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Characterization of Sodium Carboxymethylcellulose-Gelatin Complex Coacervation by Chemical Analysis of the Coacervate and Equilibrium Fluid Phases

GEOK-LAY KOH AND IAN G. TUCKER

Department of Pharmacy, University of Queensland, St. Lucia 4067, Australia

Abstract—The complex coacervation of sodium carboxymethylcellulose (SCMC) and gelatin has been characterized by chemical analyses of the coacervate and equilibrium fluid phases. The phenol-sulphuric acid (for SCMC) and Lowry (for gelatin) assays were used. Chemically analysed coacervate yield was used to predict optimum coacervation conditions, which occurred at a SCMC-gelatin mixing ratio of 3:7 at pH 3·5. The effects of pH, colloid mixing ratio and total colloid concentration on coacervate yield and composition were studied. The colloid mixing ratio, at which the peak coacervate yields occurred varied with coacervation pH. Increase in the total colloid concentration suppressed coacervation, resulting in a coacervate of higher water content. A similar coacervation mechanism was seen for two viscosity grades SCMC. However, because of the different degree of substitution of these two grades the SCMC-gelatin coacervates had different SCMC contents.

The acacia-gelatin complex coacervation system has been characterized by Bungenberg de Jong (1949) by viscosity, turbidity, coacervate volume, coacervate dry weight and chemical analysis of the colloids in the coacervate and equilibrium fluid phases. The latter process enables the construction of a phase diagram for the system. Dry weight coacervate yield has also been used by McMullen et al (1982) for the pectin-gelatin coacervation process.

In preceding work (Koh & Tucker 1988), the sodium carboxymethylcellulose (SCMC)-gelatin coacervation system was characterized through measurements of viscosity, turbidity and coacervate wet weight and volume. Therefore, it seemed desirable to develop suitable chemical analyses for SCMC and gelatin, whereby the SCMC-gelatin complex coacervation system could be characterized and optimized.

The coacervate yield, the coacervate composition (i.e. the ratio of SCMC to gelatin in the coacervate) and the electrical charge on the coacervate droplets are known to be dependent on a number of factors: colloid type, colloid mixing ratio, total colloid concentration, coacervation pH and the presence of electrolytes (Bungenberg de Jong 1949). The aim of the present study was to investigate the effects of some of these variables on the complex SCMC-gelatin coacervation system by monitoring the SCMC-gelatin composition in both the coacervate phase and equilibrium fluid. This fundamental knowledge of the complex ionic relations of SCMC and gelatin should enable the production of microcapsules with the desired wall characteristics.

Materials and Methods

Materials and characterization

The gelatin (Sigma Co.) and its characterization has been described previously (Koh & Tucker 1988). Briefly, it was alkali processed material obtained from calf skin, 225 Bloom, 9·4% moisture content and with an isoelectric point of pH 5·0.

Correspondence to: I. G. Tucker, Dept of Pharmacy, University of Queensland, St. Lucia 4067, Australia.

The sodium carboxymethylcelluloses used were of low (SCMC(LV)) and high viscosity (SCMC(HV)) grades (Sigma Co.). They were characterized in terms of infrared spectroscopy, moisture content, molecular weight, degree of substitution and purity, as described previously (Koh & Tucker 1988). They had the following properties for SCMC(LV) and SCMC(HV), respectively: moisture contents 4·0 and 5·6%; degree of substitutions 0·78 and 0·92; purities 94 and 96%; and molecular weights determined by viscosity $2{\cdot}69 \times 10^4$ and $3{\cdot}44 \times 10^5$ daltons. The manufacturer specified a degree of polymerization of 400 for SCMC(LV) and 3200 for SCMC(HV).

All other reagents were of analytical reagent grade and were used as received.

Coacervate preparation

SCMC and gelatin solutions were prepared by dispersing each colloid in distilled water and allowing them to hydrate for 12 h. The solutions were then adjusted to the desired pH (3·0, 3·5 and 4·0) with 0·1 M hydrochloric acid solution.

