	46	50	19·8	36·7	43·5	0·6	47·7	51·3
5·4	4	44	52	11·3	39·5	49·2	2·5	45·7	51·8
	4	46	50	12·9	39·7	47·4	2·1	47·6	50·3
5·6	4	46	50	8·8	42·3	48·9	2·6	47·2	50·2
	4	48	48	14·9	43·1	42·0	1·3	49·4	49·3
6·0	4	57	39	14·4	51·6	34·0	1·8	58·1	40·1
	4	59	37	19·3	50·8	29·9	0·5	60·7	38·8

* The isoionic point.

At the isoionic point, demineralized gelatin coacervates had an alcohol content of about 37% w/w, similar to that found in the coacervates of isoelectric commercial gelatin (Holleman & others, 1934, and Nixon & others, 1966). Away from the isoionic point the ethanol content of the coacervates showed a progressive increase.

Similar observations were made for the coacervates and equilibrium

TABLE 5. COMPOSITIONS OF THE COACERVATES AND EQUILIBRIUM LIQUIDS AS A FUNCTION OF pH IN THE SYSTEM: GELATIN–WATER–SODIUM SULPHATE. Gelatin used: 240 Bloom, alkali-processed. Temperature: 40° ± 0·1°.

	Percentage w/w composition								
	Total mixture			Coacervate			Equilibrium liquid		
pH	Gelatin	Sodium sulphate	Water	Gelatin	Sodium sulphate	Water	Gelatin	Sodium sulphate	Water
3·1	4	6·1	89·9	13·6	5·1	81·3	1·1	6·4	92·5
	4	6·9	89·1	16·1	5·0	78·9	0·6	7·6	91·8
	4	7·7	88·3	18·8	5·3	75·9	0·3	8·2	91·5
4·2	4	7·2	88·8	14·1	5·7	80·2	1·6	8·2	90·2
	4	8·0	88·0	16·3	5·7	78·0	1·0	8·8	90·2
	4	8·8	87·2	18·2	6·0	75·8	0·5	10·0	89·5
4·9*	4	8·2	87·8	10·8	7·1	82·1	1·8	9·3	88·9
	4	9·2	86·8	12·4	6·9	80·7	0·8	10·4	88·8
	4	10·2	85·8	14·2	6·9	78·9	0·6	11·5	87·9
6·6	4	8·8	87·2	11·1	7·3	81·6	2·1	9·8	88·1
	4	9·8	86·2	13·8	7·6	78·6	1·4	10·6	88·0
	4	10·8	85·2	15·5	7·5	77·0	0·9	11·8	87·3
8·9	4	9·4	86·6	12·1	8·2	79·7	2·8	9·9	87·3
	4	10·4	85·6	15·8	8·5	75·7	1·6	11·1	87·3
	4	11·4	84·6	17·7	8·1	74·2	0·9	11·9	87·2

* The isoionic point.

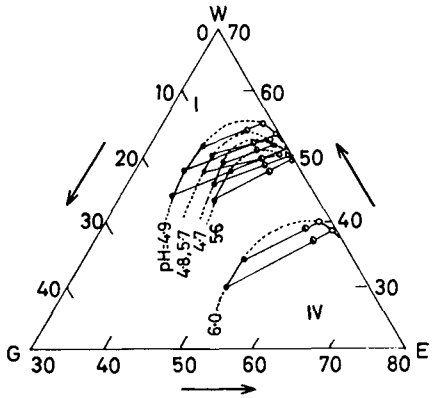

FIG. 5. Effect of pH on the composition of coacervates and the corresponding equilibrium liquids in the system: gelatin–water–ethanol. Temperature: 40° ± 0.1°. Gelatin: 240 Bloom, alkali-processed (pI 4·9). ● Coacervate. ○ Equilibrium liquid. ◑ Total mixture.

liquids of the sodium sulphate system (Table 5); the colloidal component being present exclusively in the coacervates the sodium sulphate contents of which increased on passing from an acid to an alkaline pH on both sides of the isoionic point.

Discussion

Gelatin exists in solution in a randomly coiled skein configuration. The shape of these coils is influenced by the charge on the molecules and their mutual interactions (Hermans & Overbeek, 1948). Predominance of charges of one type favours an unfolded stretched configuration. At the isoionic point the gelatin molecule attains the random coiled structure by virtue of inter- and intramolecular attractive forces. Variations in the pH result in an inbalance of charges and the force of repulsion arising causes unfolding of the coil.

Pasynskii (1958) has shown that in unbuffered solutions the shape of the gelatin molecules varied in proportion to the fourth or fifth root of the number of charges.

The role of pH in controlling gelatin coacervation in the two systems examined can be explained by the effect of pH on the two factors which appear to govern coacervation of polyelectrolyte systems: (*a*) the intra- and intermolecular attractive coulombic forces; (*b*) hydration.

The first of these favours phase separation and tends to produce floccules, whilst the second enhances redispersion of the molecular entities. The balance between these two factors gives rise to the separation of the colloid-rich isotropic liquid phase, the coacervate, which retains a certain amount of occluded liquid immobilized within the loops of the skeins. This occurs readily at the isoionic point where the hydration

effect would be balanced by the maximum attractive forces between the oppositely charged sites on the gelatin molecules.

