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Preparation and Evaluation of Gelatin-Acacia Microcapsules of Sulfamethoxazole

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Sulfamethoxazole particles were microencapsulated using the gelatin-acacia complex coacervation method. Micromeritic properties and dissolution characteristics of the microcapsules were studied. The particle size distribution followed log-normal form. As the hardening time increased, the particle size and wall thickness increased (45.3–52.0 μm, 2.02–5.12 μm, respectively).

This is considered to be due to the cross-linked wall structure of formalized microcapsules which prevents shrinking of gelatin during the dehydration and drying processes. An increase of hardening time clearly delayed the release rate. The *in vitro* 50% dissolution time (t_{50}) for unencapsulated sulfamethoxazole powder was less than 3 min.; for microcapsules hardened for 30 min, the t_{50} was 20.1 min.; for those hardened for 60 min. the t_{50} was 25.0 min.; for those hardened for 120 min., the t_{50} was 35.8 min. The surface of the unhardened microcapsules was smooth and had no cracking or pore penetration. However, the surface of the hardened microcapsules was folded and invaginated.

A variety of studies on complex coacervation have been reported in the literature[1-5]. Complex coacervation is primarily dependent upon pH. It has been reported that in gum arabic-gelatin systems, complex coacervation occurred and microcapsules formed at pH values below the isoelectric point (IEP) of the gelatin but would not occur above this pH.

The same is true of other systems containing two dispersed colloids, one of which is ampholytic[6].

Luzzi and Gerraughty reported the effects of selected variables on the extractability of oils from coacervate capsules and on the microencapsulation of solids[7,8].

Also, they reported the effects of additives and formulation techniques on controlled release of drug from microcapsules[9]. The microencapsulation of waxy solids by complex coacervation was studied by Madan *et al.*[10].

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McGniity *et al.* and Paradissis *et al.* reported the improved method for microencapsulation of soluble pharmaceuticals[12,13]. Nixon & Hassan reported the particle size distribution of microcapsules which were prepared under various stirring speeds[14]. Also, they reported the stability of oils encapsulated in microcapsules[1].

A good many studies have been reported 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 effect of the hardening time on the dissolution parameters and micromeritic properties of microcapsules.

The objective of present study is to elucidate the effect of hardening time on dissolution profile, especially on the *in vitro* 50% dissolution time. The particle size distribution, wall thickness and surface characteristics of sulfamethoxazole microcapsules were also studied to evaluate the microcapsules. 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 (Nitta Gleatin Co., Japan. isoelectric point, 4.9), Acacia (Junsei Chemical Co., Japan.), Sulfamethoxazole (Passed through a No. 200 sieve, Hong Sung Pharmaceuticals Co., Korea), Formaldehyde solution (Kanto Chemical Ind., Japan.), 2-propanol (Wako Pure Chemical Ind., Japan.).

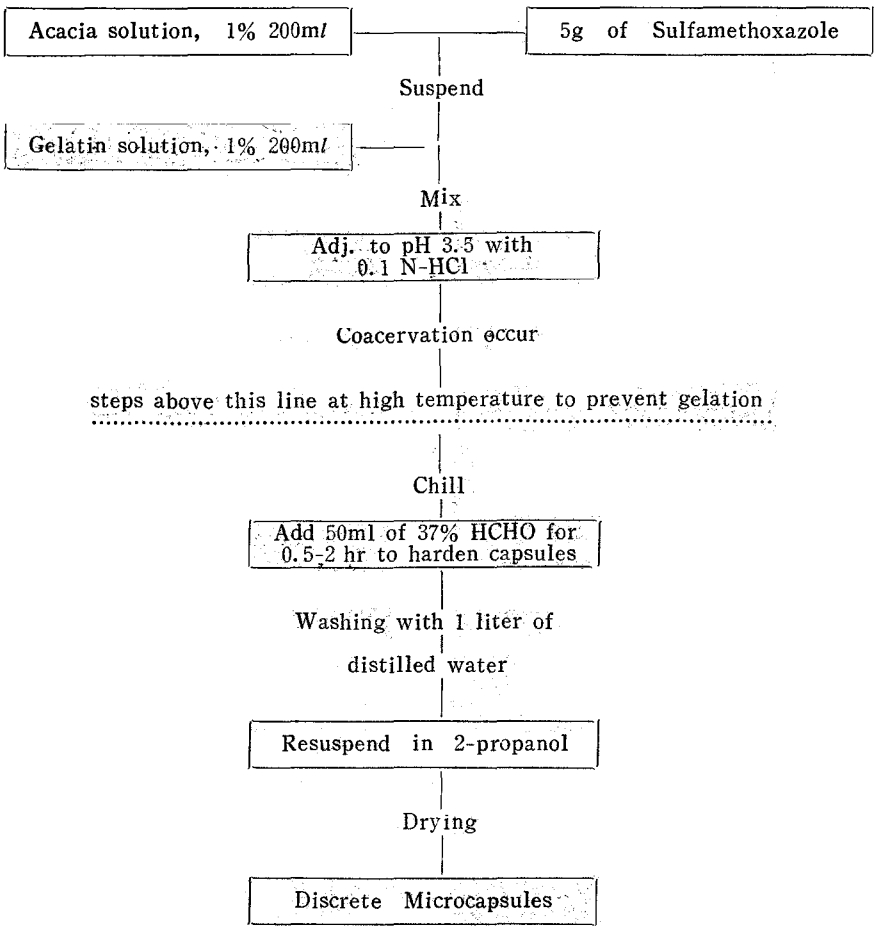
Acetylacetone reagent solution: Ammonium acetate, 150g, was dissolved in the proper volume of distilled water in a 1000 m*l* volumetric flask, 3 m*l* of acetic acid and 2 m*l* of acetylacetone were added, and solution was diluted to 1000 m*l* (prepared just prior to use).