Coacervates were prepared in duplicate at 40°C by adding the SCMC solution to the gelatin solution, with stirring (Super-mixer, Lab-line Instruments). Batches of 10 mL were made in calibrated 15 mL centrifuge tubes using 0·75 and 2·0% w/v total colloid concentrations at coacervation pH values of 3·0, 3·5 and 4·0. The SCMC percentages of total colloid studied were: 10, 20, 30, 40, 50 and 60%. After mixing, the coacervate mixture was allowed to stand for 10 min at 40°C, then centrifuged at 1500 rev min^{-1} (Super Medium Centrifuge, MSE) for 10 min, allowed to stand for 20 h at 5–10°C for complete coacervation and then again centrifuged. If the equilibrium fluid was observed to be turbid, centrifugation at 10 000 rev min^{-1} (B-20A IEC) for 10 min at 20°C was carried out. This produced a clear supernatant which was decanted, allowing the coacervate volume and wet weight to be measured.

Approximately 2–5 mL of 0·1 M NaOH solution was used to dissolve the coacervate gel. This solution and the equilibrium liquid were then diluted appropriately with distilled

water and analysed for SCMC (phenol-sulphuric acid assay) and gelatin (Lowry assay).

Phenol-sulphuric acid assay

The SCMC in the coacervate and equilibrium fluid was analysed by a modified Milwidsky (1973) phenol-sulphuric acid colorimetric method (Koh & Tucker 1986) using a Cary 219 ultraviolet/visible spectrophotometer at 490 nm. Calibration was carried out over the concentration range 0·001 to 0·005% w/v for both SCMC(LV) and SCMC(HV) solutions. Regression analyses (Draper & Smith 1968) demonstrated that the intercepts were not significantly different from zero (SCMC(LV), $0{\cdot}0077 \pm 0{\cdot}004$; SCMC(HV), $-0{\cdot}001 \pm 0{\cdot}005$), the slopes were highly significant (SCMC(LV), $140{\cdot}4 \pm 1{\cdot}2$; SCMC(HV), $131{\cdot}3 \pm 1{\cdot}5$) and there was no significant curvature in either case. The assay was also carried out on mixtures of SCMC(LV) and gelatin, with gelatin concentration 10 and 100 times higher than that of SCMC (0·001% and 0·005% w/v). Analysis of variance showed that the observed absorbance was the sum of the absorbances of SCMC and gelatin.

Lowry assay

The gelatin in the coacervate and equilibrium liquid was analysed by Lowry assay (Lowry et al 1951) using a Cary 219 ultraviolet/visible spectrophotometer at 750 nm. A calibration was carried out over the concentration range 0·0025 to 0·01% w/v. Regression analysis showed that the intercept was not significantly different from zero ($0{\cdot}0043 \pm 0{\cdot}003$), the slope was highly significant ($22{\cdot}99 \pm 0{\cdot}49$) and there was no significant curvature. The effect of SCMC on the Lowry assay was investigated at 0·0025 and 0·01% w/v gelatin concentrations. Analysis of variance showed that in the presence of SCMC at concentrations of 10 and 100 times that of gelatin, the observed absorbance was the summation of the absorbances of SCMC and gelatin.

Given the additivity of the absorbances in the two assays, it would be possible to determine the concentration of each component by solution of the two simultaneous equations. However, this subsequently proved unnecessary because the contribution of each colloid to the other colloid assay was negligible.

Results and Discussion

Characterization by coacervate yield

Bungenberg de Jong (1949) has described various criteria for the characterization of a coacervation system, of which the chemically analysed yield, that is, the yield obtained by analysis of the acacia and gelatin in the coacervate expressed as a percentage of the two colloids in the total mixture, has been employed to optimize the acacia-gelatin complex coacervation process. Similarly, dry coacervate weight has been adopted as a measure of coacervate yield to determine the maximum degree of coacervation (McMullen et al 1982; Burgess & Carless 1984).

