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Electrophoretic Properties of Sulfamethoxazole Microcapsules and Gelatin–Acacia Coacervates

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Abstract □ The electrophoretic properties of sulfamethoxazole microcapsules and the coacervates prepared by gelatin–acacia coacervation were investigated. The effects of the parameters in the microcapsule preparation, such as the coacervation pH, amount of formaldehyde used for hardening, and drying method of the coacervates, on the ζ-potential of the resultant microcapsules were clarified. The Büchner effect was observed in coacervates in an electric field, which indicated that the coacervate wall was flexible. The ζ-potential *versus* pH curves of the coacervates appeared on the upper side of the plain sulfamethoxazole, while those of the microcapsules dried conventionally shifted to the lower side due to denaturation of the gelatin in the microcapsule wall, which occurred during drying. Spray drying increased the denaturation of gelatin, which imparted a negative charge to the spray-dried microcapsules. Formalization of the coacervates refined the electrophoretic behavior of the microcapsules, depending on the amount of formaldehyde used. The ζ-potential of the plain sulfamethoxazole also was measured in the simulated coacervation solution to analyze the mechanism of coacervation electrophoretically.

Keyphrases □ Sulfamethoxazole—microcapsules and coacervates, electrophoretic properties compared □ Gelatin–acacia coacervates—sulfamethoxazole microcapsules and coacervates compared electrophoretically □ Microcapsules, acacia–gelatin—sulfamethoxazole, electrophoretic properties compared □ Coacervation, acacia–gelatin—sulfamethoxazole coacervates and microcapsules compared electrophoretically

Much attention has been paid to the electrophoretic properties of microcapsules because they are decisive parameters in stabilizing suspensions compounded with microcapsules (1). In addition, to prepare a microcapsule containing living cell fluid for use as an artificial cell, it is necessary to simulate the electrophoretic properties of the microcapsule as well as other properties such as elasticity, mechanical strength, and permeability (2).

Measurement of the ζ-potential of microcapsules is one way to assess their electrophoretic properties. The ζ-potential of microcapsules prepared by an interfacial polycondensation method was measured, and the effect of the pH medium on these properties was reported (3). The ζ-potential of gelatin–acacia coacervates, excluding the core material, also was determined (4). However, the electrophoretic properties of gelatin–acacia microcapsules containing the core material were not exhaustively investigated.

In the present study, ζ-potentials of gelatin–acacia coacervates and microcapsules prepared by drying were measured. One aim of the study was to elucidate the parameters that affect the electrophoretic properties of the coacervates and microcapsules. The amount of formaldehyde used for hardening, the coacervation pH, and the drying method of the coacervate droplets were assumed to be the parameters affecting this property. Another purpose was to manifest the coacervation process electrophoretically by measuring the changes in the ζ-potentials of plain sulfamethoxazole particles and gelatin–acacia coacervates during processing.

EXPERIMENTAL

Materials—The test samples used to measure the ζ-potential were micronized sulfamethoxazole[1], gelatin–acacia coacervate droplets, and dried gelatin–acacia microcapsules of sulfamethoxazole. The gelatin–acacia coacervates of sulfamethoxazole were prepared as described earlier (5, 6). The coacervation pH was adjusted to 2.05–4.2. The coacervate droplets were formalized with 0, 30, and 50 ml of formaldehyde.

The conventional method of drying the coacervates (using warm air at 40°) and a spray drying technique were adopted. Spray drying of the slurries containing coacervate droplets was conducted using a centrifugal wheel atomizer driven at 40,000 rpm at 140 ± 10°. The preparation procedures of the microcapsules are shown in Fig. 1.

The microcapsules used for measuring the ζ-potential were characterized by the following micromeritic properties: geometric mean diameter of 8.5–28.5 μm, wall thickness of 0.75–1.31 μm, and particle density of 1.02–1.19 g/cm³ (7).

Dispersion Medium—The dispersion media used to measure the

[1] Micronized to <6 μm by a jet micronizer, Shionogi Pharmaceuticals Co., Osaka, Japan.

