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Characterization of Albumin-acacia Complex Coacervation

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Abstract—Complex coacervation between oppositely charged albumin and acacia mixtures has been studied, and the applicability of the various theoretical treatments of complex coacervation (the Voorn-Overbeek, Veis-Aranyi, Nakajima-Sato, and Tainaka theories) to this system has been assessed. Under optimum conditions where maximum coacervate yield occurred, the Voorn-Overbeek theory appeared to apply. However, away from the optimum coacervation conditions, coacervate sol formation was observed, which is in accordance with the Veis-Aranyi and Tainaka theories. Microelectrophoretic measurements were used to determine optimum pH and ionic strength conditions for maximum coacervation, based on the method of Burgess & Carless (1984). The effects of pH and ionic strength on coacervate yield are reported. Around the optimum conditions for maximum coacervation a viscous coacervate phase and a relatively clear equilibrium phase are formed.

Complex coacervation may result on mixing oppositely charged polyions, in aqueous media. This process is the separation of the polyion mixture into two distinct phases; a dense coacervate phase, which is relatively concentrated in the polyions and a dilute equilibrium phase (Bungenberg de Jong 1949). Coacervation is a common method of microencapsulation (Bungenberg de Jong 1949; Nixon & Nouh 1978; Burgess & Carless 1985). On dispersion of the concentrated phase into the dilute phase, droplets form which can be crosslinked by chemical or thermal means to form microcapsules.

Bungenberg de Jong (1949) carried out an extensive characterization of complex coacervation between gelatin and acacia. Those studies indicated that complex coacervation was dependent on: the molecular weights, concentration and ratio of the two interacting polyions; and on the ionic strength, pH and temperature of the media. A random coil configuration for both macromolecules was also considered important. Several theoretical treatments of complex coacervation exist: the Voorn-Overbeek theory (Voorn 1956, 1959; Overbeek & Voorn 1957), the Veis-Aranyi 'dilute phase aggregated model' (Veis & Aranyi 1960) the Nakajima-Sato model (Nakajima & Sato 1972) and the Tainaka model (Tainaka 1979, 1980). These theories are contradictory on many points including the roles of electrostatic and entropy forces, the significance of Huggins interactions, and the type of charge interaction.

According to the Voorn-Overbeek theory spontaneous coacervation occurs as a result of competition between the electrical attractive forces which tend to accumulate oppositely charged polyions and entropy effects which tend to disperse them. The Voorn-Overbeek theory assumes: a random coil configuration of polyions in both phases; solvent-solute interactions are negligible; and that the electrostatic interactive forces are of a distributive nature. Overbeek & Voorn (1957) determined limiting values for polyion charge density (σ) and molecular weight (r), below which coacervation would not occur. For a two-component system, consisting of water and a polyion salt, $\sigma^3 r$ should be greater than or equal to 0·53 for complex coacervation to occur. However, Vies & Aranyi (1960) were able to form complex coacervates between oppositely charged gelatins where the critical conditions of $\sigma^3 r \geq 0{\cdot}53$ were not met. They developed their own complex coacervation theory to fit this system, the 'dilute phase aggregate model'. The Veis-Aranyi model is not limited by the assumptions of the Voorn-Overbeek theory. This model considers coacervation as a two-step process. First, spontaneous aggregation of the oppositely charged polyions takes place by electrostatic interaction upon mixing, forming aggregates of low configurational entropy. These aggregates, 'coacervate sols', then rearrange to form the coacervate phase. Rearrangement occurs slowly, taking hours or even days, and is driven by the gain in configurational entropy which occurs on the formation of a randomly mixed, concentrated coacervate phase and dilution of the aggregate phase.

Two modifications of the above theories have been reported. Nakajima & Sato (1972) and Sato & Nakajima (1974) modified the Voorn-Overbeek theory to include a Huggins interaction parameter. They also considered that the electrostatic term calculated by Voorn was much too small. The Tainaka model (Tainaka 1979, 1980) is adapted from the Veis theory (Veis & Aranyi 1960; Veis 1961, 1963; Veis et al 1967). Aggregate pairs form in the dilute phase as described by Veis, but without specific charge pairing. The aggregates condense to form the coacervate phase. In the coacervate phase the aggregates overlap resulting in an electrostatic energy gain from the increase in the ion density in the overlapped domain. Phase separation is driven by attractive forces between the aggregates. The Tainaka model is not restricted to low charge density mixtures, as the Veis model. However, the polyion charge density and molecular weights should fall within a critical range. At high charge density and molecular weight values, the formation of precipitates is predicted while at low values it is expected that

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no interaction will occur. Excellent correlation has been shown between calculated and experimental values for the phase concentrations of both polyvinyl alcohol and gelatin systems using this model (Tainaka 1979).

