Characteristics of Medicated and Unmedicated Microglobules Recovered from Complex Coacervates of Gelatin–Acacia

DAVID W. NEWTON *, JEAN N. McMULLEN ‡, and CHARLES H. BECKER ˣ

Abstract □ Medicated and unmedicated microglobules prepared from complex coacervates of Type A gelatin and acacia were recovered as water-insoluble powders consisting of discrete units, which were spontaneously revertible to highly disperse systems when suspended in water or physiological electrolyte solutions. Spherical microglobules containing up to 15% (w/w) sulfamerazine had a nominal diameter of 30 μm in aqueous suspension. Larger but irregularly shaped products containing up to 45% (w/w) sulfamerazine were also recovered. The relationships of the weight of sulfamerazine added per coacervate batch to weight yield and percent (w/w) included sulfamerazine of the microglobules were both linear.

Keyphrases □ Microglobules—medicated and unmedicated, prepared from gelatin–acacia coacervates, physical parameters evaluated for use as dosage forms □ Coacervates—gelatin–acacia, medicated and unmedicated microglobules prepared, physical parameters evaluated for use as dosage forms □ Gelatin–acacia—coacervates, medicated and unmedicated microglobules prepared, physical parameters evaluated for use as dosage forms □ Dosage forms, potential—medicated and unmedicated microglobules prepared from gelatin–acacia coacervates, physical parameters evaluated

Incipient, fundamental studies of complex coacervation, specifically of the gelatin–acacia system, were reported previously (1, 2).

Several patents were cited in reviews on microencapsulation as evidence of the first commercially successful uses of the gelatin–acacia system (3–5). Various studies involving gelatin–acacia coacervates were predominantly directed toward drug microencapsulation (6–9). Pharmaceutical uses of microencapsulated products prepared by coacervation and other processes also were reported (3–5, 10–14).

This study was undertaken to evaluate certain parameters of the complex coacervate system of Type A gelatin and acacia (gum arabic) for producing medicated microglobules suitable in various pharmaceutical dosage forms. The products were to be recovered as powders consisting of water-insoluble, discrete, nonvacuolated spherical units that would spontaneously revert to highly dispersed suspensions upon reconstitution with aqueous vehicles.

EXPERIMENTAL

Materials—Type A gelatin[1] and acacia[2] were used as 3% (w/v) solutions containing 1% (v/v) benzyl alcohol[3] for preservation. Glycerin (99.6%)[3], formaldehyde solution (37%)[3], 2-propanol (99%)[3], pancreatin[4], pepsin[4], collagenase B[5], micronized sulfamerazine[6] (majority of particles 5 μm or less), and finely powdered chloramphenicol[7] were used as received. Other solvents and reagents were analytical reagent grade or purity.

Preparation and Recovery of Microglobules—The coacervation pH (3.9–4.0), dry gelatin–acacia ratio (0.8–1.0:1.0), and sol concentration (3% w/v) were initially determined as the criteria necessary to optimize the yield of microglobules.

Except where the effects of glycerin or formaldehyde were studied in particular, microglobules were prepared by mixing at 40° 40 ml each of 3% (w/v) gelatin sol (adjusted to pH 10) and 3% (w/v) acacia sol, stirring the mixture at 250–400 rpm, and either dispersing 1.5 g of the drug for 3 min in the batch at pH 5.5 and then adjusting the pH to 3.9–4.0 or adjusting to pH 3.9–4.0 and then dispersing the drug. Over 30 min, 40 ml of glycerin was added dropwise and the batch was cooled under ambient conditions to 24–26° and then rapidly chilled to 2–5° in an ice water bath.

Microscopic examination was made to assess encapsulation. After 15 min at 2–5°, the pH was raised to 5–6, 15 ml of formaldehyde was added, and stirring was continued for 30–45 min at 2–5°. The batch was then covered and allowed to stand at room temperature (22–25°) for approximately 20 hr. The clear supernate was then discarded, the white sediment layer (50–70 ml) was brought with stirring to 75% (v/v) of 2-propanol, and the batch was allowed to stand for 10 min. Then the supernate was decanted, the batch was brought to 90% (w/v) of 2-propanol, and the flocculated system was filtered using qualitative paper on a buchner funnel. The product was washed with 99% 2-propanol and dried at 36° for 12–16 hr, and the dried yield was vibrated gently on a No. 40 screen to obtain a powder.