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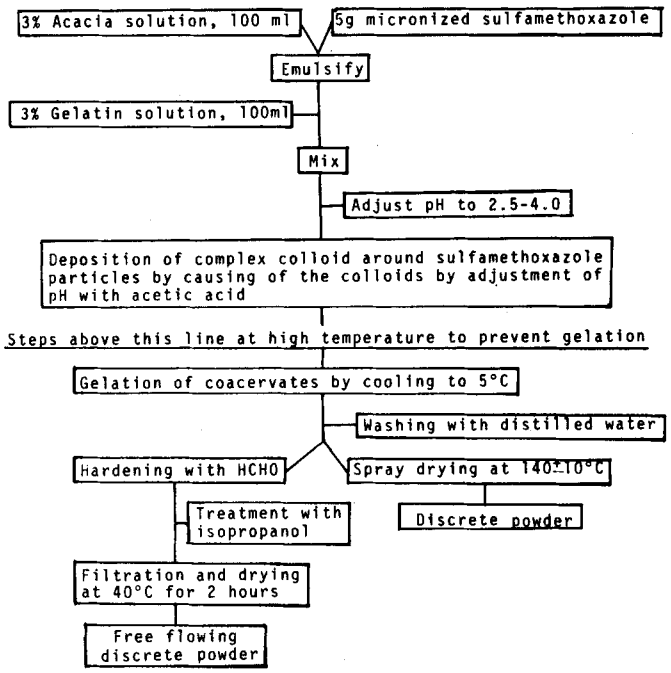

Figure 1—*Processing of microencapsulation of sulfamethoxazole by gelatin–acacia complex coacervation.*

ζ-potential were distilled water, the equilibrium coacervation solution, and the buffer solution with pH values of 2.5–9.5 and a constant ionic strength of 0.1.

Electrophoretic Mobility Measurements—The electrophoretic mobility of the test samples was measured[2]. The test particles, weighing ~10 mg, were dispersed in the required medium that was thermally controlled at 23° and were fed into the electrophoretic cell. The specific conductance of each medium was measured to select the most suitable voltage to apply across the electrophoresis cell.

The mobilities of 20 particles were measured for each batch, and the electrophoretic mobility was the average value of three batches. The experimental variations are indicated by the standard deviation bars in the figures. The ζ-potentials were calculated by inserting the mobilities into the Helmholtz–Smoluchowski equation:

$$\zeta = 9.0 \times 10^4 \left(\frac{4\P \eta u}{D}\right) \qquad \text{(Eq. 1)}$$

where ζ is the ζ-potential (millivolts), η is the viscosity of the suspending liquid (poises), D is the dielectric constant of the suspending liquid, and u is the electrophoretic mobility of the suspended solid (micrometers per second per volt per centimeter). The factor 9.0×10^4 converts electrostatic units and micrometers to practical electrical units and centimeters, respectively.

ζ-Potential Measurement of Sulfamethoxazole Particles—To elucidate the change in the electrophoretic property of sulfamethoxazole particles during formation of the gelatin–acacia coacervates, the ζ-potential of sulfamethoxazole particles was measured in the medium adjusted to simulate the coacervation procedure (Fig. 1). Five grams of sulfamethoxazole particles was suspended in 200 ml of distilled water and in 200 ml of acacia–gelatin solution (3%) thermally controlled at 50° with stirring at 620 rpm.

The pH of each suspension was adjusted gradually to 2.5–4.0 by dropwise addition of diluted acetic acid. The particles were filtered after the suspension was cooled to 5.0° by immersion in a water bath. A portion of the separated particles was redispersed in the filtrate of each suspension, and the ζ-potentials were measured.

RESULTS AND DISCUSSION

ζ-Potential of Sulfamethoxazole Particles—The pH profiles of the ζ-potential of sulfamethoxazole strongly depended on the type of polymer contained in an acetic acid solution (Fig. 2); the curves showed a positive

[2] Zeter-Meter, New York, NY 10028.

