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Studies on the Development of Sustained Release Preparation (I) Preparation and Evaluation of CAP Microcapsules of Sodium Ascorbate

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지속성 제제의 개발에 관한 연구 (I) 아스코르빈산 나트륨의 CAP 마이크로캡셀의 제조 및 평가

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Microencapsulation of sodium ascorbate with cellulose acetate phthalate(CAP) by coacervation/phase separation method were carried out. Various factors affecting microencapsulation, i.e., surfactant concentration, CAP concentration, stirring speed and treatment of spermaceti as a sealing agent were studied. Dissolution rate, particle size distribution, surface feature and stability test were investigated. CAP microcapsules prepared using 0.5% span 80 as a surfactant showed smooth and round surfaces. The release of sodium ascorbate was retarded by microencapsulation with CAP and by sealant treatment with spermaceti. When triturated with sodium bicarbonate, CAP microcapsules were more stable than unencapsulated sodium ascorbate under various RH conditions at 37℃.

Keywords—Microencapsulation, sodium ascorbate, cellulose acetate phthalate.

Cellulose acetate phthalate has been used for enteric coating of pharmaceutical dosage forms for many years. Such coatings are intended to prevent the release of the drug from the coated dosage from in the acidic gastric environment but to disintegrate and release the contents on the relatively basic intestinal medium. Recently several workers have described investigations using CAP employing either aqueous[1,2] or nonaqueous[3-6] manufacturing vehicles for the microencapsulation techniques using spray-drying[7,8] and precipitation methods.[9]

Ascorbic acid has been widely used in the food and pharmaceutical preparations as ingredients or antioxidants; however, the stability of sodium ascorbate was much concerned. To increase the stability of sodium ascorbate, microencapsulation with cellulose acetate phthalate(CAP) by coacervation method were attempted.

In the present study, we tried to find a useful, simple and rapid preparation method of microcapsules having small size because particle size of microcapsules prepared with other drugs has been quite large.[10-12]

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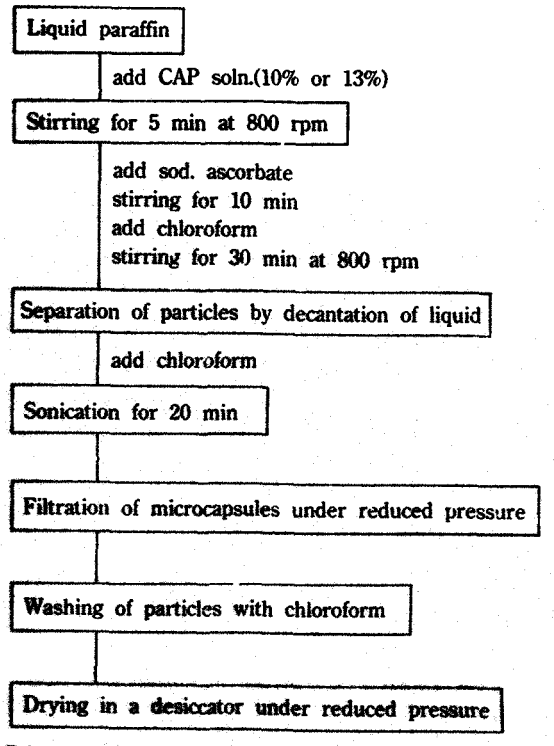


Scheme I—Preparation of microcapsules.

We studied on the various factors affecting microencapsulation, e.g., optimum conditions of stirring speed and surfactant concentration, CMC of surfactants and drug loading amounts. The release and the stability of sodium ascorbate in the microcapsules were also studied.

Experimental

Materials

Sodium ascorbate and sodium bicarbonate were pharmaceutical grade from Ilyang Pharm. Co., Ltd. (Korea). Cellulose acetate phthalate(CAP), sorbitan monooleate(span 80) and light mineral oil were reagent grade.

Apparatus

Dissolution tester(Prolabo dissolution tester), UV spectrophotometer(Perkin-Elmer, lambda 5), optical microscope(Vickers), scanning electron microscope(JSM 35C), life tester, and mechanical stirrer were used.

