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Pectin–Gelatin Complex Coacervates II: Effect of Microencapsulated Sulfamerazine on Size, Morphology, Recovery, and Extraction of Water-Dispersible Microglobules

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

Received May 11, 1982, from the **Faculty of Pharmacy, University of Montreal, Montreal, Quebec H3C 3J7, Canada*, the [‡]*Department of Pharmaceutics, College of Pharmacy, University of Nebraska Medical Center, Omaha, NE 68105*, and the [§]*College of Pharmacy, University of Florida, Gainesville, FL 32610.* Accepted for publication March 23, 1984.

Abstract □ Spherical medicated microglobules were prepared by complex coacervation of Type A gelatin with pectin, having nominal diameters of 5, 10, and 25 μm and containing 37.3, 44.9, and 45.2% (w/w) sulfamerazine, respectively. They were recovered as water-insoluble powders and were spontaneously revertible to highly disperse systems when reconstituted in water or physiological electrolyte solution. The conditions affecting microglobule formation were studied. For complete formation, the crystals must be dispersed at ≥pH 5. The effect of the sulfamerazine mass added on microglobule morphology, yield, and contents were investigated. As much as 37.3, 44.5, and 69.1% (w/w) sulfamerazine in 5-, 10- and 25-μm microglobules could be formed without loss of spherical shape. The microglobule yield *versus* drug-to-colloid ratio curves were nonlinear below the critical drug-to-colloid ratio for loss of sphericity. Addition of sulfamerazine suppressed coacervation by 10–15% but it had no significant effect on microglobule size. The extraction of medicated microglobules in various media demonstrated the existence of a porous matrix that required hydration to facilitate extraction of the microglobular drug. Fifteen percent of the encapsulated sulfamerazine was extracted from 25-μm microglobules as opposed to 9% from 10-μm microglobules after equilibration for 24 h in replacement electrolyte solution.

Keyphrases □ Microencapsulation—pectin–gelatin coacervates, sulfamerazine □ Coacervates—pectin–gelatin, sulfamerazine, microglobules, recovery, size

Little consideration of the morphological character of complex coacervate-coated particles appears in the pharmaceutical literature until Newton *et al.* (1) produced spherical microglobules of uniform size with centrally located drug. The process of medicated microglobule formation is to be differentiated from microencapsulation; the latter usually yields nonspherical particles. Complex coacervates of gelatin–acacia or gelatin–pectin readily wet and surround suspended particles and emulsified droplets. On cooling and denaturation with formaldehyde, the liquid coacervate droplets congeal, forming microglobules that are recoverable as water-dispersible powders after flocculation in 2-propanol (1, 2).

Certain physicochemical conditions for preparing and recovering nonmedicated microglobules from type A gelatin–pectin complex coacervates were detailed previously (2). The purpose of this investigation was to characterize conditions for sulfamerazine microglobule formation in complex coacervates of pectin and gelatin, yielding microglobules of uniform size with centrally located drug that were recoverable as a dry powder. Medicated microglobules with diameters of <10 μm, spontaneously revertible to a polydisperse system in aqueous fluids, were sought for potential use in parenteral dosage forms.

EXPERIMENTAL SECTION

Materials—Type A gelatin[1] and pectin NF[2] were used in sols containing 1% benzyl alcohol for preservation. Sulfamerazine USP[3], micronized powder (≤5 μm); formaldehyde solution, USP; glycerin, 99.6%; 2-propanol, 99%; replacement electrolyte solution[4]; collagenase B[5]; pancreatin[5]; and pepsin[6] were used as received. Other reagents were of analytical reagent grade.

Preparation and Recovery of Microglobules – The method of preparation and recovery of microglobules employed by McMullen *et al.* (2) was used. Coacervates were prepared at 45°C from 40.0-g batches stirred by a magnetic stirrer at a speed sufficient to produce a vortex without air bubbles. Appropriate weights of 2% (w/w) stock solutions of pectin and gelatin were individually adjusted to the mixing pH (pH 8–10) with 1.0 M NaOH at 45°C to

[1] 275 Bloom, isoelectric point 8.6; Fisher Scientific Co., Fair Lawn, N.J.
[2] Sunkist Growers Inc., Ontario, Calif.
[3] Matheson, Coleman & Bell, Norwood, Ohio.
[4] Normosol-R pH 7.4; Abbott Laboratories, North Chicago, Ill.
[5] Calbiochem, San Diego, Calif.
[6] Wilson Labs, Chicago, Ill.

