

Method of Evaluation of the Bitterness of Clarithromycin Dry Syrup¹⁾

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The degree of bitterness of clarithromycin (CAM) dry syrup was evaluated using several methods. Using the inversion method, shaking method, and paddle method, a reasonable correlation between the bitter taste and the amount dissolved was not observed. A mini-column with inner diameter of 0.76 cm and height of 5 cm packed with CAM dry syrup was used for the release test. The release rate of CAM in test solution, which passed through the mini-column, was then measured to evaluate bitterness. The release rate of CAM in the release test using the mini-column correlated well with the results of a sensory test for the bitterness of CAM dry syrup. The dissolution rate constant, defined as the percentage of CAM dissolved from the unit void surface multiplied by the void volume, was inversely proportional to the linear velocity of the test solution. The critical factors affecting evaluation of bitterness were the void volume of the column and linear velocity of the test solution. The optimum linear velocity and void volume were 0.048–0.021 cm/min and 0.27–0.12 cm³, respectively. In addition, the threshold of bitterness of CAM dry syrup was defined as the concentration at which half of the volunteers recognized bitterness in the sensory test. This threshold was found to be 135 µg/ml using the mini-column.

Key words release test; clarithromycin; spray-congealing technique; mini-column; sensory test; linear velocity

When pharmaceutical compositions comprised of ingredients with an unpleasant taste are formulated, it is important not only to mask the taste by pharmaceutical technology but also to evaluate the degree of improvement in taste. The surest method to evaluate taste is sensory testing with human volunteers.^{1,2)} However, since the sensory test is difficult to perform, a simple alternative method is required. *In vitro* release tests, such as the paddle method in the Japanese Pharmacopoeia, measurement of the amount of release in a centrifugal tube with shaking or in an injector with continuous inversion,^{3,4)} and other tests, are often substituted for sensory tests as surrogate methods of evaluation.

Clarithromycin (CAM), a macrolide antibiotic, has a strong bitter taste. Koyama *et al.* examined the effect of CAM concentration on the bitterness and reported the threshold of bitterness of CAM solution is 14 mg/l,⁵⁾ the concentration at which half of human volunteers recognize bitterness.

To mask bitterness, several pharmaceutical technologies are used for the commercial CAM pediatric dosage form (dry syrup).⁶⁾ Manufacturing conditions also affect the degree of bitterness of CAM dry syrup.⁷⁾ Since that dosage form consists of matrix comprised of CAM, the elevation of temperature during the drying process enhances the bitterness. In our first stage, the paddle method was used to evaluate the release rate of each batch manufactured under various conditions. No correlation was found between the amount of release and the degree of bitterness, however.

In this dosage form, as the amount of release of CAM from dry syrup increases, the bitter taste increases. Thus perfectly taste-masked batches do not always exhibit a low release pattern. In this study, a new method for the evaluation of bitterness of CAM dry syrup using a mini-column technique was examined and optimum measurement conditions were defined. The threshold of bitterness of CAM from dry syrup was also obtained using this method.

Experimental

Materials CAM was synthesized at Taisho Pharmaceutical Co., Ltd.

Glyceryl monostearate (GM) was of the grade specified in the Pharmacopoeia of Japan. Aminoalkyl methacrylate copolymer E (AMCE) was of commercial grade. The additives used in this study were of the grade specified in the Pharmacopoeia of Japan.

Preparation of Original CAM Dry Syrup AMCE was dissolved in melted GM at 120 °C. CAM was added to the melted solution and homogeneously dispersed. Subsequently, the dispersion was transferred to a spray dryer (CL-12, Ohkawara Kakouki Co., Ltd.), and atomized to prepare CAM matrix under cooling. The CAM matrix was mixed with D-mannitol, corn starch, hydroxypropylcellulose, and other vehicles. CAM dry syrup was prepared from this mixture with wet granulation. One gram of this dry syrup contained 100 mg of CAM.

Preparation of CAM Dry Syrups with Various Degrees of Bitterness Dry syrup was heated to 70 °C for 90 min to enhance bitterness, and the original and heat-treated dry syrup were blended at various ratios and used for the sensory test.

Measurement of Particle Size Distribution and Particle Number The particle size distribution of the dry syrup was measured using the laser diffraction method (Microtrac FRA, Nikkiso Co., Ltd.). The number of particles which remained on a 300 µm screen per 0.25 g of dry syrup was counted manually.