Formaldehyde standard solution: Prepared to be 4 μg of formaldehyde per 1m*l* of solution with hexamethylenetetramine (Hayashi pure chemical ind., Japan).

Apparatus——UV-spectrophotometer (Unicam SP 1,750), pH-meter (Corning model-7.), microscope system (Olympus models FHT & EHT with SW attachment.), microtome (American Optical AO 888 with freezing attachment.), Scanning electron microscope (Jeol model JSM-35.), mechanical stirrer (Four bladed propeller, 4 cm diameter).

Microencapsulation Procedure—The basic microencapsulation procedure was followed by the method of Green and Schleicher[2,3] as modified by Luzzi and Gerraughty[7-9]. All experiments were conducted under identical conditions with the same or similar equipment. Scheme I shows the microencapsulation procedure for sulfamethoxazole.

In these experiments, gelatin and acacia solutions were prepared by dissolving 2.0g of gelatin and 2.0g of acacia each in 200 m*l* of distilled water. Five grams of sulfamethoxazole powder, which was passed through a No. 200 sieve before encapsulation, were suspended in acacia solution at 50°C for 20 minutes (Fig. 1-A). An



Scheme I ——Microencapsulation of sulfamethoxazole via complex coacervation method.

equal amount of gelatin solution, which was thermally controlled at 50°C, was added gradually with stirring to the acacia suspension.

This suspension was maintained for 20 minutes to achieve finely dispersed suspensoin (Fig. 1-B). And pH of the mixture was adjusted gradually by dropwise addition of 0.1 N-HCl with continuous stirring. At pH 3.5, the gelatin molecules were positively charged and were attracted to negatively charged acacia molecules. Coacervation occurred, and a gelatin coating was formed (Fig. 1-C). This mixture was maintained for 10 minutes to ensure the complete encapsulation, and subsequently chilled to 5°C by immersion in an ice bath with continuous stirring. This chilled mixture was maintained for 10 minutes to ensure the complete gelation, and isolated from the equillibrium system. Then the coacervates were hardened with formaldehyde solution (50 ml) for 0.5–2 hr (Fig. 1-D). The hardened coacervates were isolated from the formaldehyde solution and washed with 1 liter of distilled water (5°C).