All batches were prepared in 250-ml glass beakers and stirred by a mixer[8] equipped with a three-blade marine-type impeller 4 cm in diameter. Gelatin and acacia sols were prepared in distilled water and refrigerated at 2–5° for not more than 30 days prior to use. The gelatin sols were warmed to, and maintained at, 45° for not more than 3 hr and were discarded after two such uses on consecutive days. Acacia sols were initially heated to 85–90° for 1 hr to destroy the activity of any potentially interfering enzymes (15, 16).

Influence of Additives and Conditions—Batches of unmedicated microglobules were prepared using 0.5, 1.0, 2.0, 2.5, and 3.0% (w/v) sols of each gelatin and acacia. Gross yields were compared by measuring the apparent volumes of liquid coacervate layers at 40° and the dry weight yields of microglobules recovered as specified earlier.

Glycerin volumes of 5, 10, 20, 30, 40, 50, 60, 70, 80, and 100 ml were added dropwise to separate unmedicated batches. Likewise, a 40-ml portion of 1,2,6-hexanetriol[6], polyethylene glycol 200[9], polyethylene glycol 400[10], 70% (w/w) sorbitol[3], and syrup USP was added to separate batches.

Batches of unmedicated microglobules were treated with 5, 10, and 15 ml of formaldehyde. Batches to which 15 ml of formaldehyde had been added also were recovered at preselected times over 0.5–4 hr to determine the minimum treatment period required for the retrieval of readily dispersible products.

For unmedicated microglobules, batches were decanted to 100 ml (about 60 ml of sediment and 40 ml of clear supernate). From these batches, a 10-ml sample was mixed individually with 5, 8, 10, 15, 20, 30, 40, 60, and 90 ml each of acetone, methanol, ethanol, 1-propanol, and 2-propanol in 100-ml cylinders. The systems were kept at room temperature for 5 min after each addition of acetone or an alcohol. The percent (v/v) of the solvents at which flocculation occurred and the apparent degree of sedimentation were recorded.

Finally, the influence of stirring rate in the 250–400-rpm range and in excess of 450 rpm on the morphology of the microglobules was compared using standard and baffled 250-ml beakers.

Extraction and Digestion of Microglobules—About 100 mg of microglobules containing approximately 15% (w/w) sulfamerazine was placed in 20-ml Type I glass vials with 10-ml portions of the following media: collagenase B solution, 20 units/ml, at pH 7.0 in 0.05 *M* tris(hy-

[1] Isoelectric point 8.6, 275 bloom, Fisher Scientific Co., Fair Lawn, N.J.
[2] USP white powder, Fisher Scientific Co., Fair Lawn, N.J.
[3] Fisher Scientific Co., Fair Lawn, N.J.
[4] Wilson Labs., Chicago, Ill.
[5] Calbiochem, San Diego, Calif.
[6] Matheson, Coleman & Bell, Norwood, Ohio.
[7] Courtesy of Parke-Davis & Co.
[8] Lightin model F, Mixing Equipment Co., Rochester, N.Y.
[9] Dow Chemical Co., Midland, Mich.
[10] Union Carbide Corp., New York, N.Y.