Preparation of CAP Microcapsules

CAP microcapsules were prepared by coacervation/phase separation method(Scheme I). The dispersion media, light mineral oil containing span 80, was added to a 500 m*l* round bottomed flask. Then 10% and 13% CAP solution in acetone: 95% ethanol(9:1) were added to the above round flask respectively. After stirring for 5 mins. sodium ascorbate powder(1 g) was added in parts and stirring was continued for 10 mins. Thereafter chloroform was added to the system as a non-solvent and then stirring was continued for 30 mins. A part of total cellulose ester available is coacervated and separated in the form of viscous liquid drops. These droups tend to deposit on the surface of sodium ascorbate particles when chloroform was added as a nonsolvent. The subsequent sonication within chloroform was carried out to prevent aggregation and agglomeration of microcapsules. The microcapsules were filtered under reduced pressure. Finally, the produced microcapsules were washed with chloroform and dried under reduced pressure. The size of produced microcapsules under various stirring speed and various concentration of span 80 as a surfactant was measured.

Determination of CMC

The first derivative absorption spectrum (FDAS) method[13,14] showed high reproducibility in the measurement of the critical micelle concentration (CMC) of the surfactant. The interfacial tension is the greatest at the interface between two immiscible liquid phase. We determined the CMC of span 80 in the system of 9:1 volume ratio of mineral oil and acetone:ethanol (9:1). The interfacial tension was measured with microbalance using ring method. A force per unit length is illustrated by means of a three side wire frame across which a movable bar is placed. When a movable bar is acrossed the interface of the two phases, frame could be stretched by applying a force of f(such as hanging mass) to movable bar, length L, which acts against the interfacial tension. The interfacial tension is calculated by equation 1.

$$\gamma=\frac{f_b}{2L} \quad (1)$$

where, γ : the interfacial tension
f : the force required to across the interface
L: the length of movable bar

Treatment of Spermaceti Solution as a Sealing Agent

To further control the permeability of the microcapsules, a spermaceti coating was applied. Spermaceti(1 g) was dissolved in 10 ml chloroform as a solvent. The formed microcapsules were then suspended in spemaceti solution for 15 mins with constant stirring. The spermaceti solution was then decanted and the microcapsules were collected on filter paper to absorb the excess spermaceti solution.

Release Rate

The release of microcapsules was determined in 500 ml of dissolution medium of simulated gastric fluid(pH 1.2) and intestinal fluid(pH 6.8) at 37℃, 100 rpm. Aliquots of 3 ml were withdrawn at various times and replaced by fresh solvent with corrections applied in the calculations. The amount of drug released was assayed spectrophotomerically at 265 nm.

Microscopic Studies of Microcapsules

Optical and scanning electron microscopy were used to evaluate the quality of coating and the particle size prepared under the various conditions.[15] The surface feature of the particles was investigated using scanning electron micrographs.

Wall Recovery Procedure

The wall material of the microcapsules was treated briefly with chloroform to dissolve any adhering debris or excessive encapsulating material. A portion of these microcapsules was then carefully dried and a known weight was refluxed with chloroform for at least 30 mins.[16] After separating the microcapsules by filtration and further washing with chloroform, the combined chloroform portion was dried and weighted.

Stability Test

The physicochemical stability of microencapsulated and unencapsulated sodium ascorbate was tested.[17] The microcapsules and unencapsulated sodium ascorbate triturated with sodium bicarbonate were stored at 37℃ of various RH conditions (40, 60, 80, 100%) for 6 days. The ascorbic acid content of stored test samples was analyzed by 2,4-dinitrophenylhydrazine method.

Determination of Wall Thickness

The average wall thickness was calculated using Madan's equation.[16] If W=Weight of microcapsules taken, W_w=Weight of wall material collected, ρ_w=density of wall material, ρ=density of sodium ascorbate and d=diameter of sodium ascorbate particles.

$$\text{Wall thickness} = \frac{W_w}{W - W_w} \cdot \frac{\rho}{\rho_w} \cdot \frac{d}{6}$$

Assay of Sodium Ascorbate Content

A 100 mg of microcapsules was transferred to 500 ml volumetric flask after grinding in a mortar. After thorough rinsing of mortar and pestle with pH 1.2 medium, the powder was suspended with about 250 ml of medium. For complete rupture of shells, the suspension was treated with ultrasonics for 10 mins.[17] The resulting solution was filtered to separate the undissolved fragments. Samples of this solution was assayed at 265 nm after proper dilution.

