

# Characterization of Sodium Carboxymethylcellulose-Gelatin Complex Coacervation by Viscosity, Turbidity and Coacervate Wet Weight and Volume Measurements

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**Abstract**—A sodium carboxymethylcellulose (SCMC) and gelatin coacervation system has been evaluated and characterized and the effects of pH and colloid mixing ratio on the coacervation process investigated. The colloid mixing ratio at which optimum coacervation occurred varied with the coacervation pH. A viscometric investigation of various isohydric SCMC-gelatin mixtures was used to predict optimum conditions for complex coacervation. Optimum coacervation occurred at pH 3.5 at a SCMC-gelatin weight ratio of 3:7 for the SCMC complex coacervation system. Turbidity data confirmed these viscometric results. Coacervate wet weight and volume measurements could not be used to predict optimal coacervation conditions due to changes in the coacervate morphology with mixing ratio. At pH values where coacervation did not occur, the viscosity showed unexpected positive deviations from additive behaviour.

The most commonly studied complex coacervation system is that of gelatin and acacia. This system has been characterized by Bungenberg de Jong (1949) through measurements of viscosity, turbidity, coacervate volume and electrophoresis. Other combinations which have been investigated are the carbopol-gelatin system (Elgindy & Elegakey 1981), the pectin-gelatin system (McMullen et al 1982) and the gelatin-gelatin system (Burgess & Carless 1985). However, to date the sodium carboxymethylcellulose (SCMC)-gelatin system has not been studied.

As coacervation can be induced in systems containing both cationic and anionic hydrophilic colloids, complex coacervation is likely between SCMC, a water-soluble anionic cellulose derivative and gelatin, an amphoteric protein which is positively charged at pH values below its isoelectric point. This system might prove useful for the microencapsulation of drugs. Furthermore, SCMC has been shown to have good mucoadhesive properties (Smart et al 1984), so such microcapsules have potential application as bioadhesive drug delivery devices.

The present work was undertaken to characterize the SCMC-gelatin system as a prerequisite to microencapsulation studies.

## Materials and Methods

### *Materials and characterization*

The gelatin (Sigma Co.) used was alkali processed Type B material (225 Bloom) obtained from calf skin. Its moisture content (9.4% w/w) was determined by the method of Eastoe & Courts (1963). Mixed-bed resin ion exchange method (Janus et al 1951) and 'U' tube viscometry (Stainsby 1952) were used to determine its isoelectric point of pH 5.0.

The sodium carboxymethylcellulose used was of low

viscosity grade (Sigma Co.). The material was characterized by infrared spectroscopy, moisture content, molecular weight, degree of substitution and purity. The moisture content was determined to be 4.0% w/w (ASTM Standards 1983). A degree of substitution of 0.78 and a purity of 94.0% of sample weight were determined by the uranyl precipitation method (Durso 1980). The molecular weight (26951 daltons) was obtained by solution viscosity measurement using the Mark-Houwink equation.

For SCMC in 0.1 M aqueous sodium chloride solution at 25°C.

$$[\eta] = 1.23 \times 10^{-4} M^{0.91}$$

where  $[\eta]$  is the intrinsic viscosity and  $M$  the molecular weight (Sitaramaiah & Goring 1962).

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

### *Coacervate preparation*

SCMC and gelation 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 with 0.1 M hydrochloric acid or 0.1 M sodium hydroxide solution.

Coacervates were prepared at 40°C by adding the SCMC solution to the gelatin solution, with stirring. The coacervate mixture was allowed to stand for 10 min before being subjected to further evaluations. For 10 mL batches, coacervations were carried out in centrifuge tubes and the mixtures stirred with a mixer (Super Mixer, Lab-Line Instruments). The 200 mL batches were prepared in 400 mL beakers and the mixtures stirred at 300 rev min<sup>-1</sup> with a mechanical stirrer (3-blade, 2 cm in diameter). The SCMC percentages of total colloid studied were 10, 20, 30, 40, 50, 60, 70, 80 and 90%.