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give the desired pectin-to-gelatin ratio. The gelatin solution was added to the pectin solution with stirring and after 2 min the pH was lowered by addition of 0.5 M HCl to pH 5, then to the pH of coacervation (pH_c). After stirring for 30 min, 5 mL of 37% (w/w) HCHO was added and stirred for 30 min under ambient conditions. After 15–20 h, the suspension was centrifuged and the supernatant was decanted. The microglobules were resuspended in 5 mL of glycerin with a vortex mixer and 35 mL of isopropyl alcohol was slowly added as a flocculating agent while mixing. The flocculated microglobules were then filtered, washed with two 100-mL portions of isopropyl alcohol and dried for 15–20 h in an oven at 36°C ± 1°C. Pectin and gelatin sol concentrations of 2.0% were selected on the basis of earlier studies (2), which demonstrated optimum yield at this concentration. The pH of mixing (pH_m)[7], pH of coacervation (pH_c)[8], and colloid ratio were varied to yield microglobules of desired morphology and size (2).

Effect of pH—A sulfamerazine sample (0.8 g) was added to 40.0-g coacervate batches at three pH_m values, at pH 5.0, and at several pH_c values. The system was stirred for 3 min before addition of 0.5 M HCl except when sulfamerazine was added at pH_c, and the recovery procedure was completed as for nonmedicated microglobules (2). Each batch was evaluated for the extent of microencapsulation of the sulfamerazine particles and for microglobule morphology. The sulfamerazine content was determined for selected batches and the particle size was analyzed using a microscopic count technique. To produce a batch of medicated microglobules having a mean diameter of ~5 μm, it was necessary to modify the procedure to eliminate sulfamerazine crystals >2–3 μm. In this case, a sample of sulfamerazine (1.87 g) was added to a 40.0-g batch at the selected pH_m, stirred to disperse the crystals, and centrifuged at 1000 rpm for 3 min. The supernatant was transferred to a 50-mL beaker and the coacervation procedure was resumed.

Effect of Mass of Sulfamerazine—Samples of sulfamerazine (≤3.2 g) were added (at pH_m 10.0) to 40.0-g coacervate batches having a pectin–gelatin colloid ratio of 30:70. Coacervates formed at pH_c of 3.8 and produced microglobules of 25 μm. Samples of sulfamerazine (1.2 g) were added (at pH_m 10.0) to 40.0-g batches having a pectin–gelatin colloid ratio of 33:67. Coacervates formed at pH_c 4.5 and produced microglobules of 10 μm. The recovered batches were evaluated for morphology, total yield, and sulfamerazine content.

Observed Extent of Extraction and Digestion of Microglobules in Aqueous Media—Microglobules (~100 mg) containing 41% (w/w) of sulfamerazine were placed in 20-mL type I glass vials containing 10 mL, respectively, of simulated gastric fluid[9]; 0.1 M HCl, simulated intestinal fluid[9]; 0.1 M NaOH; collagenase B solution, 20 U/mL at pH 7.0 in 0.05 M Tris[10] and 0.005 M calcium chloride (3); replacement electrolyte solution[4]; or distilled water. The vials were sealed with pretreated rubber closures, crimped with aluminum caps and rotated at 25 ± 3 rpm at 37°C in an apparatus similar to that used by Souder and Ellenbogen (4). The suspensions were observed visually and microscopically at 7.5, 15, 30, 60, and 120 min to determine the effect of extraction with the various media on morphology and contents of the microglobules.

Determination of Sulfamerazine Released from Microglobules Extracted with Aqueous Media—Accurately weighed 10-mg samples of microglobules [25-μm average diameter containing 33% (w/w) sulfamerazine] were extracted in 10-mL portions of replacement electrolyte solution and distilled water in 20-mL type I glass vials sealed with pretreated rubber closures and rotated at 25 ± 3 rpm at 37°C. The suspensions were sampled at 0.5, 1.0, 2.0, 4.0, 8.0, and 20.0 h, filtered through a 0.22-μm micropore filter membrane[11], and assayed. Similarly, 10-μm microglobules containing 47% (w/w) sulfamerazine were extracted in replacement electrolyte solution only.

