1. The microcapsules can be used as individual microculture flasks, making it easier to handle and manipulate culture cells, especially when they are used as an investigative tool in the study of cell metabolism and regulation and the dynamics of hormone action and production.

2. The immediate cell environment can be made more similar to solid tissue density and biochemical characteristics and, therefore, more physiological and natural.

3. *In vivo* and *in vitro* sterile conditions are easier to maintain since bacterial and viral cells cannot cross the capsular membrane.

4. When microencapsulated cells or tissues are used as injectable prostheses or implantable (or *ex vivo*) artificial organs, it should be possible to specify the capsular membrane's permeability range to suit a particular use such as prevention of immune rejection or toxin removal.

Specifically, the microencapsulated islet appears to have great potential as a better and simpler implantable and, possibly, physiologically disposable artificial pancreatic endocrine organ for the treatment of diabetes. Preliminary animal studies have begun to give encouraging results.

REFERENCES

(1) T. M. S. Chang, "Artificial Cells," Charles C Thomas, Springfield, Ill., 1972.

(2) T. M. S. Chang, "Biomedical Applications of Immobilized Enzymes and Proteins," vol. 1, Plenum, New York, N.Y., 1977, pp. 69–104.

(3) F. Lim and R. D. Moss, in "Microencapsulation, New Techniques and Applications," T. Kondo, Ed., Techno, Tokyo, Japan, 1978, pp. 167–178.

(4) F. Lim and R. D. Moss, *Clin. Chem.*, 24, 1040 (1978). *Ibid.*, 25, 1071 (1979).

(5) "Microencapsulation, Processes and Applications," J. E. Vandegaar, Ed., Plenum, New York, N.Y., 1973.

(6) "Microencapsulation," J. R. Nixon, Ed., Dekker, New York, N.Y., 1976.

(7) K. Mosbach and R. Mosbach, *Acta Chem. Scand.*, 20, 2807 (1966).

(8) R. R. Mohan and N. N. Li, *Biotechnol. Bioeng.*, 17, 1137 (1975).

(9) P. E. Lacy, E. H. Finke, S. Conant, and S. Naber, *Diabetes*, 25, 484 (1976).

ACKNOWLEDGMENTS

Preparation of Hemolysate-Loaded Poly(N^α,N^ϵ-L-lysinediylterephthaloyl) Nanocapsules

MASAYUKI ARAKAWA and TAMOTSU KONDO [x]

Abstract □ Poly(N^α,N^ϵ-L-lysinediylterephthaloyl) capsules containing hemolysate were prepared in the nanometer range by an interfacial polymerization technique using electrocapillary emulsification to obtain very fine hemolysate droplets for encapsulation. The effects of temperature, emulsifier concentration, and the applied potential on the size distribution of the polyamide capsules were investigated. The polyamide capsules prepared under proper conditions have an average diameter of ~500 nm.

Keyphrases □ Poly(N^α,N^ϵ-L-lysinediylterephthaloyl) microcapsules—containing hemolysate, prepared by interfacial polymerization using electrocapillary emulsification □ Electrocapillary emulsification—synthesis of poly(N^α,N^ϵ-L-lysinediylterephthaloyl) microcapsules containing hemolysate, effect of temperature, emulsifier concentration, and applied potential on polyamide capsule-size distribution □ Microencapsulation—symposium, preparation of nanograde-sized polyamide microcapsules containing hemolysate, effect of temperature, emulsifier concentration, and applied potential on capsule-size distribution

Previous studies on poly(N^α,N^ϵ-L-lysinediylterephthaloyl) microcapsules containing hemolysate were carried out with the aim of using them as artificial red blood cells (1–3). Most of the encapsulated hemoglobin molecules retained their oxygen absorbability while catalase and carbonic anhydrase lost some of their enzymatic activities during encapsulation; suspensions of the microcapsules exhibited flow properties similar to those of blood unless the capsule concentration exceeded 30% by volume. Because of their relatively large size (mean diameter of 10 µm) and low deformability in shear flows, the microcapsules were not expected to pass through capillary blood vessels. Thus, an attempt was made to prepare much smaller capsules loaded with hemolysate that could pass through capillary blood vessels.

