The Stability of a Novel Carbapenem Antibiotic, Meropenem (SM-7338), in a Solid State Formulation for Injection

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A formulation of meropenem, a novel carbapenem antibiotic for injection, was developed as a vial filled with a mixture of meropenem and dried sodium carbonate. During the design phase, we studied the effect of water in the formulation on the stability of meropenem in the solid state.

Meropenem is obtained as trihydrate, whose moisture content is 12.35% and is nonhygroscopic. Dehydrated meropenem, whose moisture content was 3.4%, took up moisture quickly even under low humidity (33% RH). Also, the chemical stability of dehydrated meropenem was poor compared with that of untreated meropenem, which is quite stable. Degradation of meropenem by free water was considered as a possible cause of the poor stability. Degradation of meropenem due to liberation of its crystal water to free water was also observed when meropenem was micronized by pneumatic pulverization.

Crystal water of meropenem was found to stay bound and to be almost inert in the formulation. Thus, meropenem injection formulation is stable for long time at room temperature.

Keywords crystal water; meropenem; sodium carbonate; free water; hydrolysis; thermal analysis

As shown in the preceding paper,[1] a novel carbapenem antibiotic, meropenem, is resistant to renal dehydropeptidase-I (DHP-I) mainly due to the steric hindrance of its 1β-methyl group, and is effective against both gram-positive and gram-negative microorganisms.[2] Meropenem, however, is less chemically stable than conventional β-lactam antibiotics, although it is superior to other carbapenem compounds.[3,4]

The purpose of this study was to obtain basic data on formulating meropenem for injection, to obtain good stability and dissolution behavior, either by lyophilization or aseptic powder filling.

Materials and Methods

Materials Meropenem was synthesized as reported previously.[1,2] Partially dehydrated meropenem crystals were prepared from meropenem trihydrate by drying under vacuum at 30 °C for an appropriate period. Dried sodium carbonate was prepared from commercially available anhydrous sodium carbonate of reagent grade by drying for 30 min at 250 °C. Other materials were all of reagent grade, purchased from Nacalai Tesque and were used as supplied.

Lyophilization of Meropenem Aliquots of 2 ml of meropenem solution or meropenem sodium salt solution were lyophilized in 18 ml glass vials. Lyophilization conditions were as follows: pre-freezing, −70 °C (15 min); primary drying, 0 °C, 0.1 mbar (7.5 h); terminal drying, 0 °C, 0.06 mbar (11 h). The vial was then sealed with a rubber stopper under a nitrogen atmosphere.

Measurement of Physicochemical Properties Thermal Analysis: Thermal behavior of meropenem or sodium carbonate was examined in an open or closed aluminum pan using a differential scanning calorimeter (DSC, model DSC560 Seiko Denshi Kogyo, Japan), with azobenzene as a standard. Scanning was conducted at the rate of 1 or 2 °C/min under a flow of nitrogen. Thermal gravimetry (TG) of intact and pulverized meropenem was done with a TGA-30 (Shimadzu, Japan) at the scanning rate of 1 °C/min. Relative surface area of the samples was measured by the BET method.

Hygroscopicity: Samples in weighing bottles were stored in desiccators maintained at various relative humidity (RH) values with saturated salt solutions at 25, 40 and 50 °C. The samples were weighed at predetermined times.

HPLC Conditions for Assay of Meropenem: The column, Sumipax ODS 5 μm (6 mm i.d. × 15 cm, Sumitomo Chemical Analysis Center, Japan), was kept at 40 °C during analysis. The UV detector was set at the wavelength of 220 nm. The mobile phase, 0.1% triethylamine-phosphate buffer (pH 5.0) : methanol = 5 : 1, was used at room temperature. Flow rate was 1.0 ml/min. The samples were dissolved and diluted with 0.1% triethylamine-phosphate buffer, pH 5.0. Sodium barbital was used as an internal standard.

HPLC Conditions for Assay of the Degradation Product (β-Lactam Ring-Opened Compound): The column and the detector were the same as for meropenem. The mobile phase was 0.1% triethylamine-phosphate buffer (pH 5.0) : acetonitrile = 100 : 7. Flow rate of the mobile phase was set at 1.5 ml/min.