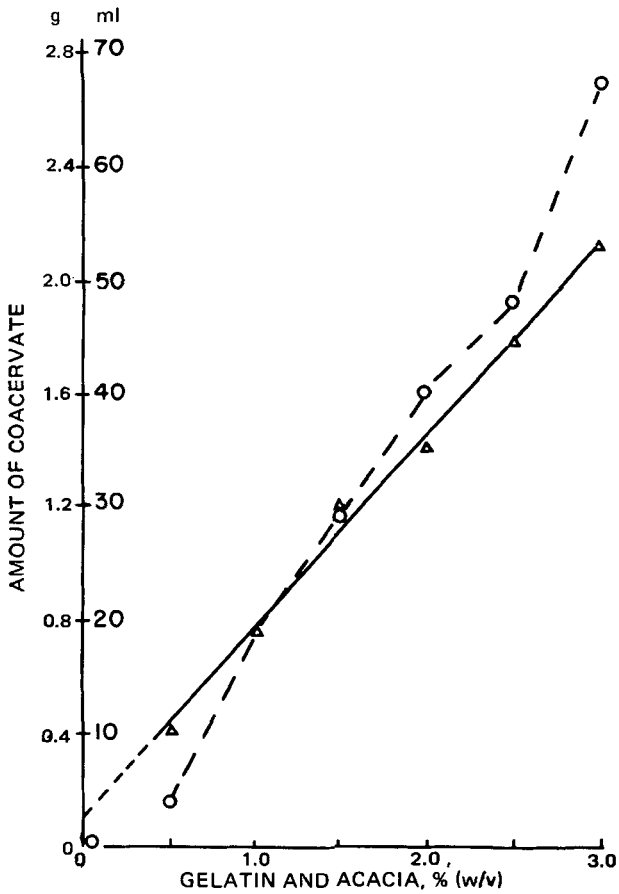

Figure 1—*Apparent volumes (○, ml) and dry weight (△, g) yields of coacervates produced at pH 3.9 from isovolumetric (40 ml) gelatin and acacia sols of selected percent (w/v) concentration.*

droxymethyl)aminomethane[11] and 0.005 *M* calcium chloride (17); 50% (v/v) ethanol; gastric fluid[12]; intestinal fluid[12]; and replacement electrolyte solution[13]. The vials were sealed with pretreated rubber closures and aluminum crimps and rotated at 25 ± 3 rpm at 37 ± 0.1° in an apparatus similar to that used by Souder and Ellenbogen (18).

The suspensions were observed visually and microscopically at 0.5, 1.0, 2.0, 3.0, 4.0, 8.0, and 20.0 hr to estimate the extent of digestion of the microglobules and/or extraction of sulfamerazine therefrom. Similarly, samples containing 12.7% (w/w) sulfamerazine were extracted for up to 12 hr in 10.0 ml of replacement electrolyte solution.

Assay of Sulfamerazine Content—The microglobules were washed in 2-propanol–acetone (1:2) by dispersing them in 150 ml of mixture per gram of sulfamerazine originally added and stirring for 5 min.

The spectrophotometric assay of sulfamerazine content was adapted from Clarke (19). An accurately weighed 100-mg sample of microglobules was digested with 15 ml of intestinal fluid for 3 hr at 37°, the mixture was adjusted to pH 3–4, and 20 ml of acetone was added. The sample was extracted with four 15-ml portions of ether, and the fractions were pooled and evaporated to dryness at room temperature.

The residue was quantitatively transferred to a 50-ml volumetric flask, first with 5 ml of distilled water and then with portions of absolute ethanol sufficient to achieve the final volume. The absorbance of the sample, appropriately diluted in 90% (v/v) ethanol, was determined at 270 nm[14], and the value was compared to a standard (Beer's) plot to calculate the percent (w/w) sulfamerazine within an aliquot of microglobules from the batch.

Sulfamerazine was isolated from the aqueous microglobule digest to preclude the error of additive UV absorbance by water-soluble residues of gelatin and pancreatin, in particular. Furthermore, the complete evaporation of acetone, which partitions into the ether phase, is necessitated because of its interfering absorbance at about 265 nm (20).

[11] THAM, Fisher Scientific Co., Fair Lawn, N.J.

[12] Simulated, TS, "The United States Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1975, p. 765.

[13] Normosol-R pH 7.4, Abbott Laboratories, North Chicago, Ill.

[14] Beckman DB-GT, Beckman Instruments, Fullerton, Calif.