### *Viscosity measurement*

Viscosities of the coacervate mixtures were determined using

a 'U' tube viscometer at a temperature of  $40 \pm 0.05^\circ\text{C}$ . The flow times of more than 200 s were measured until two consecutive readings agreed to within 0.2 s. Batches (200 mL) of coacervate mixture were prepared from isohydric 0.75% w/v SCMC and 0.75% w/v gelatin solutions, with pH values in the range 1.5 to 7.0. The pH was checked after mixing and did not differ from the starting pH value.

#### Turbidity measurement

SCMC and gelatin solutions (0.05% w/v) were used for complex coacervation. Batches (10 mL) were prepared in triplicate at coacervation pH values of 1.5, 2.5, 3.0, 3.5, 4.0 and 4.5. Turbidities of the coacervate mixtures were measured at 600 nm (Cary 219 ultraviolet/visible spectrophotometer) in 1 cm cuvettes against distilled water.

#### Coacervate weight and volume measurements

Coacervation was done in calibrated 15 mL centrifuge tubes. Batches (10 mL) were prepared in duplicate from SCMC and gelatin solutions of 0.75% w/v and 2.0% w/v concentrations. Three coacervation pH values (3.0, 3.5 and 4.0) were studied. The coacervation mixture was centrifuged at low speed of  $1500 \text{ rev min}^{-1}$  (MSE) for 10 min, allowed to stand for 20 h at  $5\text{--}10^\circ\text{C}$  for complete coacervation and then again centrifuged. If the equilibrium fluid was observed to be turbid, centrifugation at  $10\,000 \text{ rev min}^{-1}$  (B-20A IEC) for 10 min at  $20^\circ\text{C}$  was carried out. The coacervate volume was measured, then the equilibrium fluid was decanted and the coacervate weight determined.

### Results and Discussion

#### Viscometric investigation of SCMC-gelatin coacervation

Bungenberg de Jong (1949) and Nixon & Nouh (1978) reported that a considerable decrease in the viscosity of the acacia-gelatin system was observed with coacervation and this could be employed to characterize and optimize the coacervation system. Complex coacervation is known to be affected by factors such as pH, colloid mixing ratio, colloid concentration and ionic strength (Bungenberg de Jong 1949). Therefore, to determine the optimal coacervation conditions for the SCMC-gelatin system, the following variables were investigated: pH (range, pH 1.5–7.0) and colloid mixing ratio (range, 10–90% SCMC of the total colloid).

The changes in viscosity,  $\eta_{sp}\%$  (i.e. the experimentally obtained specific viscosity of the colloid mixture expressed as a percentage of the total specific viscosity contributed by each of the two colloids, assuming additivity) of a series of SCMC-gelatin mixtures as a function of the colloid mixing ratio are shown in Fig. 1. As described by Bungenberg de Jong (1949), deviations of the viscosity from additivity are closely related to the ionic interaction between the oppositely charged colloids. Sufficiently strong ionic interaction is necessary for complex coacervation to occur and if not, no coacervation will be evident even at the most favourable mixing ratio.

At pH 1.5, the viscosity of the colloid mixture was observed to be additive, indicating no SCMC-gelatin interaction, as SCMC exists mainly in the acid form at such low pH. Chowdhury & Neale (1963) reported an average pK value of 4.17 for SCMC with a degree of substitution of 0.7. Hence,

