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# EVALUATION OF UNIFORM-SIZED MICROCAPSULES USING A VIBRATION-NOZZLE METHOD

K. Shimano,[a] O. Kondo,[a] A. Miwa,[a] Y. Higashi,[a] I. Koyama, [a]
T. Yoshida,[a] Y. Ito,[b] J. Hirose[b] and S. Goto[c]
Research Center, Taisho Pharmaceutical Co., Ltd.,[a] 1-403, Yoshino-cho, Ohmiya 330, Japan, Kobe Steel, Ltd.,[b] 1-3-18, Wakinohama-cho, Chuo-ku, Kobe 651, Japan and Faculty of Pharmaceutical Sciences, Kyushu University,[c] 3-1-1 Maidashi, Higashi-ku, Fukuoka, 812, Japan



## ABSTRACT

In order to prepare microcapsules (MC) using polyvinylacetal diethylaminoacetate (AEA) gel, we have studied both the unique properties of AEA gel, in which cold-water solution forms a hard gel with exclusion of water at high temperature, and the microencapsulation technique for clarithromycin (CAM), which has an unpleasant taste (1, 2). In our previous paper (3), we described a new uniform-sized microencapsulation machine with a vibration nozzle with 536 holes 100μm in diameter designed for precise regulation of dissolution of CAM from MC.

In order to evaluate the MC thus obtained, we prepared syrups (without water) by adding sweetening agents to MC, and conducted a dissolution test at various pHs using the method described in the Japanese Pharmacopoeia (12th edition), a test for bitter taste and a test to determine bioavailability (BA) in

human subjects. At pHs below 4.0, 100% of CAM in MC was dissolved from the syrup within 5min. However, there was little dissolution of CAM from syrup at pH6.8. The results of the test for bitter taste showed that the syrup preparations, which were administered to 12 male and female volunteers with 20ml water, masked bitter taste completely during the 60min period following administration of the syrup. The mean area under the serum concentration-time curve ($AUC_{0-24}$) and the maximum serum concentration ($C_{max}$) for administration of syrup preparations to 6 male volunteers were 5.66 ± 0.73μg·hr/ml and 0.66 ± 0.13μg/ml, respectively. Blood concentration was maintained at a level above the minimum inhibitory concentration for gram-positive bacteria for a period of about 14hr after administration, demonstrating the usefulness of the syrup preparation.

## INTRODUCTION

The microencapsulation method using AEA gel is very useful for control of CAM dissolution from MC. However, the findings of a study of the relationship between particle size and CAM dissolution suggested that the bitter taste of syrup preparations was caused by dissolution of CAM from MC of small particle size in the syrup preparations. We therefore hypothesized that if a method could be developed for precise control of MC particle size to be 100μm (3), the bitter taste of syrup preparations could be decreased. As a result of investing, we developed a uniform-sized MC machine with vibration nozzle atomizer, and reported details concerning its design in our previous paper (3). On the other hand, technique for control of drug bitterness is one of the most important continuous subjects of research on pharmaceutical formulation. If the unpleasant taste of a drug can be masked, the compliance of patients (infants or children) in taking the on oral administration dosage form can be increased (4-7).

In the present study, a series of dissolution tests using syrups prepared with uniform-sized MC, sweetening agents and other ingredients were performed, in addition to a test for bitter taste and determination of BA in human subjects.

## MATERIALS AND METHODS

### Materials

Clarithromycin (Macrolide Antibiotics, Taisho Pharmaceutical Co., Ltd.) was used after it has been ground to less than 1μm average diameter with a Coball Mill (MSM-12, Shinko Pantec Co., Ltd.). AEA, a polymer soluble in gastric juice, was purchased from Sankyo Co., Ltd. Purified water was prepared with a purified water generating apparatus (lob. Ion Pure-112, Millipore Co., Ltd.). Other reagents used met the requirements of the Japanese Pharmacopoeia (JPXII) or the Japanese Standards for Food Additives.

### Preparation of uniform-sized MC

A new uniform-sized microencapsulation system was modified in a few respects related to its cooling and steaming apparatus (3). A nozzle with a total of 536 holes each with a diameter of 100μm was directly connected to a vibrator. A slurry was prepared by suspending CAM in a cold aqueous solution of AEA and transferred into a slurry tank. The slurry was maintained at constant temperature with a cooling box (a modified large refrigerator), and pumped into the nozzle with air pressure regulated by a pressure gauge. Liquid droplets were prepared as uniform-size microparticles by nozzle vibration, and were transformed into a hydrogel by steam during fall. In order to prevent coagulation, the particles were dropped into a swirling hot water tank with hot water circulating through a hot water pool. After shrinking of the hydrogel, the microparticles of the hydrogel were dried with a vibration-dryer (VU-30, Chuo Kakohki Co., Ltd.), yielding uniform-sized MC.

