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Spontaneous Formation of Small Sized Albumin/acacia Coacervate Particles

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Abstract—Microgel coacervate particles form spontaneously on mixing aqueous solutions of oppositely charged albumin and acacia, under specific conditions of pH, ionic strength, and polyion concentration, close to but not at the optimum conditions for maximum coacervate yield. The mean particle diameter of these coacervate particles is approximately 6 μm when suspended in aqueous media, as determined by HIAC/Royco particle analysis. The geometric standard deviation of the particles falls in the range 1·2–1·9 μm. The particle size was not dependent on the method of emulsification of the coacervate in the equilibrium phase, or on the stirring speed applied during the manufacturing process. The microgel particles were stable on storage, for periods up to forty-six days, without the addition of a chemical cross-linking agent, or the application of heat. Stability was measured with respect to the change in particle size of samples stored at different temperatures. The non-cross-linked microcapsules were also shown to be stable on pH change, to pH values outside the coacervation pH range. At the optimum conditions for maximum coacervate yield the albumin/acacia system formed a very viscous coacervate phase, which was unsuitable for microcapsule preparation. The rheological properties of albumin/acacia and gelatin/acacia complex coacervates optimized for maximum coacervate yield were compared. The albumin/acacia coacervate was shown to be three orders of magnitude more viscous than the gelatin/acacia system.

Complex coacervation is the separation of a binary aqueous polyion mixture into two distinct liquid phases: a dense coacervate phase, which is rich in polyions and a dilute equilibrium phase (Bungenberg de Jong 1949). Complex coacervation occurs between oppositely charged polyions under specific conditions of pH, ionic strength, molecular weight and polyion ratio. Alteration of any of these parameters outside a narrow range results in reversal of the process and re-formation of a homogeneous one-phase system. Complex coacervation is a common method of microencapsulation (Bungenberg de Jong 1949; Luzzi 1974; Burgess & Carless 1985). The coacervate phase can take up organic liquids and solid particles (Bungenberg de Jong 1949). Coacervate emulsion systems are formed by dispersing the coacervate phase in the equilibrium phase. These emulsion systems are usually unstable and the coacervate droplets will coalesce and form a separate phase when the energy applied to disperse the system is removed, unless the droplets are first cross-linked by chemical or thermal means. Stabilized coacervate droplets are known as microcapsules.

The relative stability of emulsion systems is dependent on dispersed-phase droplet size and viscosity, interfacial tension, interfacial charge, and interfacial rigidity. The smaller the droplet size, the higher the viscosity, the lower the interfacial tension, the higher the interfacial charge, and the more rigid the interface, the more stable the emulsion system will be. Interfacial tension is usually very low in coacervate systems, of the order of 1 dyn cm^{-1} (Burgess et al 1990) and therefore this parameter cannot be manipulated to enhance stability. Since complex coacervates form by charge neutralization, the coacervate droplets carry a net zero charge. Coacervate systems are in dynamic equilibrium and any attempt to alter the charge will affect the complexation of the two polyions, reducing or suppressing coacervation. The viscosity, the interfacial rigidity and the droplet size of the coacervates can be altered to enhance stability. Interfacial rigidity is related to the viscosity of the coacervate (Burgess et al 1990).

A coacervate system which is stable without the application of heat or the addition of chemical cross-linking agents would be useful as a delivery system for sensitive drugs and other materials which are unable to withstand the harsh conditions involved in these cross-linking processes. Various polyion mixtures form viscous coacervates, for example albumin and alginic acid (Singh & Burgess 1989) and albumin and acacia (Burgess et al 1991). Albumin and alginic acid mixtures form complex precipitates as well as complex coacervates and this limits the conditions under which coacervates can be prepared to very low concentrations (Singh & Burgess 1989) and hence limits the usefulness of this system for microencapsulation. It has been reported that the viscosity of albumin/acacia complex coacervates is too high to prepare microcapsules at the optimum conditions for maximum coacervate yield (Burgess et al 1991). However, since the various parameters which affect complex coacervation, such as pH and ionic strength, can be manipulated to alter viscosity, it was proposed to investigate this system with the view to preparing a relatively stable coacervate dispersion.