The objective of the present study was to characterize albumin/acacia complex coacervation and to assess the applicability of the various theoretical treatments to this system. Although the Voorn-Overbeek theory was originally based on Bungenberg de Jong's studies on gelatin/acacia coacervation, we have shown in a previous publication (Burgess 1990) that the Tainaka model is most appropriate for this system when considering a wide range of experimental conditions. Albumin and acacia should coacervate in a similar fashion to alkali-processed gelatin B and acacia as their molecular weights are very close (the molecular weight of albumin is $6{\cdot}7 \times 10^4$ and that of gelatin B is $4{\cdot}6 \times 10^4$ (Burgess & Carless 1984)).

Materials and Methods

Bovine serum albumin (mol. wt $6{\cdot}7 \times 10^4$ [osmotic pressure], isoelectric pH 5·6), acacia (mol. wt $2{\cdot}4 \times 10^5$ (Rees & Welsh 1977)), Amberlite IR-120P (cation-exchanger) and Amberlite IR-400 (anion exchanger) were obtained from Sigma Chemicals, USA. The isoelectric pH value of albumin was measured by microelectrophoresis and by ion exchange. Colloidal silica (Minusil) of particle size 2·7 μm (geometric weight-mean diameter) was obtained from Zeta-Meter Inc., New York, USA. Hydrochloric acid, sodium hydroxide, sodium chloride and other chemicals used were of analytical grade and obtained from Fisher Scientific, USA. The polyion solutions were prepared by dispersion in distilled water at $40 \pm 0{\cdot}1$°C. The macromolecules were allowed to hydrate completely; this took 30 min to 1 h. Following hydration the solutions were deionized by mixing for 30 min at $40 \pm 0{\cdot}1$°C with Amberlite resins IR-120P and IR-400 before use. This method is an adaptation of the method of Janus et al (1951).

Microelectrophoresis

The charge carried by the polyions affects coacervation. The extent of coacervation and the optimum conditions of pH and ionic strength can be predicted from microelectrophoresis data (Burgess & Carless 1984). A Lazer-Zee meter, model 501 was used in conjunction with a Plexi-glass cell. Microelectrophoresis was conducted at 1 mM NaCl, unless otherwise stated. In order to maintain constant ionic strength as the pH was varied (2–10), 1 mM NaOH and 1 mM HCl solutions were used. The polyions were adsorbed onto Minusil before microelectrophoresis (Burgess & Carless 1984). A 0·02% w/v polyion solution and 0·01% w/v Minusil suspension were used. The zeta potential was the mean of at least 20 readings.

Dry coacervate yield determination

Dry coacervate yield was used to measure the extent of coacervation as the polyion concentration, ionic strength and pH were varied. Equal volumes of the deionized polyion solutions under study were mixed with constant stirring (300 rev min^{-1}) at $40 \pm 0{\cdot}1$°C for 1 h, at the appropriate polyion concentration, pH and ionic strength conditions. The albumin/acacia mixtures were left to equilibrate for 4 h. Following equilibration, the coacervates were centrifuged (1000–2000 *g*) at their equilibrium temperatures to ensure complete phase separation. The two phases were then separated and added microions were removed, as necessary, by dilution with water followed by deionization. The coacervate and equilibrium phases were dried at 60°C for 6–10 h and weighed to obtain the coacervate yield as a percentage of the total amount of polyions added.

Polyion concentration determination. Coacervate yields were determined at different total concentrations of the two polyions (1:1 mixture), maintaining constant pH and ionic strength at the optimum values for maximum coacervation (as determined from microelectrophoresis data and previous studies).

pH determination. Coacervate yields were determined at different pH values (2–8) maintaining constant ionic strength and polyion concentration at the optimum values for maximum coacervation (as determined from microelectrophoresis data and previous studies).

Ionic strength determination. Coacervate yields were determined at different ionic strength (0–100 mM) maintaining constant pH and polyion concentration (as determined from microelectrophoresis data and previous studies).

