

## Quality of Twelve Clarithromycin Dry Syrup Formulations—Bitterness, Grittiness and Uniformity of Drug Loading

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The objective of the study was to evaluate the bitterness, grittiness and uniformity of drug loading as measures of the quality of 12 formulations of clarithromycin dry syrup (CAMDS), comprising one branded and 11 generic products. Some of the generic CAMDS formulations were more bitter than the branded product while others had similar bitterness when tested as aqueous suspensions. Only one generic product was less bitter than the branded product when tested as a suspension in acidic sports drink. The usual dissolution test described in JP XV could not be used to evaluate the bitterness of the products. A brief dissolution test using only 12.5 ml of water was used to evaluate the bitterness of the products in aqueous suspensions. There were considerable variations in the grittiness of the various products, which were independent of particle size. Changes in grittiness level seemed to be correlated with changes in the intensity of bitterness due to the disintegration of the formulation. Finally, there was less variation in the uniformity of drug loading for the branded product than for the generic products. These data may be useful when selecting which CAMDS formulation to prescribe.

**Key words** clarithromycin dry syrup; generic formulation; bitterness; dissolution test; grittiness

In recent years in Japan, following the introduction of DPC (Diagnosis Procedure Combination) as a comprehensive assessment system in many medical institutions after the amendment of the Medical Service Law, the use of generic formulations has been strongly encouraged in order to reduce healthcare costs. Accordingly, pharmacists are entrusted with the selection of a generic medicine from among many candidate products. The most important factor in selection is information on the quality of the product. Information on generic medicines mainly consists of bioequivalence data from studies required by manufacturers for regulatory approval; other information on generic medicines, such as palatability and formulation characteristics, is scarce compared with the information available on the original branded product.<sup>1–3)</sup>

Clarithromycin dry syrup (CAMDS) is frequently used in treatment of childhood infection, although the extreme bitterness of the chief ingredient, clarithromycin (CAM), is a frequent reason for noncompliance. After expiration of patent protection for CAMDS, 11 generic formulations have been brought onto the Japanese market, but little pharmaceutical information is available about these generic formulations. Not only the bitterness but also the grittiness of CAMDS is important in determining the palatability of CAMDS, while the uniformity of drug loading also seems to be important, as dry syrup formulations require elaborate taste-masking techniques. Many studies have been conducted with the aim of improving patient compliance for CAMDS,<sup>4–7)</sup> but these studies have only been performed on the branded product.

The present study was therefore performed to evaluate the bitterness, grittiness, and uniformity of drug loading as measures of the quality of 12 CAMDS formulations, in order to provide further information to help distinguish between the available generic CAMDS products. Especially for the bitterness, in the present study, we evaluated the bitterness of clarithromycin dry syrup suspended in water since the formulation was usually suspended in water and then administered to children. We already demonstrated that the bitterness

of clarithromycin suspension was almost the same as that of filtered solution in the previous article.<sup>8)</sup> The clarithromycin dry syrup formulation seems to be essentially coated particle with polymer and the significant taste masking effect for dry syrup suspension was well demonstrated in our previous article.<sup>9)</sup> According to these results, the bitterness of suspension seems to be caused by the dissolved fraction of drug in the formulation.

### Experimental

**Materials** The original branded CAMDS product (A, Taisyo Toyama Co., Ltd, Tokyo) and 11 generic formulations of CAMDS (B–L) were used in this study (Table 1). Refined water (pH 7.0), which has almost the same pH as saliva, and an acidic sports drink, pocarisweat® (pH 4) (Otsuka Pharmaceutical Co., Ltd., Tokyo) were used as diluents. Quinine hydrochloride (Sigma Chemical Co., St. Louis, MO, U.S.A.), was used as standard solution of bitterness. All other reagents were high grade.

