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Characterization of Albumin-Alginic Acid Complex Coacervation

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Abstract—Complex coacervation of albumin and alginic acid has been investigated to characterize this process, and to prepare a microencapsulation system suitable for the encapsulation of live cells, protein and polypeptide drugs. The optimum conditions of pH, ionic strength and total polyion concentration were in accordance with predictions based on the method of Burgess & Carless (1984). Albumin/alginic acid complex coacervation appears to fit the Vies-Aranyi model for complex coacervation. Coacervation was limited compared with other polypeptide/polysaccharide systems such as gelatin and acacia, with albumin/alginic acid complex precipitates rather than complex coacervates forming under certain conditions. In particular coacervation was limited to concentrations below 0·5% w/v. At concentrations between 0·35 and 0·5% w/v both complex coacervation and precipitation occurred, and at concentrations above 0·5% w/v only precipitation was detected. The albumin/alginic acid complex coacervate is very viscous and this together with the limited conditions governing the occurrence of coacervation makes this system unsuitable for the preparation of microcapsules.

Complex coacervation of oppositely charged polyelectrolytes is a common method of microencapsulation (Bungenberg de Jong 1949; Nixon & Nouh 1978; Burgess & Carless 1985). Under specific conditions of pH, ionic strength and polyion concentration the mixture of polyelectrolytes may separate into two distinct phases; a dense coacervate phase, which is relatively concentrated in the polyelectrolytes, and a dilute equilibrium phase (Bungenberg de Jong 1949). On dispersion of the concentrated phase into the dilute phase, droplets form which can be crosslinked by chemical or thermal means to form microcapsules. The potential of encapsulation by coacervate droplets was first noted by Bungenberg de Jong (1949), who showed that suspended organic, liquid droplets or solid particles were taken up by coacervate droplets. Complex coacervation of gelatin and acacia has been extensively studied for the preparation of microcapsules; high microcapsule yields have been obtained, a variety of different drugs have been encapsulated, and the microcapsule size range can be easily manipulated (Bungenberg de Jong 1949; Luzzi 1974; Burgess & Carless 1985). However, acacia is an impure heterogeneous natural carbohydrate which is unsuitable for parenteral administration.

The objective of this study was to develop a biodegradable, biocompatible microencapsulation system suitable for parenteral administration, the encapsulation of live cells, and the encapsulation of readily degradable drugs, such as peptides and proteins. Alginic acid was selected as an alternative negatively charged polyion to acacia. Purified alginic acid is biodegradable and bioacceptable and is suitable for parenteral administration (Sun et al 1983; O'Shea et al 1984). Alginic acid has a net molecular weight which is similar to that of acacia (the molecular weight of alginic acid is approximately 240 000, Rees & Welsh 1977; and the molecular weight of acacia is approximately 240 000, Anderson et al 1967). Alginic acid solutions have higher viscosities than equivalent acacia solutions. This may be related to structural differences between the molecules (Rees & Welsh 1977). Alginic acid also has a higher charge density. The charge densities may be manipulated by change in the ionic strength or the pH.

In this study albumin (bovine serum albumin, BSA) was used as an alternative to gelatin. Albumin is relatively more biocompatible than gelatin (Ratcliffe et al 1984). Both gelatin and albumin are natural proteins, have similar charge densities, pH profiles, and their molecular weights are of the same order (albumin has a molecular weight of 67 000 (Peters 1975) and alkaline processed gelatin has a molecular weight of 46 000 (Burgess & Carless 1984).

Complex coacervation between sodium alginate and gelatin has been described (Arneodo et al 1987). Ishizaka et al (1985) and Burgess & Kwok (1987) have reported complex coacervation of albumin with polyelectrolytes. The characterization of albumin/alginic acid complex coacervation has not been reported previously.