The wet mass was resuspended to 30% 2-propanol solution with stirring for 30

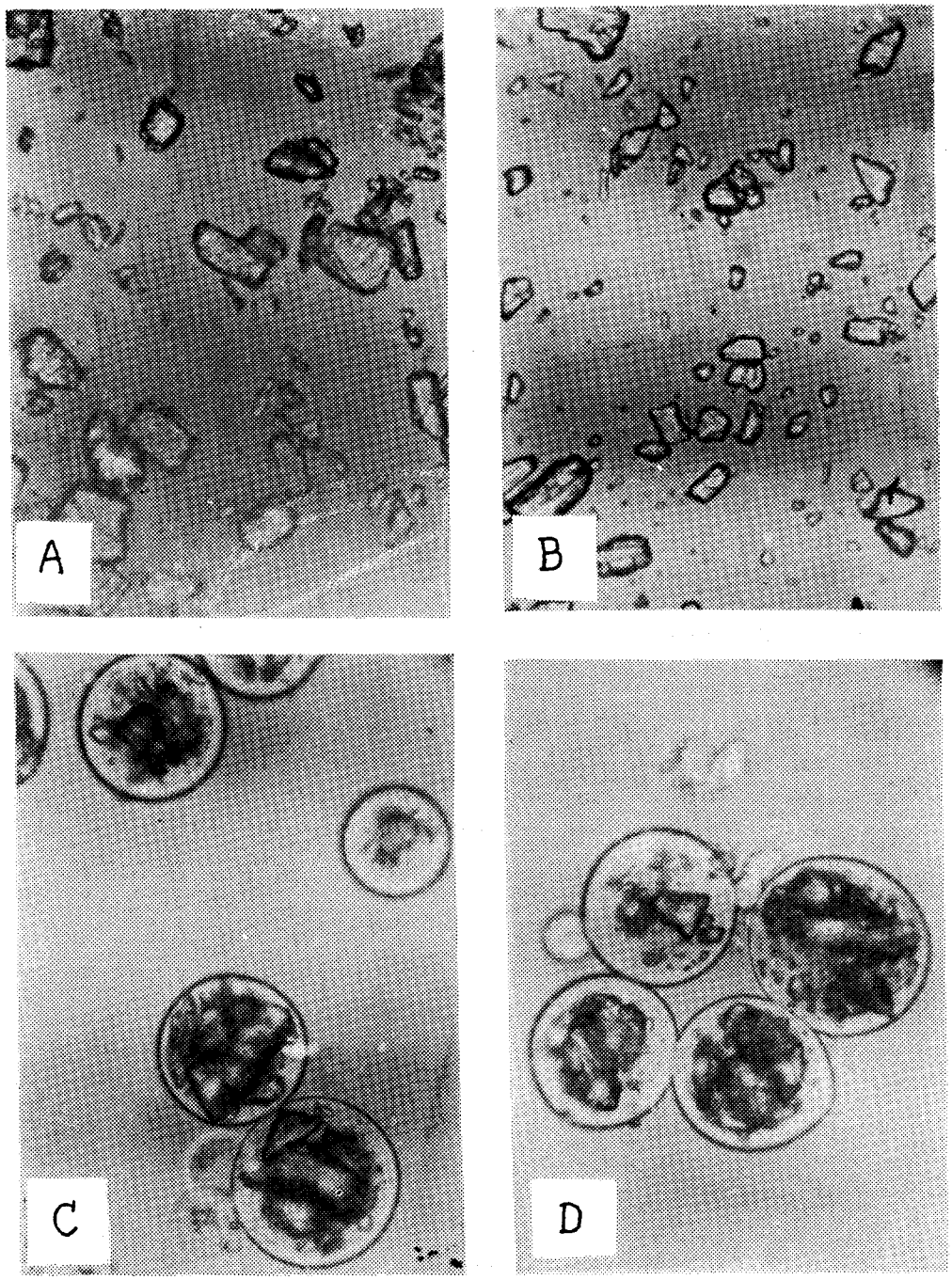


Figure 1—Photomicrographs of microencapsulation procedure.

minutes.

The sediments were dried at room temperature by vacuum drying for 10 hours to yield discrete particles and further dried at 70°C by tray dryer for 2 hours to achieve completely free-flowing discrete particles.

Particle Size Distribution——Sulfamethoxazole microcapsules were placed on an object slide glass and mounted with liquid paraffin.

The particle size of microcapsules was determined by a photographic counting method using microscope attached with calibrated micrometer. The particle size was measured along an arbitrarily chosen fixed line. This was termed horizontal diameter by Green.

The geometric mean diameter was calculated from equation as followed:

$$\log G = \frac{1}{n} \sum_i f_i \log d_i$$

where G; geometric mean diameter,
n; number of particles observed
f; number of particles in a particle size, d
d; particle size.

Not less than 600 particles were measured for each batch.

Coating Thickness—Microcapsules were embedded in a gelatin mass according to Baker[16]. Paraffin inclusion is unsatisfactory because of the solvent effect of alcohol and toluene.

Gelatin is mixed with distilled water in a 1 : 5 ratio. This mixture was kept at 37°C for 2 hours in an incubator. Microcapsules were subsequently suspended, immediately immersed into an ice bath and hardened. Cubes of gelatin obtained after hardening were kept at 4°C. A separate gelatin bath was used for each batch of microcapsules.

The gelatin cubes were subsequently sliced with a congelation microtome. Slice thickness should be about 15μ.

Each slice was placed on an object slide glass and mounted with 50% glycerin solution.

Slides were studied without staining on a microscope, fitted with a calibrated micrometer. Difference between core and coating material was apparent by the brighter zone of coating material in comparison with the darker zone of sulfamethoxazole crystals.