Determination of Sulfamerazine Released from Microglobules Extracted with Aqueous Acetone Solutions—Accurately weighed 50-mg samples of microglobules (25-μm average diameter) containing 41% (w/w) sulfamerazine were extracted for 15 min in 10 mL of aqueous acetone solutions of 0–100% (v/v) acetone in 10% increments. Each sample was filtered through a 0.25-μm membrane[12], 5 mL of filtrate was transferred to a 25-mL volumetric flask, and the solvent was evaporated to dryness. Each sample was brought to volume with 0.1 M NaOH and assayed spectrophotometrically.

Assay of Sulfamerazine Content in Microglobules—Batches of microglobules were washed with acetone, 100 mL/1.0 g of drug originally added, to dissolve any superficial drug. An accurately weighed 100-mg microglobule sample was transferred to a 100-mL volumetric flask and brought to volume with 0.1 M NaOH. A 10-mL aliquot was filtered using a 0.22-μm membrane[11]; 1.0 mL of the filtrate was transferred to a 50-mL volumetric flask and brought to volume with 0.1 M NaOH. The absorbance was read at 255 nm against a blank of 0.1 M NaOH. The concentration (mg/mL) was calculated from a standard (Beer's) curve.

Microglobule Formation of Various Drugs—Samples (0.8 g) of hydrocortisone acetate, chloramphenicol, barium sulfate, aluminum powder, phenobarbital, and cod liver oil were added (at pH_m 10.0) to 40.0-g batches having a pectin–gelatin colloid ratio of 30:70 and coacervates formed (pH_c 3.8). After recovery as a dry powder, each batch was examined microscopically at 1000× for microglobule morphology.

Table I—Effect of pH of Addition on Microencapsulation of Sulfamerazine

Pectin–Gelatin Colloid Ratio	pH_m	pH_c	Appearance[a]		
			pH_m	pH 5	pH_c
50:50	8	3.4	A	A	N
	9	3.4	P	A	N
	10	3.2	E	E	P
40:60	8	3.8	A	A	N
	9	3.8	E	P	N
	10	3.8	E	E	P
33:67	8	3.9	P	A	N
	9.5	4.6	E[b]	E	P
	10	4.5	E[c]	E	P
30:70	8	4.0	P	A	N
	9	3.8	E	E	P
	10	3.8	E[d]	E	P
25:75	8	4.4	P	A	N
	9	4.2	E	E	P
	10	3.8	E	E	P

[a] N is no affinity between drug and microglobules; A is surface adsorption; P is partial microencapsulation; E is complete microencapsulation. [b] Mean is 5.7 μm; SD = 0.93; 37.3% w/w sulfamerazine. [c] Mean is 9.2 μm; SD = 1.01; 44.9% w/w sulfamerazine. [d] Mean is 25.5 μm; SD = 4.3; 45.2% w/w sulfamerazine.

RESULTS AND DISCUSSION

Effect of pH of Addition on Microglobule Formation of Sulfamerazine—The results of studies carried out to determine the stage to add and disperse sulfamerazine are summarized in Table I. When the drug was added at the endpoint pH (pH_c), complete microencapsulation was never obtained for any of the colloid ratio–pH_m combinations studied. This confirms the highly stable nature of pectin–gelatin complex coacervate microglobules reported in a previous paper (2) which precluded their coalescence at temperatures well above the gelation point of gelatin. Adding the drug at the starting pH (pH_m) or at pH 5 resulted in uniform and complete microencapsulation in most cases (except pH_m 8). This information indicates that the drug is not included within microglobules by a surface-active phenomena of wetting and spreading as reported for the gelatin–acacia system (1, 2, 5, 6) but probably by entrapment; the adsorbed colloids on the surface react to form a coacervate layer as the pH is lowered below 5. In the cases where adsorption or partial microencapsulation was noted for the addition of drug at pH_m and pH 5 for batches with a starting pH of 8 and 9, the microglobule size was either equal to or smaller than the crystals to be formed. Consequently the forces of cohesion stabilizing the microglobules to a set size precluded the complete coating of irregularly shaped particles. It was possible to obtain medicated microglobules having mean diameters of 5.7, 9.2, and 25.5 μm as pictured in Fig. 1 containing 37.3, 44.9, and 45.2% (w/w) sulfamerazine, respectively.