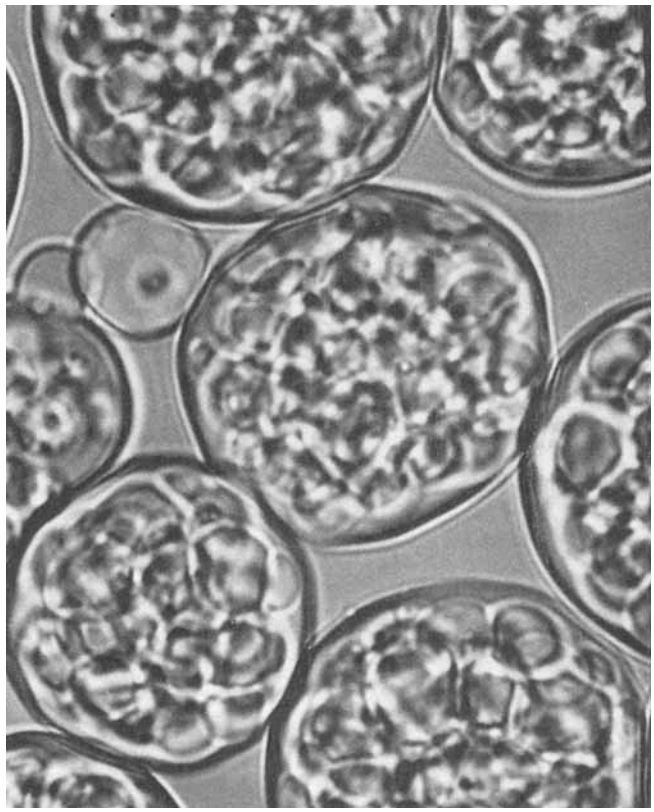

Figure 2—*Vacuolated, unmedicated gelatin–acacia microglobules (1000×).*

RESULTS AND DISCUSSION

Influence of Additives and Conditions—The advantage of measuring microglobule yields by dry weight after recovery rather than apparent coacervate volumes is evident from the plots in Fig. 1. The reliability of coacervate volume measurements is limited because the water content of gelatin–acacia coacervates increases as more concentrated sols are used (1).

The addition of glycerin and other polyols increased the viscosity and density of the coacervate systems, which appeared to inhibit deformation of the spherical droplets caused by the rotational velocity and turbulence of stirring. The spherical shape of the coacervate droplets also may be favored by an intensification of the complex ionic relations between gelatin and acacia in a system containing, for instance, 28.7% (v/v) glycerin and possessing, therefore, a dielectric constant nearer to 70 than to 80 as for water (21).

Upon cooling the coacervates to less than 28°, a glycerin concentration of 20–40% (v/v) in the system prevented vacuolation of the microglobules. Since glycerin lowers the gelation temperature and affects gelatin hydration (22), these effects may prevent vacuolation, which otherwise occurs when gelatin–acacia complex coacervate droplets are cooled to below about 28° (1). The polyols 1,2,6-hexanetriol, polyethylene glycol 200 and 400, 70% (w/w) sorbitol, and syrup USP did not prevent vacuolation of the microglobules in a concentration of 28.7% (v/v), a typical value at which glycerin was effective for this purpose.

Figure 2 shows the vacuolation caused by gelation of unmedicated microglobules from a coacervate system prepared in the absence of glycerin. In contrast, Fig. 3 shows nonvacuolated microglobules when the system contained 28.7% (v/v) glycerin.

To recover spherical microglobules, readily revertible to highly dispersed suspensions, from the coacervates, a formaldehyde concentration of 4.0% and a treatment time of at least 4 hr were required.