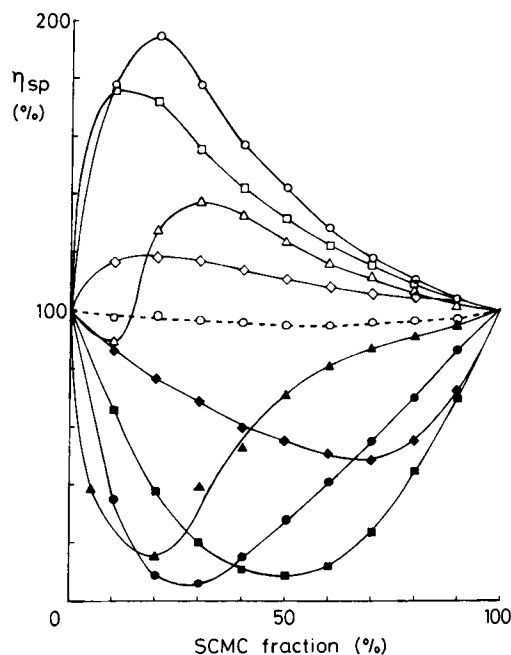


FIG. 1. The effect of colloid mixing ratio and pH on the  $\eta_{sp}\%$  (i.e. the experimentally obtained specific viscosity of the colloid mixture expressed as a percentage of the total specific viscosity contributed by each of the two colloids, assuming additivity) of SCMC-gelatin mixtures, prepared at different pH values. Key: - - - , pH 1.5; ●, pH 2.5; ■, pH 3.0; ◆, pH 3.5; ▲, pH 4.0; ▽, pH 4.5; ○, pH 5.0; □, pH 5.7; ◇, pH 7.0.

no coacervation was evident and the colloid mixtures remained clear. This was clearly demonstrated in the results obtained whereby at pH 4.5, complex coacervation occurred only at a SCMC-gelatin mixing ratio of 9:1. However, coacervation was visually evident at all colloid mixing ratios over the pH range 2.5–4.0, and negative deviations from the additive behaviour were noted. In this pH range, SCMC is negatively charged and gelatin is positively charged, resulting in strong electrostatic interaction and hence complex coacervation. As the coacervation pH is raised, the number of anionic charges on SCMC is increased while the net cationic charge on gelatin is decreased until it reaches zero, at its isoelectric point (pH 5.0). Consequently, an increasing amount of gelatin was required for an equivalent interaction with SCMC, resulting in coacervation. Thus a shift in the position of the minimum point of the curves was observed with pH change (Fig. 1). That is, at pH 2.5, 70% SCMC gave optimal coacervation as indicated by the minimum in the curve, whereas at pH 4.5 the optimal was at 10% SCMC. Maximum coacervation (i.e. maximum deviation from additive behaviour) occurred at pH 3.5 and at 30% SCMC (Fig. 2).

The observed positive deviations from additive behaviour of SCMC-gelatin mixtures over the pH range of 5.0 to 7.0 (Fig. 1) differs from the findings of Bungenberg de Jong (1949) who reported additive behaviour for the viscosity of the acacia-gelatin system at pH 1.22 and pH 5.06. It is apparent that sufficient interaction exists between the two colloids to cause the viscosity to increase but not for coacervation to occur. The maximum deviation occurred at the isoelectric point (pH 5.0) of the gelatin (Fig. 3). It is

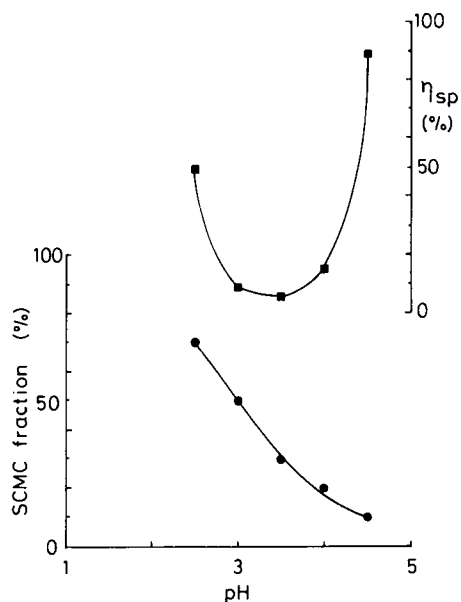


FIG. 2. Colloid mixing ratio (left ordinate) and  $\eta_{sp}$  % (right ordinate) versus pH of the optimal SCMC-gelatin coacervation mixtures.

