

## Effect of Acid Type, Acetic Acid and Sodium Carboxymethyl Cellulose Concentrations on the Formation, Micromeritic, Dissolution and Floating Properties of Theophylline Chitosan Microcapsules

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An emulsion-phase separation method was devised to prepare chitosan microcapsules containing theophylline and sodium carboxymethyl cellulose (Na CMC). The effect of acid type (acetic acid, ascorbic acid or citric acid) and Na CMC concentration on the formation, micromeritic property, release behavior and floating phenomenon of chitosan microcapsules was studied. Chitosan microcapsules prepared using an acetic acid aqueous solution as a solvent showed a more compact and less porous structure, and exhibited slow release action when compared with other chitosan microcapsules made from ascorbic acid or citric acid. We also found that the greater the amount of Na CMC used, the slower the release and the larger the particle size of the microcapsules obtained. The acetic acid concentration significantly influenced the formation, micromeritic property and release behavior of theophylline chitosan microcapsules containing Na CMC. When the acetic acid concentration was less than 30%, chitosan microcapsules did not form. The particle size of the microcapsules decreased with the increase of acetic acid concentration. Moreover, the higher the acetic acid concentration used the faster was the release rate of microcapsules. The interaction between chitosan and Na CMC in microcapsules caused the formation of a water-insoluble complex, and this complex might significantly affect the formation, micromeritic property and release behavior of chitosan microcapsules. The acetic acid and Na CMC concentrations also played an important role in controlling the floating property of the microcapsules.

**Keywords** chitosan microcapsules; emulsion-phase separation; theophylline; acetic acid; sodium carboxymethyl cellulose; insoluble complex; floating; sustained-release

### Introduction

Chitosan is a natural polyaminosaccharide prepared from chitin by N-deacetylation with alkali.<sup>1)</sup> Since chitin is insoluble in water and common organic solvents, its utility is limited. Chitosan is a weak base, and is soluble in a dilute acid solution which can convert the glucosamine units to a soluble form, R-NH<sub>3</sub><sup>+</sup>, thus increasing its utilization.<sup>2)</sup> Chitosan acts primarily as a flocculant for the treatment of waste water,<sup>3)</sup> it has recently also been used in biomedical and pharmaceutical fields because of its favorable properties of biodegradability, low toxicity and good biocompatibility.<sup>4,5)</sup> Chitosan has been used biomedically for hypocholesterolemic effects, antacid and antiulcer activities, wound and burn healing, soft and hard contact lenses, artificial organ membrane, and immobilization of enzymes and living cells.<sup>6-10)</sup> In pharmaceutical applications, it has been designed to act as a direct-compression diluent, a new vehicle or drug carrier for sustained release preparations or a floating oral drug delivery system, as well as a co-grinding diluent for the enhancement of dissolution rate and bioavailability of water-insoluble drugs.<sup>11-17)</sup>

A few investigations have been conducted on the preparation of chitosan microcapsules or microspheres. Kawashima *et al.*<sup>18)</sup> and Bodmeier *et al.*<sup>19)</sup> used a polyelectrolyte complexation method between chitosan and triphosphosphate to prepare sustained-release theophylline granules or sulfadiazine beads. Nishioka *et al.* added chitosan to albumin microspheres to retard the drug release.<sup>20)</sup> Several studies have focused on microencapsulation with chitosan and sodium carboxymethyl cellulose (Na CMC) or alginate.<sup>10,21,22)</sup> Recently, Lin and his coworkers used an emulsion-solvent evaporation method to prepare vaccine enteric-coated microcapsules in order to avoid gastric acid instabilization of vaccine.<sup>23,24)</sup> Maharaj *et al.*<sup>25)</sup> and Gupta and Rao<sup>26)</sup> prepared viral antigen, concanavallin A and vitamin B-12 microcapsules by the emulsion-solvent evaporation method in liquid paraffin

using corn starch and mannitol as diluent to form more desirable, free-flowing and spherical microcapsules. With these emulsion-solvent evaporation methods, acetone and/or ethanol are the commonly used organic solvents for easy evaporation. When chitosan is chosen to serve as the membrane for microcapsule, however, its solvent is acidic water which does not evaporate easily.

Thus in our preliminary experimentation, we found the emulsion-phase separation method could be used to prepare chitosan microcapsules in liquid paraffin. Na CMC was used as a diluent to improve microcapsule formation. The objective of this study was to investigate the effects of variables such as acid type, acetic acid and Na CMC concentrations on the formation and particle size distribution of chitosan microcapsules prepared by emulsion-phase separation method, and on the drug release from chitosan microcapsules. The effect of acetic acid and Na CMC concentrations on the morphology and floating properties of chitosan microcapsules was also examined. The possible interaction between Na CMC and chitosan in microcapsules was studied by infrared (IR) spectrophotometry.

