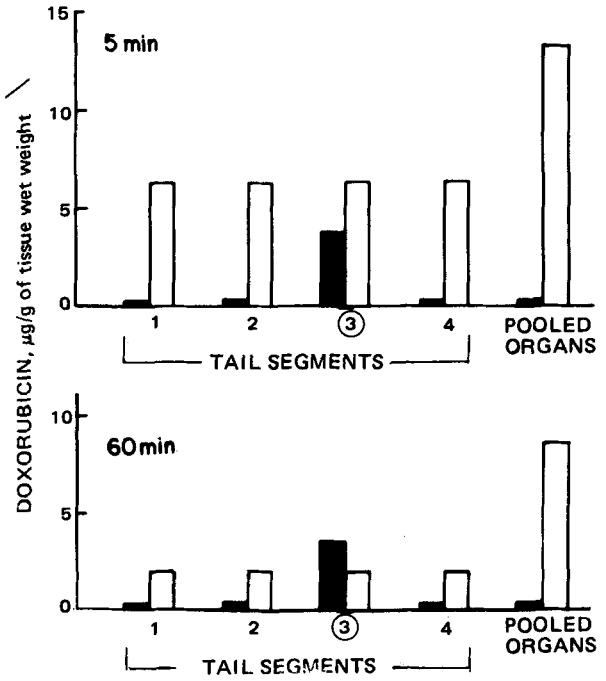

Figure 1—*Doxorubicin tissue concentration was determined spectrophotofluorometrically after infusion of either 5 mg of free doxorubicin/kg iv (□) or 0.05 mg of doxorubicin/kg encapsulated in magnetic microspheres via the caudal artery (■). Each value represents the average of two animals.*

min whereas the actual value found by spectrophotofluorometric methods was 3.7 μg/g.

The doxorubicin assay employed in these experiments was limited to determining the total tissue fluorescence. Adriamycinol, an active metabolite, accounts for a percentage of the total fluorescence. However, this metabolite, which is produced by the action of an ubiquitous intracellular aldo–keto oxidoreductase, is minimally active in rats in comparison to humans or monkeys (15). The magnetic sequestration of carrier-delivered doxorubicin from systemic circulation thus could be beneficial in eliminating many of the known acute and chronic toxic side effects. Although the data indicate a good correlation between carrier and drug distribution, the question of bioavailability of carrier-delivered doxorubicin has yet to be determined in a tumor model test system. However, analysis *in situ* by fluorescence microscopy of the microspheres 30 min postinjection at the target site suggested a radial diffusion of doxorubicin from the microspheres into the surrounding tissues. The rapid cellular uptake of doxorubicin that occurs as early as 0.5 min postinjection (10) should facilitate its diffusion into the cells once it is released from the microspheres.

In conclusion, magnetic targeting of chemotherapeutic agents to known sites of disease should be feasible with many currently available antitumor agents. As late as 60 min postinjection, 1% of a free intravenous dose of doxorubicin magnetically localized resulted in almost twice the local tissue concentration than was achieved by a 100-fold higher intravenous dose. The bioavailability of the localized drug is currently under investigation in a tumor model system.

REFERENCES

(1) T. M. Chang, *Trans. Am. Soc. Intern. Organs*, **12**, 13 (1966).

(2) G. Gregoriadis and B. D. Ryman, *Biochem. J.*, **124**, 58 (1971).

(3) D. A. Tyrrell and B. D. Ryman, *Biochem. Soc. Trans.*, **4**, 677 (1976).

(4) G. Gregoriadis and E. D. Neerunjun, *Res. Commun. Chem. Pathol.*, in press.

(5) K. J. Widder, A. E. Senyei, and D. F. Ranney, in "Advances in Pharmacology and Chemotherapy," S. Garattini, A. Goldin, F. Hawking, I. J. Kopin, and R. J. Schnitzer, Eds., vol. 16, Academic, New York, N.Y., 1979, p. 213.

(6) K. J. Widder, A. E. Senyei, and D. G. Scarpelli, *Proc. Soc. Exp. Biol. Med.*, **158**, 141 (1978).

(7) K. J. Widder, G. Flouret, and A. E. Senyei, *J. Pharm. Sci.*, **68**, 79 (1979).

(8) A. E. Senyei, K. J. Widder, and G. Czerlinski, *J. Appl. Phys.*, **49**, 3578 (1978).