**The pH Measurement of CAMDS Suspended in Water/Acidic Sports Drink and Evaluation of Their Bitterness Intensities by Gustatory Sensation Tests** One gram sample of each CAMDS formulation (A–L) was suspended with stirring (300 rev./min) in 25 ml of water or an acidic sports drink for 30 s, 2 min, or 5 min. After suspension, the pH of the suspension was measured directly using a pH meter (HORIBA, F-21, Kyoto, Japan). The bitterness intensities of products suspended in water or acidic sports drink for 30 s or 5 min were determined by gustatory sensation tests, as described in the experimental section. A formulation was categorized as 'a bitter formulation' when the bitterness was almost equal to the threshold ( $\tau_1$ ) or higher than that evaluated in the gustatory sensation test. In assessments of bitterness after suspension in an acidic sports drink, a formulation was categorized as 'a formulation with reduced bitterness' when the bitterness was below the saturation level ( $\tau_4$ ) in the gustatory sensation test performed 30 s or 5 min after suspension, as the bitterness of the original branded product was reported to reach saturation level ( $\tau_4$ ) according to a previous report.<sup>10)</sup>

**Gustatory Sensation Tests for Evaluation of Bitterness Intensity or Grittiness of Product Suspended in Water/Acidic Sports Drink** The samples used in the gustatory sensation test were tested after stirring (300 rev./min) 1.0 g of each CAMDS formulation in 25 ml of water or an acidic sports drink for 30 s or 5 min. Gustatory sensation tests were done using the equivalent density examination method of Katsuragi *et al.*<sup>11)</sup> The standard quinine hydrochloride concentrations used were 0.01, 0.03, 0.10, 0.30, and 1.00 mM and the corresponding bitterness scores were defined as 0, 1, 2, 3, and 4, respectively. Before testing, the volunteers ( $n=8$ ) were asked to keep the above standard quinine solutions in their mouths, and were told

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Table 1. pH Values in Water or Acidic Sports Drink<sup>a)</sup>

Formulation		pH value in water			pH value in acidic sports drink		
		30 s	2 min	5 min	30 s	2 min	5 min
Original brand name product	A	9.31±0.10	10.32±0.11	10.55±0.13	3.98±0.08	4.48±0.08	4.71±0.05
Generic formulations	B	10.61±0.10	10.61±0.10	10.61±0.11	4.80±0.10	7.35±0.15	9.40±0.05
	C	9.87±0.16	10.25±0.32	10.71±0.10	3.51±0.13	4.26±0.05	4.47±0.06
	D	9.59±0.10	10.08±0.10	10.25±0.05	3.94±0.05	4.51±0.09	4.60±0.05
	E	10.22±0.11	10.40±0.10	10.54±0.05	4.16±0.05	6.08±0.08	6.50±0.05
	F	9.75±0.13	9.29±0.09	9.07±0.04	3.61±0.10	3.83±0.11	3.98±0.08
	G	10.18±0.07	10.36±0.05	10.44±0.05	4.44±0.05	5.90±0.10	6.51±0.11
	H	10.04±0.15	10.11±0.10	10.23±0.05	4.13±0.06	4.65±0.05	4.89±0.08
	I	9.84±0.16	10.06±0.06	10.10±0.12	4.28±0.07	4.89±0.09	5.39±0.09
	J	6.88±0.07	6.47±0.07	5.94±0.04	3.85±0.05	4.38±0.08	4.96±0.06
	K	10.15±0.05	10.26±0.05	10.81±0.10	4.63±0.06	7.22±0.07	9.17±0.10
	L	9.60±0.10	10.26±0.06	10.45±0.05	4.32±0.10	6.37±0.15	9.53±0.11

a) Values are mean±S.D. (n=3).

the concentrations and bitterness scores of each solution. After tasting 2 ml of a test formulation suspended in water, they were asked to give the sample a bitterness score. All samples were kept in the mouth for 15 s. After testing the sample, the volunteers rinsed their mouths well and waited for at least 20 min before tasting the next sample.<sup>12)</sup>

The grittiness of the suspension in water as perceived in the oral cavity was assessed on the following rating scale: 0, no grittiness; 1, grittiness; 2, strong grittiness. The protocol and experimental designs for all gustatory sensation tests had the prior approval of the ethical committee of Mukogawa Women's University.

