Abstracted in part from a thesis submitted by I. S. Gibbs to the Graduate School, Temple University, in partial fulfillment of the Doctor of Philosophy degree requirements.

* Present address: Squibb Institute for Medical Research, New Brunswick, N.J.

x To whom inquiries should be directed.

Microencapsulation of a Waxy Solid: Wall Thickness and Surface Appearance Studies

P. L. MADAN, L. A. LUZZI, and J. C. PRICE x

Abstract □ Microencapsulation of solid stearyl alcohol particles by complex coacervation was studied. Spherical particles of solid stearyl alcohol were prepared by the vibrating capillary method. Various size fractions of these particles were encapsulated by a modified technique described in an earlier report. Particle concentration and particle-size studies revealed that only a small fraction of the total amount of colloid was used in the formation of the capsule wall. Wall thickness decreased with increasing ratios of solid particles and increased as the particle diameters increased. Scanning electron micrograph observations of the surface showed that acacia was retained on the surface of particles melted and congealed in acacia solution and that the final encapsulated particles had a scaly surface appearance. The studies tended to confirm indications from earlier work that gelatin molecules interact directly with acacia on the surface of the stearyl alcohol particles to form the capsule.

Keyphrases □ Microencapsulation—acacia-coated solid stearyl alcohol particles with gelatin solution, wall thickness, surface appearance □ Encapsulation, micro—acacia-coated solid stearyl alcohol particles with gelatin solution, wall thickness, surface appearance □ Stearyl alcohol particles, acacia coated in gelatin solution—microencapsulation, wall thickness determined, surface appearance evaluated □ Gelatin—interaction with acacia on stearyl alcohol particle surface, microencapsulation, wall thickness, surface appearance □ Acacia—interaction with gelatin on stearyl alcohol particle surface, microencapsulation, wall thickness, surface appearance

There have been a number of reports concerning encapsulation of various substances but few concerning the extent or nature of encapsulation. Undoubtedly the characteristics of the product depend, not only on the extent of encapsulation, but also on the final thickness and integrity of the deposited encapsulating material. The gelatin–sulfamerazine ratio was used (1) as an indication of wall thickness in studying encapsulation of sulfamerazine by simple coacervation. Apparently few attempts have been made to evaluate the wall thickness of microcapsules made by complex coacervation. A recent report (2) considered wall thickness and diffusion from microcapsules containing a liquid organic base.

The purposes of this investigation were to: *(a)* encapsulate spherical solid particles by complex coacervation, *(b)* evaluate the wall thickness of the microcapsules, *(c)* study the effect of particle concentration and particle size on the wall thickness of microcapsules, and *(d)* study the surface appearance of the particles at several stages of the process.

EXPERIMENTAL

Materials—Acacia USP, stearyl alcohol USP, and pigskin gelatin[1] (isoelectric point at pH 8.0) were used.

Selection of Material to Be Encapsulated—To achieve uniformity of the particles to be encapsulated, it was desirable to produce spherical particles, preferably monodisperse. The selection of the material to be encapsulated was, therefore, quickly narrowed to materials that could be easily melted and rapidly congealed to fulfill requirements of available methods of spherical particle production. Wax-like materials most nearly fulfill these requirements; stearyl alcohol was chosen, although a number of other wax-like substances, *e.g.*, beeswax and paraffin, could have been used with little difficulty.

Selection of Particle Size of Core Material—The following reasons predetermined the use of particles larger than 250 μm in diameter: *(a)* convenience, ease in handling, and microscopic observation; *(b)* the discovery that the larger wax particles when encapsulated by a modified procedure seemed to give thin, uniform capsule walls (3) from which thicknesses could be more easily estimated; and *(c)* a desire to explain the formation of the thin, uniform walls around the waxy particles.

Preparation of Particles to Be Encapsulated—The vibrating capillary method of production of spherical particles, similar to that described previously (3, 4), was used. A nest of sieves was employed for the separation of particles in various size ranges. Particles passing through one sieve and retained on the next finer sieve were assigned the arithmetic mean size of the two screens. Sieves 60, 40, 30, and 20 were used to obtain particles having average diameters of 335, 505, 715, and greater than 840 μm, respectively. The particles obtained were spherical in shape and had a predictable size distribution.