(9) N. R. Bachur, A. L. Moore, J. G. Bernstein, and A. Liu, *Cancer Chemother. Rep.*, **54**, 89 (1970).

(10) M. J. Egorin, R. C. Hildebrand, E. F. Cimino, and N. R. Bachur, *Cancer Res.*, **34**, 2243 (1974).

(11) B. A. Rhodes, I. Zolle, J. W. Buchanan, and H. N. Wagner, Jr., *Radiology*, **92**, 1453 (1969).

(12) V. M. Petrier, T. R. Bochkovz, D. G. Khachirov, and S. V. Seryi, *Med. Radiol.*, **21**, 39 (1976).

(13) N. R. Bachur, M. J. Egorin, and R. C. Hildebrand, *Biochem. Med.* **8**, 352 (1973).

(14) S. D. Reich, *Pharmacol. Ther. Part C*, **2**, 239 (1978).

(15) P. M. Wilkinson, M. Israel, W. J. Pegg, and E. Frei, III, *Cancer Chemother. Pharmacol.*, **2**, 121 (1979).

ACKNOWLEDGMENTS

Supported in part by the Northwestern University Cancer Center, the Department of Pathology, and the American Cancer Society, Illinois Division.

The authors thank Dr. Raja Bahu for preparation of the specimens for fluorescence microscopy and Leila Schneitzer for preparation of the manuscript.

Preparation and Release Characteristics of Potassium Chloride Microcapsules

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Received August 2, 1979, from the *Department of Pharmacy, Chelsea College, University of London, Manresa Road, London SW3 6LX, England.* Accepted for publication October 16, 1980.

Abstract □ The release characteristics of potassium chloride were studied when it was coated with a selection of polymers; from the results obtained, a suitable batch was microencapsulated using a gelatin–gum arabic coacervate system. The microencapsulated product offers better controlled release for this drug when compared to standard tablet and powder forms.

Keyphrases □ Potassium chloride—preparation and release characteristics of microcapsules □ Polymers—various polymers and waxes investigated for microencapsulation of potassium chloride □ Microencapsulation—symposium, preparation and release characteristics of potassium chloride microcapsules □ Drug release—potassium chloride microcapsules, preparation and release characteristics

The satisfactory control of drugs in tablet or powder form from protective membranes is often difficult to achieve (1, 2). The present work investigated the microencapsulation of a material that is usually given in a

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Table I—Release Data for Potassium Chloride from Various Coatings prior to Microencapsulation

Coating Material	Drug Released with Time, %	
	2 min	5 min
Cellulose acetate phthalate	42	80
Cellulose acetate phthalate and wax coat	20	48
Cellulose acetate phthalate over wax matrix	37	71
Hydroxypropyl methylcellulose	90	100
Hydroxypropyl methylcellulose and wax coat	30	65
Hydroxypropyl methylcellulose over wax matrix	30	66
Cellulose acetate phthalate–ethylcellulose (1:1)	40	70
Cellulose acetate phthalate–ethylcellulose (4:1)	70	90
Ethylcellulose	56	84
Cellulose acetate phthalate–ethylcellulose (1:1) and wax coat	40	68
Ethylcellulose and wax coat	28	67

slow-release form. Potassium chloride was chosen because it presents the risk that a large initial dose, or continuing smaller doses, will result in ulceration of the gut and in other side effects (3–8).

BACKGROUND

It was anticipated that a protective membrane around the drug crystals could result in a more satisfactory controlled release than is possible with conventional tableted forms, which often incorporate film polymers or sugar protective membranes in their outer portion. The gelatin–acacia coacervate microcapsule (9) was chosen as the protective membrane system because many of the coating technique parameters can be controlled. This system is normally limited to use with water-insoluble drugs. To coat a water-soluble drug with this coacervate membrane, the crystalline material first must be protected from the aqueous environment.

A selection of polymers and waxes possessing both enteric and nonenteric properties was researched for this preliminary coat. They had to offer sufficient protection to the potassium chloride crystals and retard solution time to allow coating by the coacervate. Drug coated with these polymers did not constitute a microcapsule. The gelatin–acacia coacervate coat was required for connection with tableting studies on other diuretic drugs.

