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RESEARCH PAPER

Highly Stabilized Amorphous 3-*bis*(4-Methoxyphenyl)methylene-2-indolinone (TAS-301) in Melt-Adsorbed Products with Silicate Compounds

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ABSTRACT

3-*Bis*(4-Methoxyphenyl)methylene-2-indolinone (TAS-301) is a poorly water-soluble drug showing low oral bioavailability in rats and dogs. Previously, we reported that when a physical mixture of TAS-301 and a porous calcium silicate, Florite® RE (FLR), was heated at high temperature (250°C), the drug melted and was adsorbed by the FLR in an amorphous state, and that the preparation (melt-adsorbed product) showed a significantly increased solubility and dissolution rate, and a significantly enhanced oral bioavailability of the drug. The aim of the present study was to elucidate important factors for preparing a melt-adsorbed product showing greater stability of drug in an amorphous state. We examined the effects of the kind of adsorbent, drug/adsorbent ratio, heating conditions, and drug particle size on converting drug crystal into an amorphous state, the stability of amorphous state, and chemical stability of the drug in the melt-adsorbed products under a high temperature and high humidity condition (60°C/80% RH, open). FLR, light anhydrous silicic acid and two types of hydrated silicon dioxides were tested as adsorbents. For the batch method, TAS-301 was converted into an amorphous state by heating TAS-301/adsorbents physical mixtures above the melting point of TAS-301 for more than 2 min. The amorphous state was most stabilized when FLR was used as an adsorbent and drug/FLR ratio was 1:0.5 and more. For the continuous method using the twin screw extruder that enables significantly larger scale manufacturing than batch method, TAS-301 melt-adsorbed products were able to produce when only FLR was used as adsorbent. The heating temperature was needed to be set above the melting point of TAS-301 to convert it into an amorphous state as well as batch method. The amorphous state was stabilized when

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drug/FLR ratio was 1:2 and more. The micronization of the drug decreased the stability of the amorphous state. These results indicate the importance of optimizing the above factors in the preparation of melt-adsorbed product.

Key Words: Porous calcium silicate; Twin screw extruder; Amorphous; Melt-adsorption; Particle size.

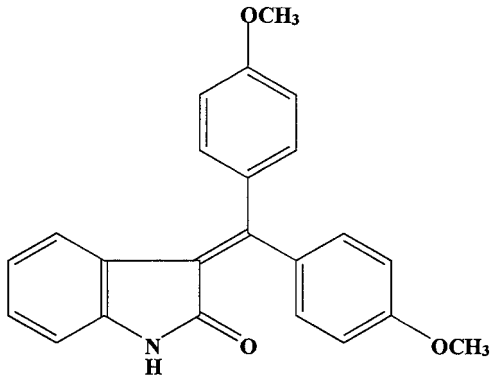
INTRODUCTION

The increase in solubility and in dissolution rate of a poorly water-soluble drug in the gastrointestinal fluid is a potential means of improving its oral bioavailability. Solid dispersion is a formulation system for improving the solubility of poorly water--soluble drugs.[1–3] In this system, the solubility of a drug is improved mainly due to its amorphization. Currently, spray-drying and layering on core particles are generally used to manufacture solid dispersions on a large scale. However, these methods require a large amount of organic solvent to dissolve the drug and a hydrophilic polymer, which serves as the matrix in most cases. Such uses of organic solvents cause several problems including environmental pollution and toxicity due to the residual solvent.[3–5]

3-*Bis*(4-Methoxyphenyl)methylene-2-indolinone (TAS-301, Fig. 1) has been developed as an antirestenosis drug for use after percutaneous transluminal coronary angioplasty. Since this compound has very poor solubility in water, only about 20 ng/mL, its bioavailability after oral administration in fasted rats and dogs is very low.[6] We already succeeded in improving its solubility and oral bioavailability by a novel melt-adsorption technique.[6] TAS-301 was melted and adsorbed on a porous calcium silicate, Florite® RE (FLR), in an amorphous state by heating at 250°C. The preparation (melt-adsorbed product) showed a significantly enhanced solubility and oral bioavailability of the drug. The melt-adsorbed product was prepared without any solvents and manufactured using a twin screw extruder that is applicable for large-scale production.

However, a detailed study on the manufacturing and stabilizing of melt-adsorbed products has never been conducted. It is known that the amorphous form is physically and chemically unstable because of a higher energy level than the crystal form.[7] Therefore, the chemical and physical stabilities of drug in an amorphous state are crucial to the commercial application of melt-adsorbed products.

