Micromeritic Properties of Sulfamethoxazole Microcapsules Prepared by Gelatin–Acacia Coacervation

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Abstract □ Micronized sulfamethoxazole particles were microencapsulated using the gelatin–acacia complex coacervation method. The effects of the coacervation pH and the amount of formaldehyde used on the micromeritic parameters of the microcapsules were investigated. The particle-size and the wall thickness distributions were log-normal forms. As the pH was increased, the particle size decreased (8.5–28.5 μm). The porosity of various pH-adjusted microcapsules was between 0.158 and 0.277. The particle size of formalized microcapsules was larger than that of the unformalized microcapsules because formalization prevents shrinking of microcapsules during the dehydration and drying process. A smooth surface appeared on the unformalized microcapsule, but a net-like wrinkled structure was observed upon scanning the formalized one. Moreover, folding and invaginating structures were found on the spray-dried microcapsules. The optimum coacervation pH value was 3.5, at which the highest core content was obtained (77.5% w/w). Approximately 6.73 μg of formaldehyde remained in 1 g of the microcapsules formalized with 50 ml of formaldehyde. The crystalline sulfamethoxazole in the microcapsules prepared by spray drying the coacervate slurries was changed into the amorphous form, while the microcapsules dried in the conventional manner showed the same sulfamethoxazole form as the starting substance.

Keyphrases □ Sulfamethoxazole—microcapsules, prepared by gelatin–acacia coacervation □ Microencapsulation—sulfamethoxazole, gelatin–acacia coacervation □ Antibacterial agents—sulfamethoxazole, microencapsulation by gelatin–acacia coacervation

Microencapsulation is an attractive tool for the preparation of dosage forms to control the release rate, to separate incompatible materials in compounding, and to protect against moisture and oxidation. Many microencapsulation processes have been described (1). One accepted technique is gelatin–acacia complex coacervation, which was developed initially for processing carbonless copy paper (2–5). Various studies were reported later (6–8) on the effect of physical factors such as stirring speed, molecular weight of gelatin, and temperature on coacervation behavior. However, few studies have been published on the effects of the optimum coacervation pH and the amount of cross-linking agents used on the micromeritic parameters such as particle size, particle distribution, wall thickness, and porosity of the microcapsules.

The objective of the present study was to elucidate the effects of these factors on the preparation of sulfamethoxazole microcapsules. The effects of spray drying as an alternative to conventional drying of the coacervates on the surface characteristics and on the crystalline form of the microencapsulated sulfamethoxazole also were investigated. The remaining amounts of formaldehyde used as a hardening agent and the content of sulfamethoxazole in the microcapsules prepared under various conditions were determined.

EXPERIMENTAL

Materials—Gelatin[1] and acacia[2] were used as 3% (w/w) solutions. Micronized sulfamethoxazole[3] was used as a model drug. Acetic acid (10%), formaldehyde solution[4] (5, 15, 30, and 50 ml), isopropanol (70%), acetylacetone reagent solution[5], and formaldehyde standard solution[6] were JP grade and were used as received without further purification or recrystallization.

Microencapsulation—The microcapsules were prepared by the method of Green and Schleicher (2, 3) as modified by Luzzi and Gerraughty (8–10). In these experiments, gelatin and acacia solutions were prepared by dissolving 3.0 g of gelatin and 3.0 g of acacia each in 100 ml of purified distilled water. Five grams of micronized sulfamethoxazole was suspended in acacia solution at 50° for 5 min; an equal amount of gelatin solution, which was thermally controlled at 50°, was added gradually, with stirring at 620 rpm, to the acacia suspension. This suspension was maintained for 5 min, and the pH of the mixture was adjusted gradually by dropwise addition of diluted acetic acid with stirring. At the desired pH (2.5–4.0), the gelatin particles were positively charged and were attracted to the negatively charged acacia particles. Coacervation occurred, and a gelatin coating was formed (Fig. 1A). This mixture was cooled to 5° by immersion in a water bath.