EXPERIMENTAL

Materials—The polymers and solvents were cellulose acetate phthalate[1] in ethanol–acetone, hydroxypropyl methylcellulose[2] in ethanol–chloroform, cellulose acetate butyrate[3] in propylene glycol–acetone, and ethylcellulose[4] in ethanol. All concentrations were kept at 8% (w/v). Carnauba wax and stearic acid were used in the wax investigations. Materials for microencapsulation were acacia BP and gelatin acid (Bloom 250) at a concentration of 2% (w/v). The solvent was distilled water.

Polymer Coating of Crystals—Potassium chloride, previously dried at 80° for 30 min, was placed in a spray-coating pan and several polymer coats were applied. The first coat consisted of 25 ml of 8% polymer solution with a 50-g charge of potassium chloride. This coat was dried for 5 min in hot air at 60°. Subsequent coats (10 ml) of polymer solution were added and dried to give a total weight increase of 10–16%.

Wax Coating of Crystals—A combination of carnauba wax (10 g) and stearic acid (5 g) was melted, and the potassium chloride (100 g) was dispersed to give a uniform coat. This mixture was allowed to cool slowly in a revolving coating pan. Discrete pellets of wax-coated drug were obtained.

Polymer and Wax Coating of Crystals—The two previous methods were combined. The polymer-coated particles were subsequently wax treated to give up to 30% increase in weight.

Microencapsulation of Coated Crystals—Equal volumes of acacia and gelatin were prepared at a concentration of 2% in distilled water. The acacia solution was stirred at ~280 rpm at 41°, and the membrane-coated potassium chloride crystals were introduced. The gelatin solution, also at 41°, was added, and the pH was adjusted to 3.9. After satisfactory coating, formaldehyde was added, and the system was cooled to 5° to fix the coat. The microcapsules then were filtered and dried with ether. The extent of potassium chloride leakage was measured by flame photometry.

Potassium Chloride Assay—A flame photometer was calibrated using standard solutions of potassium chloride in distilled water. The solutions consisted of 50, 40, 30, 20, and 10 mg of potassium chloride dissolved in 1 liter of distilled water.

In dissolution studies, the sample was diluted 1 in 10 prior to passing through the photometer. Six independent readings were taken on each sample. To determine the total quantity of potassium chloride in the coated crystals and microcapsules, necessary for expressing the percent released, a 50-mg sample was refluxed with water prior to flame photometry.

Dissolution Studies—*Preliminary Studies of Polymer-Wax-Coated Crystals*—The *in vitro* dissolution of the coated crystals was studied at pH 2.0 using 2 liters of dissolution medium at 37° and 500 mg of coated drug. The stirrer speed was 100 rpm. Suitable aliquots were taken at 1-min intervals, diluted where necessary, and assayed for potassium chloride content.

In Vitro *Dissolution of Microcapsules*—A similar technique was used for the microcapsules. Five hundred milligrams of the microcapsules containing 310 mg of potassium chloride was dispersed in 2 liters of water adjusted to pH 2.0 with hydrochloric acid. Suitable aliquots were taken, diluted where necessary, and assayed for drug content using the flame photometer.

In Vivo *Studies on Coacervate-Coated Potassium Chloride*—A baseline for potassium chloride in urine was determined in four healthy volunteers over 48 hr. No special dietary instructions were given other than the avoidance of alcoholic drinks. Subsequently, 1 g of microcapsules containing 620 mg of potassium chloride in powder form was given. Urine samples were collected, measured, and assayed over 6 hr.

RESULTS AND DISCUSSION

Prior to microencapsulation by the gelatin–acacia coacervate system, it was necessary to protect the water-soluble potassium chloride from the aqueous environment. Cellulose acetate phthalate was chosen initially because its enteric properties prevent solution at the acidic pH of coacervation. The coated material was assayed for active content and contained an average of 86% potassium chloride. The total input of core material could be accounted for within the coated crystals.

The *in vitro* dissolution of the coated crystals was studied at pH 2.0 prior to microencapsulation. Table I shows that 42% of the drug was released after 2 min and that 80% was released after 5 min. This rapid release from cellulose acetate phthalate-coated potassium chloride was due in part to the large surface area available for solution and to the fact that cellulose acetate phthalate acts as a permeable membrane in the presence of ionic solutions. Under these pH conditions, it releases potassium chloride rapidly.