### Preparation of syrups

Using the formulas shown in TABLE 1, syrups containing 10% CAM were prepared by the addition of various sweetening and other additives to MC containing CAM. Purified water was added to the mixture 30min before the beginning of each experiment.

**TABLE 1**

**Content of Slurry for Manufacture of MC**

| Composition | wt% |
|---|---|
| Polyvinylacetal Diethylaminoacetate | 6.25 |
| Clarithromycin | 2.50 |
| Sodium Dodecyl Sulfate | 0.02 |
| Magnesium Hydroxide | 1.50 |
| Purified Water | 89.73 |
| Total | 100.00 |



## Dissolution test

A series of CAM dissolution studies using the syrup preparations were carried out using the paddle method (JPXII). A 900ml volume of the test solution was used at pH1.2 (the first fluid, JPXII), 4.0 (0.1M citrate buffer), 5.8 (0.1M McIlvaine buffer) or 6.8 (the second fluid, JPXII) for elution. A 200ml or 100ml volume of the test solution and 1/10 or 1/50 of the concentration of the starting test buffer solution were used at pH4.0. CAM concentration was measured by HPLC in the fashion described in our previous paper (2).

## Test for bitter taste

Prior to the test for bitter taste, a test measuring ability to distinguish bitter taste was carried out with volunteers using CAM aqueous solution (7.2μg/ml, half the threshold concentration for bitterness of CAM) and taste blindness paper (TEST PAPER, ADVANTEC) to ensure that none had abnormalities in the ability to perceive bitter taste. Smokers were excluded from the study. The test for bitter taste was performed 2hr after breakfast. Syrup preparations (without water) containing a total of 200mg of CAM (1 adult dose) were suspended in 10ml of purified water, and then administered to 12 male and female volunteers 30min later. The container for the syrup was rinsed with 10ml of purified water, and

the washing solution was then also administered to the volunteers. The degree of bitterness was evaluated for the period beginning immediately after administration and continuing until 1hr after administration. Rating of the bitterness was made using a 5-stage rating system: extremely bitter, very bitter, bitter, slightly bitter, or not bitter. Syrup prepared by suspending 200mg of CAM in bulk form was used as a control preparation.

Test on BA in human subjects

After informed consent for participation had been obtained, and a physical examination and blood test had been performed within a month before the beginning of the BA test, 6 healthy male volunteers, aged 21 to 24 years and weighing 49-61kg, were selected for study. Each subject was administered a single dose of the syrup preparation (without water) containing 200mg of CAM, which was suspended in 10 ml of purified water immediately before administration, together with 90ml of the purified water under fasting conditions in the morning. Venous blood samples (5ml) were obtained on a predetermined time schedule at 0 (just after administration), 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 5, 8, 12 and 24hr after administration. These subjects abstained from food for 13hr before and 4hr after administration, and abstained from water for 2hr before and 4hr after administration. Blood CAM concentrations were measured by bioassay using a paper disk method.

## RESULT AND DISCUSSION

We developed a uniform-sized microencapsulation system based on the findings of study reported in our previous papers (1-3). The system is illustrated in FIGURE 1, and its appearance is shown in FIGURE 2. A slurry was prepared by suspending CAM and other additives in AEA aqueous solution. The temperature of the slurry was maintained at exactly 5°C with the use of a large refrigerator modified to form a cooling box into which a stainless steel slurry tank (60L), filtration system for the slurry and an air regulation system excluding its compressor could be placed. A vibration nozzle was set inside a transparent box

1: Vibrator
2: Nozzle
3: Stroboscope
4: Cooling Duct
5: Steaming Trunk
6: Steam
7: Hot Water Duct
8: Swirling Hot Water Tank
9: Hot Water Pool
10: Circulation Pump
11: Cooling Box
12: Filtration System
13: Suspension Tank
14: Agitator
15: Vacuum Pot
16: Pressure Gauge
17: Pressure Control Valve
18: Reservoir Tank
19: Compressor.