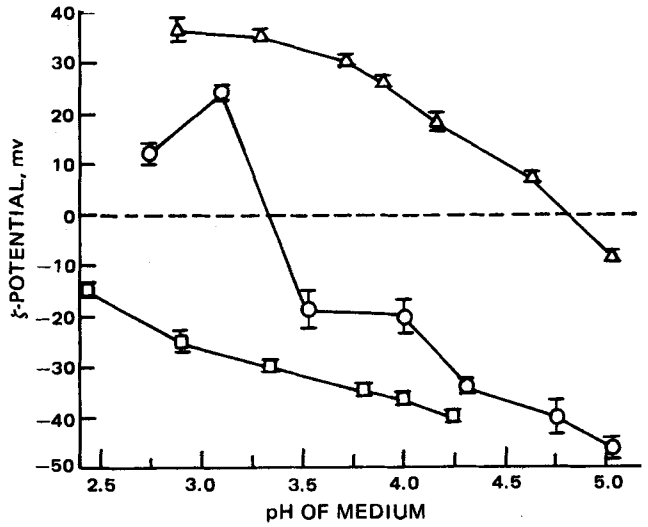

Figure 2—*ζ-Potential of sulfamethoxazole particles in acetic acid solution containing acacia and gelatin. Key (composition of medium): ○, acetic acid; □, acetic acid and acacia; and △, acetic acid and gelatin.*

charge at the lower pH range of 3.35 but a negative charge at the higher range. The apparent isoelectric point of sulfamethoxazole in this system was determined to be 3.35, which is slightly lower than the literature value of 3.8. This difference may be due to adsorption of acetate ions onto the sulfamethoxazole particle, which converts the surface charge slightly to the negative.

The ζ-potential in gelatin solution revealed a positive charge at pH 2.88–4.8. The pH value of 4.8, which intercepted the line of $\zeta = 0$, coincided with the isoelectric point of the gelatin used in this study. This result indicated that the gelatin molecules were adsorbed onto the sulfamethoxazole particles and formed a protective film around them. Thus, the particles with adsorbed gelatin molecules could behave as a gelatin molecule.

The ζ-potential of sulfamethoxazole in acacia solution was negative, which resembled the electrophoretic behavior of acacia. The absolute values of the ζ-potential decreased slightly with decreasing pH values, which can be attributed to the conversion of acacia from the salt form (such as calcium, magnesium, and potassium arabiate) to the free acid form.

The filtered sulfamethoxazole particles were redispersed directly, without washing, in distilled water for measuring the ζ-potential. Other measurements were made using particles washed with 100 ml of distilled water after filtration (Fig. 3). The ζ-potential of the washed particles from acetic acid solution was −30 mv in distilled water with a pH of 4.48–4.52. The absolute values of the ζ-potential in distilled water was slightly lower than in the acetic acid solution with a corresponding pH. This finding

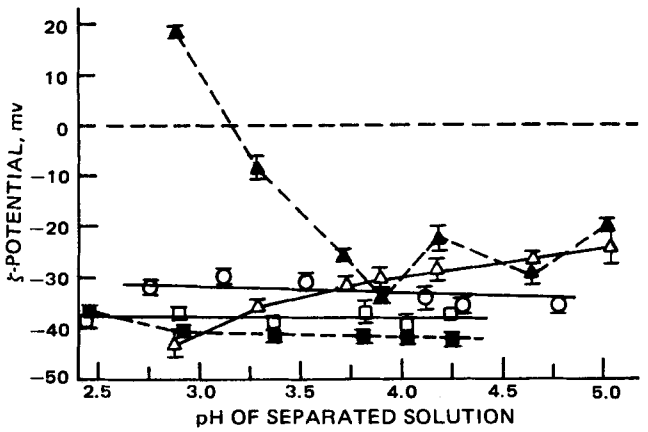

Figure 3—*Effect of washing on the ζ-potential of the sulfamethoxazole particle in distilled water. Key (type of separated solution): ■ and □, acacia without washing and washed with 100 ml of water, respectively; ▲ and △, gelatin without washing and washed with 100 ml of water, respectively; and ○, acetic acid washed with 100 ml of water.*