### Materials and Methods

**Materials** Chitosan (Flonac N, Kyowa Yushi Co., Tokyo, Japan) with 89.3% deacetylation was micronized and used after screening with a 60 mesh sieve. Theophylline and Na CMC were purchased from Nacalai Tesque Inc., Kyoto, Japan and were micronized (>100 mesh) for use. Span 20 was obtained from Wako Pure Chem. Ind., Osaka, Japan. All other materials were of analytical reagent grade.

**Studies on Compatibility of Chitosan Acidic Solution with Organic Solvent** The 1.67% (w/v) chitosan acidic solution was prepared by dissolving 0.25 g of micronized chitosan in 15 ml of 2% acidic solution, in which HCl, acetic acid, ascorbic acid or citric acid was used as acid source. An organic solvent such as methanol, absolute ethanol or acetone was respectively added to each chitosan acidic solution and the precipitation phenomenon was observed.

**Preparation of Chitosan Microcapsules Containing Na CMC and Theophylline** Theophylline chitosan microcapsules were prepared by the emulsion-phase separation method at room temperature. A certain amount

of Na CMC powder was previously suspended in 400 ml of liquid paraffin (viscosity: 32 cps) containing 0.7% of span 20, then 40 ml of different acetic acid concentrations of chitosan solution containing 20 ml of methanol and 5.3 g of theophylline were added to the liquid paraffin system to form a water-in-oil (W/O) emulsion. Stirring was done at a speed of 600 rpm for 1 h to settle the particles of emulsion. Thereafter, a total volume of 200 ml ethyl acetate was added in drops to the above system and the phase separation occurred at the interface of the W/O emulsion to form a hard-shelled microcapsule. The suspending medium was decanted, and the microcapsules were resuspended in 100 ml ethyl acetate in order to remove the residual oil. The shell of the immobilized microcapsules continued to harden in the ethyl acetate. The microcapsules were collected *via* filtration, air-dried at ambient temperature and then vacuum-dried. The processing flow-chart of microencapsulation of theophylline by the emulsion-phase separation method is shown in Fig. 1. The effect of acid type and Na CMC amount on the formation of theophylline chitosan microcapsules was also investigated. The yield of microcapsules and theophylline content in each type of microcapsules was determined.

**Determination of Micromeritic Properties of Microcapsules** The particle size of chitosan microcapsules was determined by a sieving method. The surface topography of these microcapsules was observed by a scanning electron microscope (Hitachi S-2100, Tokyo, Japan).

**Preparation and Determination of Chitosan-Na CMC Insoluble Complex** The water-insoluble complex of chitosan-Na CMC was prepared as described in Fukuda's report.<sup>27)</sup> One percent of chitosan acetic acid solution (pH 3.0) was mixed with the same pH of Na CMC acidic solution (1%), and stirred for 24 h. A water-insoluble complex was precipitated. The precipitates were washed with 0.01 N HCl, water and methanol, and then vacuum-dried to a constant weight. The IR spectra of chitosan, Na CMC, insoluble complex of chitosan-Na CMC and chitosan microcapsules containing Na CMC but without theophylline were determined by IR spectrophotometer (IR-700, Jasco, Japan).

**Dissolution Study** The drug release test of each type of chitosan microcapsules (20–30 mesh), equivalent to 100 mg of theophylline, was carried out by a rotating-basket method in 900 ml distilled water at 37°C and 50 rpm. The theophylline content in the release solution was measured spectrophotometrically at 270 nm.

**In Vitro Floating Test** An *in vitro* floating test of chitosan microcapsules was performed in the dispersing medium [pH 1.2 solution, distilled water

(pH 5.7) or pH 6.8 solution, specified in JP XI disintegration test]. Each 100 particles of microcapsules (20–30 mesh) were spread over the surface of the dispersing medium (10 ml, 25°C) with gentle stirring. At prescribed intervals, the microcapsules floating on the surface of the dispersing medium were counted. The buoyancy of the microcapsules was represented by the following equation:

$$\text{buoyancy (\%)} = \frac{\text{no. of floating microcapsules}}{\text{total no. of microcapsules}} \times 100$$

## Results and Discussion

The solvent-evaporation method is one technique of microencapsulation in which organic solvent is easily evaporated from an emulsion system so that solid microcapsules are formed. It was difficult to use the solvent-evaporation method to prepare chitosan microcapsules in this study, however, because the acidic water was difficult to evaporate even by a vacuum operation. Here, the emulsion-phase separation method was devised in a two-step form: the first to form W/O emulsion and the second to improve phase separation by the addition of ethyl acetate.

