Optimum Heat Treatment Conditions for Masking the Bitterness of the Clarithromycin Wax Matrix

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The effects of the contents of aminoalkyl methacrylate copolymer E (AMCE) in a wax matrix on the mechanism of polymorphic transformation of glyceryl monostearate (GM) were clarified by evaluating the enthalpy change defined as 1.51 ($\Delta H_1 - \Delta H_2)/\Delta H_2$, where ΔH_1 and ΔH_2 denote the enthalpies in the first and second thermal analyses, respectively. Using this value, K_1, the rate constant of transformation from α-form to β'-form, and K_2, the rate constant of transformation from β'-form to β-form, could be obtained. As the ratio of AMCE increased, K_2 increased, but a minimum point existed for K_1. K_1 was always larger than K_2, but gradually approached K_2 as the ratio of AMCE increased. The optimum temperature for the transformation of GM was 50 °C, at which the enthalpy change was maximum. To prepare the wax matrix preparation of clarithromycin (CAM), we considered 40 °C the optimum treatment temperature for the transformation of GM in a CAM wax matrix compounded from CAM, GM and AMCE, since the matrices were mutually welded at above 45 °C during the spray congealing process. Although K_1 and K_2 were almost the same at 40 °C, the rate of transformation was accelerated by tumbling. By applying the tumbling that accelerated the transformation of GM in a CAM wax matrix, almost all of the α-form disappeared, and the release of CAM from the wax matrix diminished when the enthalpy change was more than 0.8.

Key words crystal transformation; glyceryl monostearate; differential scanning calorimeter; transformation rate; spray congealing technique

Clarithromycin (CAM), a macrolide antibiotic, has a strongly bitter taste. For the purpose of developing a CAM pediatric formulation (dry syrup), we have been carrying out numerous examinations focused on masking the bitter taste of CAM.

The spray-congealing technique is a promising way to establish our objective. Through the screening of materials, we selected glyceryl monostearate (GM) and aminoalkyl methacrylate copolymer E (AMCE) as constituents of the CAM wax matrix prepared by this technique. Since GM is a low-melting-point substance which is decomposed by enzymes in the intestinal tract[1,2] and AMCE dissolves in fluid with low pH, this preparation was not dissolved in the mouth, but released CAM immediately in the gastrointestinal tract, resulting not only in masking the bitter taste of CAM but also excellent bioavailability. We also investigated the optimum manufacturing conditions for spray-congealing for a CAM wax matrix including GM.[3,4]

GM is known to exhibit four polymorphs.[5,6] Among the crystal forms, the β-form is most preferable for masking the bitter taste of the CAM wax matrix because it has poor foaming properties and low wettability. During the spray congealing process, GM (β-form) was transformed to the α-form by heat exposure and gradually returned to the β-form at ambient temperature. Furthermore, we found that a moderate temperature, optimally 50 °C, accelerated the latter transformation of GM.[7]

The objective of this study was to determine the optimum conditions for rapid formation of the β-form of GM in a CAM wax matrix during the process of manufacturing of the formulation. For this purpose, we focused on the transformation of GM in a CAM wax matrix under congealing conditions. The effects of the ratios of GM, AMCE and CAM on the transformation of GM, and of the transformation on the rate of release of CAM from wax matrix, were investigated.

Experimental

Material CAM was synthesized at Taisho Pharmaceutical Co., Ltd. GM (Taiyo Kagaku Co., Ltd.) was of the grade specified in the Japanese Pharmaceutical Excipients Directory. AMCE was of commercial grade.

Preparation of GM-AMCE Congelation AMCE of each ratio was dissolved in GM melted at 120 °C. The melted solution was dropped on the rotating wing with an approximately 5 cm blade at 1500 rpm. Dripped GM was micronized, solidified, and recovered on the bed. The formulations are shown in Table 1.

Preparation of CAM Wax Matrix[4] AMCE was dissolved in GM meltd 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 during cooling.

Method of Heat-Treatment About 2 mg of GM, GM-AMCE congelations, and CAM Wax Matrix were placed in the DSC pan and stored for fixed amounts of time (static condition). About 500 g of the wax matrix was placed in a V-blender and blended in the atmosphere of 40 °C for fixed amounts of time (tumbling condition).

Measurement of Powder Characteristics Thermal analysis was carried out using a differential scanning calorimeter (DSC-7, Perkin Elmer). About 2 mg of sample was heat-treated in an open pan in DSC and, after cooling the GM sample to 30 °C at a rate of 10 °C/min, the first run of thermal analysis was carried out at a heating rate of 10 °C/min in the temperature range of 30 to 90 °C. After cooling, the second run of thermal analysis was carried out under the same conditions. Enthalpies of first and second thermal analyses were expressed by the enthalpy change, ΔH_1 and ΔH_2, respectively.