Microencapsulation Procedure (Scheme I)—All experiments were carried out under identical experimental conditions. In all experiments, gelatin and acacia solutions were prepared by dissolving, separately, equal quantities of gelatin and acacia in 20 ml of distilled water. These solutions were allowed to hydrate for at least 12 hr before being used. Coacervation was carried out at 40° using a water bath maintained at 40 ± 1°.

A known weight of the solid stearyl alcohol particles was dispersed in acacia solution with gentle stirring, and the dispersion was heated to 60°. At this temperature, stearyl alcohol particles melted and apparently became coated with acacia. The dispersion was then cooled quickly to congeal the particles, and the acacia-coated particles were encapsulated by the procedure reported previously (3).

Particle Concentration Studies—Particle concentration studies were conducted to evaluate the effect of particle concentration

[1] American Agricultural Co., Detroit, Mich.

Table I—Effect of Particle Concentration on Encapsulation and Wall Thickness[a]

Weight of Particles, g	Calculated Surface Area of Particles, cm^2	Number of Particles (n) × 10^{-4}	Stearyl Alcohol Encapsulated, %	Wall Thickness[b] (T), μm	$T\sqrt{n} \times 10^{-2}$
1.00	208	5.9	91.5	0.44 ± 0.02	107
3.00	624	17.7	86.6	0.26 ± 0.02	109
6.00	1248	35.4	81.6	0.17 ± 0.01	102
10.00	2080	59.0	65.6	0.15 ± 0.01	115

[a] Using 335-μm diameter particles in 40 ml mixture of 5% total colloid concentration. [b] Each value represents the average of at least three experiments; ± values indicate the maximum range of values obtained.

on the wall thickness of the encapsulated particles. Particles having an average diameter of 335 μm were encapsulated in a system consisting of 1.00 g of acacia and 1.00 g of gelatin dissolved separately in 20 ml of distilled water. A series of experiments was conducted using 1.00, 3.00, 6.00, and 10.00 g of particles in each experiment while keeping all other conditions constant.

The extent of particles encapsulated was determined by briefly washing the dried encapsulated particles with toluene. Stearyl alcohol particles not encapsulated or only partially encapsulated were dissolved by the toluene, leaving encapsulated particles relatively untouched. The percent of particles encapsulated was then calculated from the original weight of particles and the dried weight remaining after the brief toluene wash.

Particle-Size Studies—Particle-size studies were conducted to determine the effect of particle size on the wall thickness of the encapsulated particles. Particles having average diameters of 335, 505, 715, and greater than 840 μm were encapsulated as described. All experiments were conducted by encapsulating 3.00 g of the particles in a system consisting of 1.00 g of acacia and 1.00 g of gelatin dissolved separately in 20 ml of distilled water. Wall material was recovered from the encapsulated particles, and wall thickness was computed as described in the following sections.

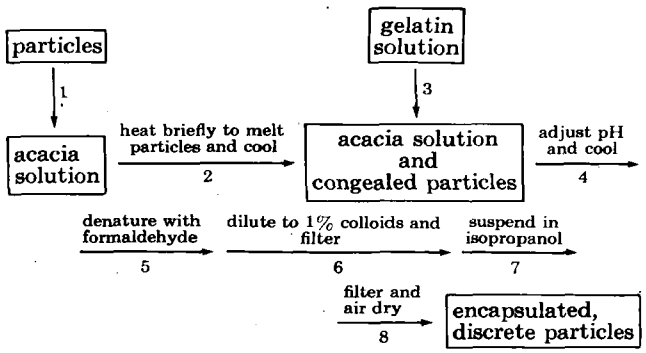
Encapsulation of Acacia-Coated Particles with Gelatin Solution—To obtain evidence concerning whether gelatin can interact directly with acacia at the particle surface to encapsulate the particles, acacia-coated particles were encapsulated by placing the pretreated particles in a 5% gelatin dispersion and proceeding as outlined previously, except that no further acacia solution was used. Encapsulation was followed microscopically, but wall thickness was not determined for these particles.

Wall Recovery Procedure—The wall material was recovered from the encapsulated particles by extraction of stearyl alcohol with toluene as follows. The dried, encapsulated material was treated briefly with toluene to dissolve any uncoated or partially coated particles. The washed and dried microcapsules were carefully separated mechanically from any adhering debris or excessive encapsulating material with the aid of a low power microscope and microspatula. A portion of these microcapsules was then carefully dried, and a known weight was refluxed with toluene for at least 30 min. Forty milliliters of toluene was used for each gram of microcapsules. This treatment completely extracted and dissolved the stearyl alcohol, leaving undissolved wall material. After separation by filtration and further washing with toluene, the wall material was dried and weighed. As a check on completeness of extraction, the weight of the stearyl alcohol extracted was determined after evaporation of the toluene washings and filtrate.