Materials and Methods

Albumin (Bovine serum albumin, BSA), sodium alginate, Amberlite 1R-120P (cation-exchanger) and Amberlite 1R-400 (anion exchanger) were obtained from Sigma Chemicals, USA. The sodium alginate was of medium viscosity (a 0·2% w/v solution, at 25°C, has a viscosity of 3500 centipoises). 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. Sodium alginate and albumin solutions were prepared by dispersion in distilled water at 40°C ± 0·1°C. The macromolecules were allowed to hydrate completely, this took 30 min to 1 h for albumin and 1 to 3 h for sodium alginate. Following hydration, the solutions were deionized by mixing for 30 min

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at 40°C±0·1°C with Amberlite resins 1R 120P and 1R 400 before use. This is an adaptation of the method of Janus et al (1951).

Microelectrophoresis

The charge carried by the polyions affects the extent of 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-glas 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 prior to 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. Coacervate volume may not give a true measurement of the degree of coacervation (Burgess & Carless 1984). Equal volumes of deionized albumin and alginic acid solutions were mixed with constant stirring (300 rev min^{-1}) at 40°C±0·1°C for 1 h, at the appropriate polyion concentration, pH and ionic strength conditions. The mixtures were left to equilibrate at 25°C±0·1°C for 12 h to ensure complete phase separation and were then centrifuged. Any precipitates formed were removed by filtration. Albumin/alginic acid coacervates do not form as rapidly as gelatin/acacia coacervates (Burgess & Carless 1984) and coacervation is enhanced by temperature reduction as occurs with gelatin/gelatin coacervation (Burgess & Carless 1985, 1986). Added microions were removed by deionization of each phase. The coacervate and equilibrium phases were dried at 60°C for 6 to 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 concentrations of alginic acid and albumin (1:1 mixture), maintaining constant pH (3·9) and ionic strength (1 mM).

pH determination. Coacervate yields were determined at different pH values (2–5) maintaining constant ionic strength (1 mM) and polyion concentration (0·15% w/v).

Ionic strength determination. Coacervate yields were determined at different ionic strength (0·1 to 50 mM) maintaining constant pH (3·9) and polyion concentration (0·15% w/v).

Results and Discussion

The complex coacervation of a binary polymer mixture requires that one polymer is positively charged and the other is negatively charged. Thus, complex coacervation is restricted to a finite pH range. To predict the effect of pH and ionic strength on coacervate yield, the zeta potentials of both the polyions as a function of pH (pH 2–10) and ionic strength (1–100 mM), were measured (Figs 1, 2). The zeta potential varies according to the ionizable groups present on the individual macromolecules. Alginic acid has a negative zeta potential which increases over the pH range 2 to 6 as the carboxylic acid groups become ionized. After pH 6·0 the zeta-potential of alginic acid remains constant. BSA which is a protein, is zwitterionic possessing both carboxylic acid and amino-groups. BSA carries a net positive charge below pH 4·9 and net negative charge above pH 4·9. A sufficiently strong ionic interaction is necessary for complex coacervation to occur (Burgess & Carless 1986). Maximum coacervation is predicted to occur at pH 3·9, the electrical equivalence point (EEP) (Burgess & Carless 1984) where alginic acid and albumin carry equal and opposite charges.

The coacervate yields were measured as the pH was varied between pH 2 and 5, at constant ionic strength (1 mM) and polyion concentration (0·15% w/v) (Fig. 3). Maximum coacervation was obtained at pH 3·9 (EEP) which is in agreement with the microelectrophoresis data (Fig. 1). At pH values higher or lower than pH 3·9 precipitation was also