Although the coated particles do not provide any delayed action, they were to be used as a basis for microencapsulation because the gelatin–acacia coacervate forms almost immediately and incorporation of the coated crystals is complete before potassium chloride leaches out. In this way, it is possible to microencapsulate a water-soluble drug within a gelatin–acacia coacervate coat.

When the normal microencapsulation process (7) was attempted, microscopic observation showed numerous small coacervate droplets. Some drug had been encapsulated, but a high percentage had gone into solution, causing salting-out effects on the coacervate. A decrease in the turbidity of the system also indicated a gradual loss of coacervate, although it was not destroyed completely. This result is similar to the influences of electrolytes on the complex coacervate system reported previously (10, 11).

Loss of drug was expected to be excessive. However, the microencapsulation was continued, formaldehyde was added prior to cooling the system to 5°, and the microcapsules were extracted in the usual way.

Assay of these microcapsules yielded only a 12% potassium chloride content. The remainder was in the separated aqueous phase, so all of the drug was accounted for. Subsequent attempts to improve the quantity encapsulated were equally unsatisfactory. Large inputs, because of the increased aqueous potassium chloride concentrations that resulted, produced complete destruction of the coacervate. Attempts to use oil

[1] Eastman Kodak Co., Rochester, NY 14650.
[2] Imperial Chemical Industries, Nobel Division, Stevenston Ayrshire, Scotland.
[3] Eastman Chemical Products.
[4] British Drug Houses, Poole, Dorset, England.

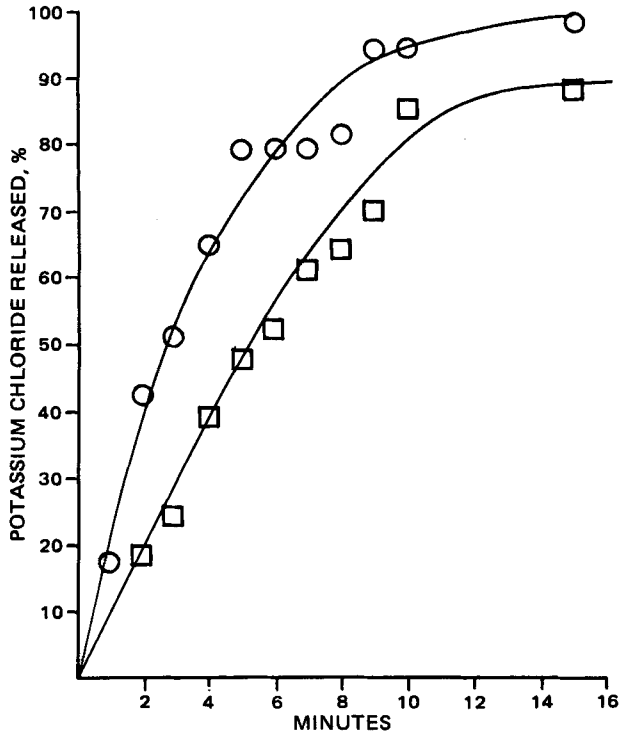

Figure 1—*Release of potassium chloride from cellulose acetate phthalate membrane and cellulose acetate phthalate membrane with outer wax coat at pH 2.0 in 2 liters at 37°. Key:* ○, *cellulose acetate phthalate membrane over potassium chloride, and* □, *cellulose acetate phthalate membrane and outer wax coat over potassium chloride.*

suspensions of the coated drug were equally unproductive.

A coating formulation employing cellulose acetate phthalate was unsatisfactory. Additional coating to retard water penetration through the polymer membrane was desirable, and carnauba wax and stearic acid were investigated. The carnauba wax would retard water penetration, and the stearic acid would act as a lubricant, which would prevent aggregation of the wax-coated particles. Both compounds also show enteric properties. The increase in weight due to this extra coating was 30%.

An *in vitro* dissolution test was performed at pH 2.0 to study the release characteristics in acidic conditions. Figure 1 shows that the application of a carnauba wax–stearic acid–cellulose acetate phthalate coat retarded drug dissolution. After 5 min, 48% of the drug was released compared to 80% with only a coat of cellulose acetate phthalate. However, when microencapsulation was attempted, except at a low potassium chloride concentration, further additions of wax coats to the polymer did not significantly improve either the percent of potassium chloride encapsulated or the release characteristics.