Determination of Wall Thickness—When assuming uniform, smooth, spherical particles, the average wall thickness (for thin-walled capsules) is given by Eq 1:

$$\text{wall thickness} = \frac{\text{volume of wall material recovered}}{\text{surface area of particles encapsulated}} \quad \text{(Eq. 1)}$$

If W = weight of microcapsules taken, W_w = weight of wall material collected, ρ_w = density of wall material, ρ = density of stearyl alcohol particles, and d = diameter of stearyl alcohol particles, then:



Scheme I—Modified encapsulation procedure

$$\text{volume of wall material recovered} = W_w/\rho_w \quad \text{(Eq. 2)}$$

The surface area, s, of a single spherical particle is:

$$s = \pi d^2 \quad \text{(Eq. 3)}$$

The number of particles per gram, N, is given by:

$$N = \frac{6}{\pi d^3 \rho} \quad \text{(Eq. 4)}$$

The total surface area, S, of N particles in a unit weight is, therefore:

$$S = (\pi d^2)\left(\frac{6}{\pi d^3 \rho}\right) \quad \text{(Eq. 5)}$$

or $S = 6/d\rho$ per unit weight of particles.

Since the weight of particles encapsulated is $W - W_w$:

$$\text{surface area of particles encapsulated} = (W - W_w)\left(\frac{6}{d\rho}\right) \quad \text{(Eq. 6)}$$

Therefore:

$$\text{wall thickness} = \left(\frac{W_w}{W - W_w}\right)\left(\frac{\rho}{\rho_w}\right)\left(\frac{d}{6}\right) \quad \text{(Eq. 7)}$$

The density of the wall material was calculated from the displacement volume of a known weight of wall material using toluene as the displacement fluid. The density of solid stearyl alcohol was determined in a similar manner using water as the displacement fluid. The calculated values are 1.341 g/cm^3 for the wall material and 0.861 g/cm^3 for the solid stearyl alcohol at 25°.

For thick-walled capsules, an encapsulated particle can be considered as two concentric spheres of volumes V_1 and V_2 having radii of r_1 and r_2, respectively. The thickness can then be determined from the volume relationships of the concentric spheres.

Optical and Electron Scanning Microscopic Studies of Capsule Wall—Photomicrographs, using an optical microscope[2], were made of small particles encapsulated by a reported procedure (5) for purposes of comparison with the large particles encapsulated by the procedure reported here. Photomicrographs of the wall of the large particles could not be made by the usual method because of the thinness of the walls. The walls of these particles were, therefore, shown by slow extraction of the wax with toluene, leaving the capsule wall suspended in the toluene. Photomicrographs were then taken, at various stages of the dissolution of the wax, while the capsules were still suspended in the toluene.

Scanning electron micrographs[3] were made of particles taken

[2] Bausch & Lomb photobinocular flat field microscope, Bausch & Lomb Optical Co., Rochester, N.Y.

[3] Cambridge Stereoscan, type 96113-2A, Cambridge Instrument Co., London, England.

Table II—Effect of Particle Size on Wall Thickness[a]

Particle Diameter, μm	Calculated Surface Area of Particles, cm^2	Number of Particles $(n) \times 10^{-4}$	Wall Thickness[b] (T), μm	$T\sqrt{n} \times 10^{-2}$
335	624	17.7	0.26 ± 0.02	109
505	414	5.17	0.49 ± 0.03	111
715	292	1.82	0.64 ± 0.02	86
840	249	1.12	1.31 ± 0.13	139

[a] Using 3 g of particles in 40 ml mixture of 5% total colloid concentration. [b] Each value represents the average of at least three experiments; ± values indicate the maximum range of values obtained.

from various stages of the process to show surface features of the particles. These particles were all shadowed with palladium-gold alloy before the micrographs were made.

RESULTS AND DISCUSSION

Effect of Particle Concentration—The effect of particle concentration on the extent of encapsulation and on wall thickness is shown in Table I. The percent of particles encapsulated decreased slightly as the weight of stearyl alcohol particles was increased, showing about a 10% decrease as the concentration of particles was changed from 20 to 75% (1–6 g in Table I) of the total solids present. However, a further increase in particles to 80% of the total solids resulted in an additional 16% drop in the ratio of particles encapsulated. Although the percent of particles decreased, the total quantity of particles encapsulated and encapsulated area increased.