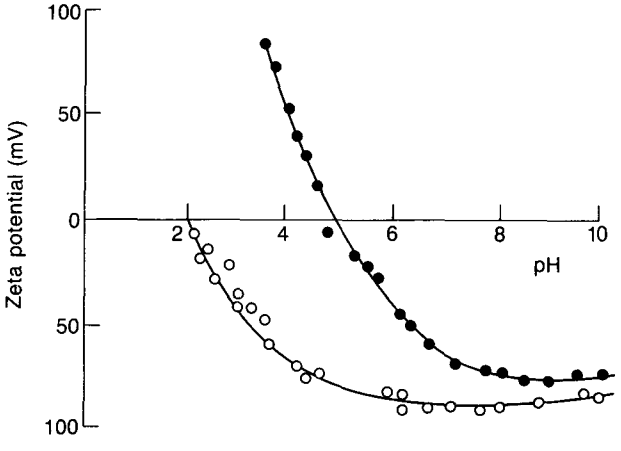

FIG. 1. The effect of pH on the zeta potential of albumin (●) and alginic acid (○) (ionic strength 1 mM).

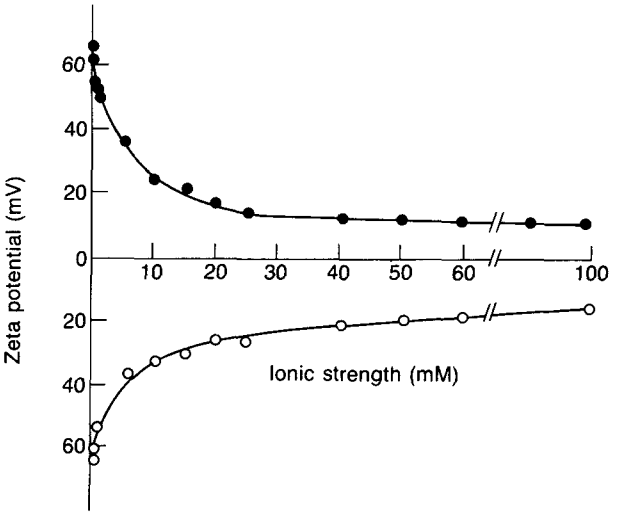

FIG. 2. The effect of ionic strength on the zeta potential of albumin (●) and alginic acid (○) (pH 3·9).

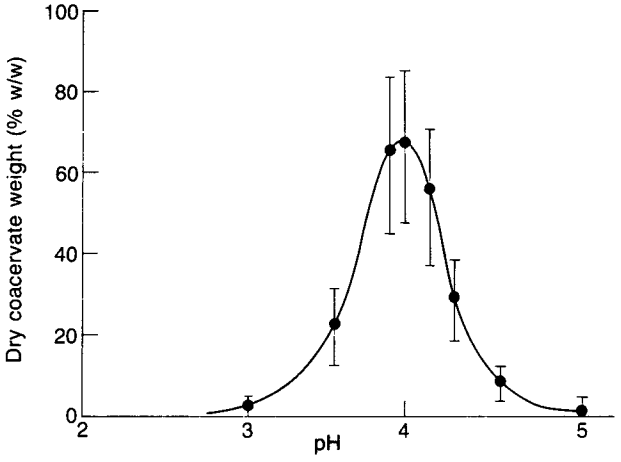

FIG. 3. The effect of pH on the percentage dry weight of albumin/alginic acid coacervate (ionic strength 1 mM, total polyion concentration 0·15% w/v).

noted. This precipitation may account for the extent of scatter observed with the albumin/alginic acid coacervate yield data. The yield data obtained for complex coacervate systems, where precipitation is not observed, such as gelatin/acacia (Bungenberg de Jong 1949; Burgess & Carless 1984) and gelatin/gelatin (Burgess & Carless 1986) are very reproducible. At pH values just outside the coacervation-pH range where neither coacervation nor precipitation were detected the solutions were opalescent.