Results and Discussion

Microelectrophoresis

The pH-zeta potential profiles of albumin and acacia (Fig. 1) were used to determine the optimum pH conditions for complex coacervation to occur, this being the electrical equivalence pH (EEP) of the polyions according to Burgess & Carless (1984). The EEP is the pH value at which both polyions carry equal and opposite charges. The EEP value of albumin and acacia is pH 3·9, according to the data presented in Fig. 1. Thus maximum coacervation is predicted to occur at pH 3·9. The pH range over which complex coacervation may occur can also be predicted from Fig. 1. The two polyions must possess opposite charges for complex

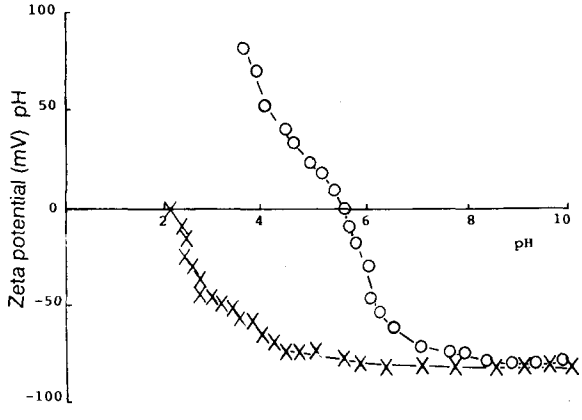

FIG. 1. The effect of pH on the zeta potential of albumin and acacia (ionic strength, 1 mM). O albumin; X acacia.

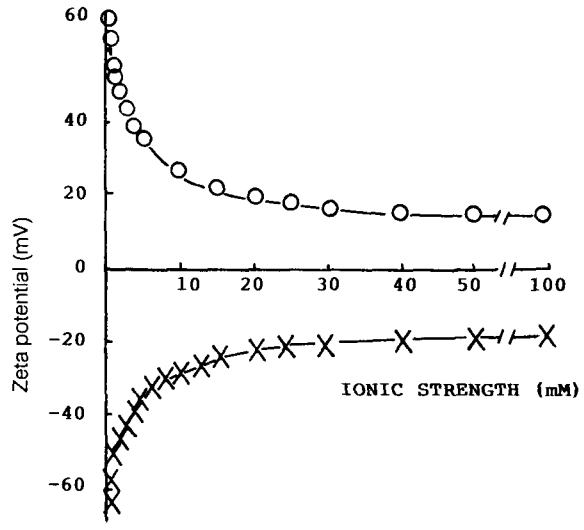

FIG. 2. The effect of ionic strength on the zeta potential of albumin and acacia at their EEP value, pH 3·9. O albumin; X acacia.

coacervation to occur, which restricts coacervation to a finite pH range (pH 2·1–5·6).

The ionic strength-zeta potential profiles of the two polyions, at their EEP value are shown in Fig. 2. The ionic strength of the medium affects the charge carried by polyions, through the screening effects of the counterions. As the ionic strength is increased, the charge on the polyions decreases. This decrease is extremely rapid between ion-free conditions and an ionic strength of 5 mM. The pH-charge and ionic strength-charge profiles of albumin are similar to Type B gelatin (Burgess & Carless 1984) and, since the molecular weights of Type B gelatin and albumin are close, albumin/acacia coacervation might be expected to follow a similar pattern to gelatin B/acacia coacervation (Burgess & Carless 1984).

Albumin/acacia coacervation

Coacervate yields were measured as the pH was varied between pH 2–6 at constant ionic strength (10 mM) and polyion concentration (1% w/v) (Fig. 3). Measurable coacervate yields were detected between pH 2·9–5·0. A maximum coacervate yield of 89% occurred at pH 3·9, which is the predicted optimum pH for coacervation according to the microelectrophoresis data. The coacervate yield decreased as the pH was altered away from this value. At pH values just outside the above range, where measurable coacervate yields were obtained, opalescence was observed (pH 2·4–2·9 and pH 5·0–5·6), while at pH values distant from this range the solutions were clear. More interestingly, the consistency and appearance of the coacervate changed markedly over the pH range studied. At pH values close to the optimum pH for maximum coacervation (pH 3·9) the mixture quickly separated into a viscous greyish coloured coacervate phase and relatively clear equilibrium phase. At pH values slightly higher or lower (pH 4·2–5·0 and pH 3·0–3·4) a cloudy dispersion of coacervate in the equilibrium fluid formed and this two-phase system could only be separated by centrifugation. The viscosity of the albumin/acacia complex coacervate around the optimum pH for maximum coacervation was high when compared with other coacervate systems such as gelatin/acacia. As noted previously, albumin/acacia and gelatin B/acacia mixtures are expected to form complex coacervates in similar fashions on the basis of their molecular weights, and their pH-charge and ionic strength-charge profiles. The higher viscosity of the albumin/acacia coacervate may be a result of the stronger interaction between albumin and water, due to the high percentage of polar side groups on albumin; aspartic acid, glutamic acid and threonine.