Effect of Mass of Sulfamerazine—The effect of mass of sulfamerazine on 10-μm and 25-μm microglobules is plotted in Figs. 2 and 3, respectively, and demonstrated in the photomicrographs (Fig. 4). The spherical nature of the microglobules was maintained at ≤69% (w/w) sulfamerazine content for the 25-μm microglobules and ≤45.5% (w/w) for the 10-μm microglobules. Above these values the spherical shape was lost as a result of the increasing volume of suspended particles being coated by a constant volume of coacervate. It is interesting to note that the curves of yield *versus* drug-to-total colloid ratio became linear with the loss of microglobule sphericity. There was a 10–15% suppression in coacervate yield as shown by the curve for calculated colloid yield[13] *versus* drug-to-colloid ratio (Figs. 2 and 3). This can be attributed to

[7] Century SS-1 pH meter; Beckman Instruments, Fullerton, Calif.
[8] Miniature glass 476031 and Calomel 476017 electrodes; Corning Scientific Products Co., Medfield, Mass.
[9] Simulated TS as per "The United States Pharmacopeia," 19th rev. U.S. Pharmacopeial Convention, Rockville, Md., 1975, p. 765.
[10] THAM; Fisher Scientific Co.
[11] 0.22-μm MF filter; Millipore Corp., Bedford, Mass.
[12] 0.25-μm UG filter; Millipore Corp.
[13] Percent suppression = [Coacervate yield (0% Drug) − Coacervate Yield (x% Drug)/Coacervate Yield (0% Drug)] × 100.

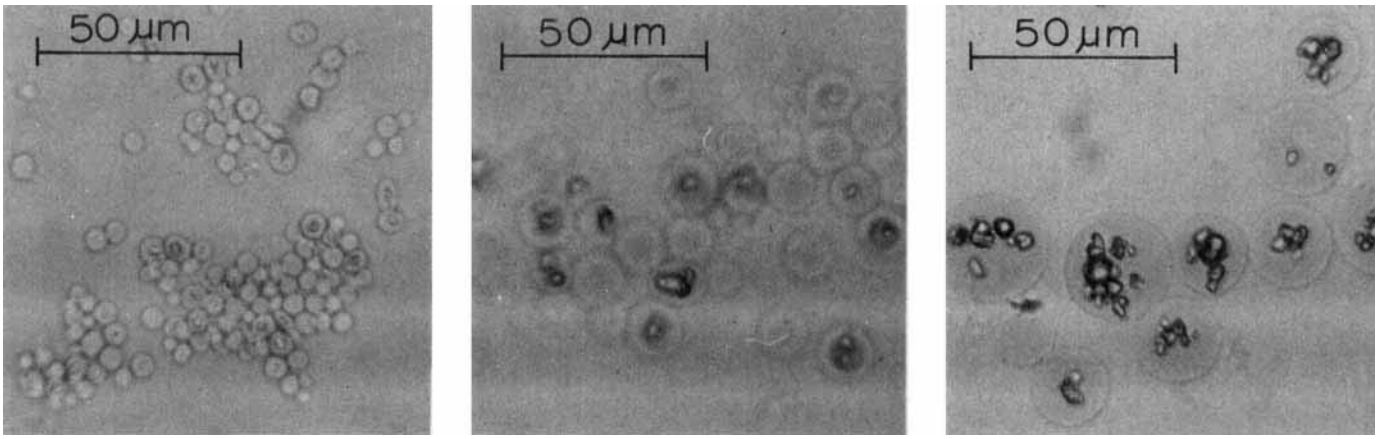

Figure 1—*Pectin–gelatin microglobules having nominal size of 5, 10, and 25 μm containing 37.3, 44.9, and 45.2% (w/w) sulfamerazine.*