Powder XRD patterns were obtained using a Rigaku Geigerflex RAD powder X-ray diffractometer under the following conditions: target, Cu; filter, nonuse; voltage, 40 kV; current, 30 mA; scanning speed, 4°/min. GM, GM-AMCE congelation, and CAM wax matrix were measured without milling.

Mini-Column Method[8] Columns with an inner diameter of 0.76 cm and a length of 5 cm were used. About 0.1 g of Wax Matrix, accurately weighed, was packed in the column. After tapping the column 10 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. A pH 6.5 phosphate buffer solution with 0.01% polysorbate 80 was supplied

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to fill the entire space of the column, and a nipple was then immediately attached to the top of the column. The upper part of the column was connected to the pump and filled with buffer solution in advance. The same solution flowed through this column at 0.5 ml/min. The eluate was collected every 1 min for 5 min. Each eluate was used as a sample solution. Separately, about 50 mg of common use standard CAM was accurately weighed and dissolved in 50 ml of the buffer solution. Five milliliters of this solution was pipetted, and fresh buffer solution was added to make 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 directed under liquid chromatography 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. Peak areas for these solutions were calculated by automatic integration.

Results and Discussion

Thermodynamic Characteristics of GM in GM-AMCE Congelations The heat energy of GM itself of α-form at melting was compared with those of GM of α-form in GM-AMCE congelations and in the CAM wax matrix. Formulas of GM-AMCE congelations and CAM wax matrix are shown in Table 1. CAM wax matrix is the product of the dispersion of powdered CAM in GM-AMCE congelation 3 (GM : AMCE=0.86 : 0.14). Table 2 shows the relationship between the GM ratio (R) and ΔH_2 per unit GM ratio ($\Delta H_2/R$), where ΔH_2 is the enthalpy of GM of α-form in the second run of thermal analyses repeated twice using a differential scanning calorimeter (DSC). There was little difference between the values of $\Delta H_2/R$ (CV=1.8%). This result showed that neither CAM nor AMCE influenced the thermodynamic characteristics of GM.

We have already reported[7] that the enthalpy change of GM can be obtained with the following equation, where ΔH_1 and ΔH_2 are the enthalpy of GM in the first and second run of thermal analyses repeated twice using DSC, respectively.

$$E=1.51(\Delta H_1-\Delta H_2)/\Delta H_2 \quad (1)$$

Equation 1 was also considered applicable to GM-AMCE congelation and CAM wax matrix, since little difference existed among the thermodynamic characteristics of GM itself, GM-AMCE congelation and CAM wax matrix.

Table 1. Formulation of GM-AMCE Congelations 1, 2, 3 and the CAM Wax Matrix

	GM-AMCE congelation 1	GM-AMCE congelation 2	GM-AMCE congelation 3	CAM wax matrix
GM	0.96	0.92	0.86	0.60
AMCE	0.04	0.08	0.14	0.10
CAM	—	—	—	0.30
Total	1	1	1	1

Table 2. Relationship between GM Ratio (R) and ΔH_2 per Unit GM Ratio ($\Delta H_2/R$)

	GM	GM-AMCE congelation 1	GM-AMCE congelation 2	GM-AMCE congelation 3	CAM wax matrix
R	1	0.96	0.92	0.86	0.60
ΔH_2	98.66	96.76	92.99	84.01	58.30
$\Delta H_2/R$	98.66	100.79	101.08	97.69	97.17
CV			1.8		

Transformation Kinetics of GM in GM-AMCE Congelation and CAM Wax Matrix According to our previous report,[7] the ratio of each crystal form of GM during the transformation process can be written as an equation of consecutive reactions as follows.

$$Y_\alpha=\exp(-K_1\cdot t) \quad (2)$$

$$Y_{\beta'}=K_1/(K_2-K_1)\cdot(\exp(-K_1\cdot t)-\exp(-K_2\cdot t)) \quad (3)$$

$$Y_\beta=1+1/(K_1-K_2)\cdot(K_2\cdot\exp(-K_1\cdot t)-K_1\cdot\exp(-K_2\cdot t)) \quad (4)$$

where Y_α, $Y_{\beta'}$, and Y_β denote the percentages of the α-form, β'-form, and β-form present at each time (t). K_1 and K_2 denote the rate constant of transformation from α-form to β'-form and from β'-form to β-form, respectively. Furthermore, the enthalpy change can be expressed by Eq. 5

$$E(t)=E(t_c)\cdot(1-Y_\alpha)+(1-E(t_c))\cdot Y_\beta \quad (5)$$

where t_c and $E(t_c)$ denote the time at which the α-form disappeared and the enthalpy change of GM at that time, respectively. $E(t_c)$, K_1, and K_2 can be calculated by the nonlinear least-squares method.