The optimum pH and ionic strength conditions for maximum albumin/acacia coacervate yield are pH 3·9 and ionic strength 10 mM (Burgess et al 1991). Albumin/acacia complex coacervates formed between pH 2·9 and 5·0, at ionic strength 10 mM, and between 0–100 mM at pH 3·9 (40°C). The consistency and appearance of the coacervate phase changed with pH and ionic strength from a thick, viscous coacervate

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at the optimum pH for maximum coacervation (pH 3·9), to a cloudy dispersion at pH values close to pH 3·9. This effect had not been previously reported for this or any other system. The cloudy dispersion of coacervate in equilibrium fluid which forms at pH values close to pH 3·9 is investigated in the present study.

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), Amberlite IR-400 (anion-exchanger) and glutaraldehyde were obtained from Sigma Chemicals, USA. Gelatin B (Bloom No. 250, mol. wt $4{\cdot}6 \times 10^4$ (osmotic pressure), isoelectric pH 4·8), was obtained from Gelatin Products Ltd, UK. The isoelectric pH values of albumin and gelatin were measured by microelectrophoresis and by ion exchange (Burgess 1990). Viscosity standards were purchased from Brookfield Laboratories, Inc., 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 polyions were allowed to hydrate completely; this took 0·5–1 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 that of Janus et al (1951).

Albumin/acacia complex coacervation, microgel and microcapsule production

Albumin/acacia coacervates were prepared by mixing equal volumes of deionized polyion solutions with constant stirring (300 rev min^{-1}) at $40 \pm 0{\cdot}1$°C for 1 h, at the appropriate pH and ionic strength conditions. The pH and ionic strength of the mixtures were adjusted using HCl, NaCl and NaOH solutions. The ionic strength was held constant at 10 mM, while the pH was adjusted and the pH was held constant at 3·9 while the ionic strength was adjusted. The concentration of the polyion solutions was adjusted to give a final total polyion concentration of 2% w/v. The appearance of the coacervates was then examined visually.

Coacervate microgel particles and microcapsules were prepared by mixing 100 mL of each polyion solution prepared as above. The pH was adjusted to either pH 3·8 or 4·2, maintaining constant ionic strength at 10 mM using HCl, NaCl and NaOH solutions. The mixtures were treated in one of the following ways: left to stand with no further treatment; stirred at 450 or 900 rev min^{-1} for 1 h using a Dyna mixer (Fisher Scientific); stirred at 450 rev min^{-1} for 10 min using a Dyna mixer, followed by sonication for 10 min using a Sonifer, Branson Sonic Co., at a power setting of 5. Each batch was separated into two portions: one portion was left to stand with no further treatment and the other portion was cross-linked by the addition of 5 mL of cross-linking agent (16% aqueous glutaraldehyde solution) while mixing for a further 5 min. In this text, non-cross-linked gel particles are described as microgel particles and cross-linked microgel particles are described as microcapsules.

As required, the coacervate microgel particles were recovered as a dry powder. The microgel particles were washed three times with cold water (6°C) and either dried under nitrogen or freeze-dried to produce a free flowing powder. The freeze-drying process involved freezing the washed coacervate microgel particles at −70°C overnight, followed by freeze-drying for 72 h at −60°C and 10 mmHg.

Table 1. Effect of pH on the appearance of albumin/acacia coacervate system (ionic strength 10 mM).

pH	Appearance
2·0	Clear
2·4	Opalescent
2·6	Opalescent
2·9	Opalescent
3·0	Slightly cloudy
3·2	Slightly cloudy
3·4	Milky-white dispersion
3·6	Milky-white dispersion
3·7	Muddy-coloured dispersion
3·8	Muddy-coloured dispersion
3·85	Muddy-coloured dispersion with some phase separation
3·9	Rapid phase separation into greyish-coloured viscous coacervate and relatively clear equilibrium phase
4·0	Muddy-coloured dispersion with some phase separation
4·1	Muddy-coloured dispersion
4·2	Milky-white dispersion
4·4	Milky-white dispersion
4·6	Cloudy
4·8	Slightly cloudy
5·0	Opalescent
5·2	Opalescent
5·4	Opalescent
5·6	Clear
6·0	Clear