**Dissolution Test**<sup>13)</sup> In accordance with Method 2 (Paddle method) of the Dissolution Test in JP XV, the dissolution of CAM products was tested after addition of the formulation to purified water (pH 7.0), or buffer at pH 4.0 and pH 6.0, from immediately after addition for up to 10 min. The dissolution test was conducted with 0.5 g of each formulation and 500 ml of each solvent at 37 °C and a paddle rotation speed of 100 rev./min. The amount of CAM dissolved after 30 s, 1, 2, 3, 5, and 10 min dissolution, was assayed by HPLC.

**Dissolution Test in Small Volumes of Water** The amount of CAM dissolved was determined as far as possible under normal administration conditions (*i.e.*, conditions mimicking the oral cavity with a small volume of saliva). This dissolution test was conducted with 0.5 g of each CAMDS formulation and 12.5 ml of water (at 25 °C) using a stirring bar (2.5 cm width) at a paddle rotating speed of 300 rev./min. The amount of CAM dissolved was assayed by HPLC at 30 s and 5 min after addition of the formulation.

**Stereoscopic Microscope Observation of Formulations Suspended in Water or Acidic Sports Drink** The surface structure of each formulation of branded product A and generic products B (categorized as 'a formulation with reduced bitterness') and E (categorized as 'a bitter formulation'), was examined under a stereoscopic microscope (Olympus SZX10) after suspension in water or acidic sports drink for 30 s or 5 min. Changes in particle size and surface structure were noted.

**Particle Size Distribution and Determination of CAM Loading in CAMDS Formulations** Determination of particle size distribution was determined in accordance with the JP XV. Samples of 5.0 g of each formulation were sub-divided according to particle size using stainless steel sieves of 75 mm inside diameter (500, 355, 150 μm).<sup>3)</sup> The weight % of particles of each size class (≥500, 355–500, 150–355, ≤150 μm, defined as large, medium, small, and very small particles, respectively) was determined. To 0.05-g samples from each particle size class were added 6 ml of ethanol and butyl *p*-hydroxybenzoate as an internal standard; the samples were then subjected to ultrasound for 30 min. Ethanol was added to make the volume up to 10 ml and the mixture was centrifuged (3500 rev./min for 10 min). A 1-ml aliquot of supernatant was taken, centrifuged again (12000 rev./min for 5 min), and assayed for CAM by HPLC. For the HPLC, 10 μl was injected onto a chromatograph (Shimadzu LC-10AT, Kyoto, Japan) equipped with a UV detector (Shimadzu SPD-10A, Kyoto, Japan), an integrator (Shimadzu C-R7A, Kyoto, Japan), a reverse-phase column (Cosmosil 5C18-AR, 4.6×150 mm, Nacalai Tesque Co., Ltd., Japan). The column temperature was 50 °C. The following mobile phase system was used: 0.2 mol/l KH<sub>2</sub>PO<sub>4</sub> (1→3) solution: acetonitrile, 13:7 (pH 4.5). The flow rate was 1 ml/min. The wavelength was 210 nm.

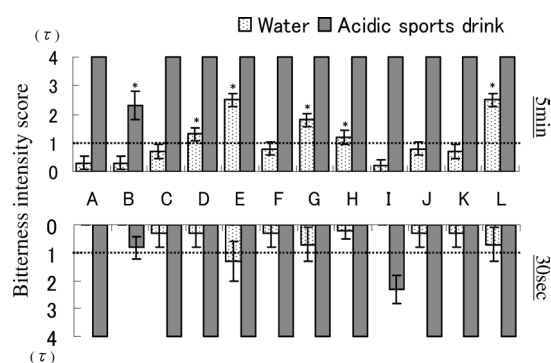


Fig. 1. Bitterness Intensities of the CAMDS Formulations after Suspension in Water or Acidic Sports Drink (at 30 s and 5 min)

\* $p < 0.05$ : significant difference in bitterness intensity compared with original branded product (A) using Mann-Whitney *U*-test. Values are mean±S.D. (n=8).