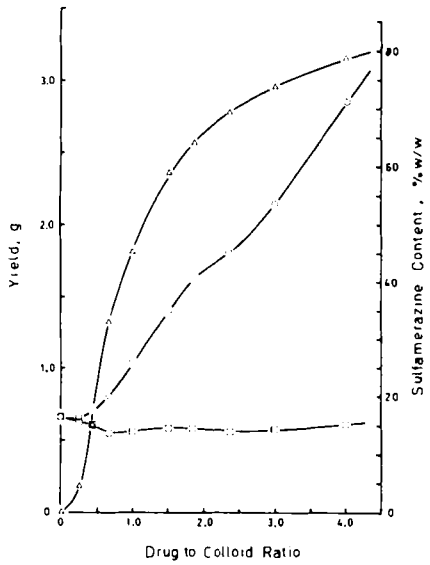

Figure 2—*Effect of mass of sulfamerazine on total dry yield, content, and coacervate yield of 25-μm microglobules. Key: (○) dry yield; (□) coacervate yield (dry yield − sulfamerazine content); (△) sulfamerazine content, % (w/w).*

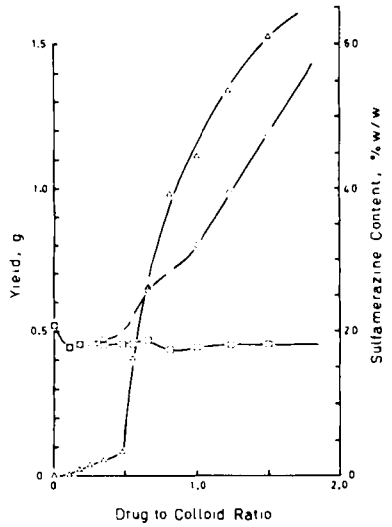

Figure 3—*Effect of mass of sulfamerazine on total dry yield, content, and coacervate yield of 10-μm microglobules. Key: (○) dry yield; (□) coacervate yield (dry yield − sulfamerazine content); (△) sulfamerazine content, % (w/w).*

salt suppression by the slight amount of dissolved sulfamerazine at pH_c, which would insulate ionic groups on pectin and gelatin and decrease their reactivity (7). For both systems the curves of percent sulfamerazine in dry product *versus* drug-to-colloid ratio show a lag phase (more pronounced in Fig. 3) resulting from the initial saturation of the equilibrium liquid with sulfamerazine.

Extraction and Digestion of Microglobules—The results of extraction studies on microglobules containing 41% (w/w) sulfamerazine are summarized in Table II. Extraction with collagenase B seems to digest the gelatin portion (3, 8) of the microglobules as evidenced by their change to near transparency in ~2 h. They completely fragmented after 8 h, having released their sulfamerazine crystals and the fragments and crystals were visible after 24 h of extraction. Complete microglobule digestion was observed with gastric and intestinal fluid test solution only. No apparent morphological change in the appearance of the microglobules was observed during extraction in 0.1 M HCl, 0.1 M NaOH, replacement electrolyte, or distilled water.

The extractions in 0.1 M HCl and 0.1 M NaOH are of particular interest in that these seem to suggest the presence of a highly porous matrix which,

Table II—Effect of Extraction on Morphology and Contents of Microglobules

Extraction Medium[a]	Minimum Time, h		Microscopic Appearance of Suspension[c]
	Digestion	Dissolution	
Collagenase B (20 u/mL)	8.0	NS[b]	f, u
Gastric fluid	8.0	0.25	d, s
Intestinal fluid	0.25	NS	d, u
0.1 M HCl	NS	0.5	i, s
0.1 M NaOH	NS	0.5	i, s
Replacement electrolyte solution (pH 7.4)	NS	NS	i, u
Distilled water	NS	NS	i, u

[a] 37°C. [b] Not sufficient time through 24 h of extraction. [c] i is intact microglobules; f is fragmented microglobules; d is digested microglobules; u is sulfamerazine crystals; s is sulfamerazine in solution.