Dissolution—The dissolution rates of pure sulfamethoxazole powder and microcapsules containing sulfamethoxazole were determined by the flask method. A round-bottomed flask with a hole cut to allow stirrer insertion was filled with 1000 m*l* of distilled water. The approximate amount of the microcapsules, equivalent to 100 mg of sulfamethoxazole, was dispersed into dissolution medium which had previously been immersed in a constant temperature bath at 37±0.5°C.

The flask of the dissolution apparatus possessed three necks and the four bladed

metal propeller, positioned at the center of the medium, was maintained at 100 rpm.

Aliquots of 5 m*l* of the dissolved solution were withdrawn at selected time intervals through a pipette with a filtertip and suitably diluted with distilled water. Aliquots of medium (same volume and temperature) were added immediately to the system after each sampling to keep the volume of the medium constant during the course of the test. A suitable cover was used to prevent loss of the dissolution medium during the experiment. The concentration of the dissolved sulfamethoxazole in the medium was determined spectrophotometrically at 260 nm, using the dissolution medium as the blank solution.

Assay of Sulfamethoxazole Content and Remaining Formaldehyde—The sulfamethoxazole content in the microcapsules was assayed by the following method. Approximately 50 mg of each sample was weighed accurately and extracted with pH 7.2 phosphate buffer for 10 hours. This extracted solution was filtered and suitably diluted.

And then sulfamethoxazole content was determined spectrophotometrically at 260 nm wavelength using pH 7.2 phosphate buffer as the blank solution. Three 50 mg sample from each batch were tested, and the mean was obtained.

The remaining amount of formaldehyde was determined by the modified acetylacetone method[16,17]. 500 mg of each was extracted with 50m*l* of distilled water by shaking for 2 hours at 40±1°C.

This extracted solution was filtered with a Millipore filter (3μ). Extracted sample solution and the formaldehyde standard solution,[22] 10 m*l* each, were added to 10 m*l* of acetylacetone reagent solution and were shaken at 40±1°C for 30 minutes.

A blank test using 10 m*l* of distilled water instead of acetylacetone reagent solution also was undertaken. The absorbance of each extracted sample was determined at 415 nm by a spectrophotometer.

Scanning Electron Microscopy—Dried samples of microcapsules were mounted on a sample stub with double-sided adhesive tape and were vacuum coated with gold film approximately 60 nm thick. And the surface topography of microcapsules was investigated by a JSM-35 Scanning Electron Microscope (Jeol).

Results and Discussion

Preparation of Microcapsules—According to previous reports[21,22], pH 3.5 is the optimum pH for gelatin-acacia coacervation. In the present system where acacia is strongly negative and gelatin is strongly positive, the positive and negative charges are equivalent at pH 3.5[23]. Since microencapsulation via complex coacervation occurs best at lower colloid concentration and the 1 : 1 acacia to gelatin ratio[8], 1–3% colloid concentrations were studied. There was no beneficial aspect at the higher concentration.

Therefore, the 1% colloid concentration was used to produce the least cohesive

microcapsules. The resuspension of the products in a 2-propanol solution for dehydration was successful with only 30% solution. Lower concentrations did not result in a free-flowing powder, and higher concentrations destroyed the shell wall integrity.

Before the conventional drying, vacuum drying technique was employed to aid the production of noncohesive discrete microcapsules.

Particle Size Distribution—Sulfamethoxazole received was pulverized by milling with mortar and pestle. This milled powder was passed through a No. 200 sieve and microencapsulated by gelatin-acacia complex coacervation method. Thus obtained microcapsules were placed on a slide glass for particle size measurement. These data are shown in Table I and Fig. 2.

Many papers have been reported on particle size distribution[18,19]. Steiner *et al.* recently demonstrated that milling of monodisperse granulations gave rise to log-normally distributed particle sizes[20]. Log-normal distributions are not uncommon for solid particles; crystallization often leads to log-normal distributions that are truncated from below and above, a fact that has been explained on theoretical grounds.

The sizes of the microcapsules prepared by present method followed a log-normal distribution as shown in Fig. 2.

In these experiments, hardening time affected its size distribution. An increase in the size of microcapsules was found as longer hardening time was employed.

The coacervates which were formed by adjustment of pH to 3.5 were deposited around suspended core material surface to yield embryonic microcapsules. Coating materials, gelatin-acacia complex coacervates, were in sufficiently swollen condition.