Results and Discussion

Stability of Lyophilized Meropenem To formulate meropenem injection, we initially studied the possibility of lyophilization. As shown in Fig. 1, the potency of lyophilized meropenem in free form or as the sodium salt decreased to less than 90% of the initial potency during storage at 40 °C for 2 weeks. This result suggested that meropenem is quite unstable in the amorphous state. Since we had obtained meropenem as a crystalline trihydrate,[5] we abandoned

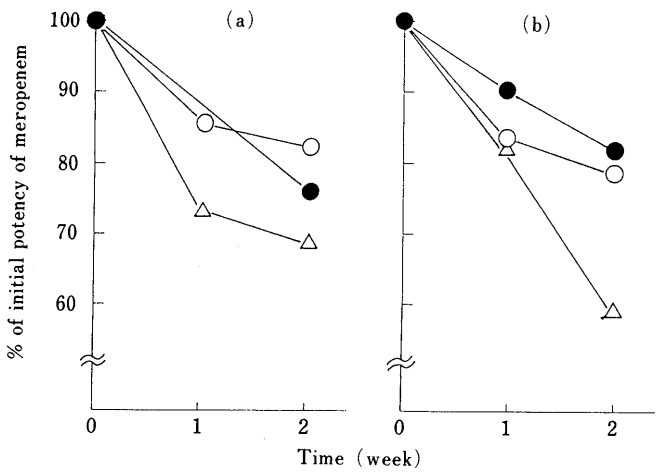

Fig. 1. Stability of Lyophilized Meropenem Stored at 40 °C

(a) free form; (b) sodium salt. Potency in a vial: 20 mg (●), 10 mg (○), 5 mg (△).

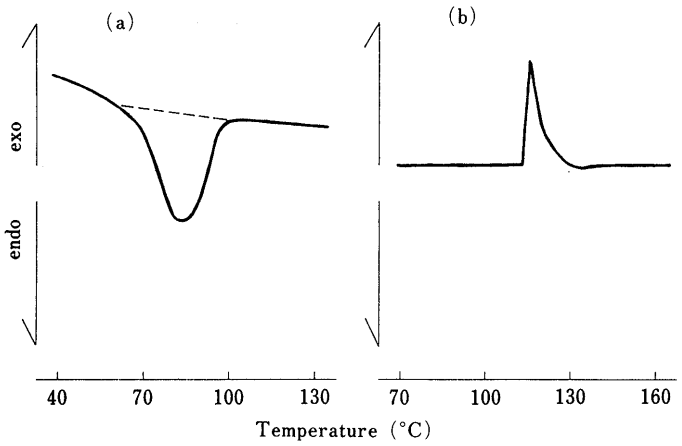

Fig. 2. DSC Curves of Meropenem Trihydrate Crystal

(a) A 5.20 mg sample was placed in an open pan. Scanning rate was 2 °C/min. (b) A 5.20 mg sample was placed in a closed pan. Scanning rate was 1 °C/min.

lyophilization and commenced studies of aseptic filling.

Physicochemical Properties of Meropenem Crystal When we decide to study the possibility of aseptic vial filling, our major concern was the behavior of crystal water of meropenem trihydrate. We first measured the latent heat of dehydration of meropenem. Figure 2a shows the DSC curve of meropenem trihydrate in an open pan. The DSC curve showed an endothermic peak at about 80 °C due to dehydration of the crystal water. The latent heat of the dehydration was calculated as 3.2×10^3 J/g H_2O. This value is similar to that of the vaporization of water at 40 °C, suggesting that the binding force of the crystal water of meropenem is not particularly strong. However, the value is larger than the latent heat of the dehydration of cephalexin (monohydrate)[6] or glucuronic acid glucuronamide monohydrate,[7] which was reported as 1.6×10^3 or 1.8×10^3 J/g, respectively.