In Fig. 1, the change in the chemically analysed coacervate yield with colloid mixing ratio is shown for SCMC(LV)-gelatin coacervation mixtures of 0·75 and 2·0% w/v concentrations at pH values of 3·0, 3·5 and 4·0 and at 0·75% w/v concentration and pH 3·5 for the SCMC(HV)-gelatin. The

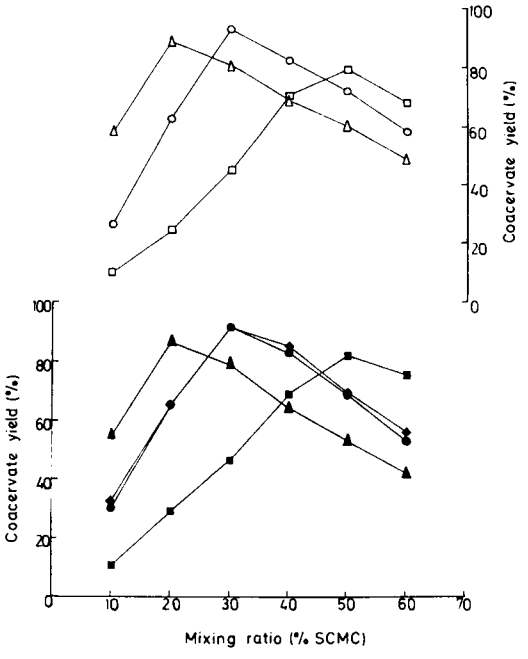

FIG. 1. The effect of colloid mixing ratio on the coacervate yield of SCMC-gelatin coacervation mixtures, prepared at different pH and total colloid concentrations. Key: total colloid concentration: closed markers, 0·75% w/v; open markers, 2·0% w/v; SCMC(LV)-gelatin mixtures: ■□, pH 3·0; ●○, pH 3·5; ▲△, pH 4·0; SCMC(HV)-gelatin mixture: ♦, pH 3·5.

yield is seen to increase as the percentage of SCMC is raised until a maximum at the optimal mixing ratio, and then to decrease. Maximum yield occurred at 30% SCMC at pH 3·5. These results are in agreement with those obtained by viscosity and turbidity measurements (Koh & Tucker 1988). It was noted that an increase in the pH resulted in a shift of the optimal mixing ratio towards a lower SCMC fraction (i.e. peak yields occurred at 50% SCMC at pH 3·0 but at 20% SCMC at pH 4·0). Complex coacervation involves electrostatic interaction between cationic gelatin and anionic SCMC. The increase in the coacervation pH causes an increase in the number of anionic charges on the SCMC while the net cationic charge on gelatin is decreased. Hence, less SCMC was required for an equivalent interaction with gelatin as the pH was raised, leading to a decrease in the SCMC content of the coacervate as pH was increased.

To determine the effect of total colloid concentration on coacervate yield, colloid mixtures at the optimal mixing ratio were studied. At pH 3·5 and 4·0, results showed a slight but significant ($P < 0{\cdot}05$) increase in the coacervate yield with increase in total colloid concentration from 0·75% w/v to 2·0% w/v. That is yield at pH 3·5 increased from $91{\cdot}6 \pm 0{\cdot}2\%$ to $93{\cdot}0 \pm 0{\cdot}3\%$ and at pH 4·0 from $87{\cdot}0 \pm 0{\cdot}2\%$ to $89{\cdot}0 \pm 0{\cdot}3\%$. This increase in degree of coacervation with increase in the total colloid concentration was observed by McMullen et al (1982) for the pectin-gelatin coacervation system. It is explicable in terms of a mass action effect. On the contrary, a slight but not significant ($P < 0{\cdot}1$) decrease in the coacervate yield was observed for colloid mixtures coacervated at pH 3·0 ($82{\cdot}0 \pm 0{\cdot}8\%$, $80{\cdot}0 \pm 0{\cdot}1\%$). This is probably due to the higher concentration of ions (from the added hydrochloric acid) which causes screening of the charges on

the colloids resulting in a weaker ionic attraction between the two oppositely charged colloids and thus suppression of coacervation.