In a final attempt to use cellulose acetate phthalate, potassium chloride was incorporated in the wax matrix, resulting in larger sized particles. These particles were film coated with the same polymer concentration as was used previously. Dissolution at pH 2.0 was studied. Table I shows that 71% of the drug was released after 5 min. This procedure was less satisfactory than the previous applications of a wax coat applied to a polymer-covered particle and, therefore, would not be expected to produce satisfactory microcapsules.

Since successful microencapsulation was not achieved with any of the cellulose acetate phthalate-coated particles, different polymer coatings were studied. It was hoped that they would show a slower drug release in the presence of aqueous ionic systems.

Cellulose acetate butyrate (17% butyrate) was investigated, but release from this polymer was significantly faster than with cellulose acetate phthalate. With hydroxypropyl methylcellulose, release was rapid but slightly slower than with the cellulose acetate butyrate. Drug release was 100% after 4 min when a simple polymer-coated particle was studied. A carnauba wax–stearic acid coat to this system produced a 65% release of drug after 5 min of dissolution. Potassium chloride incorporated in a wax matrix and film coated with hydroxypropyl methylcellulose produced a slightly slower release of drug than cellulose acetate phthalate but not to any significant extent.

Although some of the polymer formulations retained large quantities of potassium chloride during the important first few minutes of coacervation, none produced satisfactory microcapsules due to the salt effect. Thus, a more hydrophobic preliminary coating was needed. Ethylcellulose was investigated because it is more hydrophobic than cellulose acetate phthalate but will mix with the latter. It was expected to improve the release characteristics and produce a smaller salt effect.

Three formulations were prepared using four parts of cellulose acetate phthalate to one part of ethylcellulose, equal parts of both polymers, and ethylcellulose alone; the overall polymer concentrations were unchanged from those used previously. The functioning of these multipolymer systems could be compared with that of the cellulose acetate phthalate coats.

Partial *in vitro* dissolutions at pH 2.0 are shown in Table I. A mixture of the two polymers (1:1) offered the slowest release. However, the batch containing ethylcellulose alone was not significantly faster, and these two systems were studied further by coating with a wax matrix.

The batch containing ethylcellulose plus a wax coat released 67% of the drug after 5 min, and that containing the mixed polymers and wax coat released 68% (Table I). Both formulations showed similar release. However, the batch containing ethylcellulose and a wax coat released potassium chloride more slowly in the initial stages, and it was this batch that subsequently produced a satisfactory microcapsule. Although the cellulose acetate phthalate formulation had a slower release when given a wax coat (Table I), it could not be used because of problems arising during coacervation due to the ionic environment.

Before subjecting the ethylcellulose–wax-coated drug to the complex coacervate system, possible changes in the microencapsulation process itself were considered. The objective was to introduce the coated cores to the coacervate system in such a way as to ensure that they had a protective gelatin–acacia membrane surrounding them before the potassium chloride concentration leached into the equilibrium liquid could suppress coacervation. Reduction of coacervate volumes to a total of 100 ml produced a suitable handling level at the extraction stage. With the small bench quantities used in this investigation, only about 30 sec was required for the core material to be incorporated within the coacervate. Reduction of temperature to fix this coat and prevent drug diffusion from the microcapsules could be rapid; at 25°, formaldehyde was added. On reaching 5°, the microcapsules were filtered and dried with ether. The core content of potassium chloride obtained corresponded to 61% of the initial input.