In contrast, wall thickness of the encapsulated particles showed over a 60% drop (0.44–0.17 μm) when particle concentration was increased from 20 to 75% of total solids but showed an insignificant drop (to 0.15 μm) when the particle concentration was further increased to 80% of total solids. In all cases, only a small fraction of the total colloids available was incorporated into the walls of the microcapsules. The maximum colloid used amounted to about 2% of that available, and the internal or encapsulated phase exceeded 98% in all cases.

Effect of Particle Size—Table II shows the effect of particle size on wall thickness of the encapsulated particles. Results indicate that the wall thickness of the capsules increased as the diameter of the particles encapsulated was increased and the surface area of the particles decreased.

Although the results in both Tables I and II show an increase in wall thickness as the surface area of the particles decrease, correlation between the two experimental techniques on the basis of surface area does not appear to be useful. This is true, since wall thickness appears to show a different relationship to surface area in each reported experiment. However, the number of particles present appears to correlate more closely. It is not possible to draw any firm conclusions on the basis of these data, but the wall thickness times the square root of the number of particles present gives a reasonably constant number (about 110), indicating that wall thickness in these experiments is inversely related to the square root of the number of particles present when either the particle size or the weight of particles present is varied (Fig. 1).

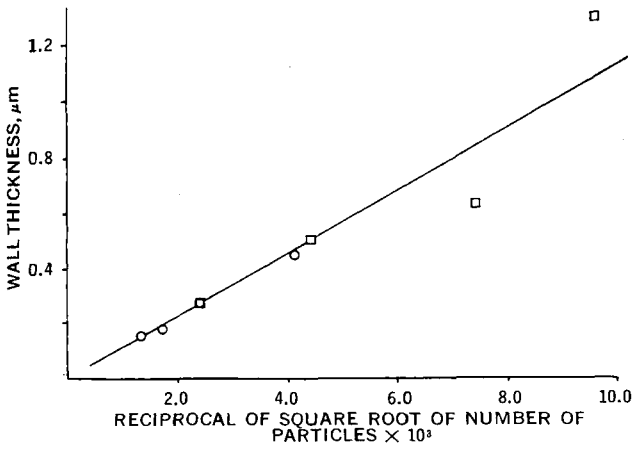


Figure 1—*Wall thickness of microcapsules as a function of reciprocal of square root of number of stearyl alcohol particles. Key: ○, particle size constant, weight of particles varied; and □, weight of particles constant, particle size varied.*

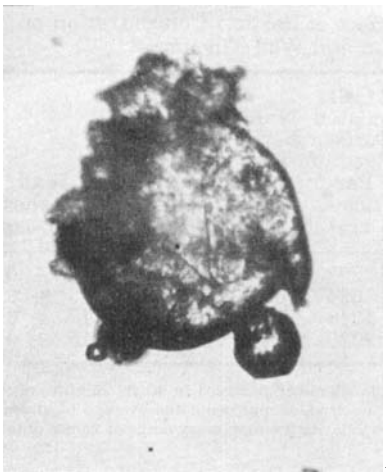


Figure 2—*Partially crushed encapsulated particle, encapsulated by the method reported by Luzzi and Gerraughty (5) (magnification 150×).*

The implication is that in this system, where only a small part of the total colloid present is used in the coating of the particle, the coating thickness is influenced by the total number of particles present. Further experiments are necessary to determine the validity of this relationship and to discern whether it applies to other systems.

Microscopic Studies—Figure 2 shows the nature of the coating on microcapsules encapsulated by the conventional method (3). In this photograph, the wall material is surrounding a 200-μm diameter particle. The wall was cracked open slightly at one end to expose a portion of the particle. The coating on these particles appears to be thick (relatively speaking), confirming the belief that encapsulation of smaller particles takes place by coalescence of one or more coacervate droplets around the particle.