Table I—Micromeritic Properties of Dried Microcapsules

Formalization (min)	D_g, μm	1, μm	ρ, g/cm^3	Drug Content(%)	Remaining Formaldehyde(μg/g)
0	45.31±1.51	2.02±1.07	1.22±0.02	72.77±0.7	—
30	46.84±1.42	3.14±1.41	1.23±0.02	59.42±0.8	8.29±2.17
60	49.76±1.20	3.98±1.70	1.22±0.02	61.29±0.3	12.95±0.46
120	52.04±1.23	5.12±1.29	1.14±0.01	56.80±0.6	19.15±1.40

Key: D_g, geometric mean diameter of dried microcapsules; 1, wall thickness of dried microcapsules; ρ, particle density.

The following processes, i.e., 2-propanol treatment and drying, contributed to the shrinkage of microcapsules. In these steps, the hardened wall of microcapsules may prevent the shrinkage that occurs during the dehydration and drying process.

Therefore, the microcapsules that were hardened for longer time may have larger particle sizes.

Geometric mean diameters of formalized microcapsules are shown in Fig. 3.

Coating Thickness—The influence of hardening agent on the wall thickness of dried microcapsules is shown in Table I. The walls of the dried microcapsules made by hardening treatments were thicker than unhardened microcapsules. And an increase

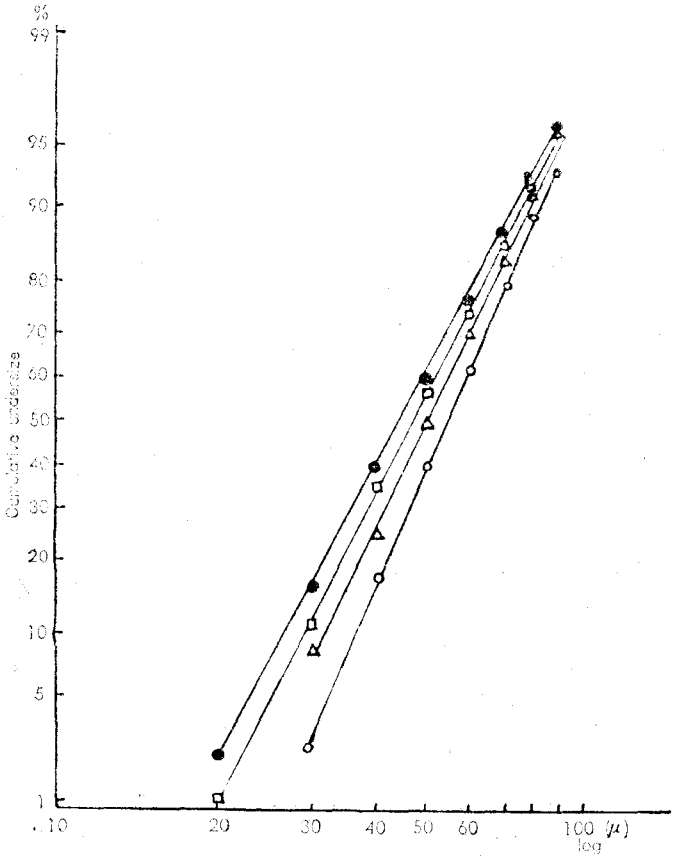


Figure 2—Particle size distribution of sulfamethoxazole microcapsules.
Key: ●, unformalized; □, formalized for 30 min; △, formalized for 60 min; ○, formalized for 120 min.

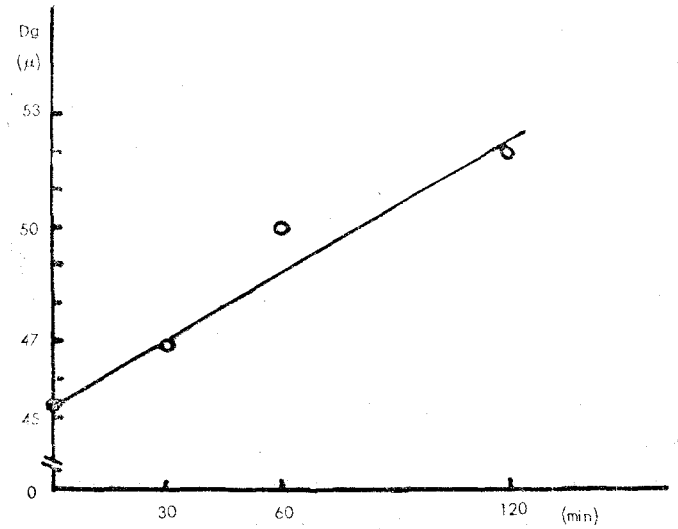


Figure 3—Geometric mean diameter of sulfamethoxazole microcapsules as a function of formalization time.

in the coating thickness was found as longer hardening time was employed.