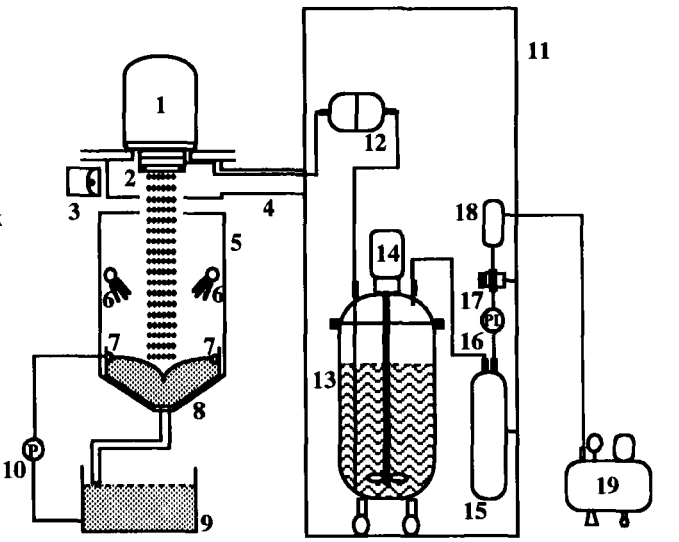


**FIGURE 1**

**Schematic Illustration of Uniform-Sized Microencapsulation Machine Using Vibration Nozzle**



made of acrylic resin in order to observe the state of uniform-sized atomization, and the nozzle and the atmosphere surrounding the nozzle were kept at about 5°C by connecting a cooling duct to the side of the cooling box.

The formulas and the conditions of manufacture of MC are presented in TABLE 1 and TABLE 2, respectively. CAM and AEA were mixed in the approximate ratio 1.0:2.5, and the slurry consisted of a 10.27:89.73 mixture of solid components and purified water. A trial preparation of MC from the slurry containing CAM was conducted with a vibration nozzle with 536 holes, and uniform-sized microparticle droplets were obtained. When these droplets were completely uniform-sized, the flow rate of atomized slurry from the nozzle was 2.77g/min, and the frequency of vibration of the nozzle was 6460Hz. These microparticle droplets were transformed into a gel by steam, during fall into a swirling hot water tank, the temperature of which was maintained at exactly 50°C. After

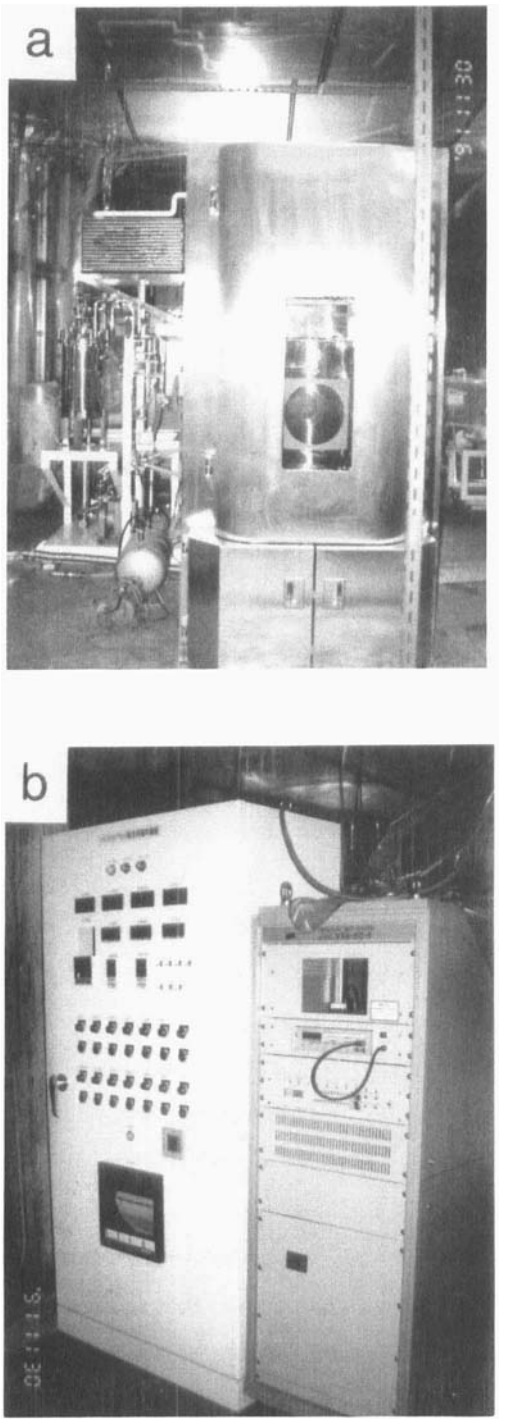


**FIGURE 2**

**Appearance of Spherical Uniform-Sized Microencapsulation Machine a: a uniform-sized droplet atomizing device and steaming apparatus for gel formation were placed inside the front door; b: regulator for this system.**

TABLE 2
Conditions of Manufacture of MC

| Manufacturing Condition | |
|---|---|
| Number of Holes in Nozzle | 536 |
| Vibration Frequency (Hz) | 6460 |
| Vibration Amplitude (μm) | 0.02 |
| Flow Rate (g/min) | 2.77 |
| Temperature of Steaming Trunk (℃) | 50.0 |
| Temperature of Circulating Hot Water (℃) | 50.0 |
| Flow Rate of Circulating Hot Water (L/min) | 20.0 |
| Drying Temperature (℃) | 70.0 |



these wet-gel microparticles had been scraped onto a stainless steel screen to remove the hot water, they were transferred into a vibration-dryer tank to dry for a period of about 1hr. In this fashion, uniform-sized MC were obtained.