The *in vitro* dissolution of these microcapsules at pH 2.0 is shown in

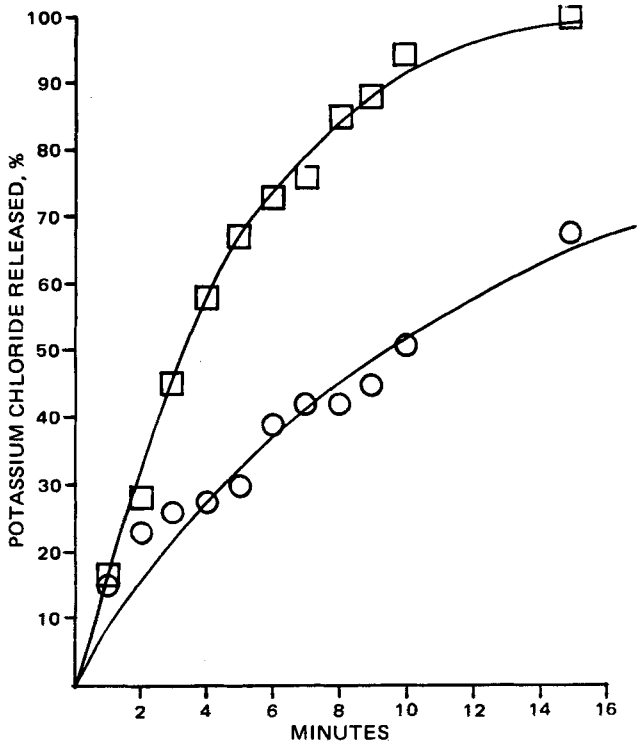

Figure 2—*Release of potassium chloride from microcapsules of ethylcellulose–wax-coated potassium chloride and release from unmicroencapsulated potassium chloride at pH 2.0 in 2 liters at 37°. Key:* ○, *microcapsules; and* □, *nonmicrocapsules.*

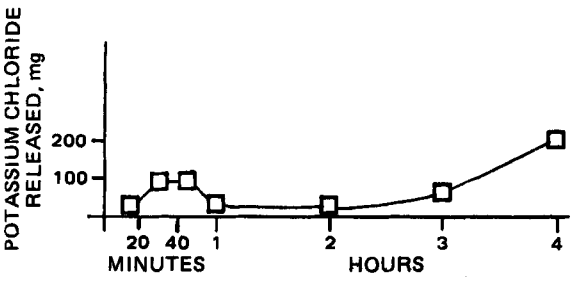

Figure 3—*Mean differential release of potassium chloride.*

Fig. 2 along with the release characterstics of the ethylcellulose–wax-coated potassium chloride. The microencapsulated material had a slower release under these *in vitro* conditions. The total potassium chloride was released from the microcapsules after 1 hr.

The *in vivo* performances of these microcapsules is being investigated. Several delivery systems are being studied including tableting, and preliminary results show a significant delayed release of potassium chloride in some cases. Some preliminary *in vivo* release results are shown in Fig. 3 where the mean differential release of potassium chloride microcapsules prior to any formulatory processes is plotted. Over the first 30–40 min after administration of the microencapsulated drug, there was a significant increase in released potassium chloride above that found under normal excretory conditions. It is believed that this increase corresponds to loosely held potassium chloride associated with the wall of the microcapsule, possibly in solid solution. This initial release shows a quiescent period when the drug is probably diffusing toward the outside of the capsule but the potassium chloride found in urine is only that normally excreted. After 2 hr, the differential potassium chloride begins to show a progressive increase as the drug in the center of the microcapsule diffuses out.

In summary, whether a drug is water soluble or not, it can be microencapsulated using the gelatin–gum arabic coacervate system. This system has many advantages due to the wide range of controllable parameters that the system allows.

REFERENCES

(1) G. K. Noren, G. K. Korpi, and G. J. England, *J. Appl. Polym. Sci.*, **24**, 2369 (1979).

(2) L. N. Sansom, R. W. Milne, and D. Cooper, *Eur. J. Clin. Pharmacol.*, **16**, 417 (1979).

(3) J. I. Kanig, *Drug Stand.*, **22**, 113 (1954).

(4) U.S. Department of Health, Education, and Welfare, cited in *Pharm. J.*, **2**, 183 (1965).

(5) B. R. McAvoy, *Br. Med. J.*, **4**, 164 (1974).

(6) F. Sandor, *J. R. Coll. Gen. Pract.*, **26**, 595 (1976).

(7) R. L. Tanner and A. Cordano, *J. Pharmacol. Exp. Ther.*, **204**, 240 (1978).

(8) B. P. Block and M. B. Thomas, *J. Pharm. Pharmacol., Suppl.*, **30**, 70p (1978).

(9) J. R. Nixon, S. A. H. Khalil, and J. E. Carless, *J. Pharm. Pharmacol.*, **18**, 409 (1966). *Ibid.*, **20**, 348, 528 (1968).

(10) H. G. Bungenberg De Jong, E. G. Haskam, and L. H. V. D. Brandhof-Schaegen, *Proc. K. Ned. Akad. Wet.*, **44**, 1104 (1941).

(11) N. Yashida and C. Thies, *J. Colloid Interface Sci.*, **24**, 29 (1967).