Flocculation and sedimentation efficiency of formaldehyde-treated microglobules as a function of concentration, percent (v/v), of the aliphatic solvents revealed the order: acetone (48.5%) > 2-propanol (52.6%) = 1-propanol (52.6%) > ethanol (71.4%) > methanol (85.1%). However, only 2-propanol and 1-propanol were capable of flocculating the micro-

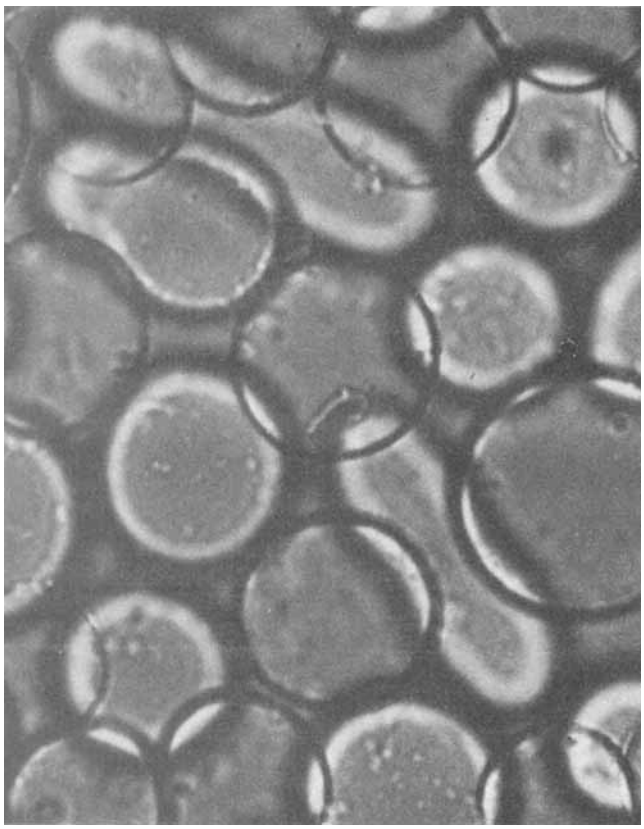

Figure 3—*Nonvacuolated, unmedicated gelatin–acacia microglobules (particulate matter unintended; 1000×).*

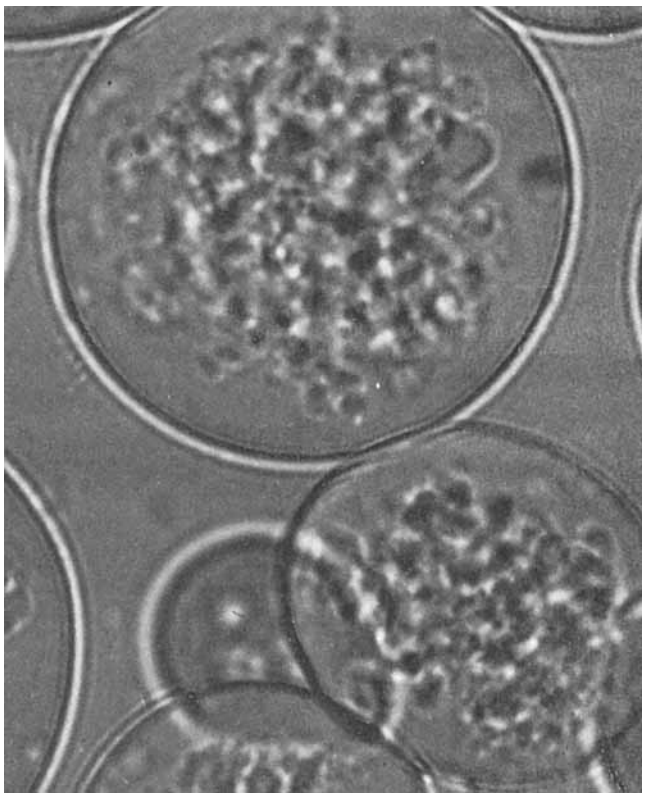

Figure 4—*Gelatin–acacia microglobules containing 12–15% (w/w) micronized sulfamerazine (1000×).*

globules to produce filter cakes, which, after drying, would spontaneously form highly dispersed suspensions in water. These results are in agreement with those of previous work (23).

When the 2-propanol concentration reached 40–45% (v/v), the microglobule suspensions changed from translucent to opaque in appearance, probably due to the precipitation of acacia within the microglobules. A 2-propanol concentration of 75% (v/v) or greater produced complete sedimentation within 10 min, and 90% (v/v) provided the greatest aqueous dispersibility of the recovered powders.