These results are considered to be due to the extent of hardening of wall. The hardened wall may be less susceptible to the shrinkage of microcapsules to lead thicker walled microcapsules. The walls of microcapsules formalized for 120 minutes may be much more hardened than those of unformalized or formalized microcapsules for 30 minutes. This extent of hardening also appears in the dissolution test.

Dissolution—The retarded release profiles of sulfamethoxazole from the gelatin-acacia coacervated microcapsules into the dissolution medium are shown in Fig. 4. Each determination is the average of at least four dissolution tests. Dissolution results for the powder and encapsulated forms of sulfamethoxazole indicated that the microencapsulated forms exhibited retarded dissolution.

An increase of hardening time clearly delayed the release rate. The microcapsules hardened for 30 minutes featured rapid initial release. The profiles in Fig. 4 were in rank order with hardening time; there was a definitely retarded release for encapsulated sulfamethoxazole.

However, it should be noted that the release rate from the microcapsules hardened for 30 minutes was faster than that of unhardened microcapsules. This was not due to a difference in the surface area of the two microcapsules, since they had almost the same size, i.e., 45.3 μm and 46.8 μm, for the unhardened microcapsules and the microcapsules hardened for 30 minutes, respectively (Table I).

The possible formation of soluble complex between formaldehyde and sulfamethoxazole may explain these peculiar results in the dissolution tests[24].

The leaching out of soluble complex through the microcapsule wall may result in an

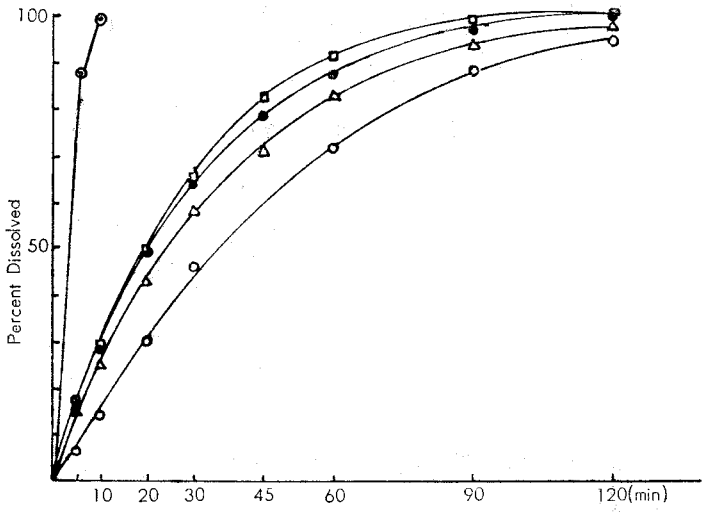


Figure 4—Dissolution profile of sulfamethoxazole microcapsules in distilled water at 37°C.

Key; ●, unformalized; □, formalized for 30 min.; △, formalized for 60 min.; ○, formalized for 120 min.; ⊙, sulfamethoxazole powder (unencapsulated).

enhanced release of sulfamethoxazole. Although the soluble complex was formed in case that other hardening time was employed, the strengthened cross-linking of the wall effectively delayed the release of sulfamethoxazole from the microcapsules.

In order to obtain the times required for 50% dissolution of sulfamethoxazole in microcapsules in bulk liquid, the released percent of sulfamethoxazole was plotted as a function of the square root residence time (Fig. 5). For all the dissolution tests, linear correlations were obtained up to 60–80% drug release, in accordance with the data of many investigators[25,23]. The *in vitro* dissolution t_{50} values obtained from Fig. 5 are shown in Table II and Fig. 6.

Fig. 6 shows a direct linear correlation between hardening times and dissolution t_{50} values.

This linearity will be useful for designing dosage forms of gelatin-acacia coacervated microcapsules.

Sulfamethoxazole Content and Remaining Formaldehyde—The extraction of sulfamethoxazole from the microcapsules showed that 72.77% (w/w) of sulfamethoxazole was contained in the unformalized microcapsules (Table I). Table I shows that the drug content of unformalized sulfamethoxazole microcapsules was higher than that of formalized sulfamethoxazole microcapsules. This result may be explained by the interaction between sulfamethoxazole and formaldehyde which was used as hardening agent.