Results and Discussion

Effect of Stirring Condition

Various factors affecting microencapsulation were investigated preliminarily. Low stirring speed below 150 rpm was not satisfactory. From 400 to 800 rpm, proper dispersion and emulsification occured and the appropriate microcapsules were produced. Smaller microcapsules were produced at the higher speed[18](Fig. 1). Fig. 2 shows photograph of microcapsules prepared at various stirring speed. Above 850 rpm, excessive splashing occured and a suitable product could not be obtained. By this preliminary experiment, we selected 800 rpm for a optimum condition of preparing microcapsules of good appearance and appropriate size range.

Effect of Surfactant Concentration

It is commonly desirable to use surfactants as small amount as possible from the viewpoints of toxicity, cost and ease of handling. In the present

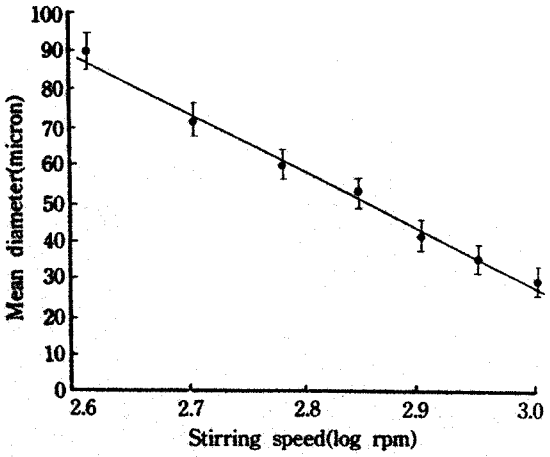


Figure 1—Effect of stirring speed on the mean diameter of CAP microcapsules prepared with 10% CAP using 0.5% span 80.

work, we used sorbitan monooleate (span 80) as a nonionic surfactant. The photographs of microcapsules prepared using different surfactant concentrations are shown in Fig. 3. The mean size of microcapsules was the smallest when 0.5% (w/w) span 80 was used (Table I). The relationships between interfacial tensions and concentrations of span 80 are shown in Fig. 4. The interfacial tension was not changed from 0 to 0.25% (w/w) of span 80, but declined rapidly from 0.3 to 0.5% (w/w) and became constant above 0.5% (w/w). The CMC and the optimum concentration of span 80 to perpare the smallest microcapsules were coincided to be about 0.5% (w/w). Therefore, the determination of CMC of a surfactant in solvent system is very important to predict the opti-