**Statistical Analysis** Multiple comparisons in bitterness intensity were made using the Mann-Whitney *U*-test. A value of  $p < 0.05$  was taken as indicating a significant difference between values.

## Results and Discussion

**Measurement of pH and Evaluation of Bitterness Intensity of CAMDS by Gustatory Sensation Tests** The pH values of CAMDS formulations after suspension in water or acidic sports drink are shown in Table 1. The pH of product J changed gradually from neutral to about pH 6 by 5 min after suspension in water. Apart from J, the other formulations made alkaline solutions (pH 9 to 11) 5 min after suspension. When suspended in acidic sports drink, the pH of all formulations was almost 4 at 30 s after suspension. By 5 min after suspension in acidic sports drink, some formulations (B, K, L) had become alkaline (pH 9), while the rest became acidic (E, G, and I were pH 6 while A, C, D, F, H, and J almost reached pH 4).

The bitterness intensities of the CAMDS formulations after suspension in water or acidic sports drink (at 30 s and at 5 min) are summarized in Fig. 1. After suspension in water for 30 s, the bitterness intensities of all formulations except E were below the bitterness threshold ( $\tau_1$ ).<sup>10)</sup> Formulations A, B, and I scored almost 0 for bitterness, while the bitterness intensity of E exceeded the bitterness threshold ( $\tau_1$ ). After suspension in water for 5 min, the bitterness intensity of five

formulations (D, E, G, H, and L), increased to  $\tau_1$  or above, their bitterness scores being significantly higher than that of the original branded product A. In particular, formulations E and L were intensely bitter (bitterness intensity of  $\tau_2$  or above). Therefore the five formulations D, E, G, H, and L, were considered to be 'bitter formulations'.

When suspended in an acidic sports drink, the bitterness of formulations other than B and I reached saturation level ( $\tau_4$ )<sup>10</sup> 30 s after suspension. The bitterness of formulation I reached saturation level ( $\tau_4$ ) after 5 min. Formulation B had a bitterness intensity at or below the threshold value ( $\tau_1$ ) after 30 s and reached a bitterness of  $\tau_2$  or  $\tau_3$  after 5 min, which was significantly lower than the bitterness of the original branded product. Therefore formulation B was considered to be 'a formulation with reduced bitterness' when co-administered with an acidic sports drink.

The results of pH determination and bitterness assessment of suspensions of each formulation in water or in acidic sports drink showed no correlation between pH and bitterness intensity.

Although an alkaline polymer coating is employed for suppression of bitterness in most formulations, the pH and bitterness intensity after suspension vary among the different formulations due to differences in additives, manufacturing process, means of suppression of bitterness, *etc.* CAMDS is used mainly for children, and bitterness is therefore the critical factor for patient compliance, children being, in general, more sensitive to a bitter taste than adults.<sup>14</sup> Further, intake by children may be rather slow and it may be necessary to suppress bitterness for up to 5 min.<sup>15</sup> In the assessment of suspensions in acidic sports drink, formulations other than B showed intensive bitterness, being at the saturation level of bitterness. With formulation B, although bitterness was increased it still remained below the saturation level, and therefore B is expected to be less bitter than the other CAMDS formulations when coadministered with acidic sports drink. In the case of formulation B, the pH value of the formulation suspended in acidic sports drink for 30 s was 4.8 and the value was larger than many other formulations as shown in Table 1. This larger pH value for the suspension of formulation B might be reason for successful taste making. The kind of sweetener or its release rate might also affect taste making effect of the dry syrup formulation.