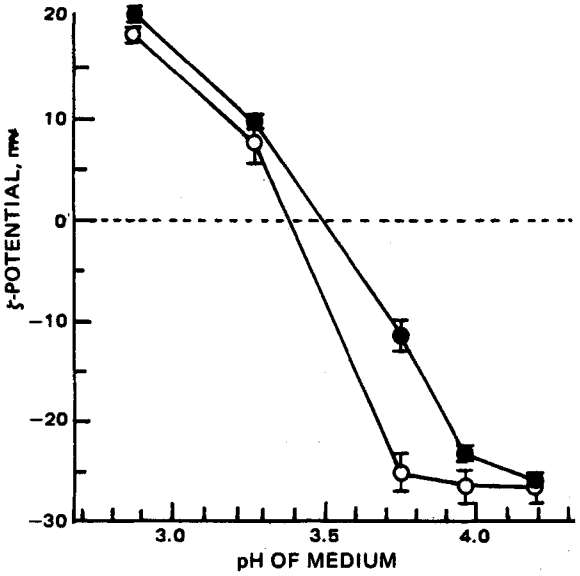

Figure 4—*ζ-Potential of the coacervate in the supernate of the coacervation solution. Key: ○, original sulfamethoxazole; and ●, coacervate.*

indicated the release of acetate ions from the particle surface by washing.

The washing effect also reflected on the ζ-potentials of particles separated from acacia and gelatin solutions. The difference in the ζ-potential curves of particles in gelatin solution before and after washing is seen in Fig. 3. Little difference was found at pH 4.0–5.0, which indicated stronger adsorption of gelatin onto the particle than at lower pH. These results suggest that, during preparation of coacervates by the present method, acacia molecules first are adsorbed onto sulfamethoxazole particles and then the coacervates are formed on the particle surface by adding gelatin and adjusting to the required pH.

ζ-Potential of Coacervate Droplets of Sulfamethoxazole—The ζ-potentials of the coacervate droplets were measured in the supernate of the coacervation solution and compared with those of the plain sulfamethoxazole particle. In an electric field, the coacervate droplets were deformed to an ellipsoid, the short axis of which was parallel to the direction of the electric field. This phenomenon, the Büchner effect (8), indicated that the coacervate wall was flexible.

Although the ζ-potential *versus* pH curve of the coacervates was above that of plain sulfamethoxazole, the form of the profile was almost the same (Fig. 4). This result suggests that the electrophoretic property of coacervates is affected by the encapsulated sulfamethoxazole. The pH profile of sulfamethoxazole almost coincided with the profile in the acetic acid solution (Fig. 2), suggesting that adsorption of the polymer to the particle was slight and that the polymer did not exist in the supernate. This finding proved that a complete phase separation occurred.

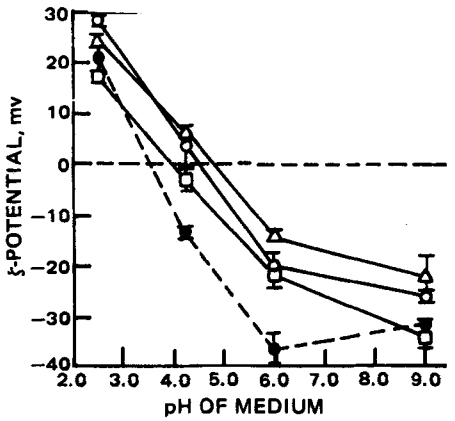

Figure 6—*Effect of coacervation pH on the ζ-potential of coacervates in the buffer solution. The buffer medium was μ 0.1. Key (coacervation pH): △, 2.5; ○, 3.5; and □, 4.0. The original sulfamethoxazole is indicated by a solid dot (●).*

The unformalized coacervate droplets were removed from the system with various pH values and were redispersed in distilled water. The change in the ζ-potential of the coacervates was derived by replacing the medium resembling the plain sulfamethoxazole adsorbed with gelatin (Figs. 3 and 5). This gelatin-like property of the coacervates suggests that the adsorbed gelatin on the particle surface can determine the electrochemical property of the coacervate. The formalized coacervate showed almost the same change in the ζ-potential as appeared in the unformalized coacervate. This result suggests that the gelatin was not denatured by formalization at this stage.

