The Physical Stability of Amorphous Nifedipine Determined by Isothermal Microcalorimetry

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The applicability of isothermal microcalorimetry to evaluation of the physical stability of amorphous nifedipine was examined. Amorphous nifedipine was obtained by fusion and subsequent rapid cooling, and its crystallization heat at a constant temperature was measured using a heat-conductive microcalorimeter. The heat production due to crystallization of amorphous nifedipine and the transformation of its metastable crystalline form into a stable crystalline one was detected at a temperature above its glass transition temperature (T_g). The time required for half an amorphous nifedipine sample to be crystallized (t_{50}) was calculated from the heat production time profile, and the reciprocal of t_{50} ($1/t_{50}$) was used as a measure of the crystallization rate, which increased as the temperature increased. The Williams, Landel and Ferry (WLF) plot of $1/t_{50}$ was linear, indicating that the crystallization rate was affected by the nifedipine matrix viscosity, which decreased as the temperature increased. Moisture decreased the T_g of the fused nifedipine and increased its crystallization rate. The effects of temperature and humidity can be interpreted by changes in the matrix viscosity, which is related to the molecular motion of nifedipine.

Key words   stability; microcalorimetry; crystallization; nifedipine; glass transition temperature (T_g)

Isothermal microcalorimetry is expected to be useful for following various changes in pharmaceutical products which occur during storage, since physical and chemical processes are accompanied by heat exchange. The chemical stability of some model drugs has been investigated using this method: the decomposition rate, estimated from the heat production time profile, has been compared with that obtained by conventional methods, such as high-performance liquid chromatography (HPLC), and the Arrhenius behavior of the drug decomposition has been examined.[1–4] The physical stability of some excipients has also been studied by microcalorimetry, namely, the moisture-induced crystallization of lactose[5,6] and the transformation of β-lactose to α-lactose.[7,8] However, no studies about the applicability of microcalorimetry to physical stability testing of drugs, such as the crystallization of amorphous drugs, have, to our knowledge, been published.

The crystallization of amorphous drugs has been studied using differential scanning calorimetry (DSC), X-ray diffractometry and other techniques. Isothermal micro-calorimetry has an advantage over these techniques, as it enables the heat of crystallization at a constant temperature to be monitored continuously, and provides information on the crystallization rate. The crystallization rate was estimated from the heat production time profile of an amorphous sample at a temperature above its glass transition temperature (T_g) and the effects of temperature and humidity on the crystallization rate in relation to the T_g are discussed.

Experimental

Materials   Nifedipine was purchased from Sigma (St. Louis, MO). Fused nifedipine was prepared according to the reported method[9]; 2 g nifedipine was placed in a vessel made of aluminum foil and kept at 180 °C for 20 min, after which nifedipine was immersed in liquid nitrogen. The degradation of nifedipine during this process was determined, by HPLC, to be less than 0.5%. The fused nifedipine obtained by this method was pulverized using a mortar and pestle and stored in an amber

glass vessel at −15 °C until required for use. All the other chemicals used were of reagent grade. All experiments were carried out in a dark room (<20 lux) where temperature and humidity was controlled at 25 °C and <50% relative humidity (RH).

Crystallinity of Fused Nifedipine   The crystallinity of fused nifedipine was examined by X-ray powder diffractometry and DSC. The X-ray powder diffraction patterns were determined using an X-ray diffractometer (model RAD-2C, Rigaku Denki Co., Tokyo) with Ni-filtered CuK_α radiation (30 kV, 10 mA) at a scanning rate of 2°/min. The DSC curves of fused nifedipine were obtained using a thermal analyzer (model DS-40, Shimadzu Co., Kyoto); samples in sealed aluminum pans were heated at 2 °C/min. The T_g was defined as the mid-point of the transition curve.