A part of the mixture was withdrawn directly onto a slide glass for analysis of the particle size of the coacervate droplets. The rest of the coacervates was isolated from the equilibrium system and washed with 1 liter of distilled water to remove the excess colloid liquid. A part of the coacervated aggregates was retained for examination of the internal texture with a scanning electron microscope. Figure 1B shows the nest-like structure in which many crystals were encapsulated by a surrounding wall. The coacervates were hardened with formaldehyde solution (5, 15, 30, or 50 ml) for ~2 hr. Then the hardened coacervates were filtered and washed with distilled water. The wet mass was added, with stirring, to 70% isopropanol solution (11); stirring was continued for 30 min.

The sediments were filtered and dried at 40° by the conventional method for 2 hr to yield free-flowing discrete particles. The spray-drying technique (12) also was applied to prepare dried discrete microcapsule powders. The aqueous slurries containing the unformalized coacervates were atomized into a drying chamber at 140 ± 10° by a centrifugal wheel atomizer. Two batches for the preparation of microcapsules were run. The maximum batch-to-batch differences in the micromeritic properties of the microcapsules are indicated in Table I.

Measurement of Micromeritic Properties—Particle size was determined by a photographic counting method using a particle-size ana-

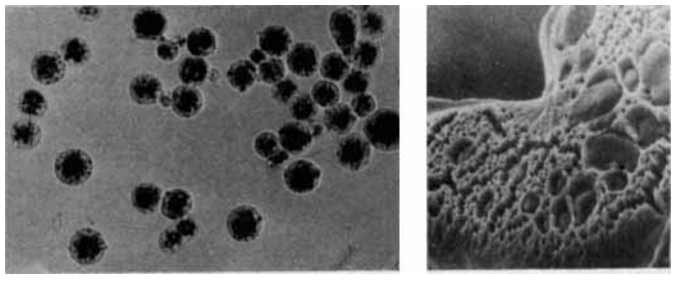

Figure 1—*Photomicrograph of sulfamethoxazole gelatin–acacia coacervates (left) and scanning electron microscopic photograph of the internal texture of coacervate aggregates (right).*

[1] Isoelectric point 4.9 and pH 6.3; the melting point of a 3% solution was 29° (Japan Leather Co., Japan).

[2] Kanto Chemical Co., Japan.

[3] Micronized to <6 μm by a jet micronizer (Shionogi Pharmaceuticals Co., Japan).

[4] Five, 15, 30, and 50 ml of 37% HCHO were added, respectively, to the coacervate suspension and diluted to a total volume of 200 ml.

[5] Ammonium acetate, 150 g, was dissolved in the proper volume of distilled water in a 1000-ml volumetric flask, 3 ml of acetic acid and 2 ml of acetylacetone were added, and the solution was diluted to 1000 ml (prepared just prior to use).

[6] Four micrograms of formaldehyde/ml of solution.