**Dissolution Characteristics of CAMDS Formulations at pH 4.0, pH 6.0 and pH 7.0** Dissolution data for 12 CAMDS formulations in water (pH 7.0) and at pH 4.0 and pH 6.0 for up to 10 min are shown in Fig. 2. As the pH increased, the amount of dissolved CAM decreased, CAM being a typical basic compound. In water, the amount of CAM dissolved from the 12 CAMDS products was negligible. Even at pH 6, the amount dissolved in the medium was low. At pH 4.0, however, the dissolved % was almost 40% in the case of formulations K and L, while for other formulations, 60–80% release was observed at 10 min. In general, the pH in the stomach is about pH 1–2. The above information suggests that all products show normal bioavailability when the conditions inside the stomach are acidic. If patients are taking H<sub>2</sub> blockers or proton-pump inhibitors, however, the bioavailability of generic products like K and L might be lower than the branded product, due to the neutral conditions inside the stomach. In conclusion, this dissolution test data

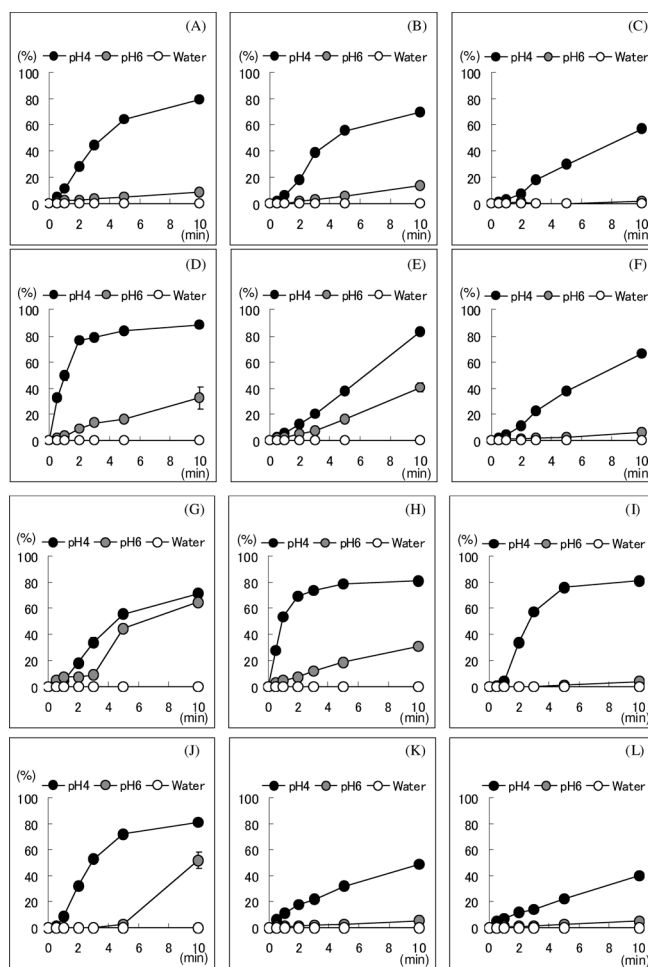


Fig. 2. Dissolution Test of CAM Formulations at pH 4.0, pH 6.0, and in Water (pH 7.0)

Values are mean  $\pm$  S.D. ( $n=3$ ).

could not predict the bitterness of each product, although it provides useful information on the bioavailability of the generic products.

#### Dissolution Test for CAM in Small Volumes of Water

In order to find a better method to predict bitterness, the CAM was dissolved in a smaller volume of water, more similar to the situation which occurs when CAMD is dissolved in saliva in the oral cavity. The results showing the bitterness of the solutions 5 min after 0.5 g samples of each formulation were dissolved in 12.5 ml of water are shown in Fig. 3. According to previous reports,<sup>6,16</sup> a CAM solution of 14  $\mu\text{g/ml}$  represents the threshold of bitterness ( $\tau_1$ ) while a solution of 240  $\mu\text{g/ml}$  represents the saturation level of bitterness ( $\tau_4$ ). Thus, formulations which can be predicted to be bitter on the basis of the dissolution test are those at a concentration of 14  $\mu\text{g/ml}$  or above after 5 min (*i.e.* D, E, G, H, and L).