An optical microscopic photograph of uniform-sized MC and the particle size distribution as measured with a particle distribution analyzer (Master Sizer, MARUBUN) are shown in FIGURE 3 and FIGURE 4, respectively. The average particle diameter was 102μm, and the minimum and maximum particle diameter were 83μm and 124μm, respectively. A cross section of a MC observed with a scanning electron microscope is shown in FIGURE 5. The surface and the interior of the MC are filled with a gel network AEA structure; and the density of the gel network on the surface was higher than that in the interior. This finding indicated that the surface was almost as susceptible to the effects of transmission of heat as the interior. The dense gel network on the surface of the MC thus appeared to play an important role in preventing dissolution of CAM from MC. The loose apparent density of the MC was 0.48g/cm$^3$, and the angle of repose as determined using the poured angle method was 33.7°. The content of CAM in the MC was 24.3%, approximately the same as the theoretical value. In contrast to

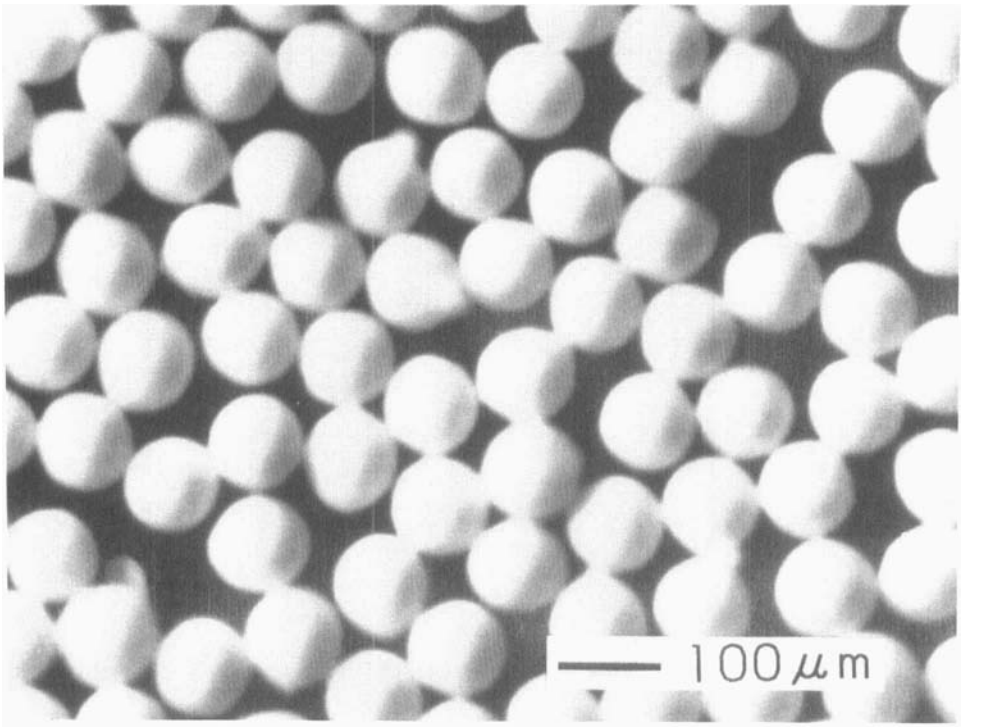


**FIGURE 3**
**Optical Microscopic Photograph of Uniform-Sized Microcapsules**

the conventional coating method using organic solvents, the microencapsulation method described here is free of the risk of explosion and problems associated with residual solvent and environmental pollution (8, 9).

As samples for testing in vitro and in vivo, syrup preparations (without water) consisting of MC containing CAM and various bulking agents were prepared. The formulation of the syrup preparation is listed in TABLE 3. Sucrose, xylitol and saccharin sodium were mixed as sweetening agents, magnesium hydroxide was added as a pH moderator for the syrup, and HPC-L and CMC-Na were added as thickeners. A strawberry essence powder was used to flavor the syrup preparations.