Release Study 1 (Inversion Method) About 1 g of CAM dry syrup, accurately weighed, was packed in a 10-ml injector. Five milliliters of water was added to the injector, which was inverted and returned to the upright position 10 times at 3-s intervals. Then the suspension was filtered through a membrane filter (pore size 0.45 µm), and the filtrate was used as the sample solution. The amount of release of CAM in 100 µl of sample solution was determined by HPLC under the following operating conditions: ultraviolet absorption photometer wavelength, 210 nm; column, reversed-phase column (L-column, 15 cm×4 mm i.d., Chemicals Inspection & Testing Institute, Japan); column temperature, constant temperature around 50 °C; mobile phase, mixture of 1/15 M monobasic potassium phosphate and acetonitrile (13 : 7); flow rate, adjusted so that the retention time of the commonly used standard CAM was about 8 min.

Release Study 2 (Shaking Method) About 0.5 g of CAM dry syrup, accurately weighed, was placed in a 50-ml centrifugation tube. Twenty milliliters of phosphate buffer solution, pH 6.5, was added to the centrifugation tube. Then the centrifugation tube was shaken at 150 times per minute (shaking width: 40 mm). After shaking for 10 or 30 min, the suspension was immediately centrifuged at 3000 rpm for 1 min. The supernatant was filtered through a membrane filter with pore size of 0.45 µm, and this solution was used as sample solution.

Separately, about 25 mg of standard CAM, accurately weighed, was dissolved in the mobile phase to exactly 100 ml, and this solution was used as standard solution. Tests with 10 µl each of the sample solution and the stan-

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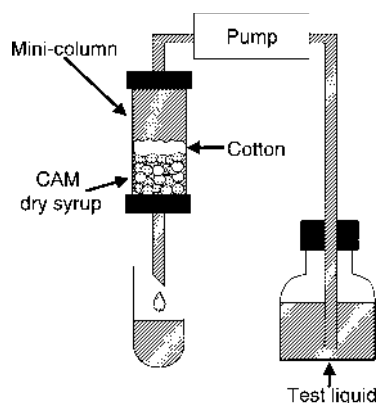


Fig. 1. Mini-column Apparatus for Evaluating the Bitterness of CAM Dry Syrup

standard solution were performed as described for liquid chromatography under the above-mentioned conditions. Peak areas in these solutions were calculated by automatic integration.

Release Study 3 (Paddle Method) The paddle test with about 1.0 g of CAM dry syrup, accurately weighed, was performed at 100 rpm according to method 2 under dissolution testing in the Japan Pharmacopoeia, using 900 ml of acetic acid and sodium acetate buffer solution, pH 5.5, and using 900 ml of phosphate buffer solution, pH 6.5. At appropriate intervals (5, 10, 20, 30, 45, 60 min), 5 ml of each solution was collected and filtered through a membrane filter with pore size of 0.45 μm . The dissolved media were then replenished with 5 ml of fresh buffer solution to maintain a constant volume. Separately, about 50 mg of standard CAM, accurately weighed, was dissolved in 50 ml of the buffer solution. Five milliliters of this solution was pipetted off, and fresh buffer solution was added to exactly 50 ml. This solution was used as the standard solution. Tests with 10 μl each of the sample solution and the standard solution were performed as described for liquid chromatography with the conditions described for release study 2. Peak areas for these solutions were calculated by automatic integration.

Scanning Electron Microscopic Observation and pH Measurement The surface of CAM dry syrup was observed using a scanning electron microscope (SEM) (S-2500, Hitachi Ltd.). The distribution of alkaline substance on the surface of CAM dry syrup was evaluated to measure the pH value on the surface of sample stored for 1 d in a desiccator saturated with water vapor using pH paper.