Particle size stability study

The microgel and microcapsule particles were stored in 10 mM NaCl solutions at their preparation pH value for periods up to forty six days, in constant temperature water baths at 5, 25 and $40 \pm 0{\cdot}1$°C. The particle size was measured on days 1, 10 and 46. The number mean diameter (dsn) and the size distribution of the particles were determined using a HIAC/Royco particle sizer (Model 4100). The particles were suspended in a 0·9% NaCl (saline) solution for size analysis. Samples were also examined by optical and scanning electron microscopy. The stability of the coacervate microgel particles to change in pH was studied by altering the pH of the final suspension to 7·4. Particle size analysis was conducted as before.

Rheological study of complex coacervates

The viscosity of albumin/acacia and gelatin/acacia coacervates was determined at the respective pH values for optimum coacervation (pH 3·9 (albumin/acacia) and pH 3·6 (gelatin/acacia) (Burgess & Carless 1984) using a Brookfield synchro-lectric viscometer, model RVT (Brookfield Laboratories Inc., Stroughton, MA. The viscometer was calibrated with different spindle numbers using viscosity standards (Brookfield Laboratories Inc.). The coacervate samples were placed in a container without a spindle guard and maintained at constant temperature ($37 \pm 0{\cdot}1$ or $60 \pm 0{\cdot}1$°C). The samples were agitated for 30 min and then equilibrated at their final temperature for 2 h before measurement. The gelatin/acacia measurements were made using a UL adapter attached to the RVT model. Three consecutive readings were taken at each rotation speed.

Table 2. Effect of ionic strength on the appearance of albumin/acacia coacervate system (pH 3·9).

Ionic strength (mM)	Appearance
0	Rapid phase separation into greyish-coloured, viscous coacervate and relatively clear equilibrium phase
10	Rapid phase separation into greyish-coloured, viscous coacervate and relatively clear equilibrium phase
20	Muddy-coloured dispersion with some phase separation
30	Muddy-coloured dispersion
40	Milky-white dispersion
50	Milky-white dispersion
60	Cloudy
80	Cloudy
100	Opalescent

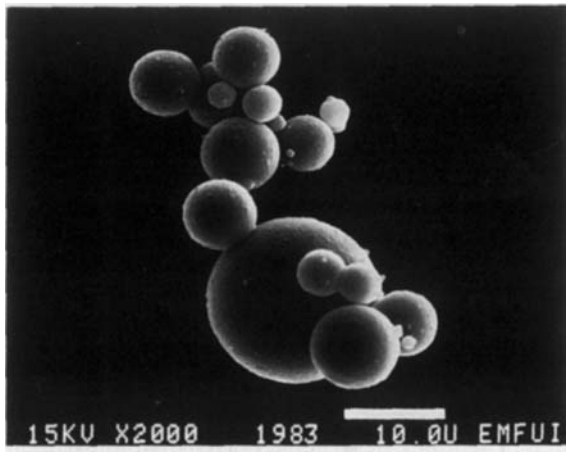

FIG. 2. Scanning electron micrograph of freeze-dried albumin/acacia coacervate particles prepared at pH 4·2.

Results and Discussion

Production of microgels and microcapsules

As reported previously, albumin/acacia coacervate microcapsules could not be prepared at pH 3·9, the optimum pH for maximum coacervate, due to the high viscosity of the coacervate phase (Burgess et al 1991). It was noted that as the pH was altered within the coacervation pH range, maintaining constant ionic strength, that the appearance of the coacervate changed markedly. The appearance of the coacervate was therefore assessed over the pH range 2·0–6·0, at ionic strength 10 mM (Table 1). At pH 3·9 the system separated rapidly into a viscous coacervate phase and a dilute equilibrium phase. At pH values close to pH 3·9 a dispersed system formed. As the pH was altered away from pH 3·9 the system became less cloudy and eventually clear. The appearance and consistency of the coacervate changed in a similar manner as the ionic strength was altered, maintaining constant pH (3·9; Table 2).