Formulation L showed poor dissolution in the dissolution test described above, due to its poor dispersion. However, its dissolution at the higher concentration and the greater rotation speed (300 rev./min) used in this experiment to mimic practical use of the formulation, reflects the result of the gustatory sensation tests well. This probably resulted primarily from improved dispersion as a result of increased rotation speed.

Intake of the bitter formulations (*i.e.*, those with CAM dis-

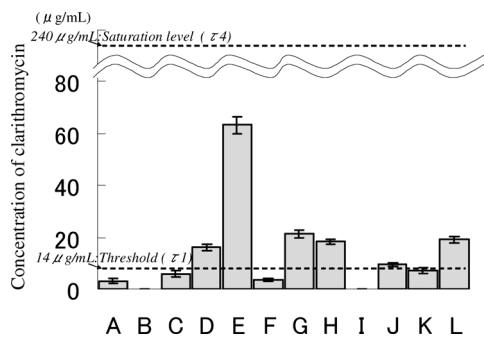


Fig. 3. Concentration of CAM in Suspension of CAMDS (0.5g) in 12.5 ml Water after Stirring for 5 min

Values are mean  $\pm$  S.D. ( $n=3$ ).



Fig. 4. Grittiness of CAMDS Formulations after Suspension in Water (after 30 s or 5 min)

Values are mean  $\pm$  S.D. ( $n=8$ ).

solution of  $14 \mu\text{g/ml}$  or above) with a greater volume of water will lower the CAM concentration, and thus reduce bitterness to some extent. On the other hand, the increased volume of water may itself present a problem with respect to intake.

**Evaluation of Grittiness for CAMDS** The results of assessment of grittiness are shown in Fig. 4. The formulations were separated into those with reduced grittiness at 5 min compared with those at 30 s (D, E, G, H, L) and those whose grittiness remained the same at 30 s and 5 min (A, B, C, F, I, J, K). The formulations whose grittiness changed between 30 s and 5 min had a bitterness intensity of  $\tau_1$  or above in the gustatory sensation test. The change in grittiness was thought to be due to disintegration of the coating of the formulation, resulting in CAM release. Formulations A, B, C, F, I, J and K, whose grittiness was the same at 30 s and at 5 min, showed bitterness intensity at the threshold value or below, suggesting no disintegration of the coating of the formulation. These results demonstrated that bitterness and change of grittiness with time correlated closely with each other.

In addition to bitterness, grittiness also seems to be an important factor influencing palatability in children.<sup>17)</sup> The results of assessment of grittiness at 5 min indicated that formulations B, F, and I have no bitterness and only low grittiness (grittiness score: 1 or below). Therefore it may be concluded that these generic formulations are particularly useful for oral administration to children.

**Examination of Surface Structure of CAMDS Formulations Suspended in Water and in Acidic Sports Drink** Stereoscopic microscopic photographs of particles after 30 s and 5 min suspension in water are shown in Fig. 5 for formulation A (bitterness at or below the threshold ( $\tau_1$ ) in suspen-