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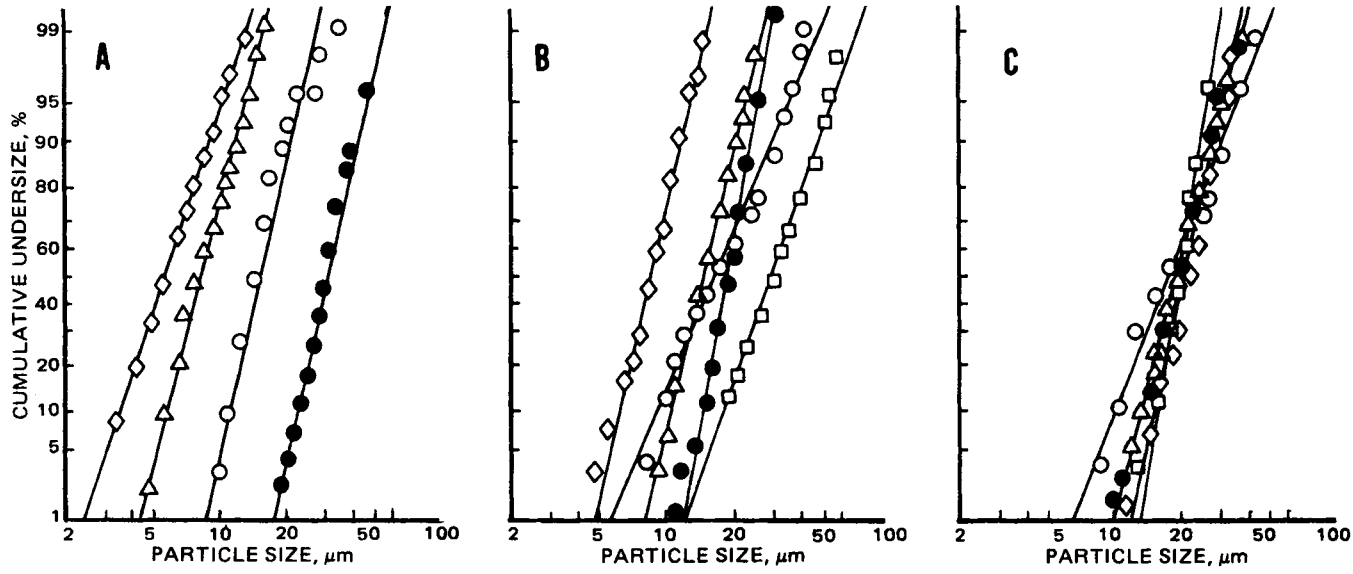

Figure 2—*Particle-size distribution of coacervates (A) and unformalized (B) and formalized (C) dried microcapsules. Key (for A and B): □, 2.5 adjusted pH; ●, 3.0 adjusted pH; ○, 3.5 adjusted pH; △, 3.7 adjusted pH; and ◇, 4.0 adjusted pH. Key (for C): ○, 0 ml of formaldehyde; ●, 5 ml of formaldehyde; △, 15 ml of formaldehyde; □, 30 ml of formaldehyde; and ◇, 50 ml of formaldehyde.*

lyzer[7]. Not less than 600 particles were measured for each batch. The coating thickness of the microcapsules was determined by direct measurement after microtome slicing of the microcapsules embedded in paraffin (mp 48–50°). The microcapsules were added to melted paraffin solution, and this mixture immediately was immersed into an ice bath and hardened. The paraffin masses subsequently were sliced with a congelation microtome. The slice thickness was ~10 μm. Each slice was placed on an object slide and mounted with liquid paraffin. Approximately 100 specimens were tested to measure the coating thickness, and the averaged value represented the wall thickness. The sections that came from the extremes of the microcapsules may have given somewhat high values for the wall thickness. However, comparison of the relative differences is valid.

The particle densities of various microcapsules and raw materials (*e.g.*, gelatin and micronized sulfamethoxazole) were measured with a helium–air comparison pycnometer[8].

The surface topography of the microcapsules was investigated by a scanning electron microscope[9]. The microcapsule samples were mounted on a sample stub with double-sided adhesive tape and were vacuum coated with carbon and gold.

The crystalline form of the microcapsulated sulfamethoxazole was determined with an X-ray diffractometer[10] with CuKα radiation (1.5418 Å). The IR spectrum was recorded with an IR spectrometer[11].

Assay of Sulfamethoxazole Content and Remaining Formaldehyde—The sulfamethoxazole content in the microcapsules was assayed by the following method. Approximately 100 mg of each sample was weighed accurately, extracted with pH 7.5 solution (simulated intestinal fluid of JP IX), and then determined spectrophotometrically at a suitable UV wavelength using a double-beam spectrophotometer. Ten 100-mg samples from two batches were tested, and the mean was obtained.