It has been reported that cephalexin shows both an endothermic peak due to dehydration and an exothermic peak due to degradation.[6] The DSC curve of meropenem trihydrate did not show an exothermic peak due to degradation in an open pan. However, when meropenem trihydrate was placed in a closed pan, an exothermic peak at 120 °C was observed (Fig. 2b). From these results, it is considered that the degradation proceeds gradually after dehydration is completed in an open pan, whereas in a closed pan the dehydration and the degradation of meropenem trihydrate occur simultaneously.

Meropenem trihydrate is essentially non hygroscopic. The weight of sample did not change during storage for 5 d in the desiccator under the condition of 11 to 87% RH at room temperature.

Figure 3 shows the recovery of the moisture content of meropenem after dehydration under vacuum. The moisture content quickly recovered to the theoretical level of the trihydrate under the condition of 33 to 75% RH at room temperature. Dehydrated meropenem crystal, however, degraded rapidly (Fig. 4). The rehydrated sample prepared from partially dehydrated meropenem also showed poor stability compared with the intact trihydrate. From these results, it is considered that once the dehydration occurs, the stable crystal structure with its hydrogen bonds changes to an unstable amorphous form, and the crystal structure

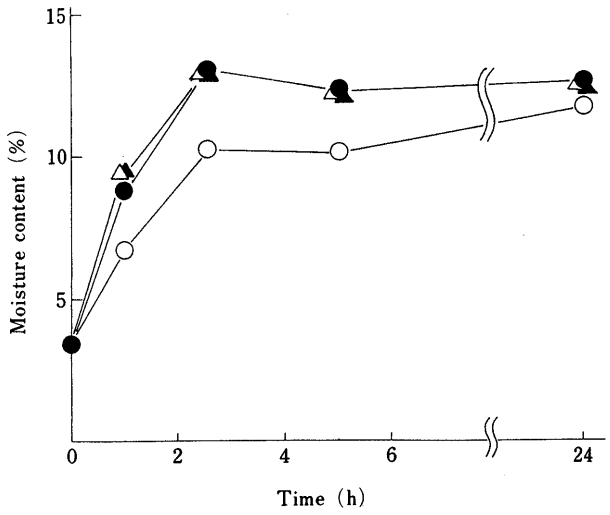

Fig. 3. Recovery of Moisture Content of Partially Dehydrated Meropenem Trihydrate

Initial moisture content of partially dehydrated meropenem was 3.4% (molar ratio to meropenem was 0.8). Samples were kept at 25 °C at the following humidity; 33%RH (○), 50%RH (●), 75%RH (△), 94%RH (▲).

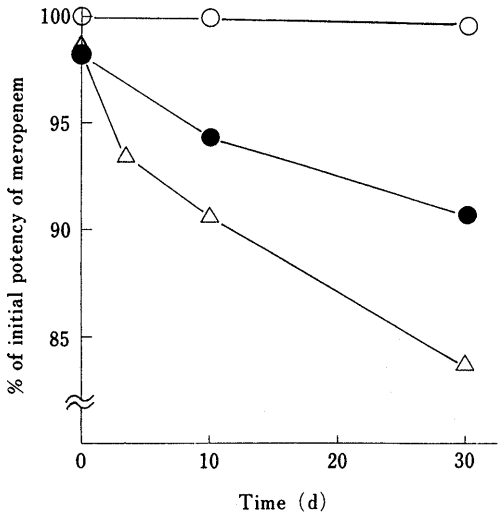

Fig. 4. Stability of Partially Dehydrated Meropenem at 50 °C

Initial moisture content was 2.4% (△), 7.6% (●) or 12.3% (○), or in molar ratio to meropenem, 0.6, 1.9 and 3.0, respectively.