Mini-column Method The mini-column method is schematically shown in Fig. 1. Columns with an inner diameter of 0.76 or 0.46 cm and with a length of 5 cm were used. About 0.5, 0.25, or 0.125 g of CAM dry syrup, accurately weighed, was packed in the column. After tapping the column 30, 10, or 3 times, absorbent cotton was packed on the sample bed to eliminate sample motion. The column was installed so that filled dry syrup was located in the bottom. Phosphate buffer solution, pH 6.5, filled the entire space of the column, and the nipple was then immediately attached to the top of the column. The upper part of the column was connected to a pump and filled with buffer solution in advance. The same solution flowed through this column at 0.7, 0.5, or 0.3 ml/min. The eluate was collected at 1-min intervals for 5 min or at 2-min intervals for 6 min. Each eluate was used as sample solution. Separately, about 50 mg of standard CAM, accurately weighed, was dissolved in 50 ml of buffer solution. Five milliliters of this solution was pipetted off, and fresh buffer solution was added to exactly 50 ml. This solution was used as the standard solution. Tests with 10 μl each of the sample solution and the standard solution were performed as described for liquid chromatography under the conditions described for release study 3. Peak areas for these solutions were calculated by automatic integration.

Sensory Test Ten volunteers participated in the sensory test. One gram of dry syrup was dispersed in 25 ml of water for 30 s. Immediately after preparation, each volunteer held about 1 ml of the dispersion in the mouth for 30 s. After expectoration, bitterness was evaluated. The degree of bitterness was classified using 4 grades, corresponding to increasing bitterness, and a comparison of bitterness among samples was performed based on the total number of persons who selected "bitter" and "slightly bitter". The ranking scheme used is shown in Table 1. The threshold of bitterness of dry syrup was determined as the point at which half of the volunteers described the taste as bitter or slightly bitter.

Table 1. Grading

Not bitter	1
Almost not bitter	2
Slightly bitter	3
Bitter	4

Table 2. Evaluation of Dry Syrups with and without Bitterness by Shaking Method ($n=3$)

n	Intact ($\mu\text{g/ml}$)	Heat-treated ($\mu\text{g/ml}$)
1	3.66	54.72
2	9.25	18.06
3	9.32	Impossible to filter

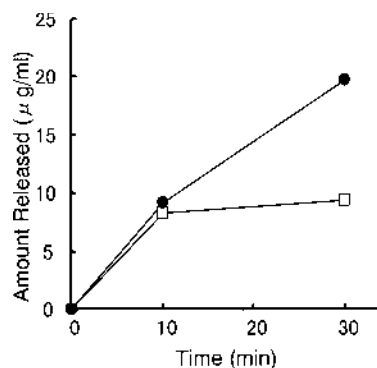


Fig. 2. Release of CAM from Dry Syrups with and without Heat Treatment in Phosphate Buffer Solution, pH 6.5, by the Shaking Method

●, Intact dry syrup; □, dry syrups with heat treatment (70 $^{\circ}\text{C}$, 90 min).

Results and Discussion

Inversion Method The effect of heat treatment on the amount of CAM released from dry syrup was examined using the inversion method reported by Sugao *et al.*,⁴⁾ and the results are shown in Table 2. The amount of CAM released from dry syrup varied in each test. The test was performed three times. Heat treatment tended to enhance the amount of CAM released. However, the test did not exhibit repeatability, probably due to the hydrophobicity of the CAM matrix. Furthermore, in one of the tests, it was impossible to filter the CAM solution completely using a membrane filter, because the liberated solid particulate clogged the pores. For this reason, the inversion method was not used for evaluation of the bitterness of CAM dry syrup.

Shaking Method Figure 2 shows the amount of CAM released from dry syrups with and without heat treatment using the shaking method. The test solution of pH 6.5 was used, because this pH is equivalent to that in the oral cavity. Although the dry syrup with heat treatment was more bitter than the intact one, its release rate was slower. Thus the fact that heat-treated dry syrup is more bitter than the intact one did not reflect the effect of shaking.

Paddle Method Figure 3 shows the amount of CAM released from dry syrups with and without heat treatment using the paddle methods. Test solutions of pH 5.5 and 6.5 were used to clarify the relationship between the amount of release and bitterness at different pH. The same results were obtained, even if pH changed, and the results were not affected

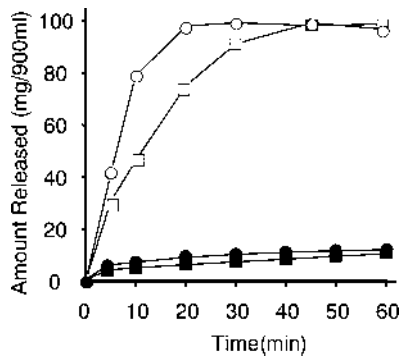


Fig. 3. Release of CAM from Dry Syrups with and without Heat Treatment by the Paddle Method

Test solution was acetic acid and sodium acetate buffer solution, pH 5.5. ○, Intact dry syrup; □, dry syrups with heat treatment (70 °C, 90 min). Test solution was phosphate buffer solution, pH 6.5. ●, Intact dry syrup; ■, dry syrups with heat treatment (70 °C, 90 min).