**Compatibility of Different Acid Types of Chitosan Solution with Organic Solvents** Our preliminary study found that using chitosan acidic solution without organic solvent it was difficult to form spherical viscous droplets in liquid paraffin in which Na CMC had previously been suspended. Since organic solvents such as methanol, ethanol and acetone possess a hydrophilic property which is immiscible with hydrophobic liquid paraffin, if these organic solvents were separately added to liquid paraffin, it was natural that spherical drops of the solvent were formed by the interfacial phenomenon. This suggests that organic solvent might improve the formation of spherical chitosan coacervates in liquid paraffin. However, it is well-known that chitosan does not dissolve in organic solvent but only dissolves in aqueous organic acids.<sup>2)</sup> Thus we firstly screened the compatibility between organic solvents and chitosan acidic solution. Table I shows the compatibility of four acid types of chitosan solution with three organic solvents. Apparently there was no any precipitation between methanol and acetic acid, ascorbic acid or citric acid. The other organic solvents and chitosan acidic solution seemed incompatible as indicated by the formation of white precipitates.

**Effect of Acetic Acid Concentration on the Formation, Micromeritic Property and Release Behavior of Theophylline Chitosan Microcapsules** Table II indicates the effect of acetic acid concentration on the possible formation of

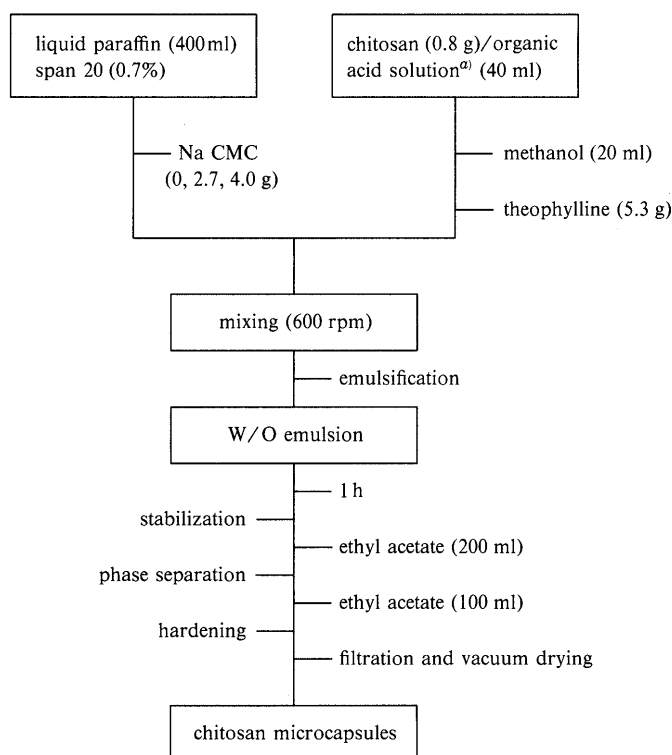


Fig. 1. Preparation of Theophylline Chitosan Microcapsules by Emulsion-Phase Separation Method

a) Organic acid; acetic acid, ascorbic acid or citric acid.

TABLE I. Compatibility between Chitosan Acidic Solution and Organic Solvents

Solvent	Methanol	Absolute ethanol	Acetone
HCl (2%, 15 ml)	+	++	+++
Acetic acid (2%, 15 ml)	—	—	+
Ascorbic acid (2%, 15 ml)	—	+	+++
Citric acid (2%, 15 ml)	—	++	++

Each acid solution contains 0.25 g chitosan. —: Indicates no white precipitate was obtained when amount of organic solvent was above 30 ml. +, ++, +++: Indicates white precipitate was obtained.