When a potential difference that is higher than a critical value, the critical voltage of emulsification, is applied to an oil–water interface, the interfacial tension is reduced almost to zero and spontaneous emulsification occurs, due to the interfacial fluctuation, in the absence of surfactant or in the presence of a very small amount (4, 5). The emulsions thus formed are monodisperse and stable, with the average particle diameter being <200 nm. The necessary condition for this electrocapillary emulsification is that the ionic strength, and hence the reciprocal double-layer thickness, of the inner phase be higher than that of the outer phase. Because of the difficulty in obtaining hemolysate droplets of <1 µm by conventional mechanical emulsification processes, the electrocapillary emulsification technique seems to be promising.

The present paper describes the preparation of poly(N^α,N^ϵ-L-lysinediylterephthaloyl) capsules in the nanometer range containing sheep hemolysate by interfacial polymerization using electrocapillary emulsification to obtain very fine hemolysate droplets for encapsulation. Factors influencing the capsule size also are described. In addition, the effect of the applied potential on the enzymatic activities of catalase and carbonic anhydrase in the hemolysate droplets is reported.

EXPERIMENTAL

Apparatus for Electrocapillary Emulsification—Figure 1 shows the schematic diagram of the apparatus used for electrocapillary emul-
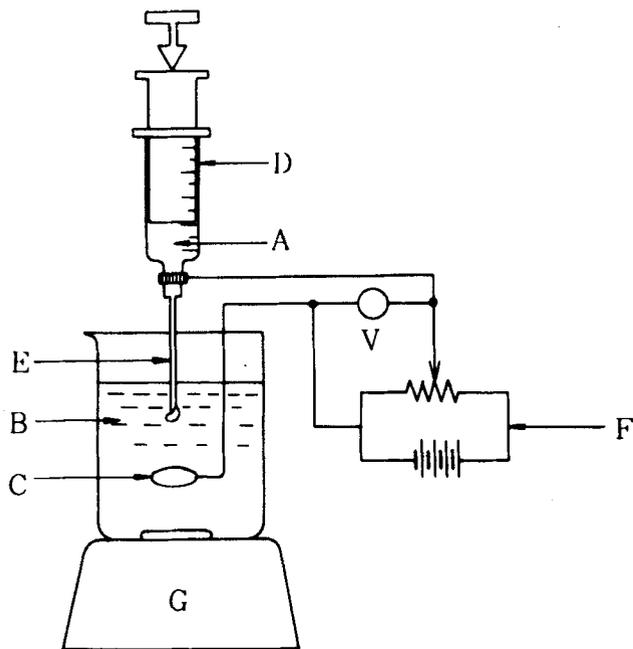
Figure 1—*Schematic diagram of the apparatus for electrocapillary emulsification.*

sification. The aqueous phase (A) in a syringe (D) was introduced into the oil phase (B) by driving a motor through a needle (E), which also was used as an electrode. This electrode and a platinum electrode (C) in the oil phase were connected to a variable dc supply (F), the potential of which is measured by a voltmeter (V). The container of the oil phase was surrounded by ice (low temperature) or water (room temperature), and the content was stirred continuously by a magnetic stirrer (G).

Preparation of Hemolysate-Loaded Polyamide Capsules—The aqueous phase to be encapsulated contained sheep hemolysate (~10% as hemoglobin), 0.4 M L-lysine, and 0.45 M sodium carbonate. The oil phase consisted of three volumes of cyclohexane and one volume of chloroform in which terephthaloyl dichloride, tetraethylammonium chloride, and sorbitan trioleate were dissolved. The concentration of terephthaloyl dichloride was 0.04 M. The quaternary ammonium salt gave electroconductivity to the oil phase, and its concentration was 1.5 × 10⁻⁴ M.
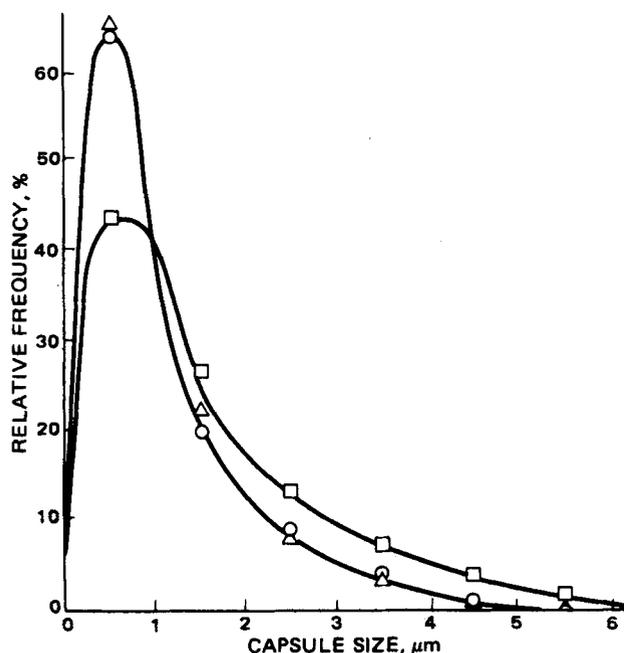