In the complex coacervation of SCMC(HV) and gelatin at pH 3·5, the coacervate yield obtained at all colloid mixing ratios was not significantly different from those for SCMC(LV)-gelatin, indicating a similar coacervation mechanism (Fig. 1).

Effect of pH and colloid concentration on the phase diagram

Phase diagrams of the SCMC-gelatin complex coacervation system at pH values of 3·0, 3·5 and 4·0 are as shown in Figs 2 and 3. Equilibrium fluid data at pH 3·5 only were shown because of closeness of data for the other pH values. The area represented by the symbol I is the region of phase separation.

A displacement of the region of phase separation towards higher SCMC percentages was seen as the pH was lowered due to the charge changes discussed above (Fig. 2). The effect of total colloid concentration on phase separation was studied at concentration of 0·75% w/v and 2·0% w/v. It was found that the region of phase separation contracted on increase of the total colloid concentration. For example, by increasing the total colloid concentration from 0·75% to 2·0% w/v at pH 3·5 and at 30% SCMC mixing ratio, the coacervate SCMC and gelatin were reduced from 4·5 and 11·7 to 3·9 and 9·4% and that of the equilibrium liquid were found to increase from 0·03 and 0·04 to 0·07 and 0·09% (Fig. 3). As the total colloid concentration is increased, the salt concentration of the system is also increased due to sodium ions from the SCMC and chloride ions from the gelatin. Shielding of the anionic groups of SCMC by the cation of the salt and the cationic gelatin by the anions, causes a reduction in the electrostatic attraction between the ionized groups of the two colloids. Hence, coacervation is suppressed and the water content of the complex coacervate is increased by the presence of the indifferent salt. These effects are in agreement with those observed in the acacia-gelatin complex coacervation system (Bungenberg de Jong 1949).

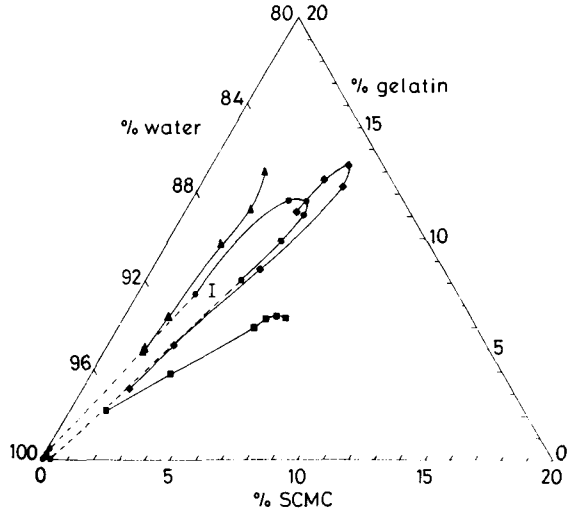

FIG. 2. Phase diagrams of SCMC-gelatin coacervation mixtures, prepared at 0·75% w/v total colloid concentration and different pH values. Key: SCMC(LV)-gelatin mixtures: ■, pH 3·0; ●, pH 3·5; ▲, pH 4·0; SCMC(HV)-gelatin mixture: ♦, pH 3·5.