The purpose of the present study was to clarify important factors in preparing melt-adsorbed products that show good stability in an amorphous state. We focused on the kind of adsorbent, drug/adsorbent ratio, heating conditions, and drug particle size, and investigated their effects on converting drug crystal into an amorphous state, the stability of amorphous state and chemical stability in the melt-adsorbed products stored under a stress condition (60°C/80% RH, open).



TAS-301: 3-*Bis*(4-methoxyphenyl)methylene-2-indolinone)
$C_{23}H_{19}NO_3$: M.W. 357.41

Figure 1. Chemical structure of TAS-301.

EXPERIMENTAL

Materials

TAS-301 was synthesized at Taiho Pharmaceutical Co., Ltd. The drug was used with and without pulverization by a hammer mill (SAMPLE-MILL KIIW-1, Fuji Paudal Co., Ltd.) and a jet mill (TJ120, Turbo Kogyo Co., Ltd.). The mean particle size of the nonpulverized drug, the pulverized drug by the hammer mill, and the pulverized drug by the jet mill were about 80, 25, and 3 μm, respectively. The drug pulverized by the hammer mill was used for preparing melt-adsorbed products unless otherwise stated. Porous calcium silicate (Florite RE, Eisai; FLR), light anhydrous

silicic acid (Sailysia 350, Fuji Silisia Chemical; SAL350), and two types of hydrated silicon dioxides (Carplex#67 and Carplex#80, Shionogi; CPX67, CPX80, respectively) were used as adsorbents. The hydrated silicon dioxides show different pHs (CPX67: pH 7.4, CPX80: pH 5.8) in 5% aqueous suspensions. All other reagents were of special commercial grade.

Preparation of Melt-Adsorbed Products

Melt-adsorbed products with different drug/adsorbent ratios (1:0.5, 1:0.75, 1:1, 1:2, and 1:3) were prepared under various heating conditions by the small-scale batch method (batch method) and twin-screw extruder method (continuous method) as reported previously.[6] For the batch method, about 100 mg of a physical mixture composed of TAS-301 and a kind of adsorbent was placed into a 2-mL glass ampoule, and without sealing it, the ampoule was heated in an electric furnace (Muffle Furnace FP41; Yamato Kagaku, Japan) or an oil bath. The heating temperatures were set at 170, 190, 210, 220, 230, or 250°C, and the heating times were about 2, 5, 10, 20, 30, or 60 min. For the continuous method, about 500 g of the physical mixture was fed into a twin screw extruder (KEX-25; Kurimoto, Japan) to produce a melt-adsorbed product. The operating conditions were as follows: screw rotation speed, 250 rpm; powder supply rate, 30 g/min; heating temperature (barrel temperature), 170, 190, 210, 220, 230, or 250°C. The fed powder was exited from the die for 20–30 sec under the conditions.

Evaluation of the Crystallinity of TAS-301

The stability of TAS-301 in the amorphous state was evaluated by DSC measurement using a Thermo Plus 8230L thermal analysis system (Rigaku, Japan). Approximately 6 mg of a sample placed in an aluminum pan was scanned at a heating rate of 20°C/min under a stream of nitrogen gas (50 mL/min). The crystallinity of TAS-301 in the melt-adsorbed products was assessed from the two endothermic peaks (around 180 and 200°C) derived from the drug crystals. The apparent crystallinity was defined as the percentage of total area of the endothermic peaks at around 180 and 200°C to that for bulk drug. The glass transition temperature was determined from an inflection point of a baseline.

Determination of Related Substances of TAS-301

The melt-adsorbed product (10 mg as TAS-301) was shaken vigorously in 50 mL of a 45% acetonitrile solution and diluted to 100 mL with a 45% acetonitrile solution. The drug solution was centrifuged at 3000 rpm for 5 min. A quantity of 10 μL of the supernatant was assayed by Shimadzu LC-10A HPLC system using a WAKOPACK 5C18 AR column (150 × 4.6 mm i.d., 5 μm, WAKO, Japan) kept at 40°C. The mobile phase was an acetonitrile/water (45:55), and its flow rate was 0.8 mL/min. TAS-301 and its related substances were detected at 210 nm. The amount of related substances (%) was calculated according to the area percentage method. The related substance is composed of the impurities in raw material and degraded products of TAS-301 and the impurities.