Using these syrup preparations, a series of CAM dissolution studies were carried out using the paddle method (JPXII). The effect of pH of test solution

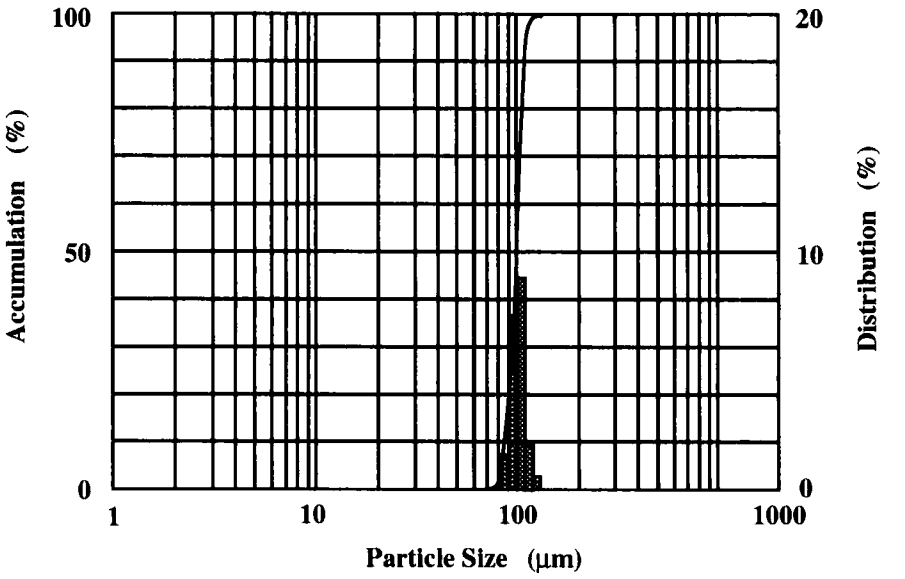


**FIGURE 4**
**Particle Size Distribution and Accumulation of Microcapsules Prepared by Vibration Nozzle Method**



(900ml) on dissolution of CAM from MC is illustrated in FIGURE 6. When the pH of test solution was 1.2 or 4.0, all CAM in MC dissolved in the medium completely within 5min. However, when the pH was 6.8, little dissolution of CAM from MC was observed. Since AEA gel comprises almost 61% of MC by weight, it appeared that dissolution of MC was strongly affected by the solubility of AEA. Therefore, in humans (infants, children and the young) with normal gastric acidity (10-12), MC can be expected to be soluble in the stomach and CAM to dissolve from MC at approximately the same time. In addition, in order to determine the in vitro-in vivo correlation of CAM dissolution (13-16) for MC, various dissolution test media at pH4.0 were prepared by changing the volume of medium and the concentration of solute. CAM dissolution was not significantly affected by the volume of the test solution, but was significantly affected by the concentration of solute (FIGURE 7 and FIGURE 8). The preparations involved CAM, a drug which is slightly soluble in water and whose dissolution is known to