At pH and ionic strength conditions close to the optimum coacervate yield conditions, where dispersed systems formed, the percentage coacervate yield was high (between 80 and 89% w/v) (Burgess et al 1991). These dispersed systems were investigated as potential microencapsulation systems. Batches of coacervates were prepared at pH 3·8 and 4·2, under different mixing conditions. The batches were divided into two portions: one portion was cross-linked with glutaraldehyde, and the other portion was left untreated. On examination by visible microscopy, small, spherical, coacervates were observed for both the cross-linked and non-cross-linked samples. The non-cross-linked coacervate microgel particles did not deform and coalesce during a 6-h examination. The application of finger pressure, via a coverslip placed over these samples on the microscope slides, caused slight elastic deformation. The microgel particles regained their shape when the pressure was removed. Fig. 1 is a scanning electron micrograph of coacervate microgel particles prepared at pH 4·2.

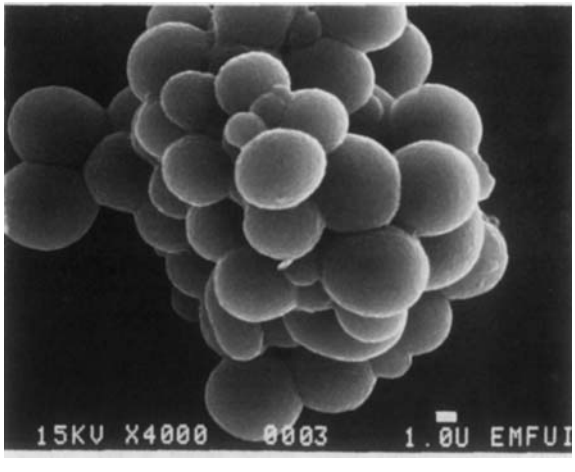

FIG. 1. Scanning electron micrograph of albumin/acacia coacervate particles prepared at pH 4·2 and stored at 40°C for forty-six days.

The albumin/acacia coacervate particles were successfully recovered as a free-flowing powder following washing with water and drying under nitrogen or freeze-drying. Samples which were air-dried tended to aggregate; however, these aggregates were broken up upon redispersion in saline solution, as observed by optical microscopy. Aggregation was not observed for the nitrogen-dried and freeze-dried coacervate particles. Freeze-dried coacervate particles were examined under scanning electron microscopy to determine whether they could withstand this type of processing. Fig. 2 is a scanning micrograph of freeze-dried coacervate particles. It appears that the albumin/acacia coacervate particles can withstand freeze-drying.

Particle size stability study

Tables 3 and 4 show the effects of stirring speed, ageing, and temperature on glutaraldehyde-treated (cross-linked) and non-glutaraldehyde-treated (non-cross-linked) samples prepared at pH 3·8 and 4·2, respectively. The effect of stirring rate on the particle size distributions of freshly prepared microgel particles and microcapsules, manufactured at pH 3·8 and 4·2, are shown in Tables 5 and 6, respectively. The majority of the particles fell within the size range 4–12 μm. The sample prepared at pH 3·8 and 900 rev min^{-1} had an

Table 3. Effect of ageing, temperature and stirring rate on the mean particle size of non-cross-linked coacervate microgel particles and cross-linked microcapsules prepared at pH 3·8 as measured by HIAC/Royco particle sizer (dsn $\pm \sigma$ g in μm).

Stirring rate (rev min^{-1})	1st day 40°C	10th day 40°C	46th day 40°C	46th day 25°C	46th day 5°C
NS	6·4 ± 1·4	6·5 ± 1·7	6·0 ± 1·8	7·4 ± 1·4	7·2 ± 1·5
450	5·9 ± 1·6	6·0 ± 1·5	5·8 ± 1·4	7·1 ± 1·5	6·8 ± 1·4
900	7·7 ± 1·5	7·4 ± 1·5	7·8 ± 1·8	7·6 ± 1·5	ND
NS*	6·7 ± 1·4	6·7 ± 1·4	6·5 ± 1·4	6·8 ± 1·3	6·6 ± 1·4
450*	7·1 ± 1·2	7·2 ± 1·3	7·2 ± 1·3	7·2 ± 1·3	7·1 ± 1·4
900*	8·2 ± 1·5	7·7 ± 1·4	ND	9·7 ± 1·6	ND
Sonicated	5·6 ± 1·4	ND	ND	ND	ND

NS = Not stirred, * cross-linked microcapsules, ND = not determined.