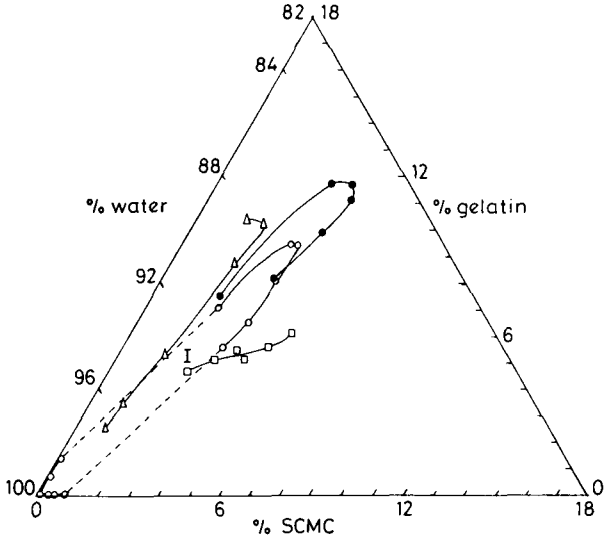

FIG. 3. Phase diagrams of SCMC(LV)-gelatin coacervation mixtures prepared at different pH. Key: □, pH 3·0; ○, pH3·5; △, pH 4·0; total colloid concentration 2·0% w/v; ●, pH 3·5; total colloid concentration 0·75% w/v.

Complex coacervation of gelatin with SCMC(HV) instead of SCMC(LV) at pH 3·5 resulted in a displacement of the region of phase separation towards higher SCMC composition (Fig. 2). This is explicable in terms of the difference in the degree of substitution of the two viscosity grades of SCMC. Chowdhury & Neale (1963) reported that with increasing degree of substitution of the SCMC, the distance between the carboxyl groups decreases, which causes increased intramolecular electrostatic interactions and hence dissociation of the carboxyl groups becomes more difficult. Thus at any pH, SCMC(HV) with a degree of substitution of 0·92 will be less dissociated than SCMC(LV) having a degree of substitution of 0·78. As a result, a lesser number of anionic groups will be available for electrostatic interaction with the cationic gelatin. So a higher concentration of SCMC is required for interaction.

Colloid composition of complex coacervate and equilibrium fluid

Changes in the colloid composition of complex coacervates and equilibrium fluids of isohydric (pH 3·5) mixtures of 0·75% w/v and 2·0% w/v total colloid concentrations are shown in alternative form in Fig. 4. Composition of the total colloid mixture is represented by the broken line. Intersection of the coacervate curve and the equilibrium fluid curve occurred at the equivalent mixing ratio where the colloid composition of the coacervate, equilibrium fluid and total mixture were the same. At 0·75% and 2·0% w/v total colloid concentrations, the equivalent mixing ratios were 27 and 28% SCMC, respectively.

As described by Bungenberg de Jong (1949), the electrophoretic reversal of charge point lies at, or nearly at, the equivalent mixing proportion. At the electrical equivalence pH and mixing ratio where the charge on the two colloids are equal and opposite, attraction forces between the charged colloids saturate each other leading to intense interaction and the highest degree of coacervation (Burgess & Carless

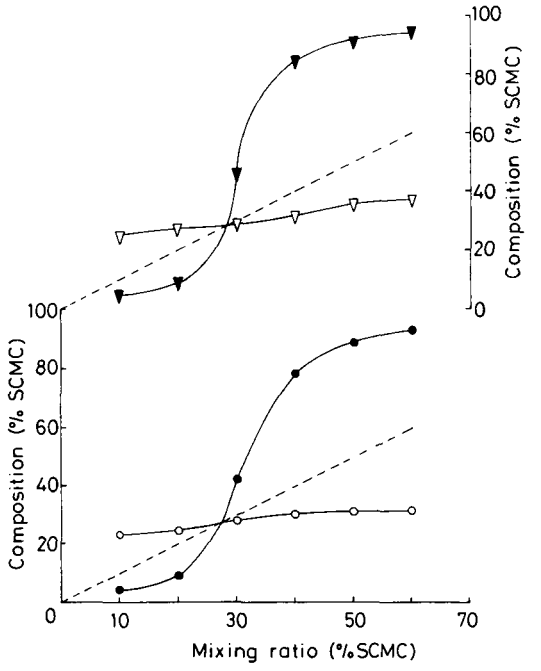

FIG. 4. The effect of colloid mixing ratio on the colloid composition of SCMC(LV)-gelatin complex coacervate and equilibrium fluid, prepared at pH 3·5 and different total colloid concentrations. Ordinate values are the SCMC content expressed as a percentage of total coacervate colloid or total equilibrium fluid colloid. Key: open markers, coacervates; closed markers, equilibrium fluids; ○●, 0·75% w/v; ▽▼, 2·0% w/v.