There was no difference in the morphology of the microglobules recovered for a given substance when powdered aluminum, barium sulfate, chloramphenicol, hydrocortisone acetate, phenobarbital, phenolphthalein, scarlet red, sulfamerazine, and sulfur were added to the coacervates either before or after the pH end-point. These observations and others, as well as previous evidence (1, 24), support the theory that drugs and other materials are included within gelatin–acacia coacervates exclusively by surface-active phenomena of wetting and spreading.

The nominal equilibrium size of unmedicated, spherical microglobules was 30 μm in aqueous suspension. The shape and diameter were also characteristic of microglobules recovered from batches to which not more than 1.0 g of micronized sulfamerazine had been added (Fig. 4). However, when a nonmicronized powder whose particles were larger than about 20 μm or more than 1 g of micronized sulfamerazine was added to the batches, the spherical shape was lost; irregular, larger microglobules resulted (Fig. 5).

Stirring is required to produce distinct microglobules from complex coacervates of gelatin–acacia. Stirring rates of 250–400 rpm resulted in the formation of spherical microglobules. However, rates at or exceeding about 450 rpm usually resulted in ellipsoidal or more irregularly distorted microglobules, as well as in the gross entrainment of air and vacuolation of the coavervate products. The use of a baffled beaker accounted for undesirable products at rates greater than 300 rpm, perhaps because of greatly increased turbulence and air entrainment.

Previous reports (9) recognized the spontaneous separation of complex coacervates of gelatin and acacia into two immiscible layers, but also discussed droplet size of the coacervates as a function of temperature. When batches at 40° or higher were rapidly sampled from a stirring system and observed microscopically, the coacervate droplets coalesced and spread over the glass slide almost instantly because of the affinity of gelatin–acacia coacervates for glass (1). Therefore, it may be unrealistic to discuss droplet size in these systems above about 35°, the approximate gelation temperature of most gelatins (25–29).

Extraction and Digestion of Microglobules—The results of extraction tests of spherical microglobules containing approximately 15% (w/w) sulfamerazine with five media are summarized in Table I. The most significant influence was demonstrated by intestinal fluid, which completely digested the formaldehyde-treated microglobules in only 30 min. Previously (23), extraction of sulfamerazine from formaldehyde-treated gelatin microcapsules was 100% complete after 2 hr with intestinal fluid but only 70% complete after 8 hr with gastric fluid.

Other studies (30, 31) found greater release of pentobarbital from formaldehyde-treated gelatin–acacia microcapsules with gastric than with intestinal fluid; the percent (w/w) pentobarbital in the products was not reported. These latter findings may have resulted because the spectrophotometric analyses of pentobarbital were conducted on filtered extracts of the aqueous media at pH 9.25. However, experimental validation from the present study showed significant absorbance below 300 nm by such digests of nonmedicated gelatin–acacia microglobules.

Although the medicated microglobules prepared in this study required 8 hr for digestion in gastric fluid, their included sulfamerazine dissolved completely after only 5 min of extraction. In the intestinal fluid, the

Table I—Effect of Extraction in Specified Media at 37° on Morphology and Contents of Microglobules Containing 15% (w/w) Sulfamerazine

Medium	Minimum Time for Complete Digestion, hr[a]	Microscopic Appearance of Suspension[b]
Collagenase B, 20 units/ml	8.0	d
Ethanol, 50% (v/v)	NS	*i*
Gastric fluid	8.0	s
Intestinal fluid	0.5	d
Replacement electrolyte solution, pH 7.4	NS	i

[a] NS = not sufficient time for digestion of microglobules through 20 hr of extraction. [b] At 100×: i = intact spheres containing sulfamerazine, d = microglobules completely digested but sulfamerazine crystals present, and s = completely digested microglobules and their sulfamerazine contents in solution.