Table III—Effect of Extraction with Acetone–Water Solutions on Microglobules[a]

Amount, % v/v	Sulfamerazine Solubility, g/10 mL	Sulfamerazine Dissolved, g/10 mL	Sulfamerazine Extracted, %
0	0.0009	0.0010	4.9
10	0.0031	0.0028	13.7
20	0.0078	0.0066	32.2
30	0.0130	0.0115	56.1
40	0.0296	0.0156	76.1
50	0.0564	0.0156	76.1
60	0.0808	0.0157	76.6
70	0.1356	0.0148	72.2
80	0.2148	0.0146	71.2
90	0.2814	0.0115	56.1
100	0.1342	0.0021	10.2

[a] Containing 41.0% (w/w) sulfamerazine (0.0205 g/10 mL).

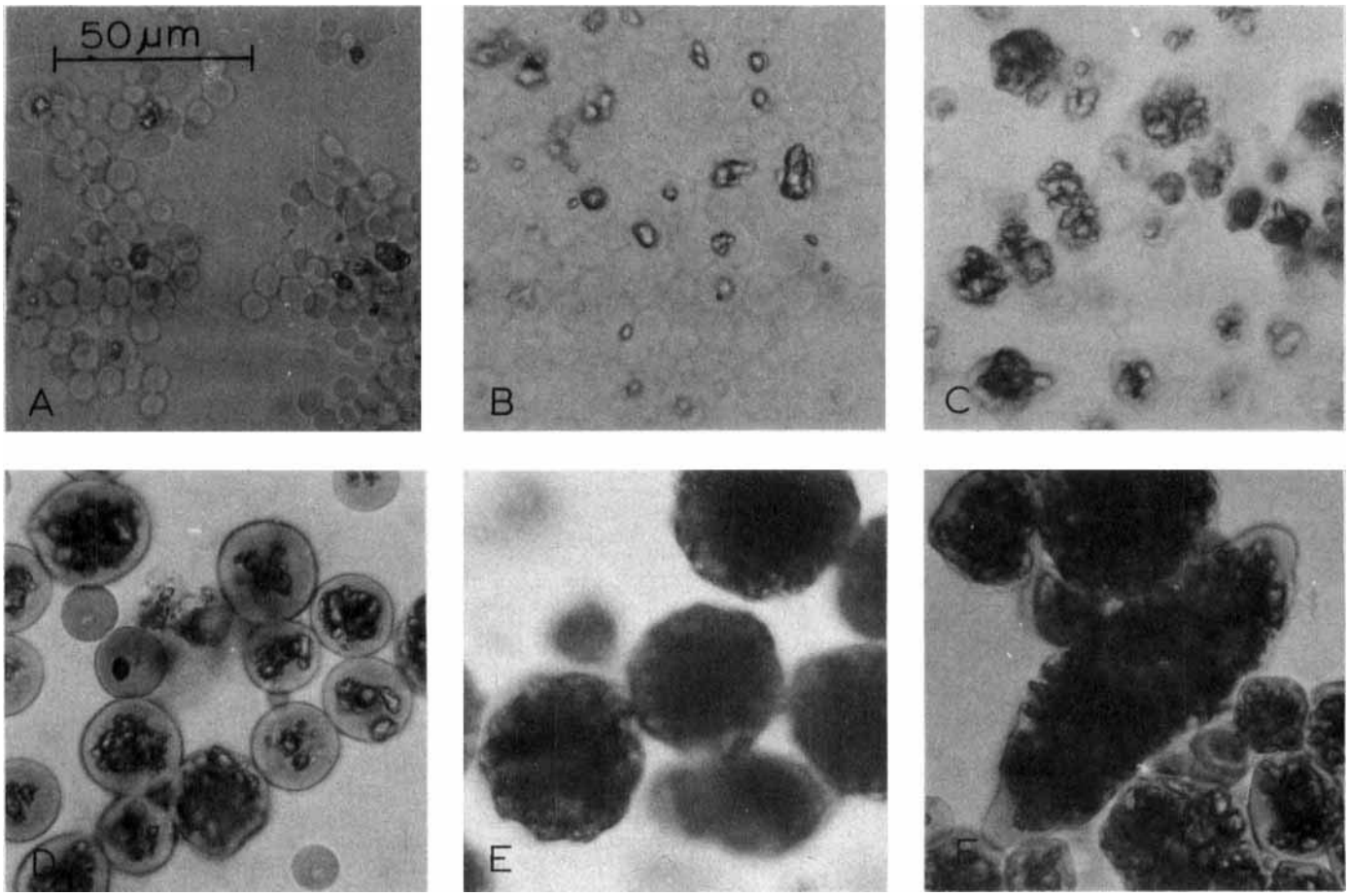

Figure 4—*Effect of mass of sulfamerazine on morphology of 10-μm microglobules containing 16.2% (A), 39.1% (B), and 53.6% (w/w) (C) sulfamerazine; 25-μm microglobules containing 30.5% (D), 58.5% (E), and 73.5% (w/w) (F) sulfamerazine.*