The remaining amount of hardening agent was determined by the modified acetylacetone method (13–15). One gram of each sample (*e.g.*, gelatin, acacia, sulfamethoxazole, and formalized microcapsules using 50 ml of formaldehyde) was extracted for ~2 hr with 100 ml of distilled water by shaking the sample at 40 ± 1° in a water bath, and the sample was filtered with a millipore filter (3 μm). Ten milliliters of the extracted sample solutions and of the formaldehyde standard solution were pipetted into separate 50-ml volumetric flasks containing 10 ml of acetylacetone reagent solution, and the flasks were shaken for 30 min at 40 ± 1° in a water bath. A blank test using 10 ml of distilled water instead of acetylacetone reagent solution also was undertaken. The absorbance of each extracted sample solution was determined at 415 nm by a spectrophotometer[12].

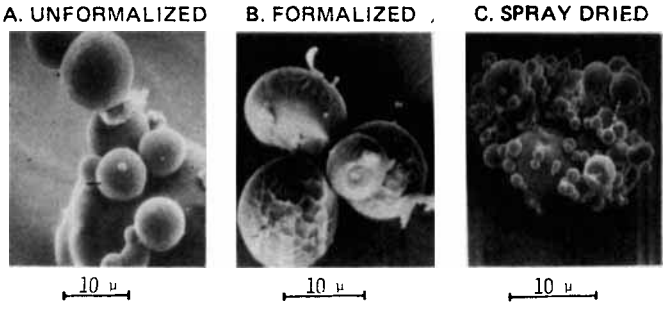

Figure 3—*Scanning electron microscopy photograph of dried sulfamethoxazole microcapsules prepared by complex coacervation.*

RESULTS AND DISCUSSION

Particle-Size Distribution of Gelatin–Acacia Coacervates and Dried Microcapsules Made Conventionally—The cooled coacervate droplets in equilibrium solution and the dried microcapsules prepared under the various pH adjustments without formalization were placed on a slide glass for particle-size measurements. These data are shown graphically in Figs. 2A and 2B, respectively. The sizes of the coacervates and the microcapsules followed a log-normal distribution. As the pH was increased from 3.3 to 4.2 in the equilibrium liquid, the geometric mean particle diameter of the microcapsules decreased (Table I). This result may have been due to the electrophoretic characteristic of gelatin–acacia coacervates. Electrophoretic data of coacervates obtained previously[13] showed that, at a pH above 3.5, the coacervates were electronegatively charged and the charges increased with increasing pH. This finding indicates that, at the higher pH range, the small coacervates could be formed due to the strong repulsive force between the coacervates. In addition, the reduced amounts of gelatin in the coacervates formed at the lower pH region enhanced the swelling of gelatin below pH 3.5–4.0 (16), leading to the formation of larger coacervates.

The unformalized microcapsules prepared at pH values higher than 3.5 were larger than the corresponding coacervates. The coacervates used for measuring the size were taken directly from the coacervation system prior to further processing. The rest of the coacervates were washed with 1 liter of distilled water, during which swelling occurred. The following drying process, *i.e.*, isopropanol treatment and air drying at 40°, contributed to the shrinkage of the microcapsules and offset the enlargement of the microcapsules caused by swelling. The coacervate with a thicker

[7] Karl Zeiss TGZ-3.
[8] Model 1302, Micromeritics Instrument Co.
[9] Nihon Denshi JDX.
[10] Nihon Denshi JSM-S1.
[11] Nihon Denshi DS-403G.
[12] Hitachi model 556.
[13] The original data for this study.