In contrast, the larger particles encapsulated by the modified method reported here were observed to have much thinner coatings than those on the small 200-μm diameter particles (Fig. 2). In fact, the coating was very difficult to see by the microscopic techniques used for the smaller particles referred to previously. To obtain visual evidence of evenness and completeness of coating, it was necessary to go to an extraction technique where the stearyl alcohol was slowly extracted with toluene at room temperature, leaving the wall material undisturbed. Figures 3*a*–3*c* were made during the extraction of stearyl alcohol from an encapsulated particle suspended in toluene. The dissolution of the stearyl alcohol in toluene seemed to leave the capsule wall undisturbed except for some initial shrinkage. These photographs, plus observation with a stereomicroscope, indicated that the wall material was uniformly distributed on the particle and that the coating on these particles was much thinner than that found on the smaller particles of stearyl alcohol encapsulated by the unmodified method. Previous experiments in these laboratories showed that wall thickness of microcapsules of other substances (such as phenobarbital) tend to be much thicker for similar sized particles.

A number of particles were treated similarly and studied microscopically to get an indication of the uniformity of the coating. It was found that almost all the particles examined were covered with a smooth thin wall over the entire surface.

It was expected that encapsulation of relatively larger particles by the modified method would probably be due to a mechanism where a number of individual coacervate droplets adsorb to and coalesce about the particles. If true, then the wall material should show a somewhat uneven coating and the coating on the particles

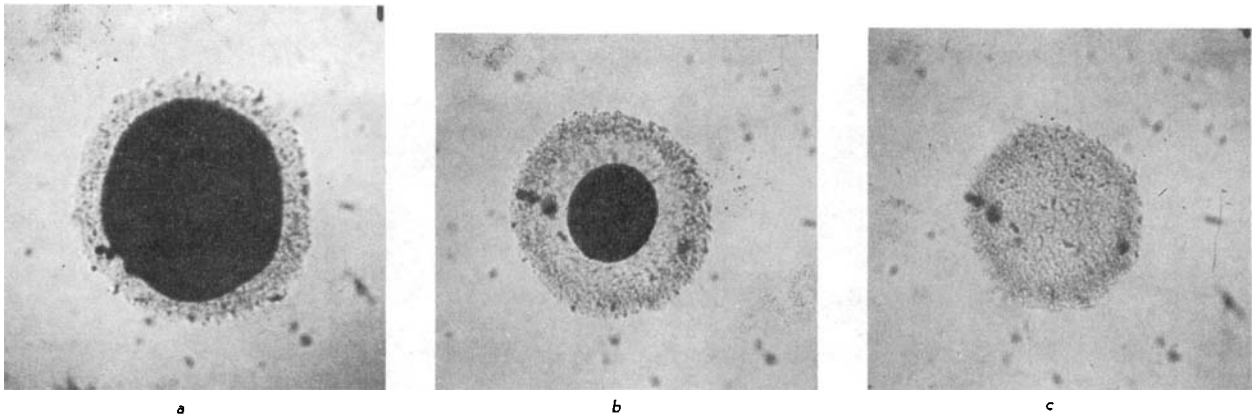


Figure 3—(a) *Encapsulated particle shortly after beginning extraction with toluene, using modified method of encapsulation and 335-μm particles.* (b) *Same particle after several minutes of extraction with toluene.* (c) *Extraction complete; only unsupported wall remains. Small round dark spots are air bubbles trapped within the wall.*

would be comparable in thickness to the one on particles shown in Fig. 2. The observation that the coating was very thin and uniform and had no adhering modules, together with a high rate of encapsulation, supports the supposition that there is a third mechanism responsible for encapsulation of the larger particles. Experiments where only gelatin was included in the encapsulation mixture using acacia-coated particles also gave a coating sufficiently stable to remain intact while the stearyl alcohol was extracted with toluene. It is probable that by first coating the particles with acacia as described, the hydrophobic portions of the acacia molecule are oriented to the relatively hydrophobic stearyl alcohol molecules, leaving the hydrophilic portion of the acacia open for reaction with gelatin. If this is true, then the initial coating on the particles is essentially a reaction of colloidal gelatin with the acacia film around the particle. Subsequently, adsorption of coacervate droplets may occur, but microscopic observation of a large number of finished particles did not indicate that it did. A few adhering droplets were observed at some stages of the process but they may have been loosely attached and were washed off during the recovery process.

To elucidate further what was happening at the surface of the particle, electron micrographs of the particles were taken at several points during the encapsulation process. These results are shown in Figs. 4–7. Figure 4 shows the surface of an uncoated stearyl alcohol particle. The surface shows some fissuring, probably because of the crystallization of the stearyl alcohol upon cooling from the molten state.