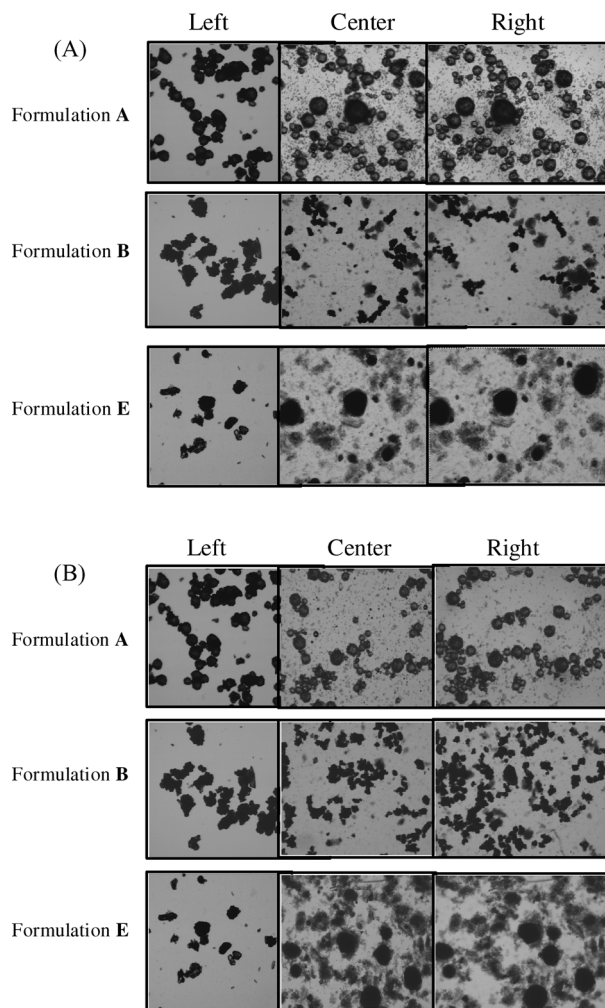


Fig. 5. Stereoscopic Microscopic Photographs of Particles after 30 s or 5 min Suspension in Water (A) or Acidic Sports Drink (B)

Left: before suspension in water; centre: after 30 s suspension; right: after 5 min suspension.

sion in water and at the saturation level ( $\tau_4$ ) in suspension in acidic sports drink), B (bitterness at or below the threshold ( $\tau_1$ ) in suspension in water and below the saturation level ( $\tau_4$ ) in suspension in an acidic sports drink), and E (bitterness at or above the threshold ( $\tau_1$ ) in suspension in water and at the saturation level ( $\tau_4$ ) in suspension in acidic sports drink). Similarly, photographs after suspension in acidic sports drink are shown in Fig. 5.

The stereoscopic microscopic photographs of surface structure revealed no remarkable changes in size and shape of particles either at 30 s or at 5 min for formulations A or B, which showed no bitterness after suspension in water, although for A it was confirmed that particles were larger at 5 min than at 30 s. In contrast, formulation E showed marked changes in both size and shape of particles at both 30 s and 5 min. An increase in size of the particles indicates that water has penetrated into the particle, causing CAM dissolution.

Formulation A was equivalent to E in bitterness intensity after suspension in acidic sports drink while the grittiness of A was greater than that of E. It may be that the particles are better protected in formulation A, due to the manufacturing technique, making them less susceptible to deformation.