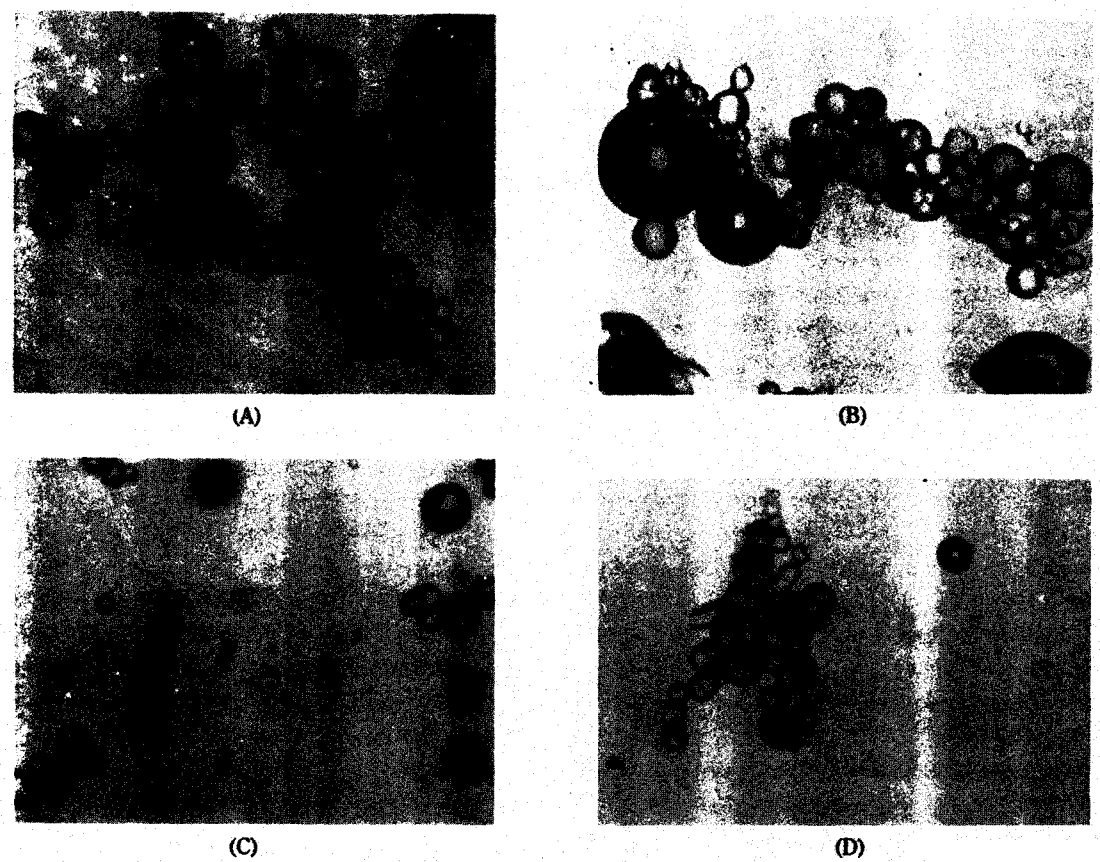
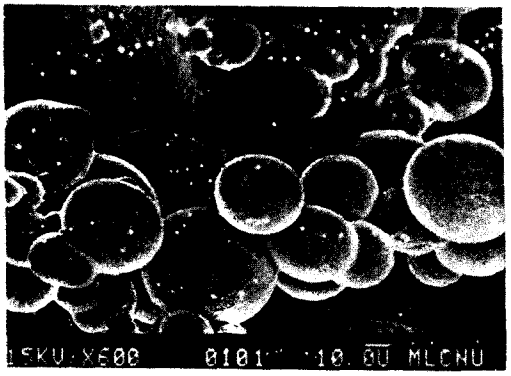


Figure 2—Photographs of microcapsules prepared with 10% CAP, 0.5% span 80 at various rpm.
Key: (A) 500 rpm, (B) 700 rpm, (C) 800 rpm, (D) 1,000 rpm

(A)



(B)

Figure 3—Scanning electron micrographs of microcapsules prepared using different surfactant concentration. (10% CAP, 800 rpm)
Key: (A) without surfactant, (B) 0.5% surfactant

Table I—*Mean size of microcapsules prepared with 10 % CAP using various % of span 80 at 800 rpm.*

Percent of span 80	Mean size (μm)
0	168.9± 11.5
0.1	82.03± 4.7
0.5	38.81± 2.25
1	80.43± 4.89
2	97.36± 6.09
3	115.3± 84.57
5	207.4± 98

mal concentration of span 80 to prepare the smallest microcapsules.