The effect of change in ionic strength on albumin/acacia coacervate yield, for a 1% w/v mixture at pH 3·9 is shown in Fig. 4. Coacervation is at a maximum at a microion content of 10 mM. As the ionic strength was altered away from the optimum value for maximum coacervation the appearance

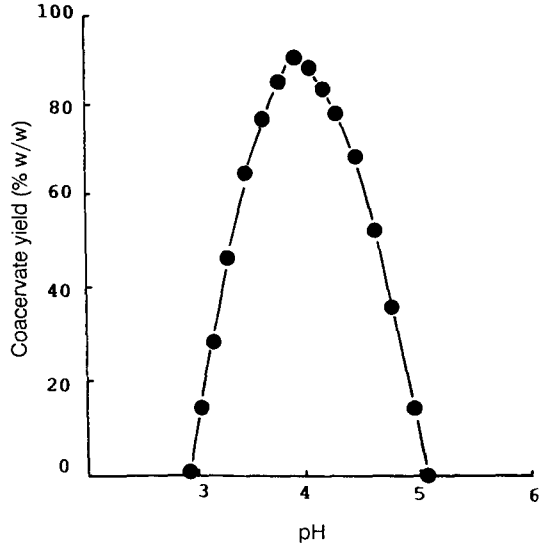

FIG. 3. The effect of pH on the coacervate yield of 1·0% w/v albumin/acacia mixtures (ionic strength 10 mM).

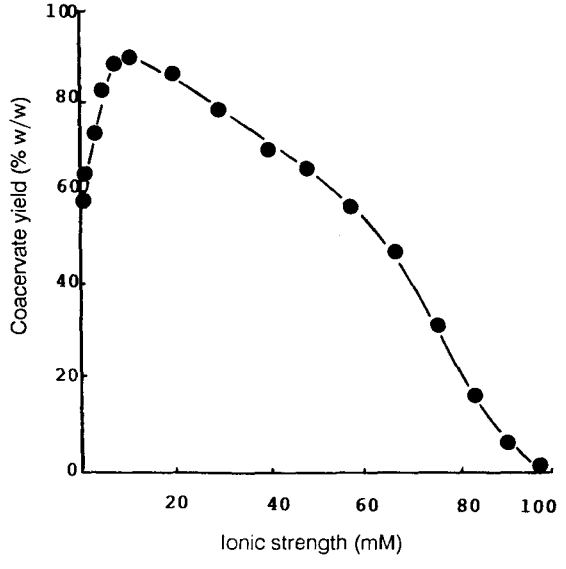

FIG. 4. The effect of ionic strength on the coacervate yield of 1·0 w/v albumin/acacia mixtures (pH 3·9).

of the mixture changed from a viscous greyish coloured coacervate phase and a relatively clear equilibrium fluid to a milky white, cloudy dispersion. At ionic strength values just above 97 mM, the upper limit of the measured coacervation range, opalescence was observed. At higher ionic strength values the mixtures were clear. As occurred with gelatin/acacia coacervation (Burgess 1991), gelatin/gelatin coacervation (Burgess & Carless 1985, 1986) and albumin/alginic acid coacervation (Singh & Burgess 1989) the coacervate yield decreased at low ionic strength as well as at high ionic strength.

Suppression of coacervation by the addition of micro-ions is predicted in all of the four theoretical treatments of complex coacervation described in the introduction. However, suppression of coacervation on reducing the micro-ion content is not predicted by any of these theories. Although, coacervate suppression at low ionic strength is not consistent with the Voorn-Overbeek theory, this phenomenon can be explained when the assumptions of the theory, with regard to the configuration of the molecules and the type of charge interaction, are taken into consideration. At very low ionic strength values the polyions are highly charged (Fig. 2) and therefore may exist in an extended rod-like configuration instead of a random coil as assumed in the Voorn-Overbeek theory. These highly charged molecules may interact at specific sites, rather than in a distributive manner. Thus, two of the assumptions of the Voorn-Overbeek model may not be met. Deviation from the Voorn-Overbeek theory also occurs just outside the limiting conditions of pH and ionic strength where opalescence was observed.