At pH values away from the isoionic point the attractive forces decrease and, as found by Jirgenson (1946) and Czerniak & Pasynskii (1948), an increase in the hydration of the gelatin molecule takes place. Both changes tend to suppress coacervation and higher concentrations of the dehydrating agent (ethanol) will be required to restore the balance. In ethanol systems, at pH values well away from the isoionic point, flocculation occurs because the gelatin molecule is fully stretched and cannot entrap the necessary occlusion liquid. At intermediate pH values the gelatin molecules retain a certain degree of flexibility, although they are not able to entrap sufficient occlusion liquid to form coacervate. Under these conditions a viscous gel is formed. Basu & Bhattacharya (1952) reported similar changes in the phases separating in gelatin–water–ethanol systems as a function of pH, where the same sequence: viscous phase, hard gel, granular precipitate was found.

The effect of ethanol in changing the ionization constants of the various groups has been examined by Jukes & Schmidt (1934) who studied its effect on pK_a values of some amino-acids. They found an increase of about one pK_a unit of the carboxyl group compared to an increase of 0·1–0·4 pK_a units for the basic groups for ethanol concentrations between 50–72% v/v. But in the present work the effects of ethanol were not taken into account because of the difficulty in predicting accurately the changes in pK_a values caused by ethanol in the system. Table 6 shows the number of active groupings per 100,000 units of molecular weight, calculated from data by Courts (1954) and Eastoe (1955) and also the net charge produced by changes in the pH. At the isoionic point, where the net charge is zero, coacervation occurs readily, but at pH values corresponding to the limiting values for ethanol coacervation, the net charge is

TABLE 6. CALCULATION OF THE NET CHARGES PER 100,000 *M* ALKALI-PROCESSED GELATIN AS A FUNCTION OF pH

Group	No. of groups* per 100,000 *M*	pK_a†	pH	% ionized	No. of charges
Carboxyl	130	3·5–5	4·4	88·8–20·1	71(−)
			4·9	96·2–55·7	94(−)
			6·9	100–98·8	129(−)
α-Amino and imidazole	7	7	4·4	99·8	7(+)
			4·9	99·2	7(+)
			6·9	55·7	4(+)
ε-Amino	37	9–10	4·4	100	37(+)
			4·9	100	37(+)
			6·9	99·2–100	37(+)
Guandino	50	12	4·4	100	50(+)
			4·9	100	50(+)
			6·9	100	50(+)

* According to Eastoe (1955).
† From values obtained at 40° (Ward & Saunders, 1958).
Net charges calculated from the above data:

Zero at pH 4·9 (pI).
23(+) at pH 4·4 (lower pH limit for ethanol coacervation).
38(−) at pH 6·9 (upper pH limit for ethanol coacervation).

23(+) or 38(—) for the deionized alkali-processed gelatin. The presence of electrolytes increased the resistance of gelatin molecules towards changes in pH. The shielding effects of both the cations and anions of the electrolyte around the oppositely charged sites on gelatin weakens the tendency for repulsion between the gelatin molecules and between the segments of the same molecule. This results in an easing of the conditions for coacervation.

Because of the electrolytic nature of sodium sulphate, the effect of pH in this system differed from that in the ethanol system. Forces of repulsion arising from pH changes would have been counteracted by the shielding effect of the sodium sulphate added as a coacervating agent. At pH values on the acid side of the isoionic point, sulphate ions are preferentially fixed at the positively charged nitrogenous groups and at such pH values coacervation occurs readily. This agrees with the fact that acid solution enhances salting out of proteins where the efficiency of the salt anions followed the lyotropic series (McBain & Kellogg, 1928).

Changes in pH, within the effective pH range for coacervation, produced variations in both the composition and volume of the separated coacervates. At the isoionic point the amount of occluded liquid was at a minimum, but the amount gradually increased with variations in the pH. The effect of pH in controlling the intra- and intermolecular spaces within which occlusion occurs may be responsible for the variation of the liquid content (water + ethanol) of the coacervates. The strong attractive forces at the isoionic point produce contraction and folding of the coil, thus giving a more dense coacervate (i.e. smaller volume). At other pH values where the attractive forces diminish, the coil expands allowing more occlusion liquid to be immobilized, thus decreasing the viscosity of the separated coacervates and increasing their volume (Fig. 3A). At each pH value there is shown to be a maximum coacervate volume, e.g. at pH 4·9 the volume increases on increasing the percentage of ethanol from 42–44% and reduces when increased to 46%, while the solvent content of the coacervate is reduced over both ranges (Table 4). There must therefore be some other factor apart from solvent occlusion contributing to the coacervate volume. The initial increase in volume at a specific pH, with increase in ethanol concentration, may be due to a decrease in the dielectric constant resulting in decreasing attraction between the molecules. The decrease in volume could also be ascribed to the desolvation effect of the ethanol becoming predominant.

The recent application of coacervation techniques for microencapsulation of oil and solid particulates, is based on the unique ability of the coacervate phase to surround suspended particles, thus forming a "coacervate coat". The success of this technique will thus mainly be dependent on working within the coacervation region (II). The results presented in this paper show the effects of pH or the relative position of the coacervation region inside the triangular diagrams.

The most important consideration is the type of gelatin used and the effect of the material to be coated on the pH of the system. In a gelatin–water–ethanol system, using acid-processed samples, no coacervation

occurs unless the pH of the gelatin solution is adjusted to a value within the effective pH range for coacervation (6·9–10·8). With alkaline gelatins no prior adjustment of pH is needed. If the materials to be encapsulated produce a change in the pH, then the location of the coacervation region under the coating conditions must be predetermined, otherwise the pH change may produce a gel-like mass or one-phase system.

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