Figure 2—*Effect of aqueous phase addition rate on the size distribution of polyamide capsules. Key: O, 0.003 ml/min; △, 0.015 ml/min; and □, 0.10 ml/min.*
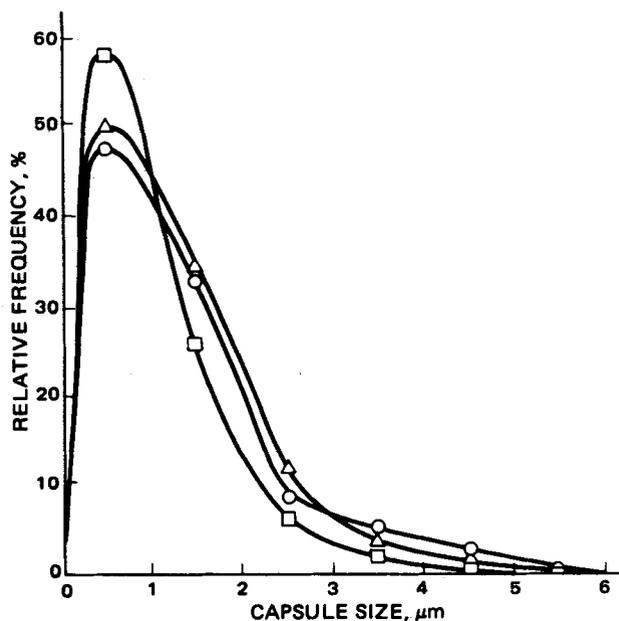

Figure 3—*Effect of emulsifier concentration on the size distribution of polyamide capsules. Key: O, 3% (v/v); △, 5% (v/v); and □, 10% (v/v).*

The aqueous phase (1 ml) was introduced through the needle to 50 ml of the oil phase in a beaker. The addition was made at a constant rate under the influence of an applied potential between the electrodes, which were separated by ~5 mm. The oil phase was gently stirred during the addition. Since the applied potential was always above a critical value, spontaneous emulsification occurred and a shower of fine hemolysate droplets was formed. The applied potential was positive on the aqueous phase side with respect to the oil phase throughout this work.

On the surface of each droplet, polycondensation took place between L-lysine and terephthaloyl dichloride to form a poly(N^α,N^ϵ-L-lysinediylterephthaloyl) membrane. The hydrogen chloride formed as a reaction by-product was neutralized immediately by the alkali in the aqueous phase. The polyamide capsules thus formed were centrifuged at 1000 rpm for 3 min. The separated capsules were dispersed in water with the aid of poly(oxyethylene) sorbitan monolaurate, a dispersing agent.

Capsule-Size Measurement—A portion of the aqueous capsule dispersion diluted with distilled water was withdrawn and placed on a hemocytometer and photographed under an optical microscope. In some cases, the capsule size also was measured by an electronic particle counter[1] or a scanning electron microscope.

Enzyme Activity Measurement—Catalase activity in the sheep hemolysate droplets dispersed in the organic phase was estimated by determining by means of iodometry at 4° and pH 7.4 the amount of hydrogen peroxide decomposed by the enzyme in the hemolysate supernate obtained after centrifuging the dispersion.

Similarly, the enzymatic activity of carbonic anhydrase in the hemolysate supernate was estimated by measuring the change in pH as a function of time in the presence of a large excess of carbon dioxide at 4° after breaking down the dispersion.

RESULTS

Capsule-Size Distribution—As the rate of addition of the aqueous phase increased, the capsule-size distribution curve widened and the relative frequency of large capsules increased. Figure 2 shows the size distribution curves of the polyamide capsules prepared under a constant applied potential (1000 v), a fixed emulsifier concentration (5%), and different rates of addition of the aqueous phase into the oil phase.