RESULTS

Amorphization of TAS-301 by Melt-Adsorption

Figure 2 shows DSC patterns of TAS-301, the physical mixtures [TAS-301/adsorbent (1:2)], and the melt-adsorbed products [TAS-301/adsorbents (1:2)], prepared by the batch method. The endothermic

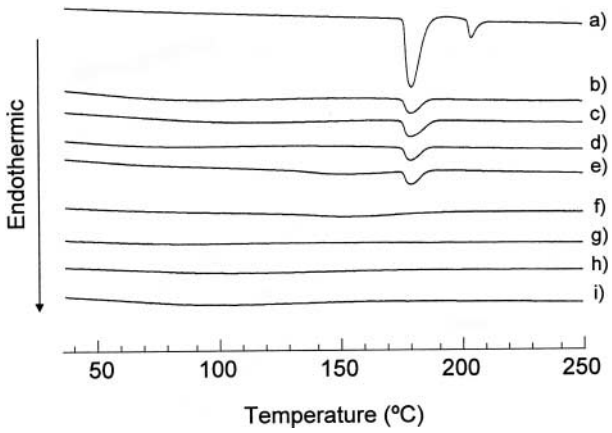


Figure 2. DSC patterns of the TAS-301/adsorbent (1:2) system. (a) TAS-301 alone, (b) physical mixture (TAS-301/FLR), (c) physical mixture (TAS-301/SAL350), (d) physical mixture (TAS-301/CPX67), (e) physical mixture (TAS-301/CPX80), (f) melt-adsorbed product (TAS-301/FLR), (g) melt-adsorbed product (TAS-301/SAL350), (h) melt-adsorbed product (TAS-301/CPX67), (i) melt-adsorbed product (TAS-301/CPX80).

peaks (around 180 and 200°C) derived from TAS-301 crystals disappeared without a trace in all the melt-adsorbed products. A glass transition temperature derived from the drug was slightly observed at near 92°C. The kind of adsorbents and drug/adsorbent ratios did not affect the glass transition temperature (data not shown). Similar results were noted for the melt-adsorbed products prepared by the continuous method.

Effects of Adsorbent Type and Drug/Adsorbent Ratio on the Amorphous State

In a previous study,[6] only FLR was used as a adsorbent for melt-adsorbed products. In the present study, three other silicate compounds, SAL350, CPX67, and CPX80, which have a large specific surface area and are capable of adsorbing a large amount of oil, were also used as adsorbents. The specific surface areas of FLR, SAL350, CPX67, and CPX80 were 128,[6] 300,[8] 429,[9] and 193 m^2/g,[9] respectively. And the amount of oil adsorbed by FLR, SAL350, CPX67, and CPX80 was 400,[10] 310,[8] 234,[9] and 237 mL/100 g,[9] respectively.

Table 1 shows the apparent crystallinity of TAS-301 in melt-adsorbed products prepared with different adsorbents and drug/adsorbent ratios before and after storage at 60°C/80% RH for 3 days. Apparent crystallinity 0% indicates the endothermic peaks derived from TAS-301 were not detected. For the batch method, when FLR, SAL350, and CPX80 were used at a drug/adsorbent ratio of 1:0.5 or more, the apparent crystallinity was 0%, indicating amorphization of the drug. A similar amorphization was observed at a drug/CPX67 ratio of 1:0.75 or more. At some of these ratios, the apparent crystallinity

Table 1. Apparent crystallinity of TAS-301 in melt-adsorbed products before and after storage at 60°C/80% RH for 3 days.

			Apparent crystallinity (%)	
Method	Adsorbent	Drug/adsorbent ratio	Initial	60°C/80% RH, 3 days (open)
Batch method	FLR	1:0.5	0	0
		1:0.75	0	0
		1:1	0	0
		1:2	0	0
		1:3	0	0
	SAL350	1:0.5	0	2.50
		1:0.75	0	1.00
		1:1	0	0
		1:2	0	0
		1:3	0	0
	CPX67	1:0.5	23.81	47.29
		1:0.75	0	10.32
		1:1	0	2.08
		1:2	0	3.46
		1:3	0	0
	CPX80	1:0.5	0	23.44
		1:0.75	0	10.99
		1:1	0	4.04
		1:2	0	0.09
		1:3	0	0
Continuous method	FLR[a]	1:1	0	3.76
		1:2	0	0.46
		1:3	0	0

Note: The conditions for the preparation of melt-adsorbed products by the batch method were as follows: heating temperature, 250°C; heating time, 2 min. The heating temperature in continuous method was 250°C.
[a]The data from a previous study.[6]

increased (i.e., the endothermic peak appeared) after the storage at 60°C/80% RH for 3 days, but the amorphous state of TAS-301 was kept at the ratios of 1:0.5 or more for FLR, 1:1 or more for SAL350, and 1:3 for CPX67 and CPX80.