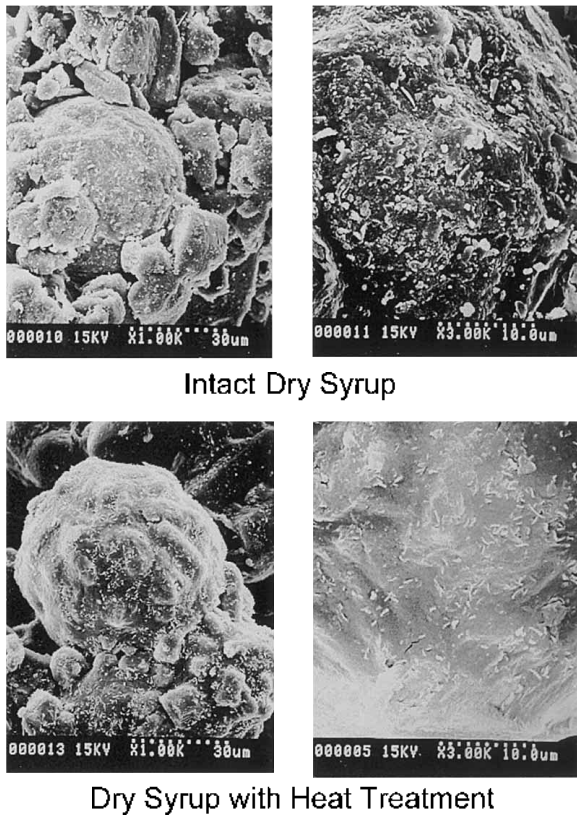


Fig. 4. SEM Micrograph of the Surface of Intact Dry Syrup and Dry Syrups with Heat Treatment at 70 °C for 90 min

by the pH of test solution. At both pH values, the release rate of dry syrup with heat treatment, which is more bitter than intact dry syrup, was slower than that of intact dry syrup.

SEM Observations and pH Measurement To explain the discrepancy between the bitterness and the release rates obtained with the shaking method and paddle methods, the surfaces of dry syrups were observed. Figure 4 shows SEM micrographs of dry syrup with and without heat treatment. With intact dry syrup, the additives adhered to the surface of the matrix, of which the surface appeared rough. On the other hand, heat treatment made the surface of the matrix smooth and coated the additive with melting monoglyceride.

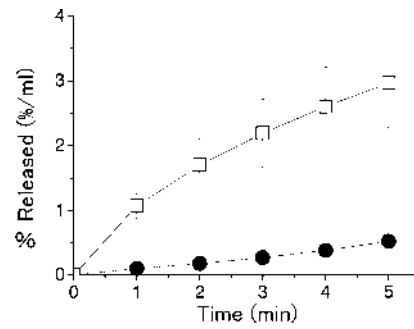


Fig. 5. Release of CAM from Dry Syrups with and without Heat Treatment with the Mini-column Method

Values are mean±S.D. of three independent tests. Tapping frequency, 10 times; flow rate, 0.5 ml/min; inner diameter, 0.76 cm; filling quantity, 0.25 g. Test solution was phosphate buffer solution, pH 6.5. ●, Intact dry syrup; □, dry syrups with heat treatment (70 °C, 90 min).

The dry syrup contains an alkaline substance to elevate the pH in the liquid dosage form, since the solubility of CAM decreases as the pH of solution increases. Addition of an alkaline substance is thus an effective way to prevent bitterness. To determine whether heat treatment influenced the distribution of alkaline substance on the dry syrup, the pH of the dry syrup was measured. The results were 7.5 and 9.0 for dry syrups with and without heat treatment, respectively. Thus the pH on the surface of the dry syrup was decreased by heat treatment. This was probably due to a decrease in the available surface area of alkaline substance exposed to the exterior. This lowering of pH in the vicinity of the surface of the matrix by heat treatment was assumed to enhance the bitterness of CAM dry syrup. On the other hand, since the surface pores of the matrix were filled with melting monoglyceride by heat treatment, the rate of penetration of test solution during the release test probably decreased, and consequently the rate of release was slower than that of intact dry syrup with the shaking method and paddle methods. In conclusion, the release of the total amount of CAM from the dry syrup is not as important as the release of that located on the surface of the matrix in correlating test results with bitterness.