An increase in the emulsifier concentration made the capsule-size distribution slightly narrower and sharper if the emulsifier concentration was higher than 1%, the minimum value needed for continuous emulsification to occur. Figure 3 shows the effect of the emulsifier concentration on the size distribution of the polyamide capsules prepared at an applied potential of 1000 v and a rate of aqueous phase addition of 0.015 ml/min.

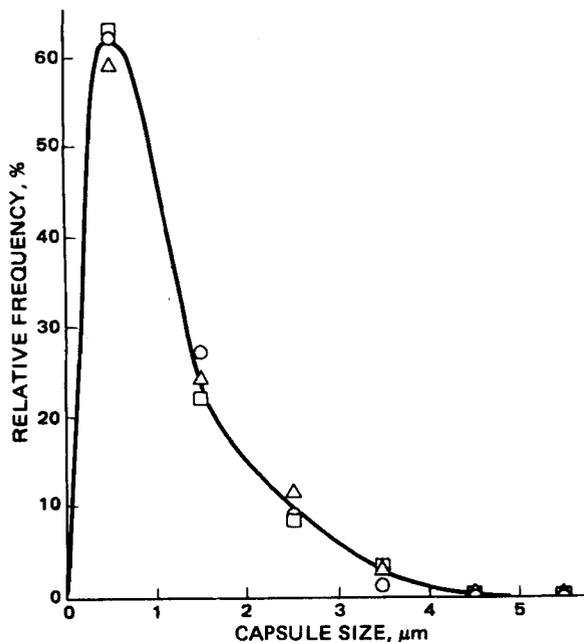[1] Coulter counter, Coulter Industrial Sales, Elmhurst, Ill.

Figure 4—*Effect of applied potential on the size distribution of polyamide capsules. Key:* O, *200 v;* △, *500 v; and* □, *1000 v.*

No significant effect of applied potential on the size distribution was observed provided that the potential was higher than the critical voltage of emulsification, which was <200 v for the system studied in this work (Fig. 4). The emulsifier concentration and the rate of addition of the aqueous phase were fixed at 5% and 0.015 ml/min, respectively.

The capsule-size distribution was affected favorably by increasing temperature. Capsules prepared at high temperature had a narrower and sharper size distribution than those prepared at low temperature. Figure 5 shows the size distribution curves of the capsules prepared at different temperatures using a 5% emulsifier concentration, a 1000-v applied potential, and a 0.015-ml/min rate of addition.

Enzymatic Activity—Since low concentrations of sorbitan trioleate did not prevent catalase and carbonic anhydrase in the hemolysate from losing enzymatic activity, the emulsifier in electrocapillary emulsification was used at 5%, which was high enough to minimize reduction in the activities caused by contact with the organic solvent.

With increasing applied potential, the enzymatic activity of catalase in the hemolysate initially decreased and then leveled off (Fig. 6). Similar results were obtained with carbonic anhydrase in the hemolysate droplets (Fig. 7).

Enzymatic activities of encapsulated catalase and carbonic anhydrase were kept at ~60% of the original values (immediately after encapsulation) after 40 days of storage in a refrigerator.
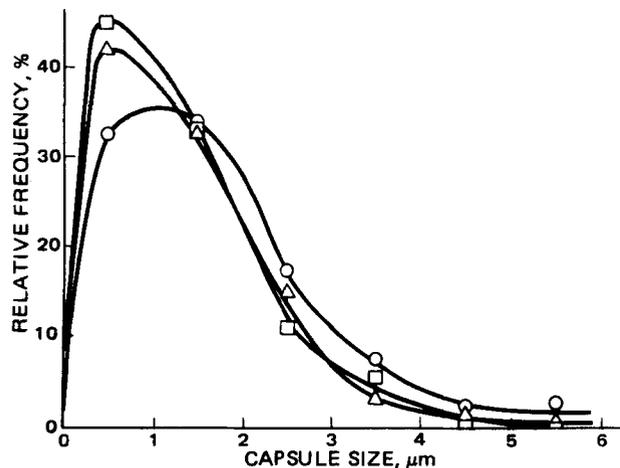