Effect of CAP Concentration

Fig. 5 shows the optical photographs of 10% or

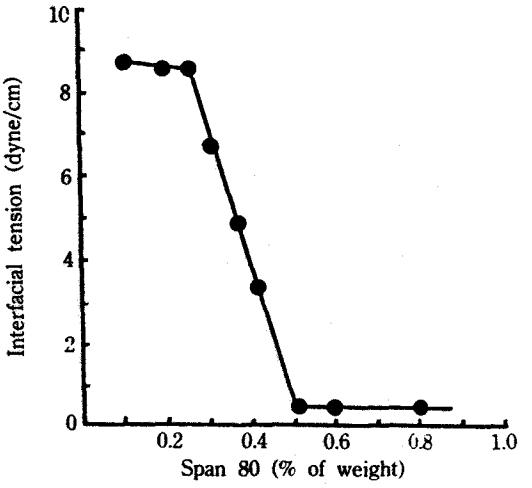
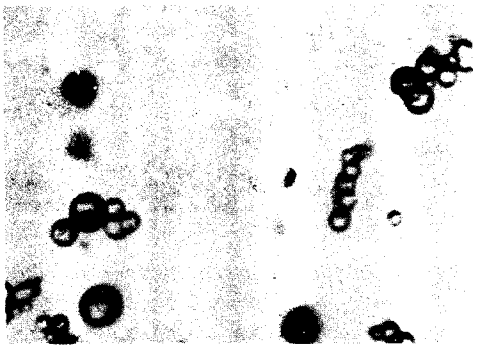
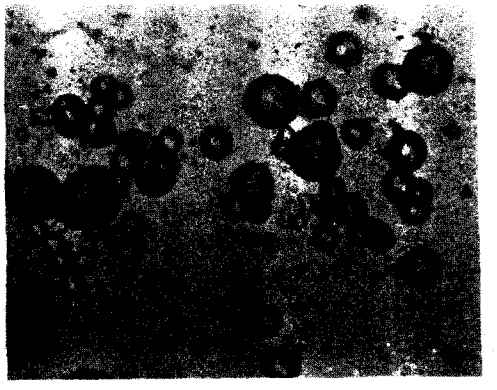


Figure 4—Interfacial tension of span 80 in the system of 9:1 ratio of mineral oil and acetone:ethanol (9:1)



(A)



(B)

Figure 5—Photographs of microcapsules prepared with different CAP concentration using 0.5% span 80 at 800 rpm.
Key: (A) 10% CAP microcapsule, (B) 13% CAP microcapsule

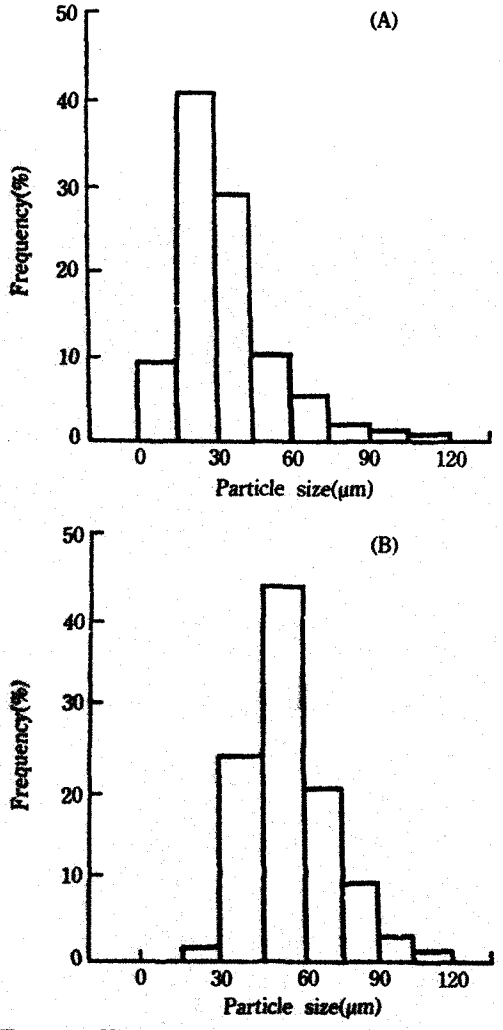


Figure 6—Histogram for the size distribution of microcapsules prepared with various CAP concentration using 0.5% span 80 at 800 rpm.
Key: (A) 10% microcapsule, (B) 13% microcapsule

13% CAP microcapsules, respectively. The shapes of both types of microcapsules were spherical. Fig. 6 shows the histogram of size distribution for the microcapsules prepared with various concentrations of CAP. The fraction of larger size increased by increasing the concentration of CAP concentration. Table II illustrates the size and the contents of sodium ascorbate in CAP microcapsules of various concentrations. The diameter was determined from these photographs of more than 200 particles. The average diameter of 10% CAP microcapsules was 38.81 μm and that of 13% CAP microcapsules was 57.3 μm. Microencapsulation of sodium ascorbate with CAP by this method was effective to prepare the small particle size.