Evaluation of Bitterness Using the Mini-column Dry syrups with and without heat treatment were evaluated by the mini-column method. The results are shown in Fig. 5. The release rate of CAM from dry syrup with heat treatment was faster than that from intact dry syrup. The release rate was faster and amount of release larger than with other test methods. That is, these results correlated well with bitterness. Since the volume of test solution in contact with the dry syrup surface was very small, it appeared that only the surface portion of the CAM matrix could be measured. The mini-column method is therefore useful in testing bitterness.

Optimization of Mini-column Method Testing Conditions The testing conditions of the mini-column method are shown in Table 3. The tapping frequency (*T*) of the column, flow rate (*F* ml/min) of test solution, filling quantity (*Q* g) of dry syrup, and inner diameter (ϕ cm) of the column were assumed to influence the mini-column method results, and their optimum conditions were therefore examined.

The inner diameter of the column, flow rate, and filling quantity were fixed at 0.76 cm, 0.5 ml/min, and 0.25 g, respectively. The tapping frequency was set at 3, 10, or 30

Table 3. Testing Conditions for Mini-column Method

No.	ϕ (cm)	Q (g)	T	F (ml/min)	D (%/ml/min)	u (cm/min)	H (cm)	Sp (cm ²)
1	0.76	0.250	10	0.3	0.223	1.249	0.985	146.1
2	0.76	0.250	3	0.5	0.222	1.931	1.080	146.1
3	0.76	0.500	10	0.5	0.068	2.033	2.025	292.3
4	0.76	0.250	10	0.5	0.173	2.082	0.985	146.1
5	0.76	0.125	10	0.5	0.190	2.152	0.475	73.1
6	0.76	0.250	30	0.5	0.166	2.667	0.790	146.1
7	0.76	0.250	10	0.7	0.117	2.915	0.985	146.1
8	0.46	0.250	10	0.5	0.166	5.388	2.865	146.1

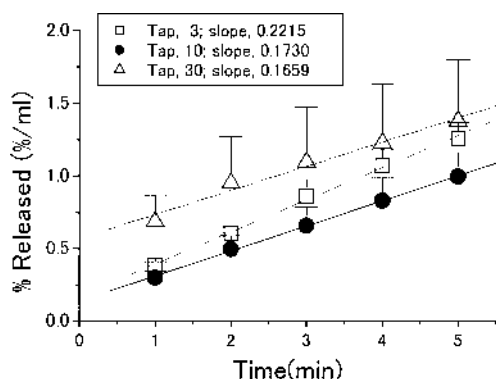


Fig. 6. Effect of Tapping Frequency on the Release of CAM with the Mini-column Method

Values are mean \pm S.D. of three independent tests. Flow rate, 0.5 ml/min; inner diameter, 0.76 cm; filling quantity, 0.25 g; tapping frequencies, 30 (Δ), 10 (\bullet), 3 times (\square). The slopes of regression lines defined as the release rate were 0.1659, 0.1730, and 0.2215%/min, respectively.

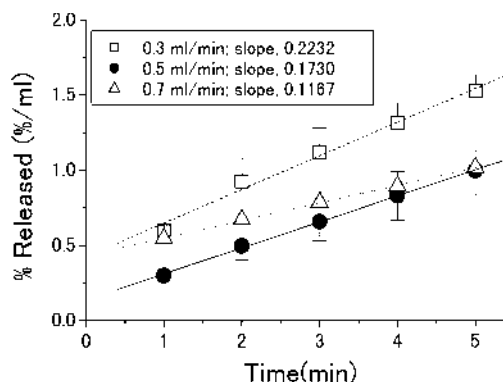


Fig. 7. Effect of Flow Rate on the Release of CAM with the Mini-column Method

Values are mean \pm S.D. of three independent tests. Tapping frequency, 10 times; inner diameter, 0.76 cm; filling quantity, 0.25 g; flow rates, 0.7 (Δ), 0.5 (\bullet), 0.3 ml/min (\square). The slopes of regression lines defined as the release rate were 0.1167, 0.1730, and 0.2232%/min, respectively.

times. The effect of tapping frequency on the release rate is shown in Fig. 6. The slopes of regression lines of these values were defined as the release rate (D %/ml/min).