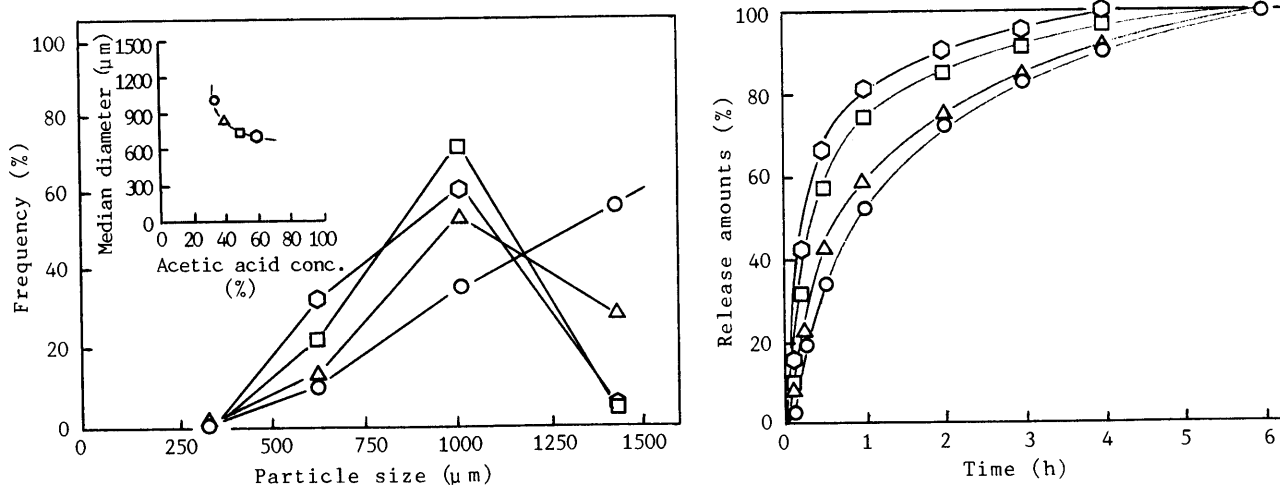


Fig. 2. The Effect of Acetic Acid Concentration on Particle Size Distribution and Dissolution of Theophylline Chitosan Microcapsules Containing Na CMC

Key: acetic acid concentration: ○, 35%; △, 40%; □, 50%; ◐, 60%.

TABLE II. Effect of Acetic Acid Concentration on the Possible Formation, Yield and Theophylline Content of Chitosan Microcapsules Prepared by Emulsion-Phase Separation Method in Liquid Paraffin

Concentration of acetic acid	28%	30%	35%	40%	50%	60%
pH	3.00	2.70	2.55	2.45	2.25	2.10
Formation of microcapsules <sup>b)</sup>	No	No <sup>a)</sup> Yes	Yes	Yes	Yes	Yes
Yield of microcapsules (%)	—	—	84.33	78.59	83.51	80.15
Theophylline content in microcapsules (%)	—	—	47.25 ±2.93	47.58 ±2.48	48.57 ±0.70	46.84 ±0.42

a) Indicates a partial microcapsule was formed. b) 2.7 g of Na CMC was previously suspended in liquid paraffin before microencapsulation.

chitosan microcapsules containing Na CMC and theophylline. When the concentration of acetic acid was <28%, W/O emulsion did not form but there was a cotton-like precipitate. When acetic acid concentration was 30%, only a partial W/O emulsion formed and this became a microcapsule after ethyl acetate was added. If acetic acid concentration was >35%, W/O emulsion was completely dispersed in liquid paraffin and finally formed a microcapsule with the addition of ethyl acetate. This suggests that acetic acid concentration and pH played an important role in the formation of chitosan microcapsules containing Na CMC and theophylline. The effect of acetic acid concentration and pH on the preparation of chitosan microcapsules might be attributable to the lower concentration of acetic acid (<35%) used being neutralized by Na CMC (with alkaline property) in liquid paraffin, leading to the precipitation of soluble chitosan. Thus W/O emulsion could not be prepared and it was impossible for microcapsules to form. However, the yield and theophylline content of microcapsules were similar, and were independent of the acetic acid concentration.

Figure 2 shows the acetic acid concentration affecting the particle size distribution and release behavior of theophylline chitosan microcapsules. As seen, the higher the acetic

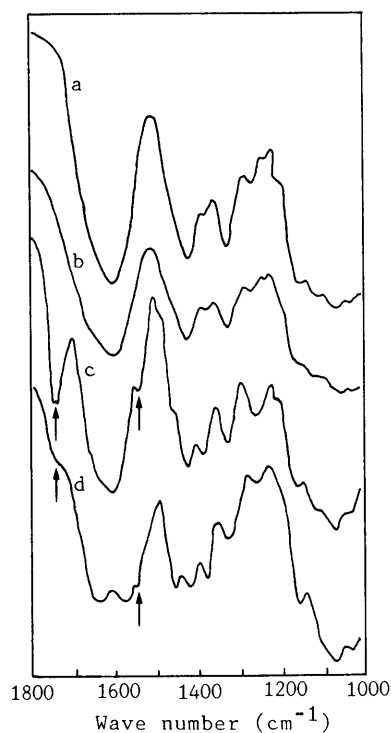


Fig. 3. IR Spectra of Chitosan (a), Na CMC (b), Chitosan-Na CMC Insoluble Complex (c) and Chitosan Microcapsules Containing Na CMC without Theophylline (d)

acid concentration used, the smaller was the particle size obtained. Moreover, the lower the acetic acid concentration, the slower was the release behavior. This might be due to the interaction of chitosan with Na CMC in the microcapsules. Fukuda<sup>27)</sup> and Monal and Covas<sup>28)</sup> have respectively reported that pH plays an important role in the preparation of the polyelectrolyte complex, *i.e.*, chitosan and Na CMC, and suggested that the optimum interaction pH should be within 2.5–5.0 for preparation of the water-insoluble complex.