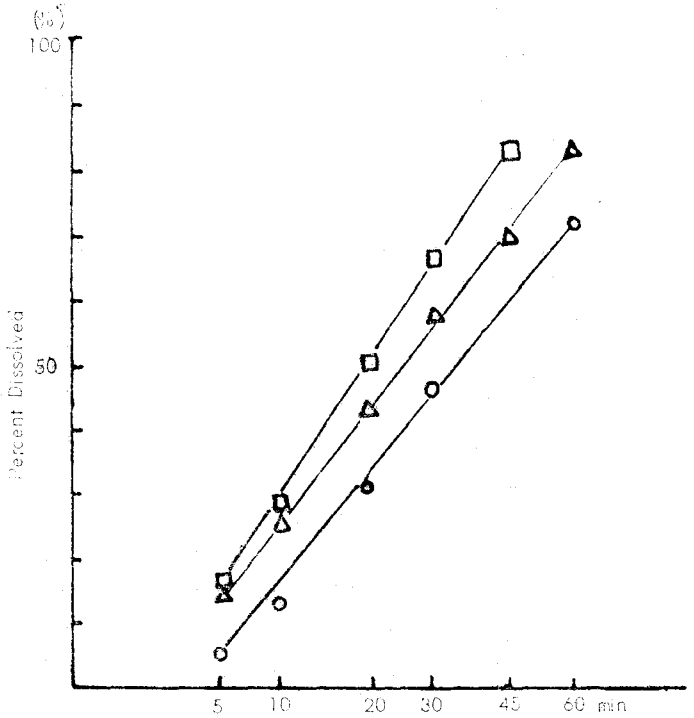


Figure 5—Drug(%) dissolved as a function of the square root of time.
Key: □, formalized for 30 min.; △, formalized for 60 min.; ○, formalized for 120 min.

Table II—*In Vitro* Dissolution t_{50} *versus* Hardening Time for Sulfamethoxazole Microcapsules*

Hardening Time, min.	In vitro Dissolution t_{50} min.
0	20.77
30	20.07
60	24.98
120	35.77

*; *In vitro* dissolution t_{50} of unencapsulated sulfamethoxazole: <3.

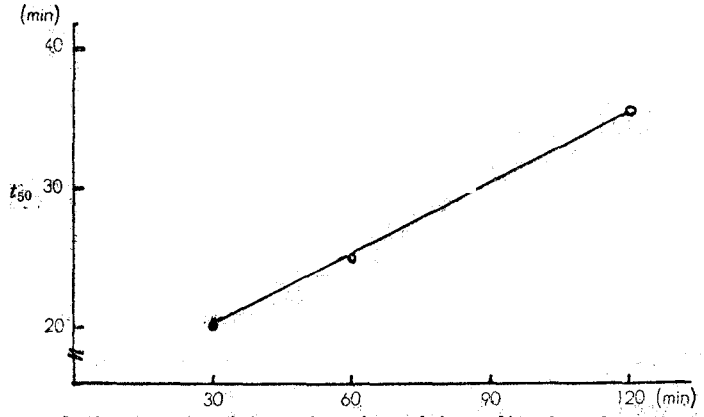


Figure 6—Dissolution time (t_{50}) as a function of formalization time.

Because the capsule wall is permeable to water and 2-propanol, the soluble complex of sulfamethoxazole and formaldehyde can be leached out into medium during the washing process and resuspending process in a 30% 2-propanol solution[27]. Therefore, the drug content of the microcapsules formalized for 2 hours was 56.80% which was less than that of unformalized microcapsules.

The remaining amounts of formaldehyde in various microcapsules are shown in Table I. In microcapsules hardened for 2 hours, 19.15 μg of formaldehyde remained in 1g of microcapsules which had been washed with 1 liter of chilled distilled water. The other formalized microcapsules contained a lower amount of remaining formaldehyde. The method of removing the remaining formaldehyde from the microcapsules is important for microencapsulation if formaldehyde is applied as a hardening agent.

Surface Topography—The scanning electron micrographs of the unformalized and formalized microcapsules are shown in Fig. 7. The surface of the unformalized microcapsules (Fig. 7-A) was smooth and had no crackings and folded portions. However the formalized microcapsules (Fig. 7 B, C, D) possessed characteristically folded and invaginated surfaces. In Fig. 7 B, the folds can be seen as smooth transitions rather than sharp discontinuities such as would result from cracks. But the craters and pore penetrations were found in the surface of microcapsules hardened for 1–2 hours (Fig. 7 C, D).