In the case of the continuous method, melt-adsorbed products were able to produce when only when FLR was used as adsorbent, because the physical mixtures with other adsorbents were clogged at tips of the screws (furthest from the paddle portion) of the extruder. The apparent crystallinity was 0% at drug/FLR ratios of 1:1, 1:2, and 1:3. The crystallinity was increased slightly at 1:2 after the storage, but not at 1:3.

Effect of Heating Conditions on the Amorphous State

We used FLR as an adsorbent and a drug/adsorbent ratio of 1:2 to evaluate the effects of heating conditions on the amorphous state of TAS-301 in melt-adsorbed products prepared by both the batch method and continuous method.

Table 2 shows the apparent crystallinity of TAS-301 in the melt-adsorbed products [TAS-301/FLR (1:2)] prepared under different heating conditions by the batch method, before and after storing at 60°C/80% RH for 3 days. Apparent crystallinity was 4.1 and 0.03%, respectively, when melt-adsorbed products were prepared by heating the physical mixtures at 170 and 190°C for 60 min, whereas apparent crystallinity was 0% at 210°C for 5 min and at 220, 230, and 250°C for 2 min. The result suggests that TAS-301 was amorphized by heating above its melting point (180–200°C) for a short time. These melt-adsorbed products prepared above 210°C remained amorphous even after the storage at 60°C/80% RH for 3 days.

Table 3 shows the apparent crystallinity of TAS-301 in melt-adsorbed products [TAS-301/FLR (1:2)] prepared at 170–250°C by the continuous method, before and after storage at 60°C/80% RH for 3 days. By heating above the melting point, TAS-301 was amorphized in the melt-adsorbed products as well as the batch method (Table 2). Slight crystallization was observed after storage at 60°C/80% RH for 3 days (the apparent crystallinity: 0.46–1.86%).

Table 2. Apparent crystallinity of TAS-301 in melt-adsorbed products [TAS-301/FLR (1:2)] produced by the batch method before and after storage at 60°C/80% RH for 3 days.

Heating conditions		Apparent crystallinity (%)	
Temperature (°C)	Time (min)	Initial	60°C/80% RH, 3 days (open)
170	5	10.40	—[a]
	10	11.79	—
	20	9.64	—
	30	9.64	—
	60	4.10	—
190	5	0.01	—
	10	0.13	—
	20	0.06	—
	30	0.20	—
	60	0.03	—
210	2	0.03	—
	5	0	0
	10	0	0
	20	0	0
	30	0	0
	60	0	0
220	2	0	0
230	2	0	0
250	2	0	0
	5	0	0
	10	0	0

[a]—: Not studied.

Table 3. Apparent crystallinity of TAS-301 in melt-adsorbed products produced by continuous method before and after storage at 60°C/80% RH for 3 days.

	Apparent crystallinity (%)	
Heating temperature (°C)	Initial	60°C/80% RH, 3 days (open)
170	28.28	—[a]
190	4.78	—
210	0	0.76
220	0	1.48
230	0	1.86
250	0	0.46

[a]—: Not studied.

Effect of Drug Particle Size on Stability in the Amorphous State

Table 4 shows apparent crystallinity of TAS-301 melt-adsorbed products [TAS-301/FLR (1:1)] prepared at 250°C by the continuous method using different particle sizes of the drug. The melt-adsorbed products prepared with larger particles showed better stability of the amorphous state at 60°C/80% RH.

Table 4. Effect of particle size on the stability of amorphous state of melt-adsorbed products [TAS-301/FLR (1:1)] produced by continuous method.

	Apparent crystallinity (%)	
TAS-301 Particle size	Initial	60°C/80% RH, 3 days (open)
Large[a]	0	0.98
Medium[b]	0	4.53
Fine[c]	0	14.66

Note: The heating temperature in continuous method was 250°C.
[a]TAS-301 nonpulverized (D_{50}: about 80 μm).
[b]TAS-301 was pulverized by a hammer mill (D_{50}: about 25 μm).
[c]TAS-301 was pulverized by a jet mill (D_{50}: about 3 μm).

Table 5. Amount of related substances in melt-adsorbed products [TAS-301/FLR (1:2)] during preparation by batch and continuous methods.

	Heating conditions		
Method	Temperature (°C)	Time (min)	Related substances (%)
Batch method	Physical mixture		0.6
	210	5	0.9
		10	1.4
		20	2.3
		30	3.0
		60	5.2
	220	2	0.3
	230	2	0.3
	250	2	0.6
		5	1.6
		10	5.6
Continuous method	Physical mixture		0.6
	210	—	0.3
	220	—	0.5
	230	—	0.4
	250	—	0.5

Chemical Stability of TAS-301 in Melt-Adsorbed Products

Table 5 shows the amount (%) of TAS-301-related substances in the melt-adsorbed products [TAS-301/FLR (1:2)] produced under different heating conditions by the batch and continuous methods. For the batch method, the amount increased with heating time and exceeded 0.9% (0.5% as increased amount of related substances) by heating at 210 and 250°C over 5 min. However, there was no increase in related substances in the case of heating for 2 min. For the continuous method, the related substances did not increase at any heating temperatures between 210 and 250°C. The continuous method was able to suppress the increase of related substances during the preparation compared with the batch method because the physical mixture passed through entire barrels of the twin screw extruder for a short time (20–30 sec).