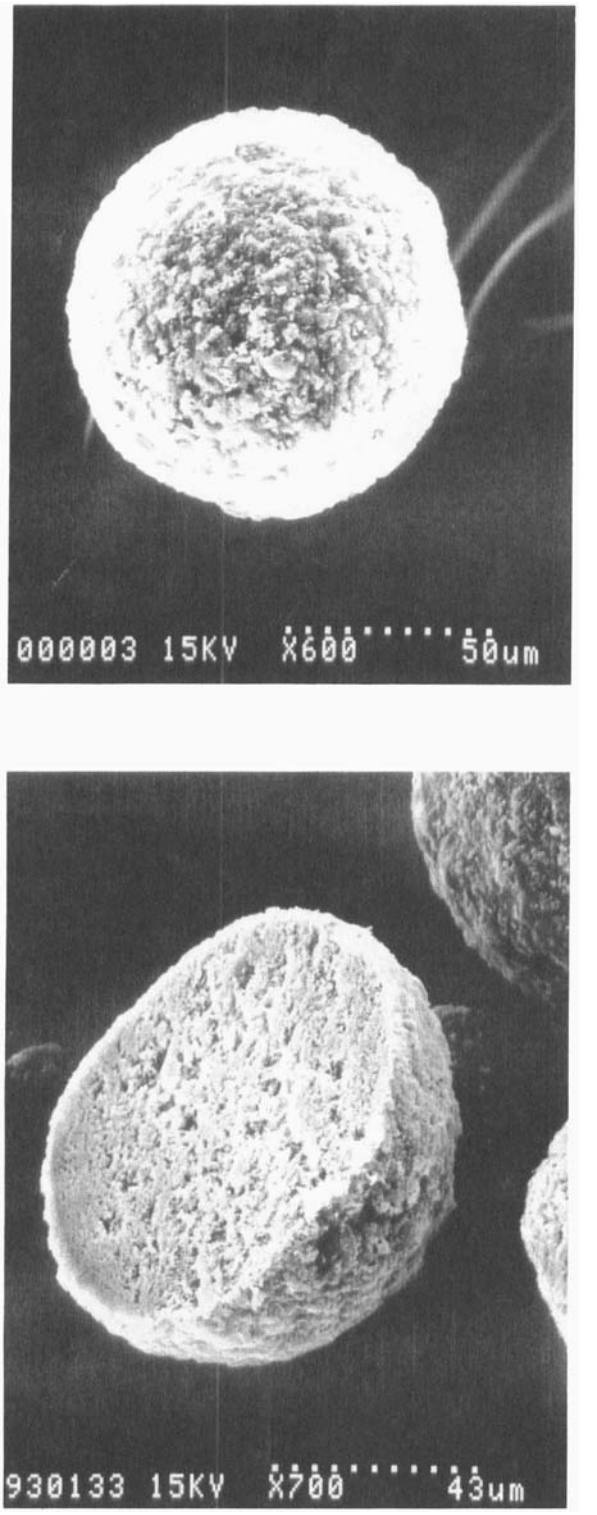


**FIGURE 5**

**Scanning Electron Micrographs of a Microcapsule, and Microcapsule Cross-Section**

TABLE 3

Formulation of Dry Syrup (Oral Suspension)

| Composition | Weight |
|---|---|
| AEA Microcapsules | 412 mg |
| (Clarithromycin) | (100) |
| Sucrose | 113 |
| Xylitol | 400 |
| Saccharin Sodium | 3 |
| Magnesium Hydroxide | 40 |
| HPC-L | 20 |
| CMC-Na | 10 |
| Lubricant | 1 |
| Strawberry Essence | 1 |
| Total | 1000 mg |

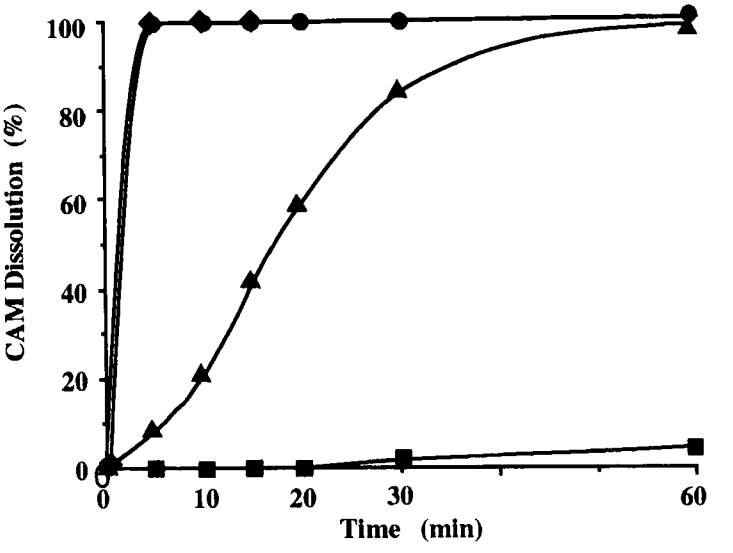


FIGURE 6

Clarithromycin Dissolution Profiles of Syrup Preparations Containing AEA Microcapsules in pH1.2, pH4.0, pH5.8 and pH6.8 Buffers

◆, pH1.2 Buffer; ●, pH4.0; ▲, pH5.8; ■, pH6.8. A 900ml volume of buffered solution was used in all experiments. Each value represents the mean of results of three experiments.