Table II—*Drug content in microcapsules prepared with 10% or 13% CAP-acetone-ethanol solution using 0.5% span 80 at 800 rpm.*

Microcapsules	Content of sod. ascorbate mean± SD(%)	Mean size ± SD(μm)
10% CAP microcapsule	43.64± 0.5333	38.81± 2.254
13% CAP microcapsule	41.04± 0.473	57.3± 2.04

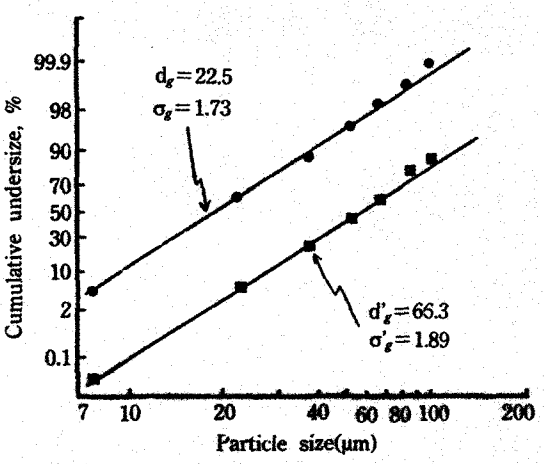


Figure 7—Log-probability plots of 10% CAP microcapsule.
Key: ●: number distribution, ■; weight distribution

Number and Weight Distribution

The mean logarithmic probability plot of percent cumulative undersize for 10% CAP microcapsules is shown in Fig. 7 and 13% CAP microcapsules in Fig. 8. The reference point used is the logarithm of the particle size equivalent to 50% on the probability scale, i.e., the 50% size.[19,20] This is known as the geometric mean diameter and is given the symbol d_g. The slope is given by the geometric standard deviation, d_g, which is the quotation of the ratio(84% undersize or 16% oversize)/(50% size) or (50% size)/(16% undersize or 84% oversize). In log-probability of 10% CAP microcapsules, d_g=22.5 μm and σ_g=1.73 for the number distribution and d'$_g$=66.3 μm and σ'_g=1.89

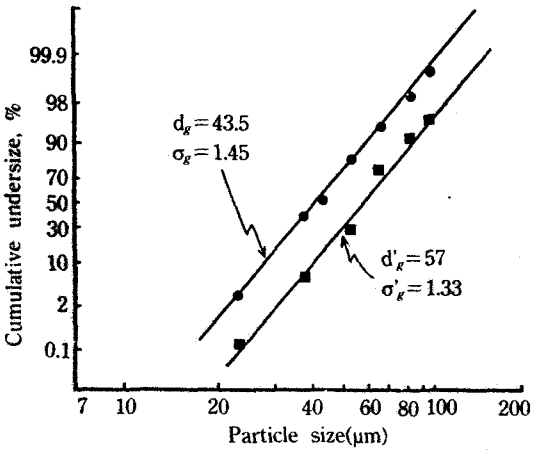


Figure 8—Log-probability plots of 13% CAP microcapsules.
Key: ●: number distribution, ■: weight distribution

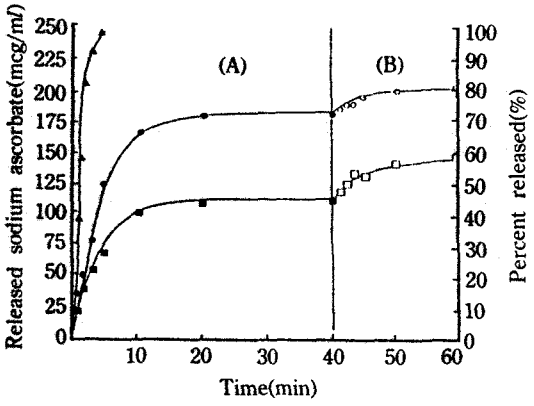


Figure 9—Release of sod. ascorbate from microcapsules prepared using 10% CAP, 0.5% span 80 in dissolution medium (pH 1.2 and 6.8) at 200 rpm, 37℃.
Key: ▲: Sod. ascorbate, ●·○: 10% CAP microcapsules, ■·□: Spermaceti treated microcapsules, (A) dissolution medium(pH 1.2), (B) dissolution medium(pH 6.8)