Figure 5 shows a similar particle which has been melted and quickly recongealed in water. The surface is somewhat rougher than with a particle congealed in air. Particles so treated show no improvement in tendency to be encapsulated over air-congealed particles.

A particle that had been melted and recongealed in 5% acacia solution is shown in Fig. 6. This particle shows areas that are smoother than the uncoated particles interspersed by surface crack lines. The particle appears to be coated with a thin film of acacia and thus gives visual evidence for earlier suppositions. Such acacia-coated particles do not appear to be retarded in their dissolution in toluene, and no intact film can be seen when the dissolution is observed with an optical microscope. Since acacia films are rather brittle, it is probable that the coating breaks up as soon as a small amount of the supporting wax is dissolved from beneath the coating film.

The surface of a particle coated by the modified coacervation procedure is shown in Fig. 7. In this photograph, the coating has a flaky or scale-like surface. This appearance was not expected since observation with the optical microscope seemed to indicate a smooth surface. The depth of focus of the optical microscope may have been insufficient at high power to reveal significant details of the surface, or the coating may have been raised to some extent by the vacuum during the metal shadowing treatment. If it is assumed that the scale-like surface is not an artifact caused by the metallizing process, it is possible that it is caused either by a brief toluene wash of the particles used to remove any remaining uncoated stearyl alcohol or by the alcohol treatment after encapsulation.

The calculated wall thickness (Tables I and II) is liable to certain experimental variations, including weight fluctuations encountered in weighing gelatinous material due to absorption of moisture, variations in particle diameter during coating with

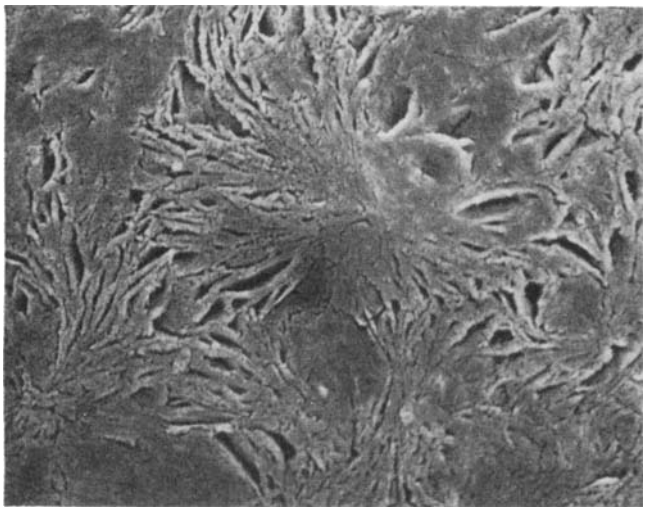


Figure 4—*Electron scanning micrograph of the surface of an uncoated stearyl alcohol particle (magnification about 475×).*

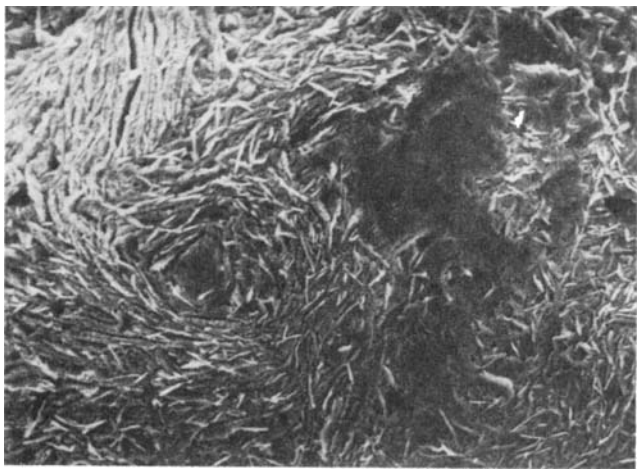


Figure 5—*Electron scanning micrograph of the surface of a stearyl alcohol particle melted and recongealed in water (magnification about 480×).*