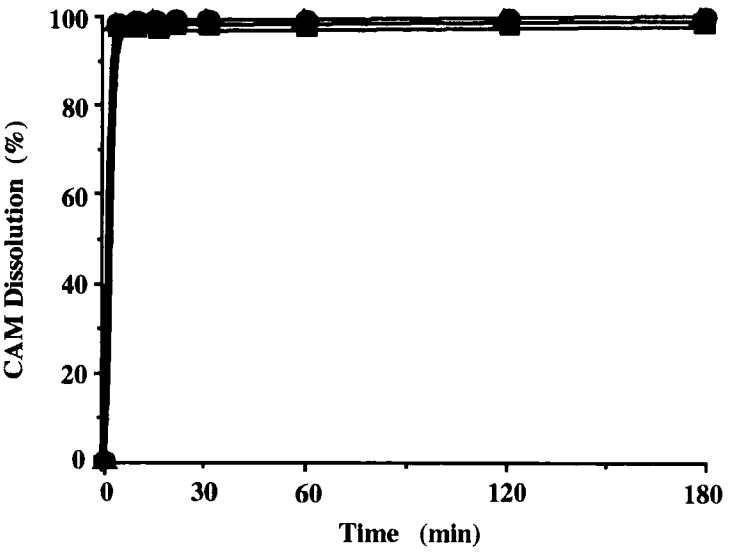


**FIGURE 7**

**Clarithromycin Dissolution Profiles of Syrup Preparations Containing AEA Microcapsules Prepared Using Different Volumes of Buffered Solution at pH4.0 ●, 900ml buffer solution; ▲, 200ml; ■, 100ml. Each value represents the mean of results of three experiments.**

be unaffected by volume of medium (17, 18). The results of our experiments supported those of the above-noted studies. On the other hand, since the total hydrogen ion concentration in 100ml of medium was higher than that in themedium with 1/10 the concentration of control medium, it appeared that extent of CAM dissolution from MC was quite sensitive to concentration of hydrogen ion.

The test for bitter taste was performed every Monday morning for four weeks. The volunteers was divided into 4 groups and administered two preparations (syrup preparations, and CAM intact drug preparations, which were mixed with sweetening agent and other additives as a control) every week after a 5-day rest period, following a predetermined administration schedule. The mean values of bitterness during the 60min period following control preparation administration or syrup preparation administration to 12 male and female

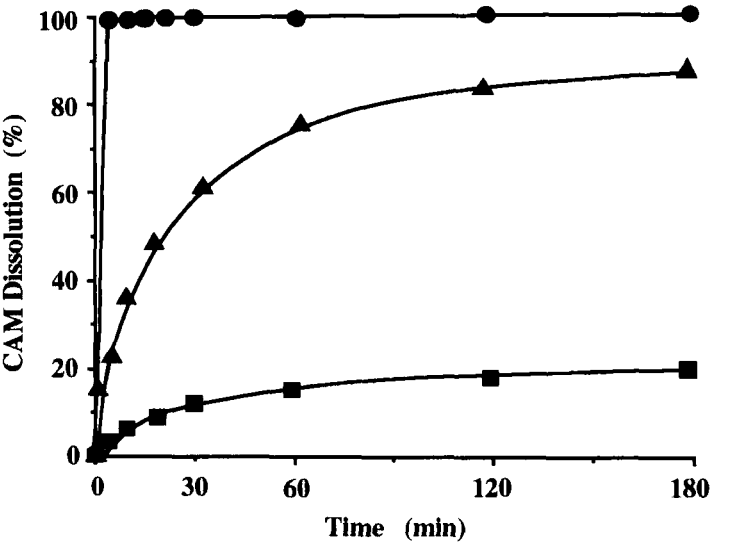


**FIGURE 8**

**Clarithromycin Dissolution Profiles of Syrup Preparations Containing AEA Microcapsules Prepared Using Different Concentrations of Solute in Buffered Solution at pH4.0**

**●, 0.1M citrate-buffered solution as control; ▲, 0.01M citrate-buffered solution; ■, 0.002M citrate-buffered solution. Each value represents the mean of results of three experiments.**

volunteers are shown in FIGURE 9. These volunteers noted extremely bitter taste immediately after administration of CAM intact drug preparations, and bitter taste continued for over an hour. On the other hand, the syrup preparations of uniform-sized MC containing CAM completely masked bitter taste for one hour. Thus, the pronounced bitter-taste masking effect of uniform-sized MC with AEA gel as base was demonstrated.

FIGURE 10 shows the time course changes of mean serum concentration of CAM as well as the area under the serum concentration-time curve ($AUC_{0\text{-}24}$) and the maximum serum concentration ($C_{max}$) calculated for administration of the syrup preparations to 6 male volunteers. The $AUC_{0\text{-}24}$ calculated using the