Table 4. Effect of ageing, temperature and stirring rate on the mean particle size of non-cross-linked coacervate microgel particles and cross-linked microcapsules prepared at pH 4·2 as measured by HIAC/Royco particle sizer (dsn $\pm \sigma$ g in μm).

Stirring rate (rev min^{-1})	1st day 40°C	10th day 40°C	46th day 40°C	46th day 25°C	46th day 5°C
NS	6·2 ± 1·4	5·3 ± 1·5	6·6 ± 1·5	5·9 ± 1·3	6·2 ± 1·3
450	4·6 ± 1·7	4·7 ± 1·5	6·4 ± 1·5	7·0 ± 1·4	6·6 ± 1·4
900	5·3 ± 1·9	5·2 ± 1·5	6·6 ± 1·5	5·5 ± 1·4	6·2 ± 1·3
NS*	5·0 ± 1·3	5·0 ± 1·4	4·8 ± 1·4	5·0 ± 1·4	4·8 ± 1·4
450*	5·7 ± 1·4	5·6 ± 1·4	5·7 ± 1·4	5·5 ± 1·4	5·5 ± 1·4
900*	5·4 ± 1·5	5·4 ± 1·5	5·6 ± 1·4	5·6 ± 1·4	5·4 ± 1·4
Sonicated	5·5 ± 1·3	ND	ND	ND	ND

NS = Not stirred, * cross-linked microcapsules, ND = not determined.

Table 5. Effect of stirring rate on the particle size distribution of freshly prepared non-cross-linked coacervate microgel particles and cross-linked microcapsules (pH 3·8) as measured by HIAC/Royco particle sizer.

Stirring rate (rev min^{-1})	Mean (dsn)	80% > (μm)	5% > (μm)	% > 4 μm	% > 12 μm
NS	6·4	4·6	12	90	5
NS*	6·7	5·1	12	94	5
450	5·9	4	14	80	9
450*	7·1	5·8	11	99	1
900	7·7	5·3	17	95	22
900*	8·2	5·9	18	95·5	22
Sonicated	5·6	4·3	8·9	88	1

NS = Not stirred, * cross-linked microcapsules.

Table 6. Effect of stirring rate on the particle size distribution of freshly prepared non-cross-linked coacervate microgel particles and cross-linked microcapsules (pH 4·2) as measured by HIAC/Royco particle sizer.

Stirring rate (rev min^{-1})	Mean (dsn)	80% > (μm)	5% > (μm)	% > 4 μm	% > 12 μm
NS	6·2	4·4	12·5	86	5
NS*	5·0	3·9	8·0	78	0·1
450	4·6	3·6	9·5	71	1·6
450*	5·7	4·4	9	89	0·4
900	5·3	2·7	17	62	11
900*	5·4	3·6	10	70	2
Sonicated	5·5	4·7	8·5	94	4

NS = Not stirred, * cross-linked microcapsules.

unusually large percentage of both cross-linked and non-cross-linked microcapsules > 12 μm. Optical microscopy confirmed that this was due to the formation of aggregates in these samples. However, the mean particle diameters and particle size distributions of samples prepared at different stirring speeds did not vary significantly. There was no apparent correlation between the stirring rate and the particle size. Increase in shearing forces usually results in the production of smaller coacervate emulsion droplets and therefore smaller microcapsules (Burgess & Carless 1985).