Precipitation of the alginic acid/albumin system may be related to the structure of alginic acid. Alginic acid has an extended coil configuration in solution, and in the condensed phase it has a stiff ribbon-type of structure, which has restricted behaviour. The alginic acid crosslinked chains pack together under conditions of high ionization. As a consequence of these structural effects alginic acid is very viscous. Acacia is globular in nature and has a relatively low viscosity in solution. The albumin/alginic acid coacervate is therefore more viscous than an equivalent albumin/acacia or gelatin/acacia coacervate system. The ribbon-type structure of alginic acid is likely to promote precipitation at high polyion concentration and under conditions of high charge density (low ionic strength). The ribbon-type molecules will interact in such a way that there is very little water occluded between the chains of the macromolecules. Water occlusion is necessary to form a liquid coacervate rather than a solid precipitate. A random coil type structure such as acacia allows for a considerable quantity of water of occlusion and therefore promotes coacervation.

We wished to attempt to fit this system to one of the coacervation theories. The Vies-Aranyi theory states that coacervation occurs in two steps: spontaneous aggregation by ion pairing, followed by a rearrangement of these aggregates to form a coacervate phase (Vies & Aranyi 1960). The complex coacervation theory derived by Overbeek & Voorn (1957), states that coacervation is a spontaneous process resulting from competition between electrical effects which tend to accumulate the charged polyions and entropy effects which tend to disperse them. The Overbeek-Voorn theory assumes a random coil distribution of molecules, a distributive charge interaction, and that solvent-solute interactions are negligible. The Vies-Aranyi theory is more practical than the Overbeek-Voorn theory as solvent-solute interactions are considered and the electrostatic interaction term used is dependent on both the charge density and the polyion concentration. The opalescence observed in the albumin/alginic acid system suggests that spontaneous aggregation is taking place and the enhanced coacervation on temperature reduction suggests that a slow rearrangement of aggregates to form a coacervate phase may be occurring. The albumin/alginic acid system appears to fit the Vies-Aranyi theory of complex coacervation.

Effect of ionic strength on coacervation

The addition of salt causes screening of the charges on the polyions resulting in a weaker attraction and thus a reduction in coacervation. Coacervation may be completely suppressed at a critical salt concentration (Bungenberg de Jong 1949). It is therefore important to deionize the polyions before interaction. The optimum ionic strength for maximum coacervation is low (1–10 mM) for this albumin/alginic acid system (Fig. 4), which is in agreement with the microelectrophoresis data (Fig. 2). At low ionic strength, both of the polyions carry a high charge, hence there is a sufficiently strong ionic attraction for coacervation to occur. At very low ionic strength (< 1 mM), coacervation is suppressed to some extent. The charges carried by the polyions are very high at ionic strength values < 1 mM, as there will be almost no screening effect. The alginate molecules will take on an extended ribbon-type of structure under these conditions. Complex precipitation rather than complex coacervation may then occur as the molecules may closely adlineate on interaction excluding water which is

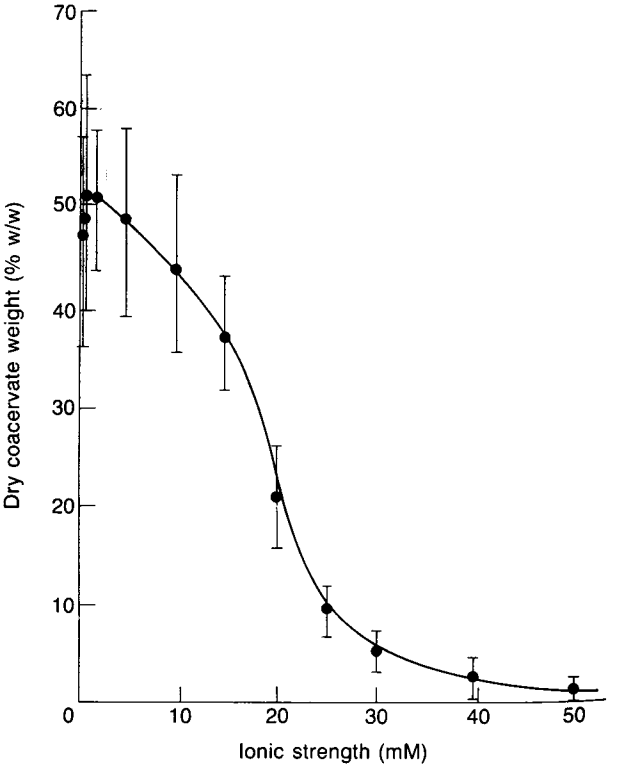

FIG. 4. The effect of ionic strength on percentage dry weight of albumin/alginic acid coacervate (pH 3·9, total polyion concentration 0·15% w/v).