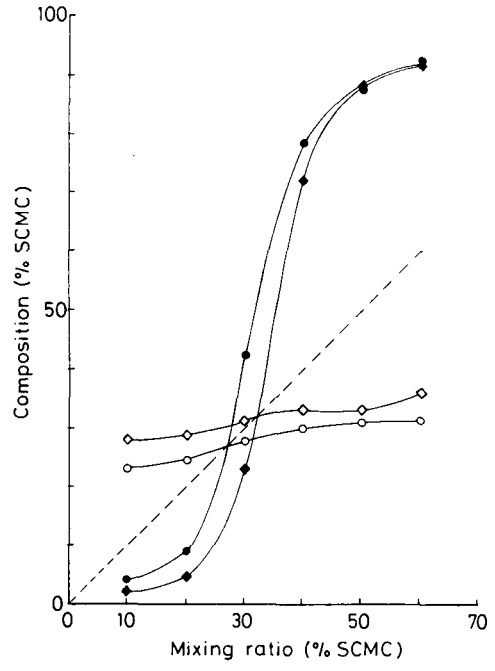

FIG. 5. The effect of colloid mixing ratio on the colloid composition of SCMC-gelatin complex coacervate and equilibrium fluid, prepared at 0·75% w/v total colloid concentration and different pH values. Key: open markers, coacervates; closed markers, equilibrium fluid; ○●, SCMC(LV); ◇◆, SCMC(HV).

1984). Maximum coacervation (in terms of highest coacervate yield) occurred at a colloid mixing ratio very close to the equivalent mixing ratio. At other colloid mixing ratios where the charges are no longer balanced, there is a reduction in the interaction between the oppositely charged colloids and thus a lesser degree of coacervation. In these regions, the coacervate is electrophoretically charged, assuming the charge of the colloid component present in excess. The colloid composition of the coacervate, equilibrium fluid and total mixture are no longer the same. Thus, the excess colloid component distributes itself between the coacervate and equilibrium fluid phases, very much in favour of the latter. Consequently, the coacervation process tends to maintain the separation of an equivalent coacervate, as evidenced by the relatively flat coacervate composition curve compared with that of the equilibrium fluid (Figs 4, 5).

Curves for the pH 3·0 and pH 4·0 coacervation were similar except the points of intersection of the coacervate and equilibrium fluid curves were displaced to 47% SCMC at pH 3·0 and 18% SCMC at pH 4·0, in accordance with the theory described above. An increase in the total colloid concentration from 0·75 to 2·0% w/v showed no significant change in the equivalent mixing ratio at each particular pH.

For SCMC(HV) at pH 3·5, the equivalent mixing ratio was 32% SCMC, which is significantly higher than that for the SCMC(LV)-gelatin complex coacervate (27% SCMC) (Fig. 5). The SCMC(HV)-gelatin coacervate was found to have a higher SCMC content at all colloid mixing ratios (Fig. 5). Once again, this is explicable in terms of the lower degree of ionization of the SCMC(HV) compared with SCMC(LV) at a particular pH, due to the higher degree of substitution of the SCMC(HV).

Conclusions

The foregoing results indicate that the SCMC-gelatin complex coacervation is fundamentally the same as the well characterized acacia-gelatin system. The results provide a basis for the design of microcapsules with walls of variable polymer content by manipulation of coacervation pH and mixing ratio. However, because the latter method has only slight effect on the polymer ratio in the coacervate coacervation pH is of primary importance.

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