Ageing over forty-six days at 40, 25 and 5°C did not have a significant effect on the particle size of the microgels and microcapsules. At the end of forty-six days the studies were terminated. The largest variation in mean particle size on storage, was an increase from 4·6 ± 1·7 to 7·0 ± 1·4 μm for the non-cross-linked sample prepared at pH 4·2, stirred at 450 rev min^{-1} and stored at 25°C. This slight increase in size appeared to be due to the formation of a few aggregates, as observed by optical microscopy. Other non-cross-linked and cross-linked samples showed either no increase in particle size or slightly smaller increases. Non-cross-linked liquid coacervate droplets generally coalesce rapidly and form a separate phase (Bungenberg de Jong 1949; Luzzi 1974; Burgess & Carless 1985). The particle size distribution probit plots of cross-linked and non-cross-linked microcapsules prepared and stored under identical conditions are shown in Fig. 3. Four sets of data were selected to show the effects of stirring rate and ageing. Freshly prepared systems were compared with systems aged for forty-six days (pH 3·8, stirring rate 450 rev min^{-1} at 40°C and pH 4·2, no stirring, 40°C). It is apparent that no significant change in particle size occurs on cross-linking. The particle size data suggests that

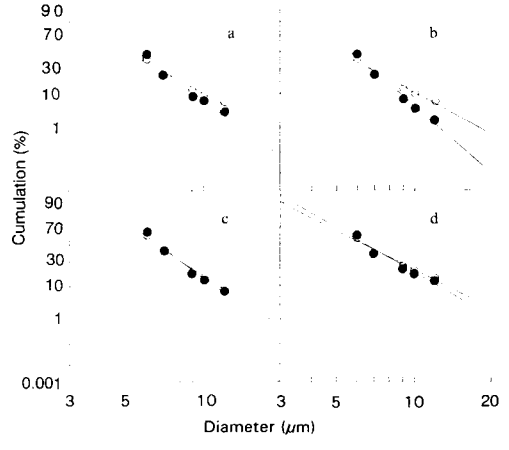

FIG. 3. Cumulative size distribution of albumin/acacia coacervate microgel particles and microcapsules obtained using a HIAC/Royco instrument and plotted as a log-probit graph: ○ non-cross-linked microgel particles, ● cross-linked microcapsules. (a) Freshly prepared, stirred at 450 rev min^{-1}, 40°C, pH 4·2; (b) aged for forty-six days, stirred at 450 rev min^{-1}, 40°C, pH 4·2; (c) freshly prepared, no stirring, 40°C, pH 3·8; (d) aged for forty-six days, no stirring, 40°C, pH 3·8.

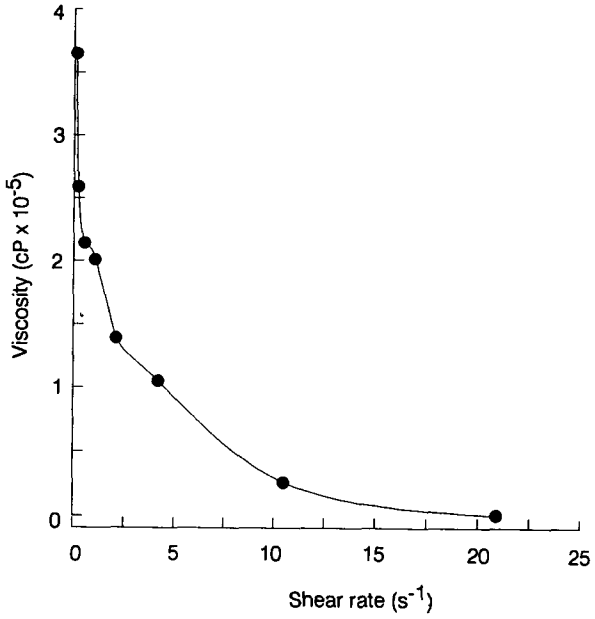

FIG. 4. Viscosity vs shear rate of albumin/acacia complex coacervate systems (pH 3·9, 10 mM, 37°C).