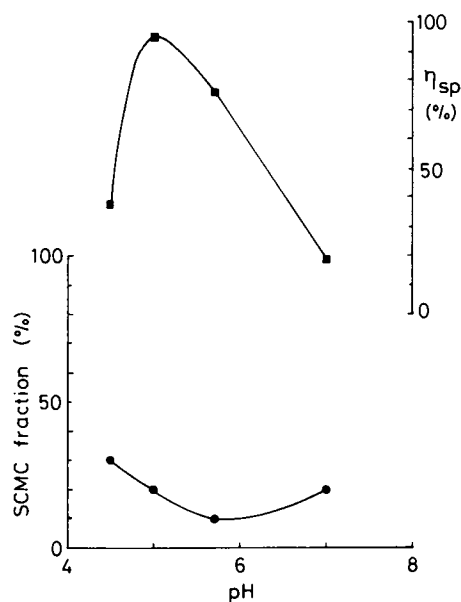


FIG. 3. Colloid mixing ratio (left ordinate) and  $\eta_{sp}$  % (right ordinate) versus pH of the non-coacervating SCMC-gelatin mixtures with maximum interaction.

suggested that the behaviour is the result of an interplay between two factors: the degree of dissociation of the carboxyl groups on the SCMC molecules and the number of positive charges on the gelatin molecules. At pH 5.0 (the isoelectric point), there exists an equal number of anionic and cationic charges on the gelatin molecules. Thus an ionic interaction with the anionic SCMC molecules results in the formation of negatively charged soluble SCMC-gelatin complex. Electrostatic repulsion leads to unfolding of the complex and the viscosity show a large positive deviation from additivity since the gelatin is normally tightly coiled at this pH.

Complexes formed at pH 4.5 have a lower net negative charge due to the net positive charge on the gelatin and lower degree of ionization of the SCMC. Furthermore, the gelatin alone is partially uncoiled at pH 4.5 with the result that deviations from additivity are not as great as at pH 5.0. As the pH is raised, the number of positive charges on the gelatin diminishes and consequently ionic interaction with SCMC will be reduced and eventually not occur. This causes the viscosity of the SCMC-gelatin mixture to approach additive behaviour.

#### Characterization by turbidity measurement

Turbidity measurement can be used to study complex coacervation at low colloid concentrations of 0.05% w/v (Bungenberg de Jong 1949). The turbid appearance of the coacervation mixture is attributable to inhomogeneity of the medium as it contains coacervate particles dispersed throughout the equilibrium fluid. When a beam of light is passed through such a medium, its intensity is decreased as a result of absorption and scatter. Light scattering is determined by the size of the dispersed particles relative to the wavelength of the light, the refractive index of the particles relative to that of the medium and the particle concentration (Oster 1972). Hence, morphology, size and concentration variations in the coacervate formed at different pH and colloid mixing ratios might impose limitations on the use of this technique in the optimization of coacervation system. However, the results obtained in this study were in agreement with those of the viscometric investigation.

The pH range for coacervation of SCMC and gelatin when estimated by turbidity measurement was 2.5 to 4.5, in agreement with the viscometric data (Figs 1, 4). Furthermore, maximum turbidity occurred at pH 3.5 and 30% SCMC also in agreement with the viscometric data. Thus, in