The coacervates removed from the system at various pH values were washed sufficiently with distilled water and were redispersed in the buffer solution, which was adjusted to various pH values and a constant ionic strength of 0.1. The pH profile of ζ-potentials of the coacervates and plain sulfamethoxazole in buffer are represented in Fig. 6. The ζ-potential *versus* pH curves of the coacervates appeared mainly on the upper side relative to the plain particles. These results indicate that the electrical property of gelatin–acacia coacervates is controlled by gelatin and the encapsulated core material. With decreasing coacervation pH, the pH

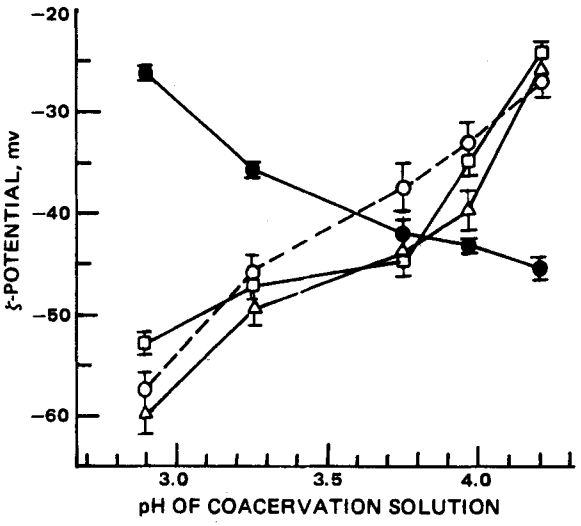

Figure 5—*Effect of washing on the ζ-potential of the coacervate in distilled water. Key: □, formalized coacervate with 15 ml of formaldehyde and washing with 1200 ml of water; ●, unformalized coacervate without washing; △, unformalized coacervate washed with 100 ml of water; and ○, unformalized coacervate washed with 1200 ml of water.*

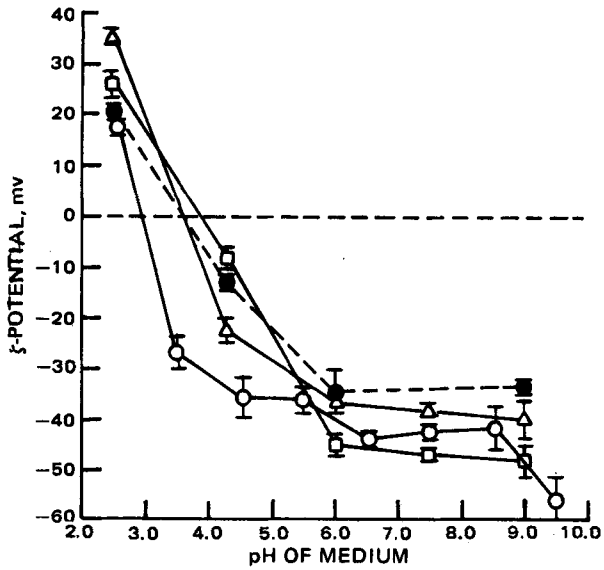

Figure 7—*Effect of coacervation pH on the ζ-potential of microcapsules in the buffer solution. The buffer medium was μ 0.1. Key (coacervation pH): △, 2.5; ○, 3.5; and □, 4.0. The original sulfamethoxazole is indicated by a solid dot (●).*