Isothermal Microcalorimetry   The heat production of fused nifedipine was measured using an isothermal microcalorimeter (model 2277, Thermometric AB, Sweden) at various constant temperatures from 50 to 60 °C. Ten to 20 mg fused nifedipine was weighed into a 3-ml glass ampule, which was sealed, placed in the equilibration position of the microcalorimeter for 10 min and then lowered to the measuring position of the instrument. The output from the microcalorimeter was collected using a computer (model 9801VM, NEC, Tokyo) through an AD converter (model EC-2325, Elmec Inc., Limited, Tokyo). When the effect of humidity on the crystallization rate was studied, a glass tube (4.2 mm internal diameter) containing an appropriate salt solution, which was saturated at 25 °C, was added to the ampule and the relative humidity in the ampule was controlled. Relative humidity of the salt solution was determined at 50 °C using a hygroscope (model DT, Rotoronic ag, Switzerland).

Determination of the Water Content of Fused Nifedipine   Twenty mg fused nifedipine was weighed into a 3-ml glass ampule for the isothermal calorimeter, in which a glass tube (4.2 mm internal diameter), containing magnesium chloride solution which was saturated at 25 °C, was placed. The ampule was sealed and kept at 50 °C for an appropriate time. The glass tube was removed from the ampule. One ml of anhydrous dichloromethane was added to the ampule. After nifedipine and the water absorbed by the nifedipine sample were dissolved, the water content of the dichloromethane solution was determined by the Karl Fischer method using a coulometric Karl Fischer titrater (model E684, Metrohm, Switzerland).

Results and Discussion

Effect of Temperature on the Crystallization Rate   The crystallinity of the fused nifedipine was investigated by DSC and X-ray powder diffractometry. Figure 1 shows the X-ray diffraction patterns of the fused samples. No

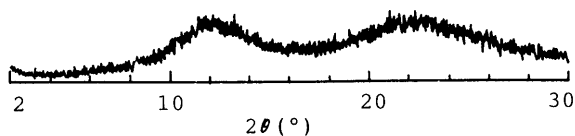* To whom correspondence should be addressed.

Fig. 1. X-Ray Powder Diffraction Diagram of Fused Nifedipine Obtained by Fusing and Subsequent Rapid Cooling
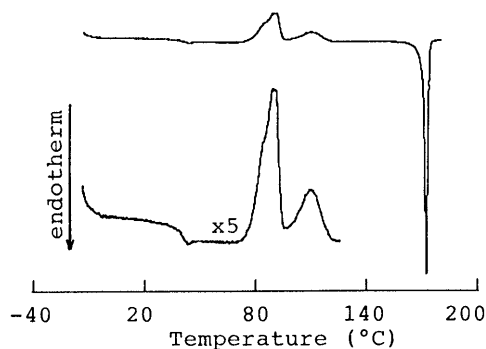

Fig. 2. DSC Curve of Fused Nifedipine Obtained by Fusing and Subsequent Rapid Cooling
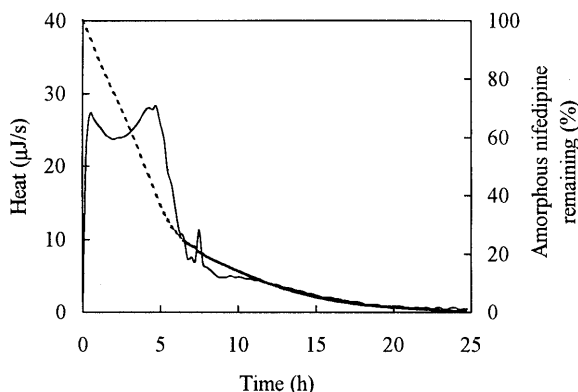

Fig. 3. Typical Time Profiles of Heat Production (——) and Amorphous Nifedipine Remaining (-----) for Fused Nifedipine at 58 °C

diffraction peaks were observed, indicating that the fusing and subsequent rapid cooling rendered nifedipine amorphous. The DSC scans of the nifedipine samples also showed that they were amorphous. A break in the baseline at 40 °C was observed (Fig. 2), which could be ascribed to the glass transition of amorphous nifedipine. Exothermic peaks at 90 and 110 °C were due to the crystallization of amorphous nifedipine and its transformation from a metastable crystalline form into a stable crystalline one, respectively.[9] The total exothermic peak area at 90 and 110 °C was about 85% of the endothermic peak area at 172 °C, indicating that most of the fused nifedipine was amorphous.