TABLE I. Thermal Behavior of Intact and Pulverized Meropenem Trihydrate Observed by TG

Sample	Relative surface area (m^2/g)	Particle size[b] (μm)	% of initial weight loss[c]	
			RT to 40 °C	RT to 50 °C
Intact	0.4	11	0.15±0.05	0.38±0.07
Pulverized[a]	5.6	0.8	1.13±0.05	1.47±0.07

a) Meropenem was pulverized in a centrifugal mill (type ZM-1, Retsch, GmbH). *b*) Particle size was calculated from relative surface area supposing that the particle is spherical or cubic. *c*) Percent of initial weight loss is represented as the mean±S.D. RT: room temperature.

is not restored by the subsequent increase of moisture content. Although the moisture content is restored to that of the original trihydrate, the reabsorbed water molecules may exist as free water and may participate in solid-state hydrolytic reaction. Therefore the conversion of the

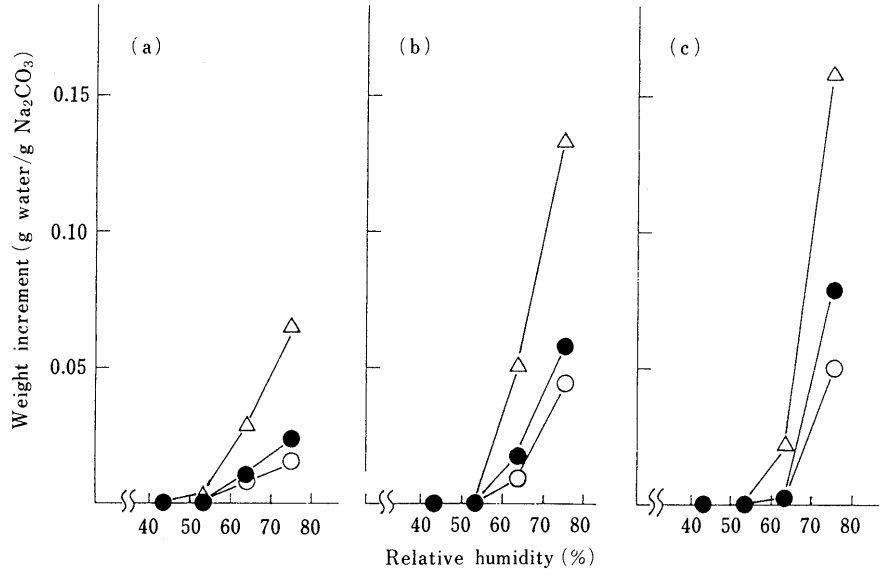

Fig. 5. Hygroscopicity of Dried Sodium Carbonate at (a) Room Temperature, (b) 40 °C and (c) 50 °C
Weight increment during storage under various humidity levels for; 6 h (○), 12 h (●) and 24 h (△).

trihydrate to dehydrated meropenem is concluded to be an irreversible phenomenon with respect to the breakdown of the strong crystal lattice.

Reduction of particle size is usually effective to facilitate the dissolution of drugs, and pulverization is commonly conducted for this purpose. However, an increase of free water was observed when meropenem was pulverized pneumatically as shown in Table I. This sample also showed poor chemical stability when stored as a formulation. The content of the degradation product (β-lactam ring-opened compound) increased to 1.1% during storage at 40 °C for 3 months. These results are in good accordance with the previous report that pulverization of cephalexin causes irreversible disturbances in the crystal lattice.[8] The increment of the degradation product seemed independent of storage period, suggesting that degradation of meropenem occurred immediately after pulverization until all of the free water was consumed.

As described in the previous papers,[1,5] meropenem trihydrate forms an extensive matrix structure with intramolecular hydrogen bonds, which results in an excellent solid state stability. When the water of crystallization is removed by physical treatments, such as drying and pulverization, the matrix structure is disordered and the trihydrate crystal is partly converted to amorphous powder, which results in instability in the solid state. On the other hand, lyophilized meropenem is obtained as an amorphous powder, and it is considered that the water molecules in this amorphous powder react as free water (the drug is labile to hydrolysis).

Physicochemical Properties of Dried Sodium Carbonate Based on the above results, we decided to employ an additive to aid solubilization in the formulation for meropenem injection. Considering the balance between dissolution rate and stability, we selected dried sodium carbonate as a primary candidate and measured its physicochemical properties.

We first measured the critical relative humidity (CRH) of dried sodium carbonate. Figure 5 shows the weight increment of dried sodium carbonate under the conditions of 44 to 75% RH. From these data, the CRH of dried sodium carbonate is estimated to be around 50% RH at room temperature, as well as at 40 and 50 °C.