Table I—Micromeritic Properties of Dried Microcapsules by a Conventional Method [a]

Adjusted pH	D_g^1, μm	l, μm	ρ, g/cm³	ϵ (−)	Drug Content, %
4.0 (4.2)[b]	8.5 ± 0.3 (5.7 ± 0.3)[c]	1.31 ± 0.02	1.02 ± 0.01	0.270 ± 0.006	53.6 ± 0.6
3.7 (3.9)[b]	15.2 ± 0.4 (8.4 ± 0.3)[c]	1.15 ± 0.02	1.03 ± 0.01	0.277 ± 0.005	63.9 ± 0.5
3.5 (3.76)[b]	17.1 ± 0.4 (15.0 ± 0.4)[c]	1.00 ± 0.03	1.19 ± 0.03	0.186 ± 0.006	77.5 ± 0.8
3.0 (3.26)[b]	19.1 ± 0.5 (31.2 ± 0.6)[c]	0.87 ± 0.01	1.08 ± 0.01	0.217 ± 0.004	46.8 ± 0.4
2.5 (2.85)[b]	28.5 ± 0.5	0.75 ± 0.02	1.03 ± 0.00	0.264 ± 0.001	54.1 ± 0.5

Amount of Formaldehyde, ml	D_g^2, μm	ρ, g/cm³	ϵ (−)	Remaining Formaldehyde, μg/g	Drug Content, %
50	22.0 ± 0.4	1.17 ± 0.02	0.167 ± 0.004	6.73 ± 0.06	56.4 ± 0.5
30	20.1 ± 0.4	1.11 ± 0.02	0.203 ± 0.005		51.7 ± 0.5
15	19.0 ± 0.2	1.19 ± 0.03	0.158 ± 0.003		59.5 ± 0.4
5	19.1 ± 0.5	1.18 ± 0.02	0.161 ± 0.002		57.1 ± 0.6
0	17.1 ± 0.4	1.19 ± 0.03	0.186 ± 0.003		77.5 ± 0.8

[a] All data show the average values from two batches. Key: D_g^1, D_g^2, geometric mean diameter of unformalized and formalized dried microcapsules, respectively; ρ, particle density; l, wall thickness of unformalized dried microcapsules; and ϵ, porosity. [b] Final pH of equilibrium solution. [c] Geometric mean diameter of coacervates.

wall at the higher pH region (Table I) might retard shrinkage during the drying process, leading to the described results (Figs. 2A and 2B and Table I). For the microcapsule with a thinner wall at pH 3.0, the shrinking effects compared to the swelling might result in a size that is smaller than the coacervate (Table I). This expectation also was confirmed using a scanning electron microscope by the detection of a smooth surface on the microcapsule at the higher pH and a wrinkled surface at the lower pH.

The particle-size distributions of the unformalized and formalized microcapsules are seen in Figs. 2B and 2C, respectively. Upon formalizing the coacervates, some differences were observed in the particle-size distribution of microcapsules. An increase in the size of the microcapsules was found as more hardening agent was used (Table I). The hardened wall may prevent the shrinkage that occurs during the dehydration and drying process.

Microcapsule Wall Thickness and Porosity—The influence of pH on the wall thickness of dried microcapsules is shown in Table I. The wall of the dried microcapsules made at the higher pH was thicker than that of microcapsules made at the lower pH. Previous investigators (17–19) concluded that gelatin–acacia coacervates have a gelatin structure in which acacia is attracted by ionic bonds only and does not participate in the gel structure formation. At high pH values, NH_3^+ groups of gelatin are comparatively few but COO^- groups of acacia are numerous. To prepare coacervates, a larger amount of gelatin is required to interact with acacia, therefore leading to a thicker wall.

The particle densities (1.02–1.19) of the microcapsules, as measured by a helium–air comparison pycnometer, were smaller than those of the raw materials, *i.e.*, gelatin (1.27) and sulfamethoxazole (1.53). This result is reasonable because the microcapsule film may prevent helium penetration into the internal matrix of the microcapsule. If it is assumed that the microcapsule film is composed of gelatin alone (16, 17), the porosity, ϵ, of the microcapsule can be determined by inserting the density values into:

$$\epsilon = 1 - \frac{(C_g\rho_s + C_s\rho_g)\rho_m}{\rho_s\rho_g} \qquad \text{(Eq. 1)}$$

where C_g is the weight percent of gelatin, C_s is the weight percent of drug, ρ_s is the density of the drug particles, ρ_g is the density of the gelatin particles, and ρ_m is the density of the microcapsule particles. The porosity of the microcapsules was 0.158–0.277 (Table I).