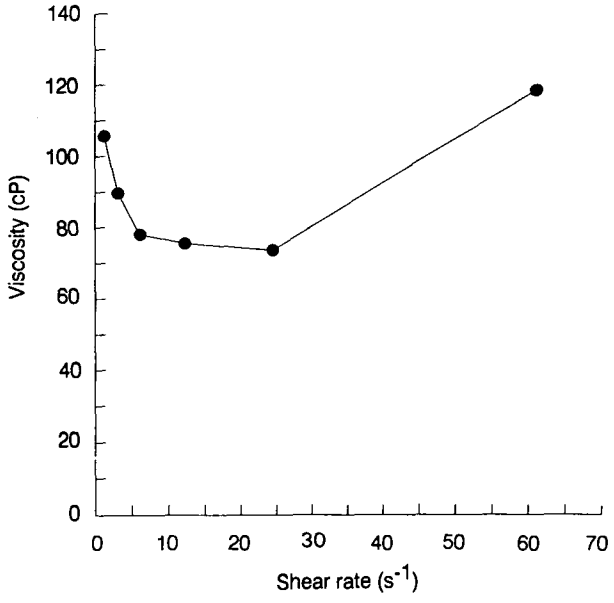

FIG. 5. Viscosity vs shear rate of gelatin/acacia complex coacervate systems (pH 3·6, 10 mM, 37°C).

the non-cross-linked system is extremely stable to coalescence. The slight changes observed in the mean particle diameter with time are probably a consequence of aggregation. The relative stability of the coacervate microgel system may be due to the high viscosity which results from strong electrostatic interactions between albumin and acacia and between albumin and water. The high percentage of polar side groups on albumin (aspartic acid, glutamic acid and threonine) contributes to these interactions. The coacervates apparently form spontaneously and aggregate up to a critical size. The consistency of these aggregates is such that the system gels and any further aggregation results in flocculation rather than coalescence. Applied energy (stirring, ultrasound) does not affect the particle size as the shearing forces are unable to break the microgel particles.

The pH of the non-cross-linked microgels was altered to pH 7·4, which is outside the coacervation-pH range (pH 3·0–5·0). The microgels remained intact with no variation in particle size, as determined by HIAC/Royco analysis and microscopy. Liquid coacervate systems, such as gelatin/acacia, are unstable to change in pH, and on alteration of the pH to values outside the coacervation-pH range the coacervation process reverses and a one phase system is formed.

The albumin/acacia coacervate microgels have a rigid structure and hence applied energy such as vibrational (ultrasound), mechanical (stirring) and heat do not affect the particle size. This structure is also able to withstand pH change. It therefore follows that although the formation of the gel is initiated by electrostatic interactions the structure is reinforced by other types of bonding such as van der Waals, hydrophobic and hydrogen bonding.

Rheological study of complex coacervates

The viscosity of albumin/acacia complex coacervates was determined at pH 3·9 and ionic strength 10 mM at 37°C. Coacervate yield is optimized under these conditions and the coacervate formed is a viscous liquid which readily phase separates. Gelatin/acacia coacervates, prepared at the optimum pH and ionic strength conditions for maximum coacervate yield (pH 3·6, and ionic strength 10 mM (Burgess & Carless 1984)), were also studied for comparative purposes. The viscosity of the albumin/acacia system was three orders of magnitude higher than that of the gelatin/acacia system (Figs 4, 5). At a shear rate of 1·05 s^{-1} the viscosity of the albumin/acacia system was 200 800 cP and that of the gelatin/acacia was 105·6 cP at a shear rate of 1·224 s^{-1}. The high viscosity of the albumin/acacia system is responsible for the inability of this system to form a coacervate emulsion at the optimum yield conditions and is also responsible for the formation of small coacervate microgels at pH values close to the optimum conditions.

Conclusions

Albumin/acacia coacervate microgels, with mean particle diameters of approximately 6 μm, have been prepared at pH values close to but not at the optimum conditions for maximum coacervate yield. These microgels are stable with respect to ageing, temperature, pH, and glutaraldehyde cross-linking, as determined by particle size analysis. The relative stability of the coacervate microgels is apparently a consequence of the high viscosity of this system. Gelation occurs without the use of cross-linking agents or temperature change. The coacervates aggregate until the system gels and at this point further growth by coalescence is inhibited as the system is in a semi-solid state. Since particle size is not affected by stirring speed during the mixing of the two polyions, growth of the gel droplets must be rapid and the process can be considered spontaneous. The microgel coacervate particles can be recovered as a free flowing powder following washing with water, drying under nitrogen or freeze-drying. The coacervate particles are able to withstand the freeze-drying process.

Albumin/acacia microgel coacervate particles may be suitable for the encapsulation of relatively unstable drugs, biological agents and cells, which are unable to withstand the harsh conditions involved in other microencapsulation processes.

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