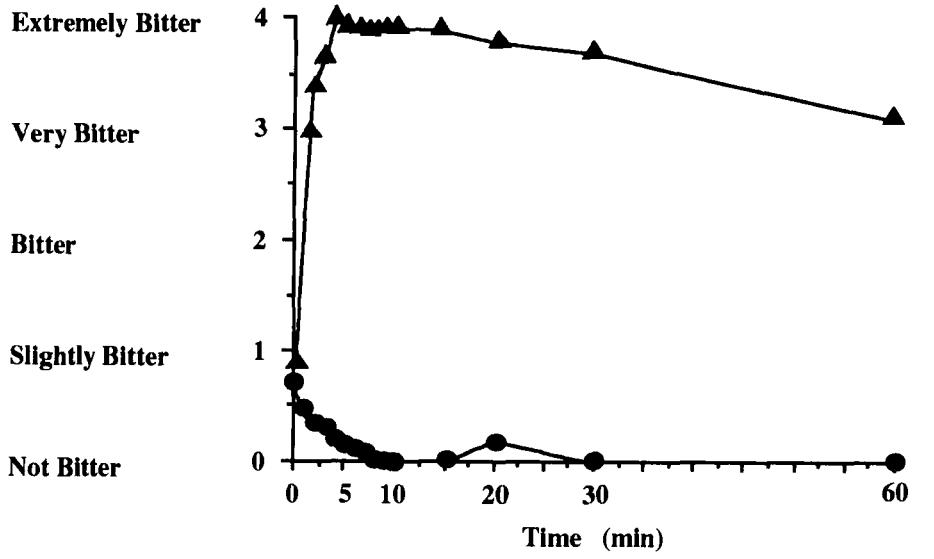


FIGURE 9

**Results of the Test for Bitter Taste after Administration of Syrup Preparations Containing 200mg of Clarithromycin**

**Each point represents the mean of results for 12 male and female volunteers.**

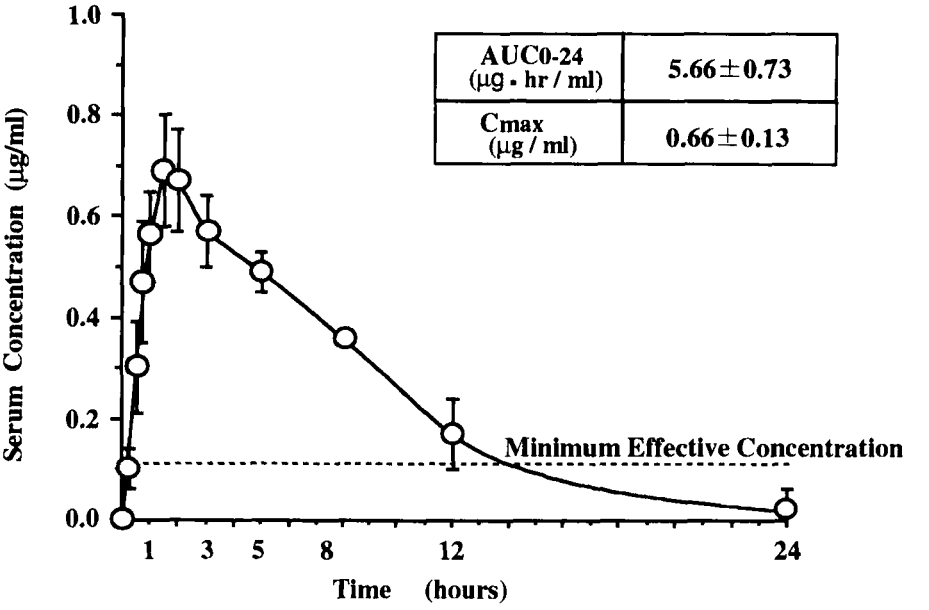


FIGURE 10

**Mean Serum Clarithromycin Concentration-Time Curves Obtained for a Single 200mg/man A dministration of Syrup Preparations Containing Uniform-Sized Microcapsules**

**Each point represents the mean of results for 6 male volunteers.**

trapezoidal rule and $C_{max}$ value were 5.66 ± 0.73μg·hr/ml and 0.66 ± 0.13μg/ml, respectively (19-21). Furthermore, the mean first-order absorption rate constant (Ka) and first-order elimination rate constant (Kel) for the 6 volunteers computed with a one-compartment model using NONLIN84 were 1.164 and 0.110$h^{-1}$, respectively (22). The blood concentration of CAM was maintained at a level above the minimum inhibitory concentration ($MIC_{50}$) for gram-positive bacteria (23-25), i.e., *Staphylococcus aureus* (0.2μg/ml), *Staphylococcus epidermidis* (0.1μg/ml), *Streptococcus pyogenes* (0.05μg/ml), and *Streptococcus pneumoniae* (≤0.025μg/ml), for a period of about 14hr after administration. These findings demonstrated the usefulness of this preparation.

The new uniform-sized microencapsulation system described here enabled the production of 30 kg of uniform-sized MC per 5hr on a dried basis, and afforded an approximately about 90% yield, the same as that of our previous machine. We consider it necessary in the future to increase the yield still more and improve the microencapsulation system.

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