When the tapping frequency was 30 times, the amount of CAM released from dry syrup varied due to an increase in the discharge pressure of the pump. It was thus considered difficult for the test solution to penetrate the dry syrup under these conditions. However, the release rate did not vary when the tapping frequency was 3 or 10 times. The release rate decreased as the tapping frequency increased.

Next, the tapping frequency, inner diameter, and filling quantity were set at 10 times, 0.76 cm, and 0.25 g, respectively, and flow rates at 0.3, 0.5, and 0.7 ml/min. The results are shown in Fig. 7. The release rate decreased as the flow rates increased. When the tapping frequency or the flow rate increased, the release rate decreased. This was probably due to a delay in liquid penetration into the matrix, since the liquid flow rate on the matrix surface increased.

The effect of the inner diameter of the column on the release rate of CAM was investigated. The inner diameter was changed from 0.76 to 0.46 cm. Figure 8 shows the results of the test with the conditions described above (tapping frequency, 10 times; flow rate, 0.5 ml/min; filling quantity, 0.25 g). The change in the inner diameter of the column did not influence the release rate.

The effect of the filling quantity of dry syrup on the release rate of CAM was investigated using a column of which the inner diameter was 0.76 cm. The filling quantity of dry syrup was set at 0.5, 0.25 or 0.125 g under the conditions described above (tapping frequency, 10 times; flow rate,

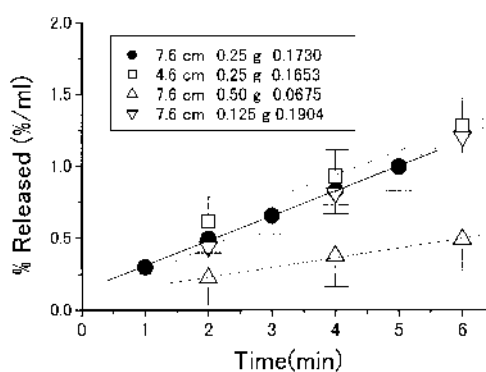


Fig. 8. Effect of Inner Diameter and Filling Quantity on the Release of CAM with the Mini-column Method

Values are mean \pm S.D. of three independent tests. Tapping frequency, 10 times; flow rate, 0.5 ml/min; filling quantity, 0.25 g; inner diameter, 0.76 (\bullet), 0.46 cm (\square). The slopes of regression lines defined as the release rate were 0.1730 and 0.1653%/min, respectively. Tapping frequency, 10 times; flow rate, 0.5 ml/min; inner diameter, 0.76 cm; filling quantities, 0.5 (Δ), 0.25 (\bullet), 0.125 g (∇). The slopes of regression lines defined as the release rate were 0.0675, 0.1730, and 0.1904%/min, respectively.

0.5 ml/min; inside diameter, 0.76 cm). The results are shown in Fig. 8. The amount of CAM released from dry syrup varied and was slow when the filling quantity was 0.5 g. There was little difference between release rates when the filling quantity was 0.125 or 0.25 g.

Relationship between the Release Rate of CAM from Dry Syrup and Linear Velocity of Liquid Flow The percentage (W^0 /ml) dissolved from the filling quantity of CAM in a column is obtained from Eq. 1, when a column is regarded as 1 unit.

$$W = D \cdot R \tag{1}$$

where D (%/ml/min) is the release rate of CAM from the surface of the matrix in the column. R (min) is the residence time in the column, written by the following Eq. 2

$$R = V_e / F \tag{2}$$

where V_e (cm³) and F (ml/min) are the void volume and the flow rate of test solution which flows in the column, respectively.

Then, K , which is W per unit void surface area, can be obtained as

$$K = W / S_e \tag{3}$$

where S_e (cm²) is the void surface area defined by the surface area of test solution in contact with the powder.

From Eqs. 1—3, Eq. 4 is obtained.

$$K = D \cdot (V_e / F) / S_e = (D / F) \cdot (V_b - V_p) / S_p \tag{4}$$

where V_b (cm³), V_p (cm³), and S_p (cm²) denote the volume of the powder bed, powder volume, and surface area of powder, respectively. In addition, S_p and S_e are equal.