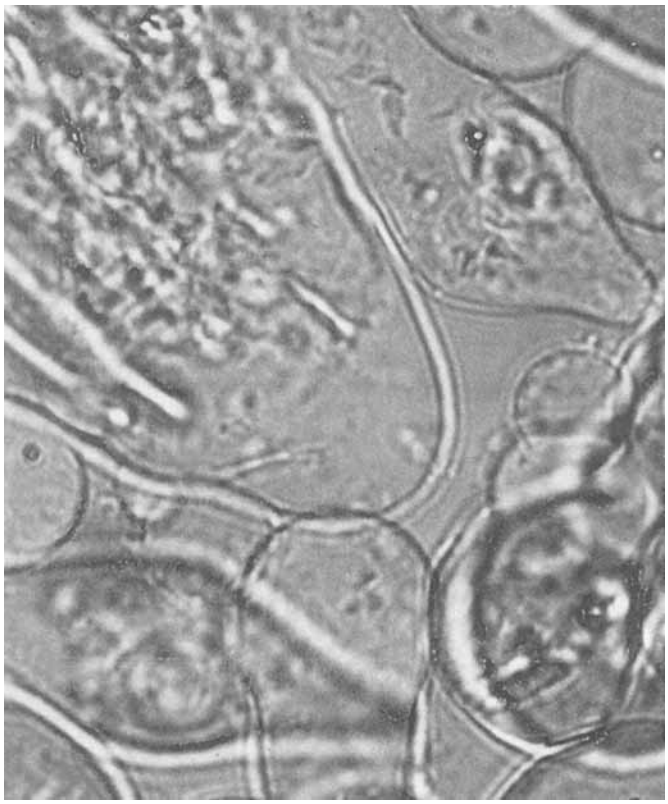

Figure 5—*Nonvacuolated gelatin–acacia microglobules containing an undetermined fraction of chloramphenicol (1000×).*

microglobules were completely digested within 30 min, but with incomplete dissolution of the included sulfamerazine. Other extractions of the microglobules showed less than 5% loss of included sulfamerazine when suspended and stirred for 5–10 min in pure acetone. Furthermore, extensive or complete loss of sulfamerazine from the intact microglobules in similar tests was observed in binary systems composed of 20–80% water and acetone, suggesting that the microglobules lack a system of "fixed pores or channels," thus requiring hydration before access to included sulfamerazine by a solvent such as acetone can be afforded *via* imbibition and a "wicking type of action."

The pKa value for the loss of a proton by the sulfonamido moiety of sulfamerazine to form a sodium salt is 7.06 (32). Therefore, at pH 7.4, such as in intestinal fluid[12], the drug would be 69% ionized. However, the pKa of the anilino group on sulfamerazine may be approximated at about 3.0 (33). Thus, at pH 1.2, such as in gastric fluid[12], sulfamerazine would be 98% protonated, thereby favoring greater drug solubility.

The extraction of spherical microglobules containing 12.7% (w/w) sulfamerazine with replacement electrolyte solution, pH 7.4, at 37° showed little variation in the fraction of sulfamerazine released. Only 26.8 ± 2.2% was extracted over periods of up to 12 hr. The results are in agreement with a previously published report (34).

REFERENCES

(1) "Colloid Science," vol. II, H. R. Kruyt, Ed., Elsevier, New York, N.Y., 1949, pp. 338–384, 433–450.

(2) "Industrial Gums," R. L. Whistler, Ed., Academic, New York, N.Y., 1959, pp. 256–263.

(3) L. A. Luzzi, *J. Pharm. Sci.*, **59,** 1367 (1970).

(4) "The Theory and Practice of Industrial Pharmacy," 2nd ed., L. Lachman, H. A. Lieberman, and J. L. Kanig, Eds., Lea & Febiger, Philadelphia, Pa., 1976, pp. 420–438.

(5) "Encyclopedia of Chemical Technology," vol. 13, A. Standen, Ed., Wiley, New York, N.Y., 1967, pp. 436–456.

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

(7) S. Javidan, R. U. Haque, and R. G. Mrtek, *ibid.*, **60,** 1825 (1971).

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

(9) A. B. Dhruv, T. C. Needham, Jr., and L. A. Luzzi, *Can. J. Pharm. Sci.*, **10,** 33 (1975).