for the weight distribution (Fig. 7) and in that of 13% CAP microcapsules, d_g=43.5 and σ_g=1.45 for the number distribution and d'_g=57 μm and σ'_g=1.33 for the weight distribution, respectively (Fig. 8).

Drug Release

Dissolution tests were carried out to compare the release rates of microcapsules with those of spermaceti treated microcapsules. The data were plotted using the following equation to compensate

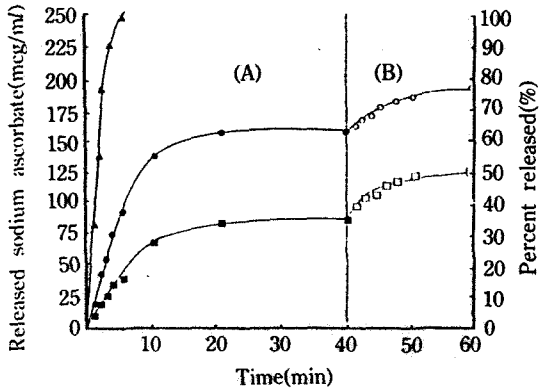


Figure 10—Release of sod. ascorbate from microcapsules kprepared using 13% CAP, 0.5% span 80 in dissolution medium (pH 1.2 and 6.8) at 200 rpm, 37℃.
Key: ▲: Sod. ascorbate, ●·○: 13% CAP microcapsules, ■·□: Spermaceti treated microcapsules, (A) dissolution medium(pH 1.2), (B) dissolution medium(pH 6.8)

Table III—*Stability of sod. ascorbate from CAP microcapsules stored with sod. bicarbonate for 6 days under various RH conditions at 47°C*

RH(%)	Remained amount (%)	
	Microcapsule	Sod. ascorbate
40	81.2	61.7
60	76.3	48.6
80	63.1	32.1
100	58.1	5.3

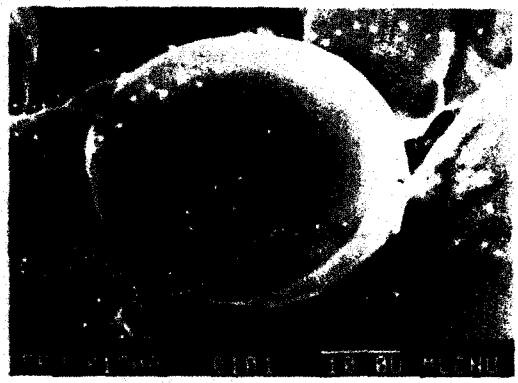
*Wall thickness of 10% CAP microcapsule was about 4.15 μm.

for the portion of the drug removed from the dissolution flask at each withdrawal of the sample for concentration determination.

$$C_{corr}=C_{read}+\frac{3}{500}\sum_{s=1}^{n-1}C_{uncorr}$$

Fig. 9 and 10 show the dissolution patterns of sodium ascorbate from different CAP microcapsules and that microcapsules treated with spermaceti in pH 1.2 and 6.8 dissolution medium at 37℃, 100 rpm. The release of sodium ascorbate from microcapsules was retarded in dissolution medium of pH 1.2 and 6.8 as compared with the unencapsulated powder. The release of microcapsules sealed with 10% spermaceti was retarded considerably

(A)