The linear velocity (u , cm/min) is written as

$$u = (F / V_e) \cdot H \tag{5}$$

where H (cm) is the height of the powder bed.

From Eqs. 4 and 5, Eq. 6 is obtained.

$$K = (D \cdot H) / S_p \cdot (1 / u) \tag{6}$$

To determine S_p and V_p values of dry syrup, the numeral particle size distribution was measured using the laser diffraction method. Using the particle size distribution data, S_p and V_p can be obtained with Eqs. 7 and 8.

$$S_p = \sum a_i \cdot (\pi \cdot X_i^2) \tag{7}$$

$$V_p = \sum a_i \cdot (\pi \cdot X_i^3 / 6) \tag{8}$$

where a_i denotes number of particles with diameter between X_i and X_{i-1} . Since the largest diameter (X_0) measured by the apparatus (Microtrac FRA) is 296 μm, 0.25 g of the dry syrup was sieved through a 300-μm screen and the number of particles larger than $X_0(N)$ was counted manually.

Then a_i of each fraction was determined by the following equation.

$$a_i = N \cdot (b_i / b_0) \cdot (Q / 0.25) \tag{9}$$

where b_i and b_0 are the ratio of particle number to the total particle number for the fractions between X_i and X_{i-1} and larger than X_0 , respectively, which are automatically obtained by the laser diffraction method. Q is the filling quantity. The effects of the tapping frequency, flow rate, inner diameter of column, and filling quantity on the above-mentioned parameters are summarized in Table 3. The relationship between the release rate constant and linear velocity is shown in Fig. 9. The release rate constant increased as linear velocity decreased. This can also be explained from Eq. 6.

Furthermore, a linear relationship was observed between the release rate constant and linear velocity except for the data when the inner diameter of the column was 0.46 cm or the filling quantity was 0.5 g. When the inner diameter of the

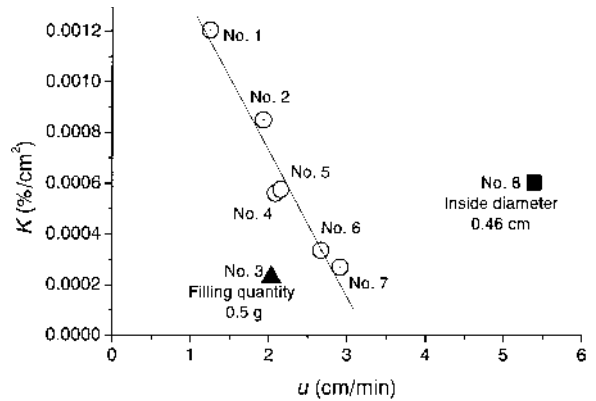


Fig. 9. Relationship between Linear Velocity of Liquid Flow and Release Rate Constant

The numbers are the experimental conditions shown in Table 3. Inner diameter, 0.46 cm φ (■); filling quantity, 0.5 g (▲); other conditions (○).

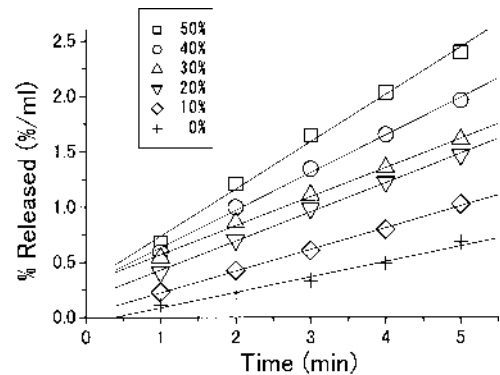


Fig. 10. Relationship between Blending Ratio of Bitter Dry Syrup and Amount of CAM Released

Tapping frequency, 10 times; flow rate, 0.5 ml/min; inner diameter, 0.76 cm; filling quantity, 0.25 g; blending ratios, 50 (□), 40 (○), 30 (△), 20 (▽), 10 (◇), 0% (+).

column was 0.46 cm, the linear velocity of liquid flow was considered to be sufficiently fast that turbulence of liquid flow might occur between the matrix and test solution, resulting in enhancement of the release rate constant. The filling quantity of 0.5 g was considered excessive and therefore the test solution did not contact the powder perfectly, which is probably due to a delay in the release rate.