In the present study, however, higher acetic acid concentration showed a lower pH value which was outside

of the optimal pH (2.5–5.0) (Table II), leading to a reduction of the interaction of chitosan and Na CMC.<sup>27)</sup> This resulted in small particle size as well as rapid release of chitosan microcapsules, and suggests that the water-insoluble complex was important in drug release. Figure 3 shows the IR spectra of water-insoluble complex made by chitosan and Na CMC, and chitosan microcapsules containing Na CMC but without theophylline. A strong absorption band around 1740 cm<sup>-1</sup>, assigned to -COOH, was found in the water-insoluble complex but a weak absorption band was also evident in the chitosan microcapsule. Another weak absorption band around 1520 cm<sup>-1</sup>, assigned to -NH<sub>3</sub><sup>+</sup>, was also found in both samples. This indicates that the wall of the microcapsules consisted of a small amount of insoluble-complex, chitosan and Na CMC, which, in turn, suggests that the water-insoluble complex of chitosan-Na CMC was formed in the preparation of chitosan microcapsules when Na CMC

was added as a diluent.

Figure 4 shows the surface topography of chitosan microcapsules prepared with different acetic acid solutions. Since the microcapsule wall was mixture of cellulose-like polymers such as chitosan, Na CMC and insoluble complex and was phased out by ethyl acetate, little encapsulating smooth film was obtained. Instead, there was a less porous and cracked phenomenon on the surface of chitosan microcapsules prepared with the lower concentration of acetic acid was obtained. The interaction between chitosan and Na CMC occurring in the microcapsules might be responsible for this compact structure.

**Effect of Acid Type and Na CMC Concentration on the Micromeritic Properties and Release Behavior of Chitosan Microcapsules** Effect of acid type on the particle size and release amount of theophylline chitosan microcapsules is shown in Fig. 5. It is apparent that the microcapsules prepared with ascorbic acid were larger in particle size than

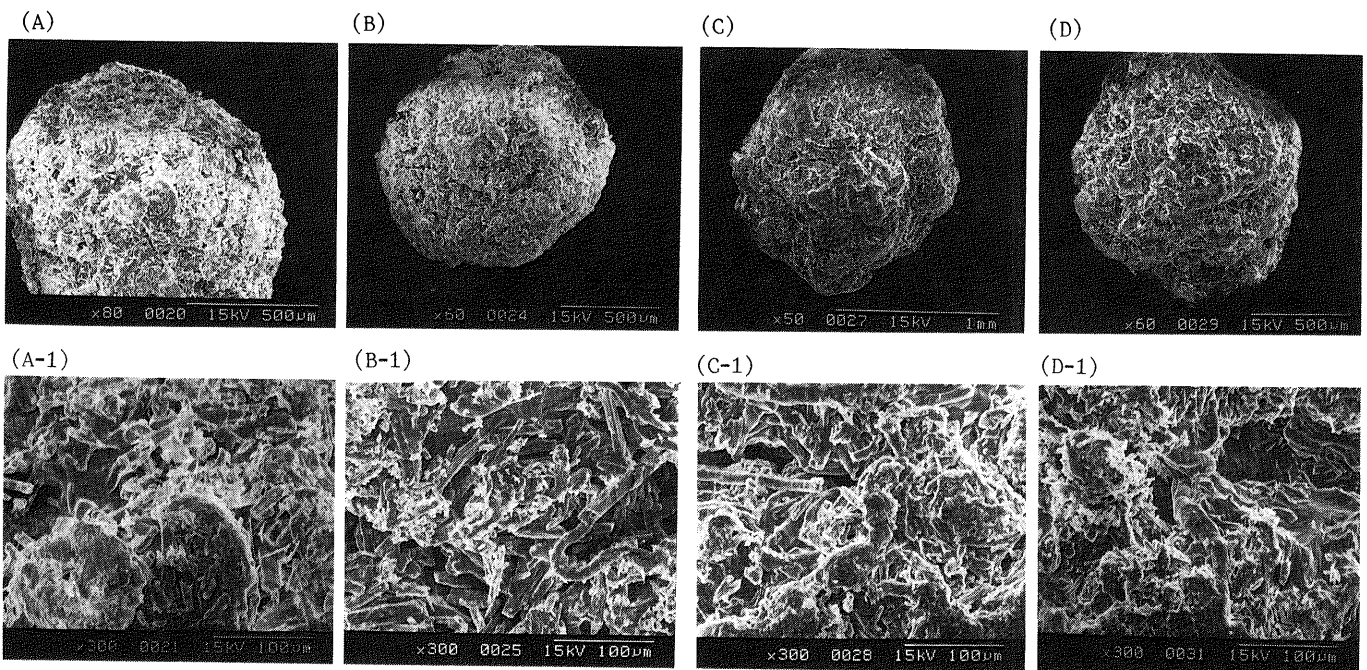


Fig. 4. Effect of Acetic Acid Concentration on the Surface Topography of Theophylline Chitosan Microcapsules Containing Na CMC  
Key: acetic acid concentration: (A, A-1), 35%; (B, B-1), 40%; (C, C-1), 50%; (D, D-1), 60%.