Figure 5—*Effect of temperature on the size distribution of polyamide capsules. Key:* O, *2°;* △, *20°; and* □, *40°.*
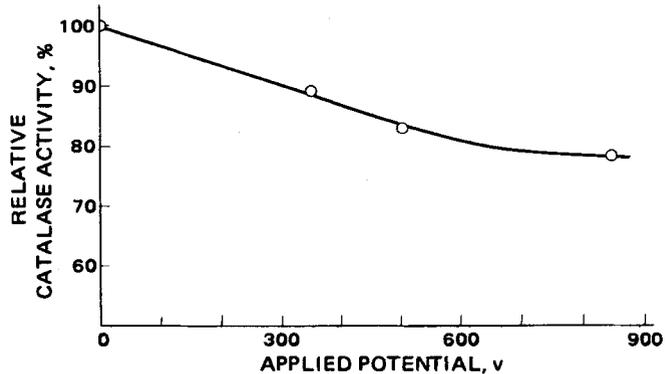

Figure 6—*Effect of applied potential on catalase activity in hemolysate.*

DISCUSSION

An increase in the addition rate of the hemolysate probably increases the drop size and the drop formation rate due to gravity acting on the drops being formed at the tip of the needle. This result causes incomplete electrocapillary emulsification, which leads to capsules with a broad size distribution. Consequently, to obtain capsules with a narrow size distribution and a small mean diameter, the rate at which hemolysate is introduced into the oil phase should be lower than a certain definite value.

In general, the emulsifier concentration is important in preparing an emulsion with a narrow and sharp size distribution. However, the role of emulsifier in this case is to prevent coalescence of primary hemolysate droplets formed in electrocapillary emulsification, rather than allowing the aqueous phase to disperse easily as fine droplets into the oil phase, since the interfacial tension is almost zero due to the applied potential. Therefore, the emulsifier concentration should only be enough to cover the surface of the primary droplets.

Since the interfacial tension between the hemolysate and the organic phase is lowered almost to zero by an applied potential that is higher than the critical voltage of emulsification in the presence of a small amount of emulsifier, there is no significant effect of the applied potential on the size distribution when the potential is increased beyond the critical value.

Several factors are affected by temperature change. The interfacial fluctuation increases as the temperature rises, which leads to formation of hemolysate droplets of smaller size at high temperature than those obtained at low temperature. However, this effect will not be significant if a sufficiently high potential is applied to the interface and a small amount of emulsifier is present.

The rise in temperature also decreases the viscosity of the oil phase, suggesting that hemolysate droplets coalesce to form larger drops through collision of the primary droplets. This effect is countered by the formation of a polymer membrane on the surface of the primary droplets. The rate of polymer formation is dependent on the temperature and transfer of water-soluble reactant into the oil phase from the aqueous phase. These factors can affect the capsule size to a considerable extent (6).

Transfer of L-lysine is affected by the emulsifier concentration in the oil phase and temperature (Fig. 8). At a fixed concentration of sorbitan
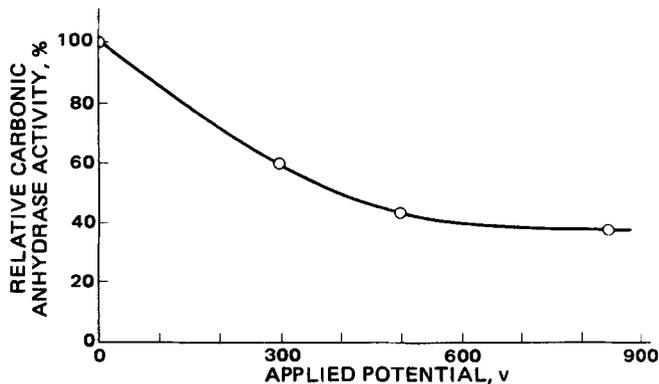