Microcapsule Surface Topography—The surface of the unformalized microcapsules (Fig. 3A) was smooth and had no cracking or pore penetration. Evidence of folding and invagination was absent. The formalized microcapsules (Fig. 3B) also had a partially smooth surface and were free from structural defects. However, miscellaneous matter was seen occasionally on the capsule surface, which may have been due to crystals or polymer remaining in the medium during hardening. Another partial surface appearance showed a net-like wrinkled structure, which also was observed on the simple coacervated microcapsules during formalizing (23).

Adhesive sundries and crystals on the surface can be attributed to the soluble complex formed by the interaction between the formaldehyde and the primary amino groups of sulfamethoxazole, which was leached out to the surface during the washing process (24). These surface aberrations also may be the partially ruptured coacervates formed during hardening or dehydration, with the unencapsulated crystals adhering to the surface. The shrinking structure, which was not found on the unformalized microcapsules, may suggest that crossover linkage occurred during the formalizing procedure. Reaction of the gel with formaldehyde caused a high degree of insolubility as a result of intermolecular crosslinkage formation. Adhesive polymer sundries could be due to the ruptured fractions of coacervates or the remaining gelatin fractions that were crosslinked with formaldehyde upon hardening.

Drug Content and Remaining Amounts of Hardening Agents—The extraction of sulfamethoxazole microcapsules showed that >45% (w/w) of sulfamethoxazole was contained in the microcapsules (Table I). The microcapsules adjusted to pH 3.5 had the highest content. This result may have been due to the high volume of coacervates formed when the pH was adjusted to 3.5, at which point the positive and negative charges were equivalent (25). According to these data and those from a previous report (26), pH 3.5 is the optimum pH for coacervation in the present system where the acacia is strongly negative and the gelatin is strongly positive. Table I illustrates that the content of the unformalized microcapsules was higher than that of the formalized microcapsules. Because of the interaction between formaldehyde and sulfamethoxazole, this soluble complex was leached out and lost in the medium, resulting in a drug content of ~55% in the formalized microcapsules, which was 22% less than the unformalized content.

Approximately 6.73 μg of formaldehyde remained in 1 g of the formalized microcapsules prepared with 50 ml of formaldehyde. If 710 mg of these microcapsules (56.4%) is administered orally, according to the

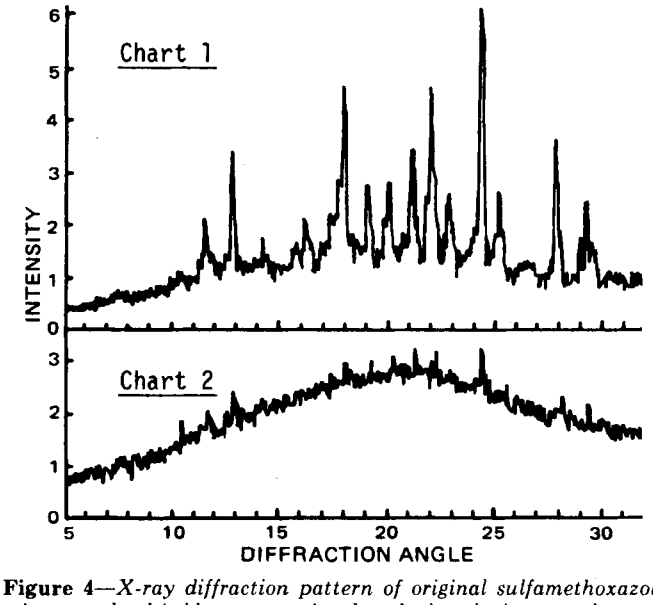

Figure 4—*X-ray diffraction pattern of original sulfamethoxazole, microcapsules dried by a conventional method, and microcapsules spray dried after coacervation. Key: Chart 1, original sulfamethoxazole or dried microcapsules by a conventional method; and Chart 2, spray-dried microcapsules. (Intensity scale: 1 = 10 Hz.)*