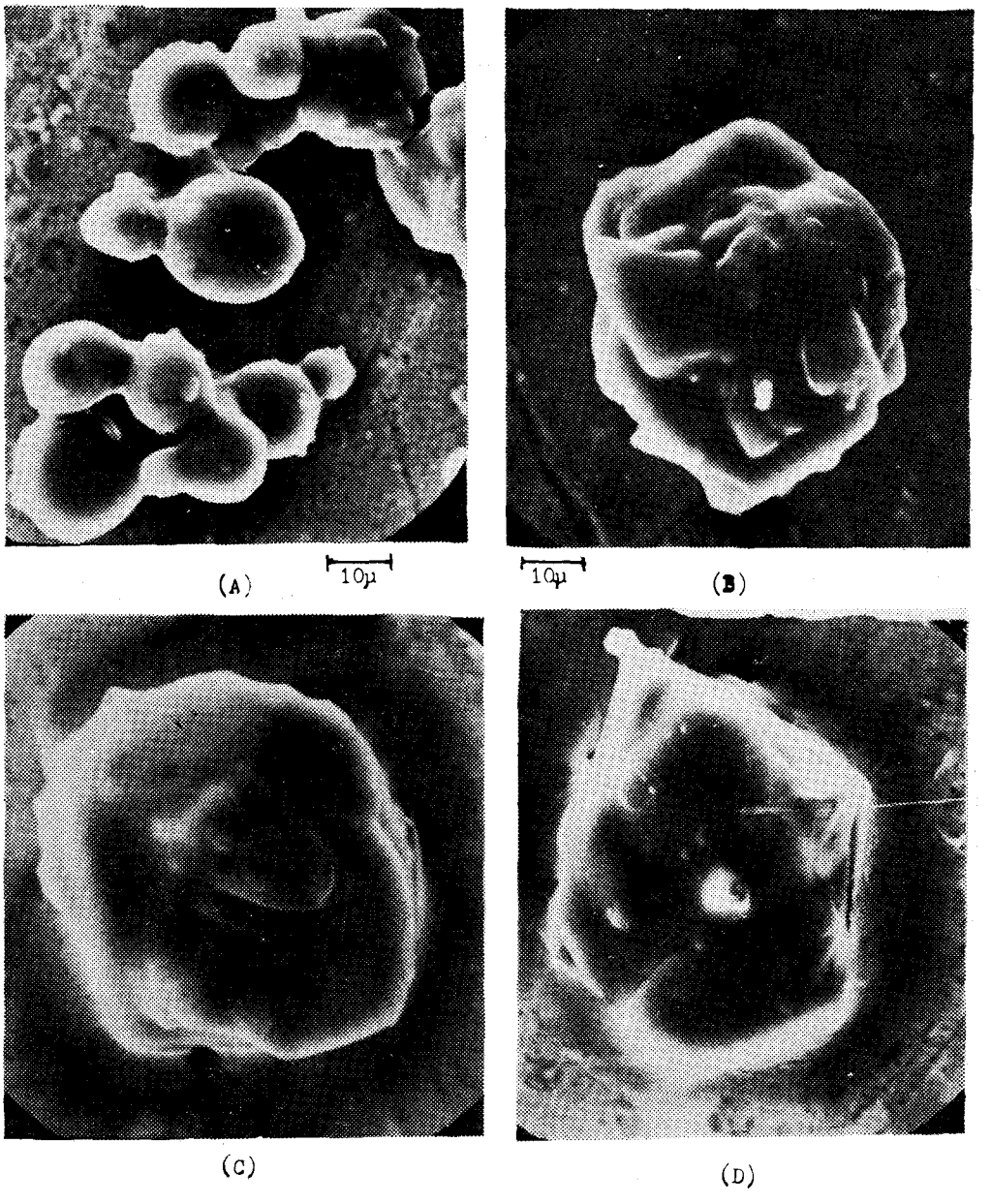


Figure 7—Scanning electron microscopy photographs of dried microcapsules prepared by complex coacervation.
Key: (A), Unformalized; (B), Formalized for 30 min.; (C), Formalized for 60 min.; (D), Formalized for 120 min.

The micellaneous matters were seen occasionally on the surface of microcapsules, which might be attributed to the ruptured fraction of coacervates or the remaining polymer fractions that had been cross-linked with formaldehyde upon hardening.

Summary

1) The microencapsulation of sulfamethoxazole via gelatin-acacia complex coacervation was successful with only 1% colloid concentration and 30% 2-propanol solution.

2) The size of the microcapsules prepared by gelatin-acacia complex coacervation method followed log-normal distribution. And the geometric mean diameter of the hardened microcapsules was larger than that of the unhardened microcapsules.

3) The relationship between hardening time and *in vitro* 50% dissolution time (t_{50}) was found to be a direct linear correlation.

This linearity will be useful for designing the sustained release dosage forms of gelatin-acacia coacervated microcapsules.

4) The surface of the unformalized microcapsules was smooth and had no crackings or folded portions. However, the formalized microcapsules possessed folded and invaginated surfaces.

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