These equations were applied to the transformation of GM in GM-AMCE congelation. Each sample was stored in a DSC pan at 50 °C for 0, 0.5, 1, 1.5, 2, 4, 6 and 8 h, and duplicate thermal analyses were carried out. Figure 1 shows the first-order plots for enthalpy change versus processing time of GM in the DSC pan.

Each profile of $-\ln(1-E)$ exhibited a two-phase pattern with a point of refraction, the same result as for GM itself. The effects of AMCE ratio on the rate constant of transformations (K_1, K_2) are shown in Fig. 2. As the ratio of AMCE increased, the K_2 value increased, but a minimum point existed for K_1. K_1 was always larger than K_2, but gradually approximated K_2 as the ratio of AMCE increased. These results indicate that during the transformation from the α-form to β'-form, at one point the movement of GM molecules in GM-AMCE congelation should decrease as the ratio of AMCE increases. However, with further addition of AMCE, the movement of GM molecules should gradually recover. On the other hand, the movement of GM molecules should monotonically increase as the ratio of AMCE increases dur-

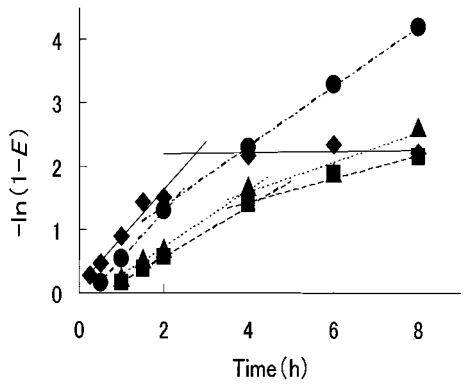

Fig. 1. First-Order Plots of Enthalpy Change (E) *versus* Processing Time for GM and GM-AMCE Congelations in a DSC Pan at 50 °C

◆: GM, $y=0.7577x+0.1192$ ($R^2=0.9601$), $y=0.0109x+2.1741$ ($R^2=0.0502$), ■: congelation 1, $y=0.4014x-0.2281$ ($R^2=0.9982$), $y=0.1869x+0.6939$ ($R^2=0.9709$), ▲: congelation 2, $y=0.4535x-0.1812$ ($R^2=0.9771$), $y=0.2337x+0.6662$ ($R^2=0.9109$), ●: congelation 3, $y=0.7614x-0.2191$ ($R^2=0.9999$), $y=0.4771x+0.4075$ ($R^2=0.9994$).

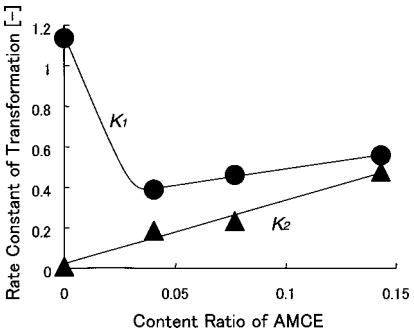

Fig. 2. Effect of AMCE Ratio on Rate Constant of Transformation (K_1, K_2)

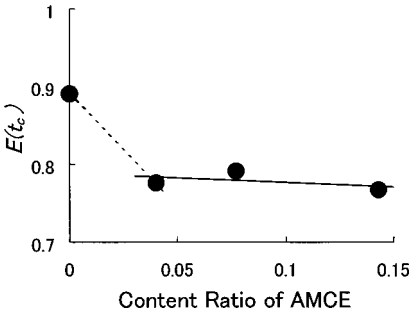

Fig. 3. Relationship between $E(t_c)$ and AMCE Ratio

$E(t_c)$: enthalpy change at the point at which the α-form disappeared.

ing the transformation from β'-form to β-form.

Maruyama *et al.* reported the following.[9] The speed of the crystal transformation of GM slows down in the solid solution of the short chain GM with the long chain GM so that the long chain GM may obstruct the transfer of the short chain GM. On the other hand, transfer of the molecule is accelerated in the solid solution of the long chain GM with the short chain GM, since holes are formed between arranged molecules of the long chain GM. In the case of crystal transformation in the GM-AMCE congelation, it can also be considered.