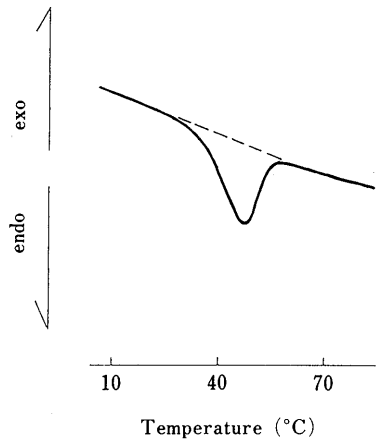

Fig. 6. DSC Curve of Moisturized Sodium Carbonate
Sodium carbonate was stored under 54% RH for 24 h. A 7.45 mg sample was placed in an open pan.

Figure 6 shows the DSC curve of dried sodium carbonate moisturized at 54% RH for 24 h. An endothermic peak was observed at above 34 °C due to the vaporization of the adsorbed water. On the other hand, the DSC curve of sodium carbonate monohydrate showed an endothermic peak at above 67 °C (Fig. 7), which is higher than that of moisturized dried sodium carbonate. These results suggest that dried sodium carbonate takes up water from the atmosphere at more than 50% RH but loses it easily under normal conditions.

Physicochemical Properties of Meropenem/Dried Sodium Carbonate Mixture We finally decided to employ dried sodium carbonate in meropenem injection with the molar ratio of 0.75 to meropenem. The reason for selecting this

ratio was to obtain a fast dissolution rate and optimum stability in water.[4]

Hygroscopicity of the mixture was studied by measuring the weight increment of the mixture under conditions of 45 to 75% RH. As shown in Fig. 8, the CRH of the mixture is the same as that of dried sodium carbonate, that is around 50% RH. The rate of the weight increment is different between dried sodium carbonate and the mixture, which is probably attributable to the difference of effective surface area of dried sodium carbonate exposed to the atmospheric moisture.

Chemical Stability of Meropenem Formulation To clarify the basic properties of meropenem formulation, the effect of atmospheric moisture in the vial on the stability of meropenem in the mixture was studied. The mixture, containing 500 mg potency of meropenem, was exposed to air at 11 to 44% RH for 24 h. Table II shows the quantity of degradation product, β-lactam ring-opened compound, present after storage at 50 °C for 1 month. The mixture exposed to 44% RH was unstable, whereas the mixture exposed to 33% RH was stable for 3 months. The hygroscopicity of the mixture is thus closely related to the stability of meropenem. It is also suggested that the true CRH of the mixture is around 40% RH, although the mixture was practically nonhygroscopic at this humidity (Fig. 8).

To study the possible cause of instability of the formulation further, the effect of moisture content of sodium

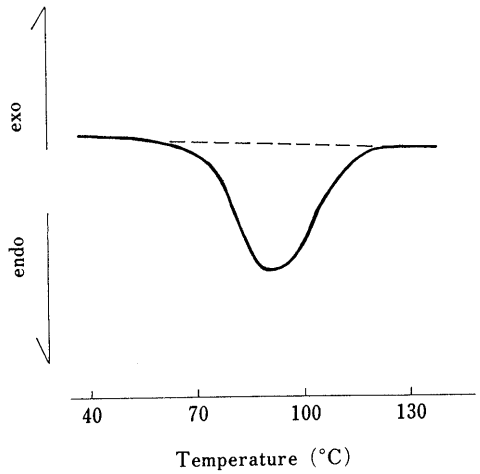

Fig. 7. DSC Curve of Sodium Carbonate Monohydrate
A 5.70 mg sample was placed in an open pan.

Table II. Effect of Humidity on Stability of Meropenem in Meropenem/Na_2CO_3 Mixture

Exposed humidity[a] (%RH)	Content of degradation product (%)[b]		
	1 month	2 months	3 months
11	0.2	0.1	0.2
22	0.2	0.1	0.2
33	0.2	0.1	0.4
44	3.3	3.3	3.3

a) Meropenem/Na_2CO_3 mixture was exposed to 11—44%RH at room temperature for 24 h and stored in closed vials. *b*) Degradation product; β-lactam ring-opened compound (percentage of initial potency of meropenem after storage at 50 °C).