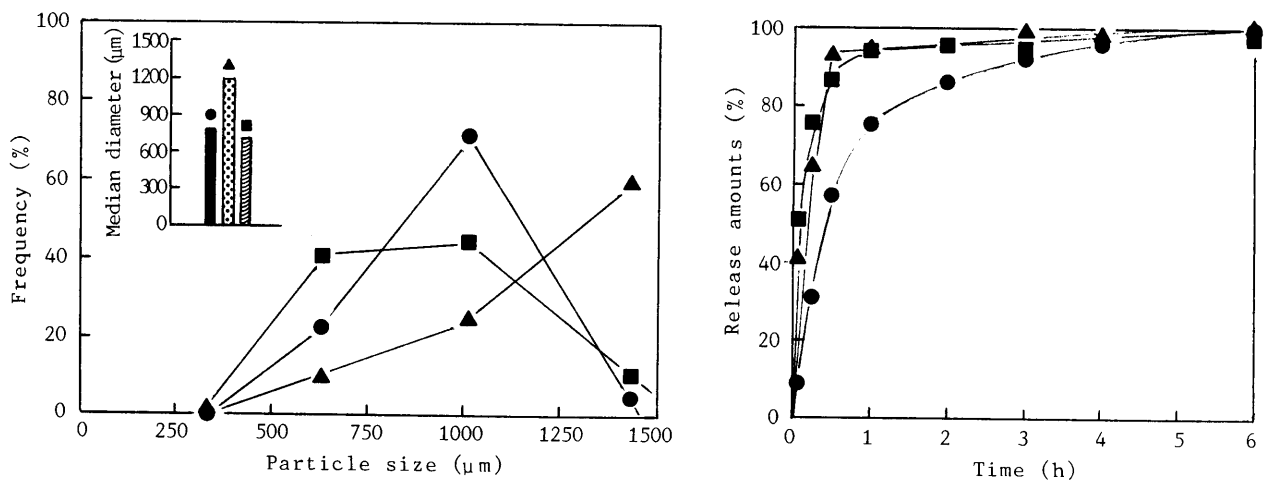


Fig. 5. Effect of Acid Type on the Particle Size Distribution and Dissolution of Theophylline Chitosan Microcapsules Containing Na CMC  
Key: type of acid: ●, 50% acetic acid; ▲, 50% ascorbic acid; ■, 50% citric acid.

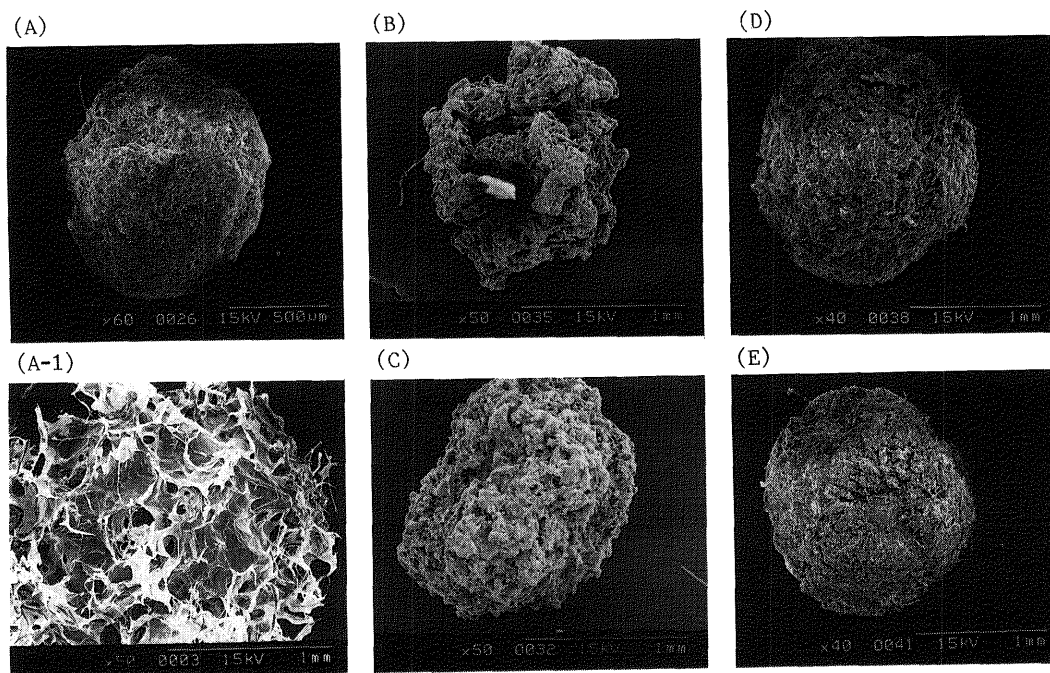


Fig. 6. Effect of Acid Type and Na CMC Concentration on the Surface Topography of Theophylline Chitosan Microcapsules  
 Key: (A, A-1), acetic acid; (B), ascorbic acid; (C), citric acid; (D), 0 g Na CMC; (E), 4 g Na CMC.