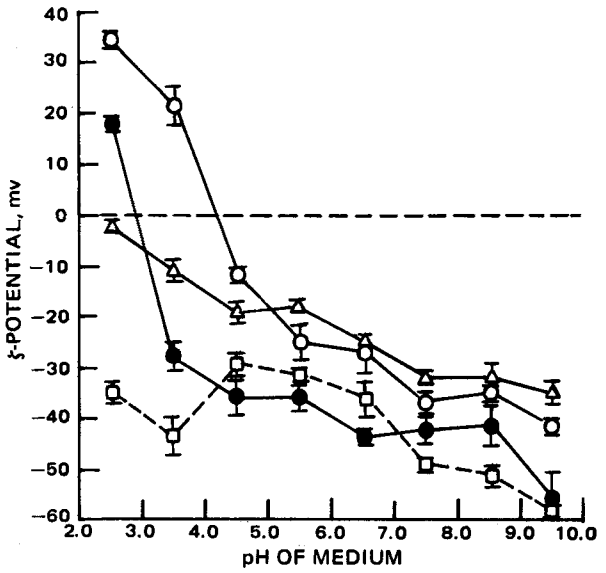

Figure 8—*Effect of formalization and spray drying on the ζ-potential of microcapsules in buffer solution. The buffer medium was μ 0.1. Key (milliliters of formaldehyde used): ●, 0; ○, 30; and △, 50. The spray-dried microcapsules are indicated by an open box (□).*

profile of the ζ-potential shifted more to the right, and the isoelectric point moved toward that of gelatin. The ζ-potential–pH profile of the coacervate prepared at pH 2.5 almost coincided with that of gelatin.

ζ-Potential of Microcapsules—The unformalized microcapsules prepared by drying the coacervates in the conventional way were dispersed in the coacervation medium adjusted to the required pH. The distinct difference in the pH profiles of the microcapsules from those of the corresponding coacervates is illustrated in Fig. 7. All of the curves moved to the left of the plain particles. This result was due to the denaturation of gelatin in the microcapsule wall which occurred during drying. Because of the gelatin denaturation, the contribution of acacia compared to that of gelatin became dominant, resulting in the negative charge.

The ζ-potential in the buffer solution *versus* pH curves of the microcapsules prepared at pH 3.5 was a function of the amount of formaldehyde used for hardening (Fig. 8). Upon formalizing with 30 ml of formaldehyde, the ζ-potential curve shifted to the upper side relative to that of the unformalized microcapsule. This result might be due to liberation of acacia from the microcapsule wall, which was induced by formalization. Thies (9) reported that glutaraldehyde treatment liberated acacia from gelatin–acacia coacervates. The release of acacia from the microcapsule wall caused the electrical equilibrium on the microcapsule surface to become unbalanced. The gelatin remaining in the microcapsule wall imparted a positive charge to the microcapsule, whereas stronger formalization, *e.g.*, 50 ml of formaldehyde, pushed the ζ-potential curve to the negative side for all of the pH ranges. In this case, the denaturation of gelatin compared to the liberation of acacia contributed to the negative conversion of the ζ-potential. The electrical property of the microcapsule was determined by a balance of the two actions. The spray-dried microcapsules revealed negative ζ-potentials at pH 2.5–9.5. The pH profile of the spray-dried microcapsules as seen in Fig. 8 resembled that of acacia in Fig. 2. The denaturation of gelatin, which occurred during drying, led to this result. The denaturation of gelatin in the microcapsule wall and the liberation of acacia from the wall were the main parameters used to determine the electrical properties of the microcapsules.

Conclusion—The mechanism of coacervation with gelatin and acacia was elucidated electrochemically, and the parameters that affected the electrophoretic properties of the microcapsules were found by measuring the ζ-potential of plain sulfamethoxazole, coacervates, and microcapsules.

Coacervation occurred on the surface of the sulfamethoxazole particle with adsorbed acacia, as shown by the fact that the ζ-potential–pH profile of the coacervates coincided with that of gelatin.

The Büchner effect appeared when the coacervates were in an electric field, thus indicating that the wall of coacervates was flexible.

With decreasing coacervation pH, the ζ-potential–pH curve of the coacervate became closer to that of gelatin and eventually coincided with it at pH 2.0.

The denaturation of gelatin caused by formaldehyde did not occur at the coacervation stage but during the succeeding drying process, which completed the hardening of the microcapsules.

Used as a hardening agent, formaldehyde can release acacia from the coacervate and denature the gelatin left in the microcapsule, thus determining the ζ-potentials of the formalized microcapsules. When the amount of formaldehyde was insufficient or sufficient for denaturing the gelatin, the ζ-potential of the formalized microcapsules became higher or lower, respectively, than that of the unformalized microcapsules.

The gelatin in the coacervates was denatured during spray drying, which depressed the ζ-potentials of the dried microcapsules.

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