Table III. Effect of Moisture Content of Na_2CO_3 on Stability of Meropenem in Meropenem/Na_2CO_3 Mixture

Moisture content of Na_2CO_3 (%)[b]	Content of degradation product (%)[a]		
	40 °C	50 °C	60 °C
0.1	0.1	0.1	0.2
0.2	0.1	0.5	2.2

a) Degradation product; β-lactam ring-opened compound (percentage of initial potency of meropenem after storage for 10 d). *b*) Water/Na_2CO_3 ratio.

Table IV. Effect of Void Volume in Vials on Stability of Meropenem

Initial g potency of meropenem in vials[a]	Void volume ratio in vials[b]	Content of degradation product (%)[c]
2 g	0.90	0.1
1 g	0.95	0.1
0.5 g	0.98	0.1
0.25 g	0.99	0.1
0.1 g	>0.99	0.1

a) Meropenem/Na_2CO_3 mixture was kept in 18 ml glass vials. *b*) Void volume ratio in vials was calculated from the true density of the mixture. *c*) Degradation product; β-lactam ring-opened compound (percentage of initial potency of meropenem after storage at 60 °C for 3 months).

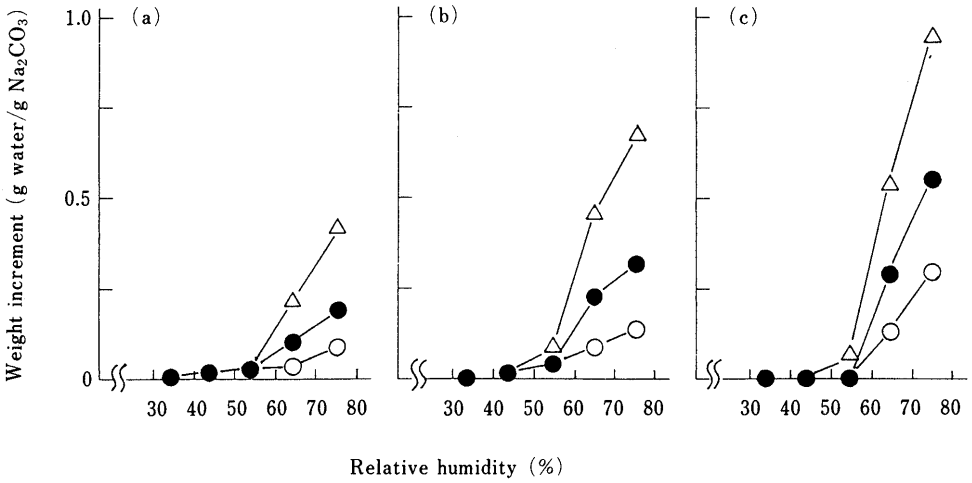

Fig. 8. Hygroscopicity of Meropenem/Sodium Carbonate Mixture at (a) Room Temperature, (b) 40 °C and (c) 50 °C
Molar ratio of sodium carbonate to meropenem was 0.75. Weight increment after storage for; 6 h (○), 12 h (●), and 24 h (△).

carbonate on the stability of meropenem in the mixture was examined. Dried sodium carbonate was moisturized under 54% RH for 24 h to get a moisture content of 0.2% and then mixed with meropenem. Soon after, the mixture containing 500 mg potency of meropenem was placed in 18 ml vials and stored at various temperatures. During storage for 10 d, meropenem was significantly degraded (Table III). In contrast, the mixture of meropenem and dried sodium carbonate, whose moisture content was 0.1% (w/w), was very stable.

Various potencies of meropenem/sodium carbonate mixture were kept in 18 ml vials under an atmosphere of less than 40% RH and were stored at 60 °C for 3 months. The void volume of the vial is 99.6% for 100 mg potency and 90.2% for 2 g potency. As shown in Table IV, degradation of meropenem was not detected in any vial. This strongly suggests that the formulation is quite stable at room temperature, independent of the filling dose, as long as the formulation is prepared under a controlled humidity of less than 40% RH.

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