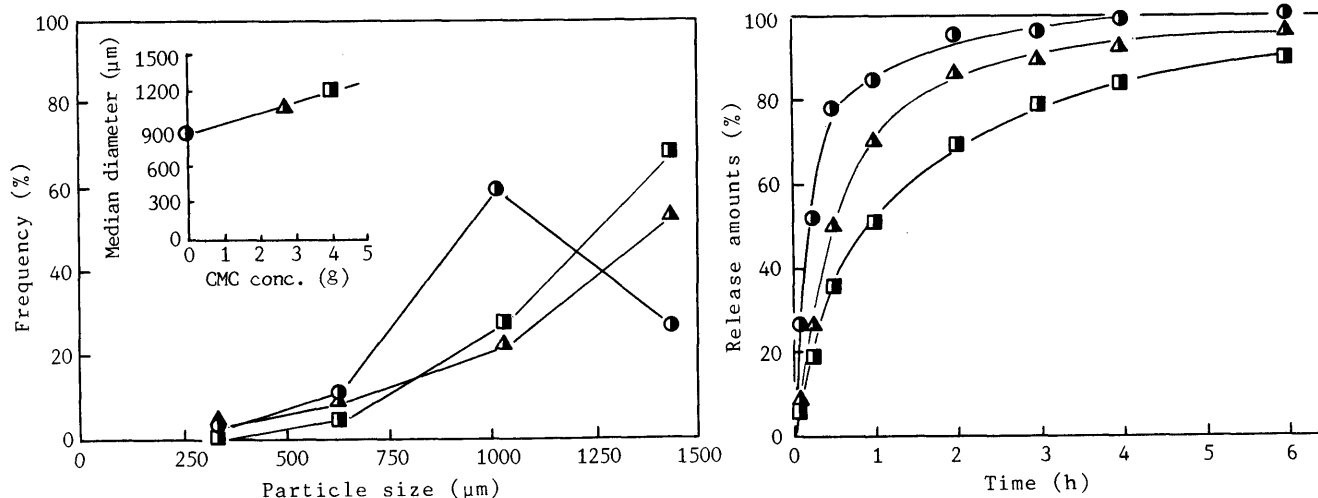


Fig. 7. The Effect of Na CMC Concentration on Particle Size Distribution and Dissolution of Theophylline Chitosan Microcapsules  
 Key: Na CMC concentration: ●, 0 g; ▲, 2.7 g; ■, 4.0 g.

those prepared with acetic acid or citric acid. However, chitosan microcapsules made from acetic acid might result in slower release of theophylline as compared with those prepared by citric or ascorbic acid, probably because the surface topography of chitosan microcapsules prepared with the latter acids was more porous in structure and in appearance resulting in faster dissolution of theophylline (Fig. 6). Furthermore, a chitosan microcapsule made with acetic acid solution was swollen in dissolution medium and doubled in particle size, this lengthening its diffusion pathway and slowing its release action (Fig. 6A-1). Figure 7 shows the effect of Na CMC concentration on the particle size and release behavior of theophylline chitosan microcapsules prepared using 50% acetic acid as medium. It was found that the greater the Na CMC concentration used, the larger was the particle size obtained. The in-

soluble complex formed and the excess of Na CMC in the chitosan microcapsules might be responsible for such larger particle size and slower release behavior.

**In Vitro Floating Behavior of Chitosan Microcapsules** The *in vitro* floating property of chitosan microcapsules prepared with different acetic acid concentrations was investigated in pH 1.2 solution, distilled water (pH 5.7) and pH 6.8 solution with gentle stirring (Fig. 8). Chitosan microcapsules prepared by 35% and 40% acetic acid were still floatable (> buoyancy 80%) even after 24 h of three aqueous media. Other chitosan microcapsules prepared by 50% and 60% of acetic acid quickly sank into the distilled water and pH 6.8 aqueous medium, settled at the bottom of the beaker and were partially swollen. Interestingly, the particles of chitosan microcapsules prepared by 40%, 50% and 60% of acetic acid sank quickly into pH 1.2 aqueous medium