DISCUSSION

For a dosage form containing an amorphous drug dispersed in a carrier, both the choice of carrier and the drug/carrier ratio are important considerations for stabilizing the drug. It is well known that the recrystallization of an amorphous drug in a solid dispersion often causes a decrease in its dissolution rate. Sugimoto et al. reported that when a solid dispersion [nifedipine/polyvinylpyrrolidone (1:3)] was stored under 40°C/75% RH, amorphous nifedipine was recrystallized and its dissolution rate was decreased, whereas the solid dispersion [nifedipine/hydroxypropylmethyl-cellulose (1:3)] was resistant to humidity and its dissolution rate was unchanged.[11]

We explored adsorbent type and drug/adsorbent ratios to prepare a TAS-301 melt-adsorbed product that shows excellent stability in an amorphous state. From the results of the batch method (Table 1), TAS-301 recrystallization in the melt-adsorbed products stored at 60°C/80% RH tended to be suppressed with the increase in the ratio of the adsorbent. Particularly, as no obvious crystallization was noted even after storing under severe conditions when FLR was used at a drug/adsorbent ratio of 1:0.5 or more, FLR was considered to be highly effective at retaining the drug in an amorphous state. Khalil et al. reported that a corticosteroid–PEG solid dispersion prepared with a drug/carrier ratio of 1:99 showed no decrease in dissolution rate on aging, because most of the drug was molecularly dispersed in the large amount of carrier.[12] Therefore, if the adsorbent has enough capability to adsorb amorphous drug, the energy state of the drug in the melt-adsorbed product may be kept in a high level. The amounts of oil adsorbed by adsorbents were reported as follows: FLR (400 mL/100 g) > SAL350 (310 mL/100 g) > CPX67 (237 mL/100 g), CPX80 (234 mL/100 g). The difference in stability of the amorphous drug among these adsorbents may depend on the amount of

oil adsorbed, that is the capacity to adsorb a melted drug. And FLR (porous calcium silicate) is less hygroscopic than other adsorbents (silicon dioxide).[8–10] The difference of hygroscopicity between adsorbents may affect the stability of amorphous TAS-301 under the humid condition. The extent of TAS-301 recrystallization after storage at 60°C/80% RH was less in melt-adsorbed products prepared by the batch method than by the continuous method (Tables 1–3). It was considered that the difference in stability in the amorphous state was due to the difference in heating time between the batch method (2 min) and the continuous method (20–30 sec).

The melt-adsorbed product could be prepared using the twin screw extruder only when FLR was employed as the adsorbent. We considered that FLR, which has a mean particle size of about 32 μm and shows good fluidity, passed through entire barrels of the extruder without clogging it, whereas SAL350, CPX67, and CPX80, which have mean particle sizes of a few micrometers and are likely to aggregate, became stuck in the extruder. The preparation of melt-adsorbed products containing SAL350, CPX67, and CPX80 as adsorbents may become possible by modifying the screw pattern or using a single screw.

The stability of the amorphous drug in the melt-adsorbed product prepared by the continuous method was also affected by particle size (Table 4). It was considered that the difference in drug particle size influenced the heat conduction or kneading energy in twin screw extruder.

In this study, the stability of the drug in the amorphous state was evaluated under high temperature and high humidity conditions (60°C/80% RH, open) for 3 days. The stressed condition was employed to detect drug recrystallization for a short period. The melt-adsorbed product [TAS-301/FLR (1:2)] prepared by the continuous method showed slight recrystallization under the condition (Table 1). However, it has not recrystallized for 3 years under room temperature in an airtight container (data not shown).

It was concluded that the kind of adsorbent, drug/adsorbent ratio, heating condition, and drug particle size are important factors for preparing the melt-adsorbed product showing good stability of the TAS-301 amorphous state. A highly stabilized amorphous TAS-301 was obtained by optimizing these preparation conditions in both the batch and continuous method. It is considered that the melt-adsorption is a useful method of amorphization for enhancing the solubility and oral bioavailability of poorly water-soluble drugs in the pharmaceutical field.

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