Figure 3 shows the relationship between $E(t_c)$ and the AMCE ratio. The $E(t_c)$ value of GM itself was about 0.9, while that of GM-AMCE congelation was about 0.8 and independent of the ratio of AMCE. It appears that adding AMCE in GM decreased $E(t_c)$.

Optimum Treatment Temperature for Transformation Congelation 3 was stored at 35, 40, 45, 50, 55 and 60 °C for 90 min, and duplicate thermal analyses were carried out for each storage sample. Figure 4 shows the enthalpy change of each sample. The optimum temperature for the transformation was 50 °C, at which the enthalpy change was maximal. The optimum temperature was equivalent to that of GM itself.[7] The addition of AMCE thus did not influence optimum temperature.

However, the CAM wax matrix consists of congelation 3 and CAM, which dispersed in congelation 3. The melting point of CAM is over 200 °C. It thus appeared that the optimum treatment temperature for the transformation of GM in CAM wax matrix is 50 °C. However, SEM observation revealed that the wax matrices were mutually welded by the melting of GM above 45 °C during the spray congealing process. We therefore considered 40 °C the optimum treatment temperature for transformation, taking handling into consideration. Examination has since been carried out at 40 °C.

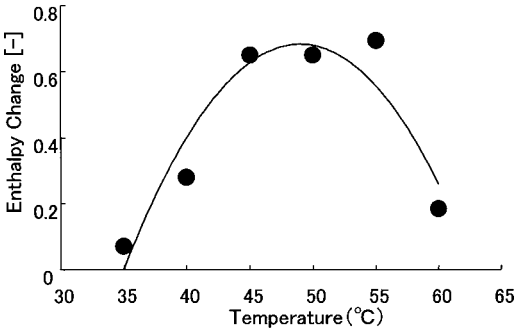

Fig. 4. Effect of Processing Temperature on Enthalpy Change of Congelation 3

Processing time: 90 min. AMCE : GM = 1 : 6.

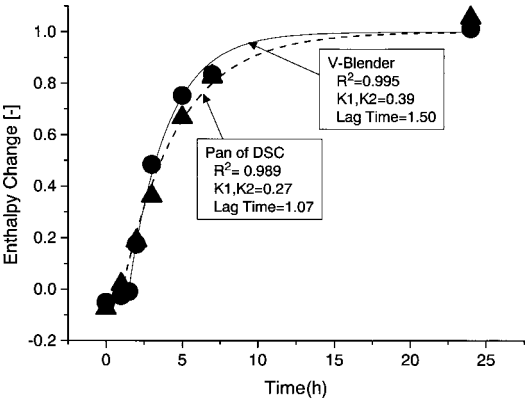

Fig. 5. Enthalpy Change of CAM Wax Matrix at 40 °C in Static and Tumbling Conditions

--▲--: static, —●—: tumbling.

Effect of Tumbling on the Transformation of GM in CAM Wax Matrix Mechanical tumbling is expected to accelerate the transformation of polymorphs. The CAM wax matrix was therefore stored in a DSC pan (static condition) and in a rolling V- blender (tumbling condition) at 40 °C, and the effect of tumbling on the enthalpy change of GM in the CAM wax matrix was observed. The result is shown in Fig. 5. A lag time existed for the enthalpy change of the sample in the tumbling condition, because a long time was required in order to heat 500 g of the wax matrix. The K_1 and K_2 of enthalpy change in the tumbling condition was larger than those in the static condition.

***E*(t_c) of CAM Wax Matrix** To determine the $E(t_c)$ of the CAM wax matrix, we used powdered XRD. Figure 6 shows the X-ray diffraction patterns of samples stored at 40 °C in tumbling conditions. The specific peaks of the α-form disappeared at 5 h. $E(t_c)$ could then be determined to be about 0.8 from Fig. 5. This value was almost equivalent to that for GM-AMCE congelation in Fig. 3.

Release of CAM from Wax Matrix The effect of crystal transformation on masking the bitter taste of CAM wax matrix was evaluated by the mini-column method.