once the microglobules are hydrated, permits the formation and subsequent diffusion out of the microglobules of the soluble hydrochloride or sodium salts of sulfamerazine, respectively. The dependence on hydration for the pore or

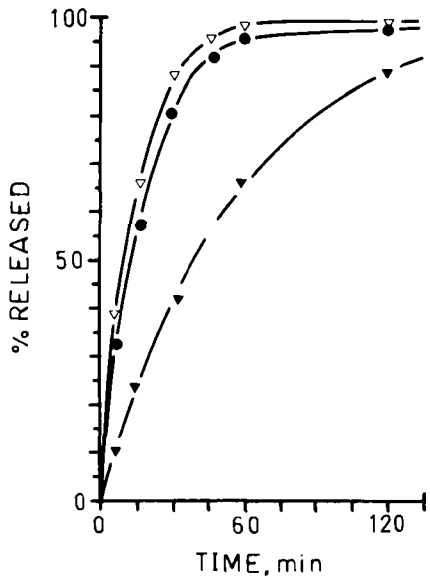

Figure 5—*Effect of extraction with replacement electrolyte solution and distilled water on 10- and 25-μm microglobules. Key: 25-μm microglobules containing 33% (w/w) sulfamerazine extracted in (▼) replacement electrolyte solution and (▽) distilled water; (●) 10-μm microglobules containing 47% (w/w) sulfamerazine extracted in replacement electrolyte solution.*

channel formation was confirmed by extraction of microglobules with aqueous acetone solutions of various concentrations. These results (Table III) indicate that with ≤30% acetone, the amount released is limited by the solubility of sulfamerazine in the binary mixture. For greater concentrations of acetone (≤60%) the microglobules are sufficiently hydrated to allow as much as a 76% release of the drug. At still higher concentrations, the amount of sulfamerazine released decreases although its solubility increases to a maximum at ~90% acetone. The higher acetone concentrations are accompanied by a progressive morphological change from uniform smooth microglobules with a smooth surface to smaller microglobules with a wrinkled surface, an indication of progressive dehydration of the hydrophilic colloids. In pure acetone the extraction of 10.2% of the sulfamerazine from the microglobules is attributed to solubilization of sulfamerazine imbedded superficially in the microglobules (1).

Determination of Sulfamerazine Released from Microglobules Extracted with Aqueous Media—The curves plotted in Fig. 5 demonstrate rapid dissolution of sulfamerazine from the microglobule matrix. The dissolution profile is first order with half-lives of 38.7 min and 9.8 min for 25-μm microglobules in replacement electrolyte and distilled water, respectively, and 12.2 min for 10-μm microglobules in replacement electrolyte. The reduced dissolution rate for microglobules containing sulfamerazine in replacement electrolyte as opposed to distilled water can be attributed to reduced hydration of the microglobule matrix. The higher dissolution rate for sulfamerazine from the 10-μm microglobules in replacement electrolyte results from the higher surface area. These results indicate a very porous matrix which would be more suitable to high molecular weight drugs, drugs microencapsulated as an oil or waxy dispersion, or drugs chemically linked to the polymer matrix.