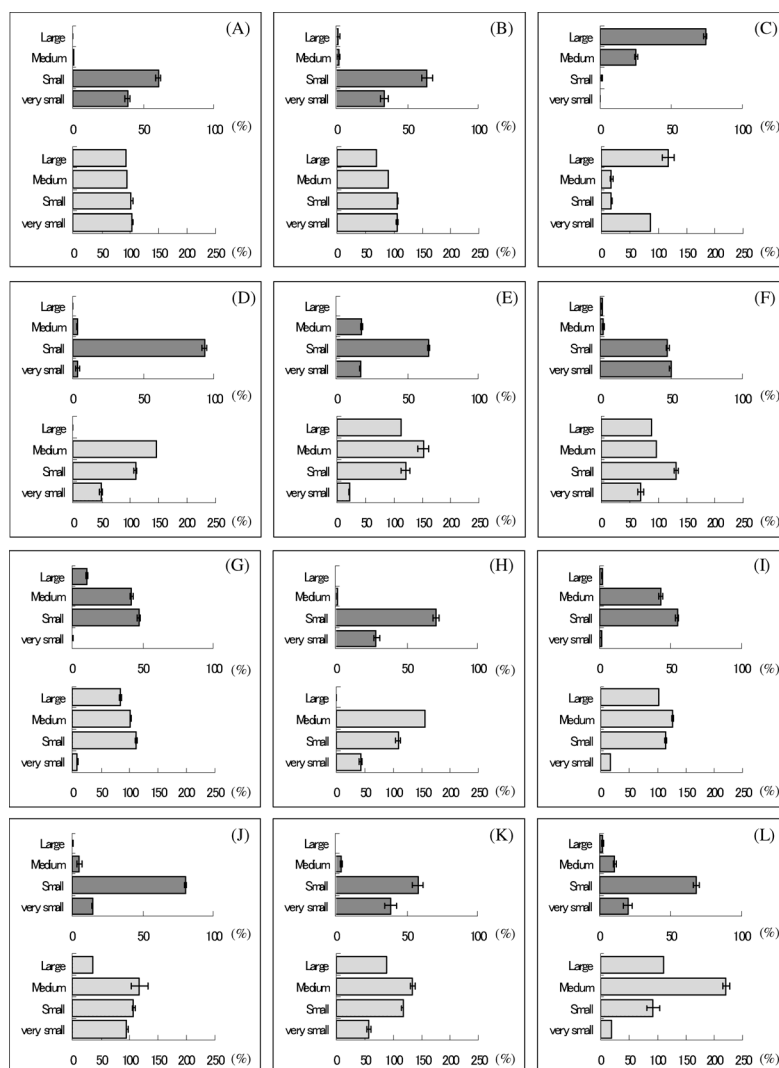


Fig. 6. Particle-Size Distribution and Drug Loading of CAM in CAMDS Formulations

Above (shadowed columns): weight of particles of each size class (%); below (dotted columns): CAM extracted from particles with different particle size (%). Values are mean  $\pm$  S.D. ( $n=3$ ).

**Particle-Size Distribution and Drug Loading in Different CAMDS Formulations** The bitterness of CAMDS formulations was assessed on the basis of particle-size distribution of the formulation, and content and dissolution of the active ingredient.

The particle-size distribution and active ingredient content by particle size of the 12 CAMDS formulations are shown in Fig. 6. The particle-size distributions of the generic drugs were different from the branded product. Good uniformity of drug loading in different diameter particles was confirmed in the branded product while all the generic products except B showed large variance in drug loading among particles of different diameter (50–200%).

**Conclusion**

1. Five of the generic CAMDS formulations were more bitter than the branded product when suspended in water, while one generic formulation was less bitter than the branded product when suspended in acidic sports drink.
2. The dissolution characteristics of generic products at pH 4, pH 6 and water (pH 7) was not significantly different from that of the branded product, although two generic

products showed less dissolved % of drug compared to the branded product, which might suggest a risk of reduced bioavailability in patients taking H2 blockers or proton pump inhibitors.

3. The CAM concentrations found when the formulations are dissolved in a small volume of water, can predict the bitterness intensity of the product measured in human gustatory sensation tests.
4. The grittiness of the products was not directly correlated with the mean diameter of particles in each formulation.
5. The particle size distribution of the generic formulations was different from that of the branded product. Good uniformity of drug loading among different diameter particles was confirmed in the branded product while almost all the generic products showed large variance in drug loading among different diameter particles (50–200%).

Based on these results, it can be concluded that formulations B, F, and I may be useful in the pediatric field due to their relatively high suppression of bitterness and good palatability. In particular, the bitterness of B was successfully suppressed when suspended in acidic sports drink. Formula-

tion A showed some grittiness but the high quality of its manufacturing process provided good suppression of bitterness.

Some generic products are no less useful than the original branded product with respect to suppression of bitterness and palatability. The results obtained in the present study provide useful information for patients with respect to selection of formulations. Currently, many pharmacists working at medical institutions provide information collected from companies, academic papers, presentations at academic meetings, *etc.*, however, little information is available to them in which generic products are compared with the original branded product. Therefore pharmacists are required to analyze and compare products from multiple sources, depending on whether the product is an original or a generic copy. The authors believe that medical and research & education institutions (universities) should work in closer cooperation to make such comparisons easier.

**Acknowledgement** We are grateful to Miss Mayuko HAYASHI and Kana NIOKA for their technical assistance.

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