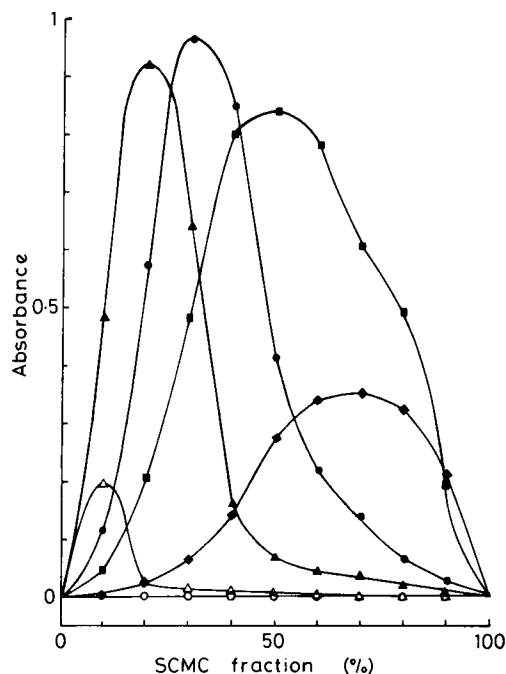


FIG. 4. Effect of colloid mixing ratio and pH on the absorbance (600 nm) of SCMC-gelatin mixtures, prepared at different pH values. Key:  $\circ$ , pH 1.5;  $\diamond$ , pH 2.5;  $\blacksquare$ , pH 3.0;  $\bullet$ , pH 3.5;  $\blacktriangle$ , pH 4.0;  $\triangle$ , pH 4.5.

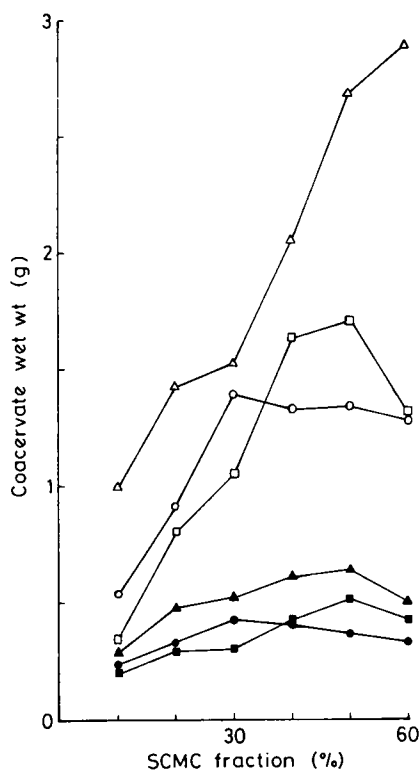


FIG. 5. Coacervate wet-weight versus colloid mixing ratio of coacervate mixtures of different pH and total colloid concentrations of 0.75% w/v and 2.0% w/v. Key: Closed markers, 0.75% w/v; Open markers, 2.0% w/v; ■ □, pH 3.0; ● ○, pH 3.5; ▲ △, pH 4.0.

spite of the various factors which might affect light scattering, turbidometric measurement appears to be a suitable rapid technique for locating optimal coacervation conditions.

#### Coacervate weight and volume measurements

The effect of pH, colloid mixing ratio and colloid concentration on coacervate weight are as shown in Fig. 5. Coacervate volume curves were similar. The coacervate wet weights and volumes were measured after centrifugation. For complex coacervation at pH 3.0 and pH 3.5 and total colloid concentrations of 0.75 and 2.0% w/v, both the coacervate weight and volume were observed to peak at 50 and 30% SCMC fractions, respectively. These results are in agreement with data from the viscometric and turbidity studies.

It was noted, however, the results obtained at pH 4.0 and 2.0% w/v total colloid differed in that no optimum coacervation condition was seen. The coacervate formed at colloid

mixing ratios away from the optimum (as determined in the viscometric and turbidity studies) were in a highly dispersed form, making volume and wet weight measurements impractical. The coacervate drops of these opalescent coacervation mixtures were very small, their stability being promoted by the unfavourable mixing ratios, the low electrolyte strength of the medium and the net charge on the coacervate particles (Bungenberg de Jong 1949). Increasing coacervate volumes have been attributed to a change in coacervate morphology, resulting in lower packed density and greater hydration (McMullen et al 1982). Thus, it is evident that coacervate wet weight and volume cannot be used, in general, to predict optimal coacervation conditions. Coacervate dry weight or yield has been used by McMullen et al (1982) to optimize the pectin-gelatin coacervation system.

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