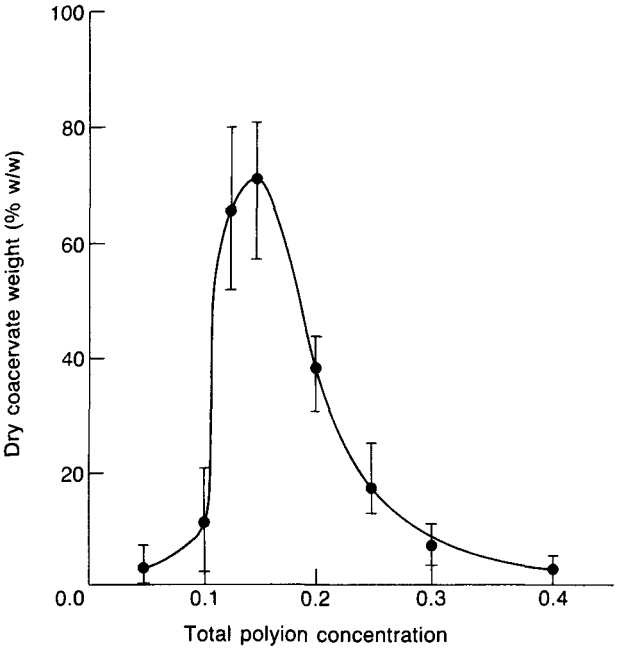

FIG. 5. The effect of total polyion concentrations on the percentage dry weight of albumin/alginic acid coacervate (pH 3·9, ionic strength 1 mM).

necessary for coacervation. At intermediate ionic strength both coacervation and precipitation were observed (5–50 mM). With increase in ionic strength coacervation is suppressed due to the screening effect. Total salt suppression occurs at ionic strength values > 50 mM. At ionic strength values between 50-75 mM, opalescence was observed.

Effect of polyions concentration on coacervation

Complex coacervation was observed between 0·05 and 0·5% w/v total polyion concentration. At optimum pH and ionic strength, the total polyion concentration for complex coacervation was 0·15% w/v (Fig. 5). At concentrations above 0·35% w/v, precipitation also occurred and above 0·5% w/v only precipitation was observed. Alginic acid takes on the ribbon-type structure as the concentration is increased. At total polyion concentrations above 0·5% w/v the mixtures were opalescent.

Conclusions

The effects of pH, ionic strength and total polyion concentration on albumin/alginic acid complex coacervation have been investigated experimentally. The results are in agreement with predictions based on microelectrophoresis data, according to the method of Burgess & Carless (1984). pH 3·9, ionic strength 1 mM and 0·15% w/v total polyion concentration were shown to be the optimum conditions for maximum coacervate yield. Albumin/alginic acid complex coacervation appears to fit the Vies-Aranyi model of complex coacervation.

The coacervation conditions for alginic acid and albumin are limited, compared to other polypeptide/polysaccharide systems such as gelatin/acacia, since complex precipitation tends to occur rather than complex coacervation. This is a consequence of the structure of the alginate molecules. The extent of precipitation increases, the farther the system is from optimum coacervation conditions. In particular, high polyion charge density and high polyion concentration promote precipitation. At the low total polyion concentrations studied, the alginic acid/albumin coacervate is too viscous to form microcapsules. These limitations make this system unsuitable as a microcapsule drug delivery system.

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