formulation of co-trimoxazole tablets[14] (BP 1973), it is predicted that no side effects would occur since only 4.75 μg of formaldehyde is contained in this formulation. The other formalized microcapsules may have a lower amount of remaining formaldehyde, although no such data are available. The method of removing the remaining formaldehyde from the formalized microcapsules also is important for microencapsulation if formaldehyde is applied as a hardening agent.

Effect of Spray-Drying Procedure on Microcapsule Surface Topography and Crystalline Drug Form—On the surface of the spray-dried microcapsules prepared from the unformalized coacervates (Fig. 3C), a smooth surface with some fissures of the coating was observed. Moreover, a few microcapsules possessed a characteristically folded and invaginated surface. This phenomenon may be deduced from a diagram (20–22) of a spray-dried slurry droplet.

X-ray diffraction patterns of the ground microcapsules were obtained to investigate the crystalline forms of sulfamethoxazole in the microcapsule prepared by conventional and spray-drying techniques. The intensities of the diffraction peaks of the spray-dried sulfamethoxazole were weaker than those of the original material. A rapid evaporation of solvent from spray droplets might change the crystalline form to the disordered form (Fig. 4). On the other hand, the intensities of the peaks found in the microcapsules dried by the conventional method coincided with those of the original sulfamethoxazole. This result may be explained in that, although the coacervation temperature was 50°, the crystalline forms could not be disordered due to the mild drying process employed. In addition, the IR spectrum of all of the microcapsules coincided with that of the original sulfamethoxazole, indicating that complexes between sulfamethoxazole and the polymers (gelatin or acacia) were not formed through spray drying and the complex coacervation processes.

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[14] One tablet of co-trimoxazole contains 400 mg of sulfamethoxazole and 80 mg of trimethoprim.

Effects of Dansylated Acetylcholine Analogs on *Schistosoma mansoni*

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Abstract □ A series of dansylated fluorescent analogs was synthesized and tested for cholinergic and anticholinergic activity in *Schistosoma mansoni*. The compounds were compared with a previously reported analog, 5-(dimethylamino)-*N*-(2-dimethylaminoethyl)naphthalenesulfonamide hydrochloride (I). All of the compounds produced some fluorescent labeling of a structure in the head region of the worms. Major differences were not seen among the compounds. The compounds also were tested as antagonists of carbachol-induced paralysis in an activity monitor. Significant carbachol antagonism was seen for all compounds. Effects on the serotonin response also were recorded. Only one compound, a dibutylaminopropylamine derivative, produced striking antagonism of serotonin-induced motor activity.

Keyphrases □ Cholinergics—antagonists, dansylated acetylcholine analogs, *in vitro* scanning fluorescence method, neuroactive drugs in schistosomes □ Carbachol—effect on schistosomes, effect of anticholinergic agents, scanning fluorescence method, motility studies □ Serotonin—effect on schistosomes, anticholinergic agents, scanning fluorescence method □ Structure–activity relationships—dansylated acetylcholine analogs, effect on motility and neuroactivity in schistosomes, scanning fluorescence method, motility studies

Various cholinomimetic and anticholinergic drugs affect the nervous system of schistosomes. However, some drugs, such as muscarine, nicotine, and tubocurarine, which are highly active at mammalian cholinergic synapses, are conspicuously inactive in schistosomes (1), although acetylcholine and carbachol are effective immediately. This finding suggests that sensitivity to cholinergic drugs in schistosomes is different from that in nicotinic or muscarinic mammalian synapses.

Dimethylaminonaphthalene - 5 - sulfonamidoethyltri-

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