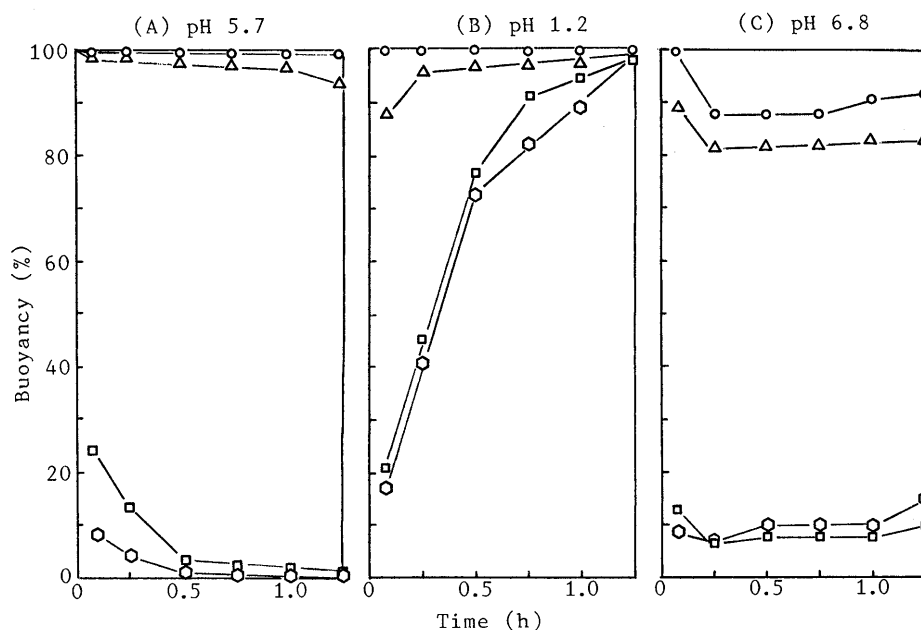


Fig. 8. *In Vitro* Floating Property of Theophylline Chitosan Microcapsules Prepared with Different Acetic Acid Concentrations  
Key: see Fig. 2.

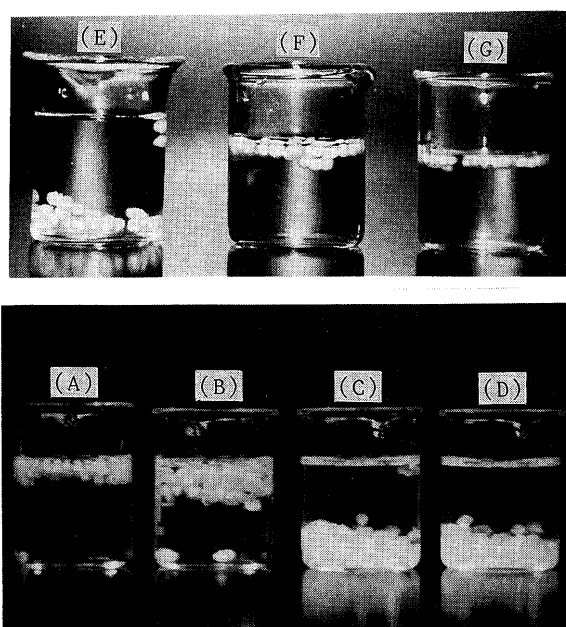


Fig. 9. Effect of Acetic Acid and Na CMC Concentrations on the Floating Behavior of Chitosan Microcapsules in Water after 1 h

Key: acetic acid concentration: (A), 35%; (B), 40%; (C), 50%; (D), 60%. Na CMC concentration: (E), 0 g; (F), 2.7 g; (G), 4.0 g.

but floated again right away. This different floating behavior was perhaps related to the content of water-insoluble complex and excess of Na CMC in the microcapsules; the reactive pH range might be responsible for the amount of this complex formed.<sup>27)</sup> We also found that chitosan microcapsules prepared by higher acetic acid concentration might swell in the floating medium, but less swelling was found in those microcapsules made from lower acetic acid concentration. The less acetic acid used, the more floatable the chitosan microcapsule was. Figure 9 shows the floating behavior of different types of chitosan microcapsules in distilled water for 1 h. The buoyancy phenomenon of

chitosan microcapsules prepared with different concentrations of acetic acid was discussed above. The microcapsules without Na CMC sank immediately into the distilled water and became swollen. The density of theophylline ( $> 1$ ) and the large quantity contained in chitosan microcapsules might explain this behavior. When a large amount of Na CMC was contained in chitosan microcapsules, a part of the Na CMC located beside the microcapsules was rapidly dissolved to form a gel barrier which resulted in the entrapped air hardly being able to escape, leading to floating the chitosan microcapsules.

### Conclusions

An emulsion-phase separation method in liquid paraffin was devised to prepare chitosan microcapsules containing theophylline and Na CMC. We found that acetic acid concentration significantly influenced the formation, micromeritic property, release behavior and floating property of these microcapsules, since the interaction between chitosan and Na CMC occurring in the preparation of the microcapsules produced a water-insoluble complex of chitosan-Na CMC. Chitosan microcapsules prepared using acetic acid aqueous solution as a solvent exhibited capsule wall with a more compact and less porous structure, and a delayed action of release as compared with microcapsules prepared with ascorbic acid or citric acid aqueous solution. The amount of Na CMC in chitosan microcapsules was also important in controlling the particle size and the release rate of the microcapsules.

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