A 0.1 g portion of CAM wax matrix (equivalent to 30 mg of CAM) stored at 40 °C in a rolling V-blender was packed in a mini-column, and release tests were performed. The results

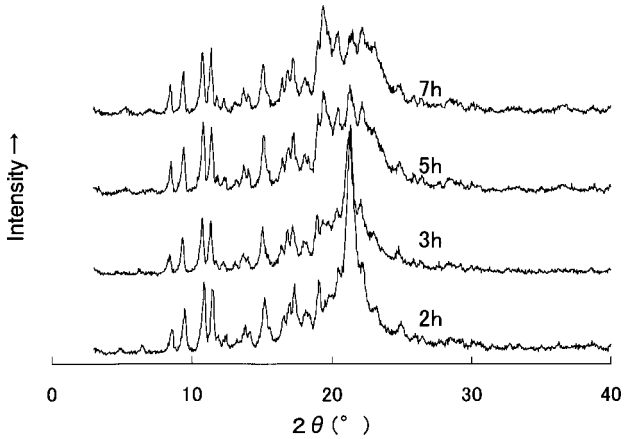

Fig. 6. Powdered XRD Patterns of the CAM Wax Matrix Stored in a Tumbling Condition (V-Blender)

Processing temperature: 40 °C.

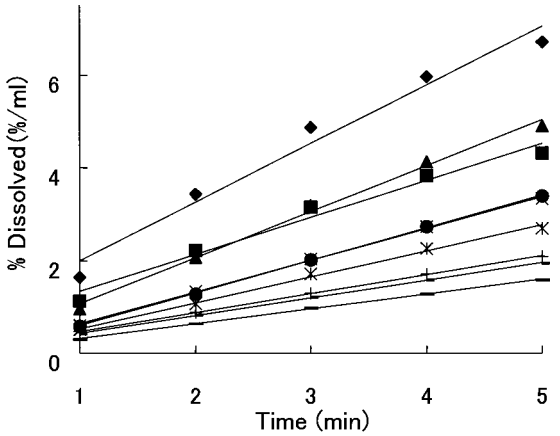

Fig. 7. Percentage of CAM Dissolved from the Wax Matrix in the Mini-Column Method

Processing temperature: 40 °C, processing time: ◆: 0 h, slope=1.27, R^2=0.975, ■: 0.5 h, slope=0.79, R^2=0.976, ▲: 1 h, slope=0.99, R^2=0.994, ×: 1.5 h, slope=0.69, R^2=0.999, ∗: 2 h, slope=0.56, R^2=0.996, ●: 3 h, slope=0.70, R^2=0.999, +: 5 h, slope=0.41, R^2=0.999, −: 7 h, slope=0.38, R^2=0.999, —: 24 h, slope=0.32, R^2=0.999, sample amount: 0.1 g as CAM.

are shown in Fig. 7. The rate of release is denoted by the slope of % release in Fig. 7. As processing time increased, the amount released and the rate of release decreased.

Figure 8 shows the relationship between the rate of release and processing time. As processing time increased, the rate of release decreased quickly. However, the rate was nearly constant at more than 5 h of processing time, since most of the α-form had been transformed to the β'-form. We therefore considered 5 h a sufficient amount of time for masking the bitter taste of CAM in the wax matrix. When the CAM wax matrix was stored at 40 °C for 5 h in a rolling V-blender, the enthalpy change was about 0.8, as shown in Fig. 5. This result indicates that the optimum treatment conditions for masking the bitterness of the CAM wax matrix are to store it at 40 °C in a rolling V-blender until the enthalpy change is above 0.8.

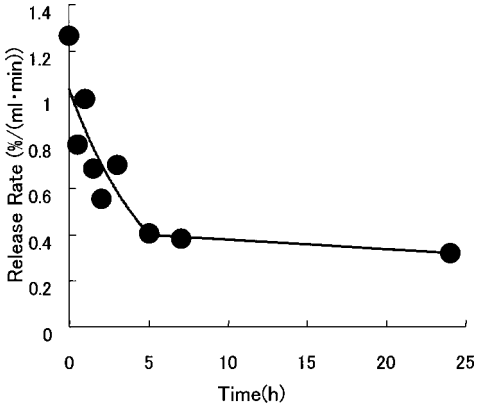

Fig. 8. Relationship between Processing Time and Rate of Release from CAM Wax Matrix

Conclusion

Duplicate thermal analyses were effective for determining the endpoint of the transformation of a GM polymorph. The optimum treatment temperature for the transformation of GM in a CAM wax matrix was 40 °C. By applying the tumbling that accelerated the transformation of GM in the CAM wax matrix, almost all of the α-form disappeared, and the release of CAM from the wax matrix diminished, when the enthalpy change was more than 0.8.

It is considered that the crystal transformation of GM is accelerated with an increase of AMCE. However, the viscosity of the GM-AMCE melt is high, and it is not possible to carry out the spray congealing agglomeration when AMCE is over 14%. Therefore, the optimum quantity of AMCE is 14%.

Thus, the optimum conditions for the transformation of a CAM wax matrix include treatment at 40 °C in a tumbling condition and completion of the heat treatment when the enthalpy change is above 0.8.

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