For these reasons, two data points were considered to deviate from the regression line in Fig. 9. We then determined the optimum operating conditions to be: inner diameter, 0.76 cm; filling quantity, 0.25 g; tapping frequency, 10 times; and flow rate, 0.5 ml/min.

In conclusion, the critical factors affecting the operating conditions for evaluating bitterness using the mini-column were the void volume in the column and the linear velocity of liquid flow. The optimum ranges for the linear velocity and the void volume were 2.9—1.3 cm/min and 0.27—0.12 cm³, respectively.

Validity of the Test Method Intact dry syrup and dry syrup with heat treatment were blended at several ratios and the release from these mixtures was evaluated using the mini-column. The results are shown in Fig. 10. The relationships between the amount of release of CAM after 1 min and blending ratio of dry syrup with heat treatment, and between the release rate and blending ratio of one are shown in Fig.

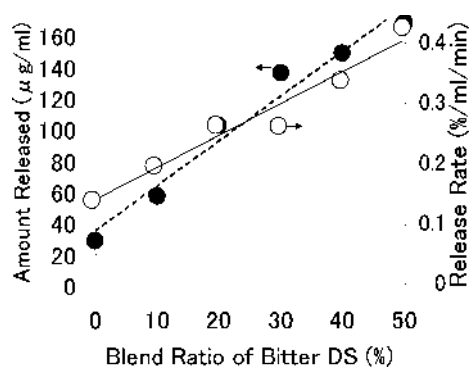


Fig. 11. Relationships between the Amount of CAM Released after 1 min and Blending Ratio of Dry Syrup (DS) with Heat Treatment, and between the Release Rate and Blending Ratio of One

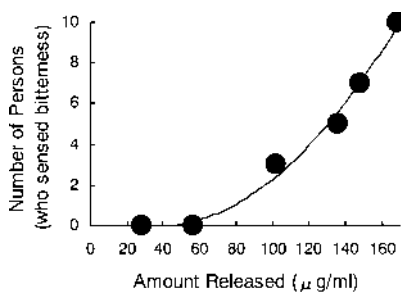


Fig. 12. Relationship between Amount of Release and Results of Sensory Test of Each Sample Containing Dry Syrup with Heat Treatment

11. The amount of CAM released and release rate increased as the blending ratio of dry syrup with heat treatment increased, and the amount of release and release rate correlated well with the ratio of blending of dry syrup with heat treatment. These results confirmed that the mini-column method is useful for evaluating the bitterness of CAM dry syrup.

Determination of the Threshold of Bitterness Using the Mini-column Method Sensory tests were performed on samples containing various ratios of dry syrup with heat treatment. Figure 12 shows the results of the sensory tests and amount of CAM released after 1 min with the mini-column method. The amount of CAM released increased as the number of persons who sensed bitterness increased. With the sample containing 30% dry syrup with heat treatment, half of

the volunteers sensed bitterness. The corresponding amount of CAM released after 1 min for this sample ($135 \mu\text{g}/\text{ml}$) was defined as the threshold of bitterness for CAM dry syrup. The value ($135 \mu\text{g}/\text{ml}$) was about 10 times larger than that ($14 \mu\text{g}/\text{ml}$) in solution.³⁾ This result is probably due to the effect of sweetener and flavor contained in dry syrup.

Conclusion

The measurement of CAM released in the vicinity of the matrix surface is important for the evaluation of the bitterness of CAM dry syrup. The mini-column method was therefore developed and validated. The results obtained with the mini-column method correlated well with those of the sensory test, although good correlations were not obtained with the inversion method, shaking method, or paddle method.

A good correlation was observed between the release rate constant and the linear velocity of test solution. The release rate constant decreased as linear velocity increased. The critical factors affecting the operating conditions for evaluating bitterness using the mini-column were the void volume in the column and linear velocity of test solution; $2.9\text{--}1.3 \text{ cm}/\text{min}$ and $0.27\text{--}0.12 \text{ cm}^3$ were optimum regions for linear velocity and void volume, respectively.

Based on the results obtained with the mini-column method and sensory method, the threshold of bitterness for CAM dry syrup was determined to be $135 \mu\text{g}/\text{ml}$. The mini-column method is a specific method for measuring the concentration of the drug released in the vicinity of the particle surface where masking processing has been conducted.

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