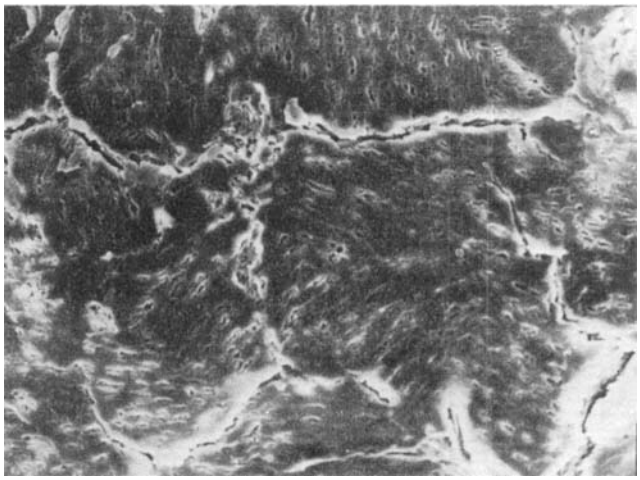


Figure 6—*Electron scanning micrograph of the surface of a stearyl alcohol particle melted and recongealed in acacia solution (magnification about 550×).*

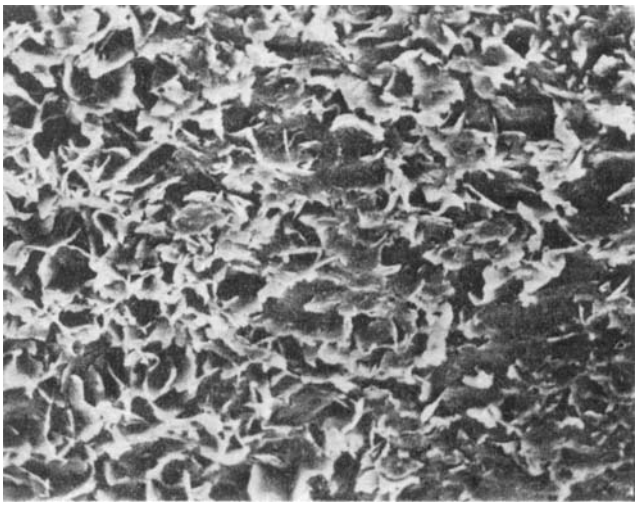


Figure 7—*Electron scanning micrograph of the surface of a stearyl alcohol particle encapsulated by the modified method (magnification about 500×).*

acacia solution in preparing particles for encapsulation by the modified method, use of average particle diameter in a distribution of sizes, and small errors in the determination of density of the wall material. Nevertheless, it seems logical to believe that the thin capsule walls formed on particles encapsulated by the modified method reported here strongly indicate that the initial wall was formed by molecular interaction between gelatin and acacia previously attached to the stearyl alcohol during the brief melting in acacia solution rather than by coalescence of preformed coacervate droplets around the particle.

CONCLUSIONS

From the results of this study, the following conclusions can be drawn.

1. Only a small fraction of the total amount of colloid was used in the formation of the capsule wall.

2. The extent of encapsulation (percent particles encapsulated) was affected only slightly up to the point where the particles constituted 75% of the total solids but decreased somewhat at higher concentrations.

3. The wall thickness decreased with increasing ratios of solid particles.

4. The wall thickness increased as particle diameters increased and total surface areas of the particles decreased.

5. An inverse relationship between the square root of the number of particles present and the wall thickness was noted.

6. Optical and electron scanning microscopic observations of the encapsulated particles indicated that treatment by melting in acacia solution left a thin fragile acacia coating on the particle and that the final coated particle had a scale-like appearance. The final coating was sufficiently strong to remain intact during dissolution of the wax in toluene.

7. The evidence presented strongly supported earlier conclusions (3) that the capsule wall can be formed as a result of a molecular interaction between colloidal gelatin in the coacervate mixture and acacia held at the surface of the particles.

REFERENCES

(1) J. R. Nixon, S. A. H. Khalil, and J. E. Carless, *J. Pharm. Pharmacol.*, **20**, 538(1968).

(2) S. N. Luu, P. F. Carlier, P. Delrot, J. Gazzola, and D. LaFont, *J. Pharm. Sci.*, **62**, 542(1973).

(3) P. L. Madan, L. A. Luzzi, and J. C. Price, *ibid.*, **61**, 1586(1972).

(4) B. J. Mason, O. W. Jayarante, and J. D. Wood, *Rev. Sci. Instrum.*, **40**, 247(1963).

(5) L. A. Luzzi and R. J. Gerraughty, *J. Pharm. Sci.*, **53**, 429(1964).

ACKNOWLEDGMENTS AND ADDRESSES

Received February 6, 1973, from the *School of Pharmacy, University of Georgia, Athens, GA 30601*

Accepted for publication September 14, 1973.

[x] To whom inquiries should be directed.