Microencapsulation of Various Drugs—For successful drug microencapsulation by complex coacervates of pectin and gelatin, the solubility of the drug in the equilibrium liquid and the coacervate must be sufficiently low, it must not have any surfactant activity (9), it must have adequate stability in alkaline and acidic media, and it must have a uniform particle size lower than the desired microglobule size to maintain a spherical shape. Various organic and

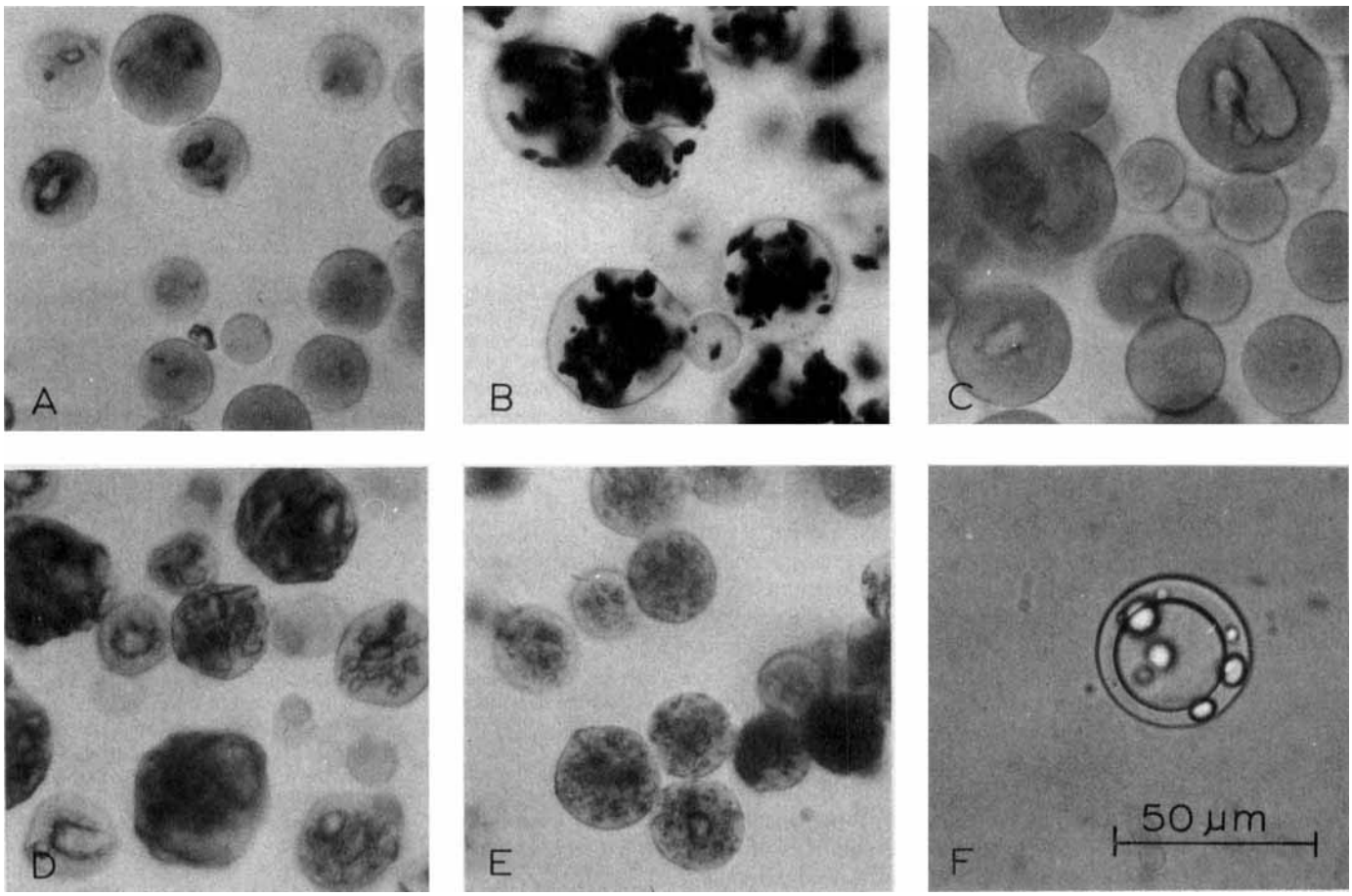

Figure 6—*Pectin-gelatin microglobules containing an undetermined amount of chloramphenicol (A), aluminum powder (B), phenobarbital (C), hydrocortisone acetate (D), barium sulfate (E), and cod liver oil (F).*

inorganic substances complying with these requirements were successfully microencapsulated (Fig. 6).

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