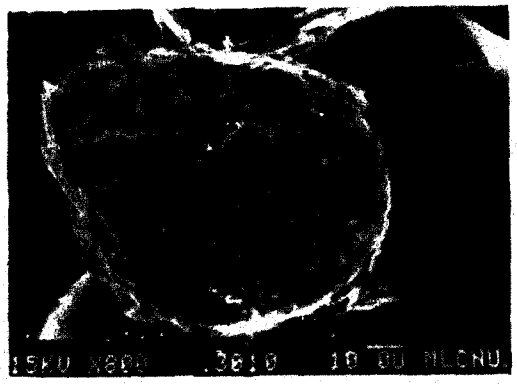
(B)

(C)

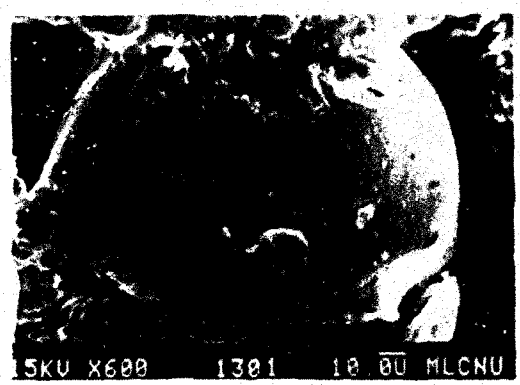
Figure 11—Scanning electron micrographs of microcapsules prepared with 10% CAP using 0.5% span 80 at 800 rpm.
Key: (A) before dissolution, (B) after dissolution in medium, pH 1.2, (C) after dissolution in medium, pH 6.8

comparing with CAP microcapsules (Fig. 10).

Stability of Sodium Ascorbate in Microcapsules



(A)



(B)

Figure 12—Scanning electron micrographs of 10% microcapsules sealed with 10% spermaceti.
Key: (A) before dissolution, (B) after dissolution

To check the physicochemical stability of sodium ascorbate by microencapsulation, the sodium ascorbate and the CAP microcapsules were triturated with sodium bicarbonate, respectively and stored at various RH conditions. The color of sodium ascorbate was changed to dark brown at high RH condition showing complete degradation, while that of microcapsules was not altered at low RH conditions and altered to slightly yellowish brown at high RH conditions (Table III). Microencapsulation using CAP is a useful method for prevention of decomposition of sodium ascorbate.

Surface of Microcapsules

Optical microscopic observations were performed on microcapsules. Using the optimum concentration and conditions, all core particles were individually and completely coated, suggesting uni-

formity of thickness around the cores. CAP microcapsules prepared using 0.5% span 80 showed smooth and round, while irregular and rough surface in microcapsules prepared without surfactant (Fig. 3). Before the drug release test, the surface of microcapsules was smooth, spherical and dense as demonstrated by the scanning electron micrographs. After the drug release test in dissolution medium of pH 1.2, the shape of spherical microcapsules recovered was found to be almost unchanged, but macro-pores were observed after dissolution in pH 6.8 medium (Fig. 11). There were no pores in CAP microcapsules sealed with 10% spermaceti even after release test in dissolution medium of pH 6.8 medium. It is suggested that CAP is ruptured in pH 6.8 medium, however, rupture of CAP film was protected by sealing with sptemaceti (Fig. 12).

Conclusions

The present investigation on the microencapsulation of sodium ascorbate showed the following results.

1. The optimum stirring speed for microencapsulation was between 400 and 800 rpm and smaller size microcapsules were produced at the higher speed.

2. CMC of span 80 in the mineral oil/acetone system was about 1.5% (w/w).

3. CAP microcapsules retarded the release of sodium ascorbate. The release rate increased by increasing the amounts of drug loaded.

4. Surface of CAP microcapsules prepared without span 80 was irregular, rough and highly aggregated but that of microcapsules prepared using 1.5% span 80 was smooth, round and small particle size. The shape of spherical microcapsules in dissolution medium of pH 1.2 was almost unchanged, but macro-pores were observed after dissolution in pH 6.8 medium.

5. The size of microcapsules of sodium ascorbate was at the range of powder size.

6. Preparation method used for this work was simple and rapid and microencapsulation of sodium ascorbate with CAP could be useful for sustained release preparations or protection from degradation of sodium ascorbate.

Acknowledgement

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