According to the microelectrophoresis data the two polyions carry opposite charges over the pH range 2·5–5·6, which is much wider than the pH range where coacervation was detected. Within the detectable coacervate ranges, pH 2·9–5·0 and ionic strength values below 97 mM the polyions have relatively high charge densities. Outside those ranges the charge density of at least one of the polyions must be below a critical minimum required to bring about coacervation (Overbeek & Voorn 1957; Burgess & Carless 1984). The opalescence observed just outside the pH-coacervation range and the ionic strength-coacervation range may be due to the presence of 'coacervate sols' as described in the Veis-Aranyi theory.

An attempt was made to form complex coacervates under the conditions where opalescence was observed, using the method for gelatin/gelatin coacervation (Burgess & Carless 1985, 1986). The temperature was reduced from 40 to 15°C to aid coacervate formation and the mixtures were allowed up to 24 h to equilibrate. Coacervation was observed in most cases. Coacervate yields of up to 78% w/w were detected (Table 1). Thus, the aggregates or 'coacervate sols' must have rearranged with time, on temperature reduction to form the coacervate phase. This phenomenon is consistent with the Veis-Aranyi and Tainaka theories.

Table 1. Albumin/acacia coacervation yield determined on equilibration of 1% w/v total polyion (1:1) mixtures at 15°C for 24 h.

Albumin/acacia mixture	Coacervate yield (% w/w)
pH 2·8 10 mM	64
pH 5·2 10 mM	78
pH 3·9 100 mM	37

Application of albumin/acacia coacervation to the theories

The Voorn-Overbeek and Veis-Aranyi theories are contradictory on many points, as described in the introduction, and apply to systems which coacervate under different conditions. The Voorn-Overbeek theory applies to systems of high charge density, in which the coacervate phase forms spontaneously. The Veis-Aranyi theory applies to systems of low charge density, in which the coacervate phase does not form spontaneously. The albumin/acacia systems described here formed coacervates at both high and low charge density. Therefore neither theory can fully explain complex coacervation of albumin and acacia.

The Voorn-Overbeek theory does not explain the decrease in coacervate yield at low ionic strength nor does it explain the formation of coacervates upon temperature reduction in systems where the charge density is too low for coacervate formation at 40°C. The assumptions of this theory (that the polyions are in a random coil state in both phases, that solvent-solute interactions are negligible and that the interactive forces are of a distributive nature) must be set aside to explain the reduction in coacervation at low ionic strength. However, this model cannot be adapted to explain the opalescence observed under conditions where the charge density is too low for coacervate phase formation, or to explain the formation of a coacervate phase in these systems, upon temperature reduction.

The Veis-Aranyi model does not explain spontaneous albumin/acacia coacervation under conditions of high charge density. The Tainaka model which is an adaptation of the Veis-Aranyi model appears to be the most appropriate theory to fully explain complex coacervation of albumin and acacia under all practical conditions. Unlike the Veis-Aranyi model, the Tainaka model does not require specific charge pairing to occur in the initial aggregate pairs and is not restricted to low charge densities and systems which do not spontaneously coacervate. It is applicable over a broad range of conditions encompassing both spontaneous and non-spontaneous coacervate phase formation. This model does assume, as does the Veis-Aranyi model, that small aggregates form initially which rearrange and condense into a coacervate phase. However, unlike the Veis-Aranyi model it does not restrict the rearrangement to a non-spontaneous process.

The effects of pH and ionic strength on albumin/acacia complex coacervation are in agreement with predictions based on microelectrophoresis data, according to the method of Burgess & Carless (1984). The optimum conditions for maximum coacervation are pH 3·9 and an ionic strength of 10 mM. Complex coacervation was suppressed at both high and low ionic strength. Coacervate suppression at low ionic strength is inconsistent with the theoretical treatments of complex coacervation of Voorn (1956, 1959), Overbeek & Voorn (1957), Veis & Aranyi (1960), Nakajima & Sato (1972) and Tainaka (1979, 1980). However, it is consistent with data previously obtained in this laboratory (Burgess & Carless 1985, 1986; Singh & Burgess 1989; Burgess 1991). The overall best theoretical fit to this system is the Tainaka model of complex coacervation. The high viscosity of the coacervate phase at pH values around the optimum pH for maximum

coacervation (pH 3·9) makes it difficult to homogenously disperse the coacervate phase in the equilibrium phase, as is required for the formation of small spherical microcapsules.

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