(10) J. Nack, *J. Soc. Cosmet. Chem.*, **21,** 85 (1970).

(11) J. A. Bakan and J. L. Anderson, *Drug Cosmet. Ind.*, **110,** 34 (1972).

(12) *Manuf. Chem.*, **33,** 16 (1962).

(13) P. A. Kramer, *J. Pharm. Sci.*, **63,** 1646 (1974).

(14) J. Whitmore, Ph.D. thesis, University of Florida, Gainesville, Fla., 1966.

(15) "Industrial Gums," R. L. Whistler, Ed., Academic, New York, N.Y., 1959, p. 266.

(16) C. L. Mantell, "The Water-Soluble Gums," Reinhold, New York, N.Y., 1947, pp. 35–39.

(17) "Methods in Enzymology," vol. V, S. P. Colowick and N. O. Kaplan, Eds., Academic, New York, N.Y., 1962, pp. 659–665.

(18) J. C. Souder and W. C. Ellenbogen, *Drug Stand.*, **26,** 77 (1958).

(19) "Isolation and Identification of Drugs," E. G. C. Clarke, Ed., Pharmaceutical Press, London, England, 1969, pp. 550, 551.

(20) K. A. Connors, "A Textbook of Pharmaceutical Analysis," Wiley, New York, N.Y., 1967, p. 179.

(21) D. L. Sorby, R. G. Bitter, and J. G. Webb, *J. Pharm. Sci.*, **52,** 1149 (1963).

(22) K. H. Gustavson, "The Chemistry and Reactivity of Collagen," Academic, New York, N.Y., 1956, pp. 204–207.

(23) J. R. Nixon, S. A. H. Khalil, and J. E. Carless, *J. Pharm. Pharmacol.*, **20,** 528 (1968).

(24) G. N. Paradisis and E. L. Parrott, *J. Clin. Pharmacol.*, **8,** 54 (1968).

(25) "Encyclopedia of Chemical Technology," vol. 10, A. Standen, Ed., Wiley, New York, N.Y., 1966, p. 501.

(26) A. Veis, "The Macromolecular Chemistry of Gelatin," Academic, New York, N.Y., 1964, pp. 255, 267.

(27) K. H. Gustavon, "The Chemistry and Reactivity of Collagen," Academic, New York, N.Y. 1956, pp. 288, 289.

(28) H. Boedtker and P. Doty, *J. Phys. Chem.*, **58,** 968 (1954).

(29) "Treatise on Collagen," vol. I, G. N. Ramachandran, Ed., Academic, New York, N.Y., 1967, p. 261.

(30) L. A. Luzzi and R. J. Gerraughty, *J. Pharm. Sci.*, **56,** 634 (1967).

(31) *Ibid.*, **56,** 1174 (1967).

(32) A. N. Martin, J. Swarbrick, and A. Cammarata, "Physical Pharmacy," 2nd ed., Lea & Febiger, Philadelphia, Pa., 1969, p. 194.

(33) A. Albert and E. P. Serjeant, "Ionization Constants of Acids and Bases," Wiley, New York, N.Y., 1962, p. 148.

(34) J. R. Nixon and S. E. Walker, *J. Pharm. Pharmacol.*, **23,** 147S (1971).

ACKNOWLEDGMENTS AND ADDRESSES

Received June 3, 1976, from the *College of Pharmacy, University of Florida, Gainesville, FL 32610.*

Accepted for publication December 15, 1976.

Abstracted in part from a dissertation presented by D. W. Newton to the University of Florida in partial fulfillment of the Doctor of Philosophy degree requirements.

The authors thank Mrs. Ruby Wilson for the preparation of the manuscript, Mr. George Perry for the construction of Fig. 1, and Mr. Louis Mallory for the photomicrographs in Figs. 2–5.

* Present address: Department of Pharmaceutics, Massachusetts College of Pharmacy, Boston, MA 02115.

‡ Present address: Glaxo Laboratories, Toronto, Ontario, M8Z 4W1, Canada.

ˣ To whom inquiries should be directed.