Figure 7—*Effect of applied potential on carbonic anhydrase activity in hemolysate.*
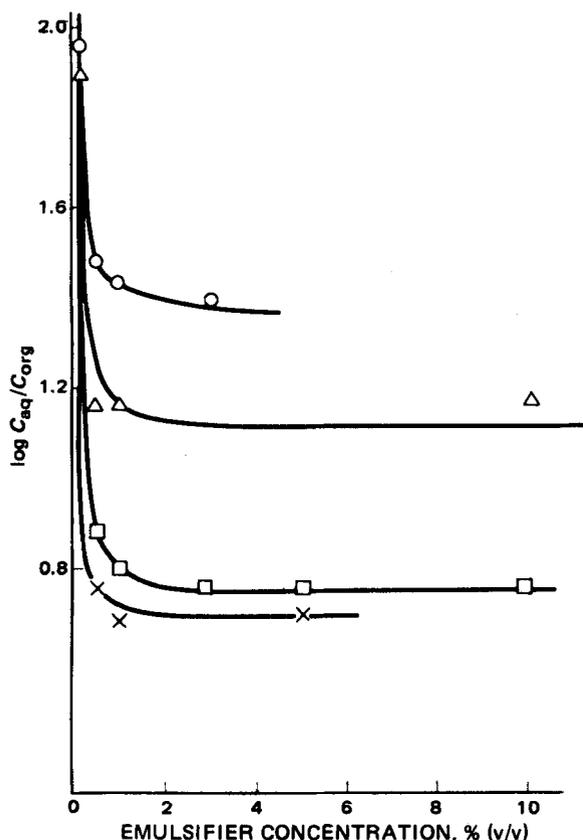
Figure 8—*Transfer of L-lysine into the oil phase from the aqueous phase as a function of temperature and emulsifier concentration. Key: O, 20°, 800 v; Δ, 20°, 1000 v; □, 20°, 1000 v, stirred; and X, 2°, 1000 v.*

Figure 9—*Scanning electron micrograph of polyamide capsules.*

Application of electric potential to the interface between the aqueous and oil phases causes an oriented adsorption of the enzymes at the interface. The adsorption should increase the chance for the enzymes to contact the organic solvent and lose their activities (7).

trioleate, transfer of the amino acid is more noticeable at low temperature than at high temperature. However, since the amino acid concentration in the oil phase is determined a few minutes after the completion of emulsification, only cumulative values of the amino acid concentration are given in the the figure. In other words, no useful information is obtained from these data on the rate of transfer of the amino acid in a very short period, which presumably will be high at high temperature. In addition, the rate of polycondensation also will be high at high temperature. Therefore, it is not unreasonable to expect that the capsule-size distribution becomes narrower and sharper as temperature rises if a small amount of emulsifier is present to promote the amino acid transfer.

Based on the previous discussion, hemolysate-loaded polyamide capsules with an average diameter of ~500 nm can be prepared. Figure 9 gives an example of a scanning electron micrograph of the capsules.
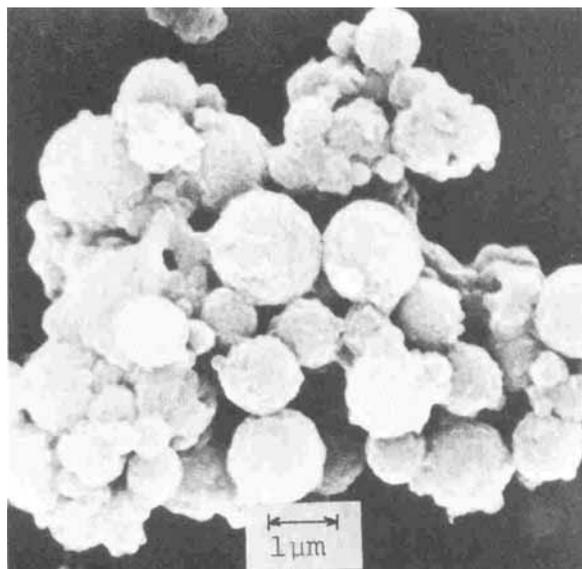
REFERENCES

(1) T. Kondo, M. Arakawa, and B. Tamamushi, in "Microencapsulation," J. R. Nixon, Ed., Dekker, New York, N.Y., 1976, p. 163.

(2) M. Arakawa, T. Kondo, and B. Tamamushi, *Biorheology*, 12, 57 (1975).

(3) M. Arakawa and T. Kondo, *Can. J. Physiol. Pharmacol.*, 55, 1378 (1977).

(4) A. Watanabe, K. Higashitsuji, and K. Nishizawa, in "Colloidal Dispersion and Micellar Behavior," K. L. Mittal, Ed., American Chemical Society, Washington, D.C., 1975, p. 97.

(5) A. Watanabe, K. Higashitsuji, and K. Nishizawa, *J. Colloid Interface Sci.*, 64, 278 (1978).

(6) Y. Shigeri, M. Koishi, T. Kondo, M. Shiba, and S. Tomioka, *Can. J. Chem.*, 48, 2047 (1970).

(7) T. Kondo and N. Muramatsu, in "Microencapsulation," J. R. Nixon, Ed., Dekker, New York, N.Y., 1976, p. 67.

ACKNOWLEDGMENTS