Fused nifedipine produced heat at a temperature above the T_g of amorphous nifedipine. Figure 3 shows a typical time profile of the heat production of fused nifedipine measured at 58 °C (solid line). Considerable heat production was observed immediately after the measurements began, and this continued for about 5 h. The heat production of fused nifedipine in the equilibrium position of the microcalorimeter was neglected, since the time in the equilibrium position was much shorter than the total

measuring time. The time profile of heat production changed with the temperature, but the areas under the curves were estimated to be about 65 J/g and were independent of temperature. This value was consistent with the sum of the crystallization heat and transformation heat of the metastable to the stable crystalline form, which were both determined by DSC. After measuring heat production using the isothermal microcalorimeter, the DSC of fused nifedipine showed only an endothermic peak at about 170 °C, which could be ascribed to the melting of crystalline nifedipine. Crystalline nifedipine, the raw material used to produce fused nifedipine, produced no heat under the experimental conditions used. These results suggest that the heat production observed using isothermal microcalorimetry corresponded to the total heat of crystallization of amorphous nifedipine and its transformation to a stable crystalline form.

We assumed that the heat production observed in a unit of time was proportional to the amount of amorphous nifedipine crystallized in that unit of time, as the transformation of its metastable crystalline form into a stable crystalline one was faster than the crystallization of amorphous nifedipine.[9] The amount of amorphous nifedipine remaining in each fused sample at a certain time was calculated from the area under the heat production–time curve from time zero to the certain time (proportional to the amount of nifedipine crystallized from time zero to the certain time), and from the total area under the curve (proportional to the initial amount of amorphous nifedipine). From the time profile of amorphous nifedipine remaining, shown by a dashed line in Fig. 3, the t_{50} value, the time required for half the amorphous nifedipine to be crystallized, was calculated. The reciprocal of t_{50} ($1/t_{50}$) was used as a measure of the crystallization rate.

Williams, Landel and Ferry (WLF) proposed an empirical equation (Eq. 1) to express the temperature-dependence of mechanical and electrical relaxation times. Over a temperature range of T_g to $T_g + 100$ °C, this equation was found to be applicable to a wide variety of polymers, polymer solutions, organic glass-forming liquids and inorganic glasses.[10]

$$\log a_T = -17.44(T - T_g)/(51.6 + T - T_g) \quad (1)$$

where a_T is the ratio of the mechanical or electrical relaxation times at temperature T to their values at the T_g and can be approximately related to the ratio of the viscosity at temperature T to that at the T_g, η/η_g.[10]

$$\log \eta/\eta_g = -17.44(T - T_g)/(51.6 + T - T_g) \quad (2)$$

Because the crystallization is brought about by molecular reorientation, the crystallization rate of nifedipine is determined by the mobility of its molecules and is a function of its viscosity. If η/η_g in Eq. 2 is replaced with $\{(1/t_{50})/(1/t_{50})_g\}^A$, Eq. 2 can be rewritten as follows:

$$\log(1/t_{50}) = \log(1/t_{50})_g + \{-17.44(T - T_g)/(51.6 + T - T_g)\}/A \quad (3)$$

where $(1/t_{50})$ and $(1/t_{50})_g$ are the crystallization rate constants at temperatures T and T_g, respectively, and A is constant. Figure 4 shows the temperature-dependence of $1/t_{50}$, according to Eq. 3. The linear plot obtained
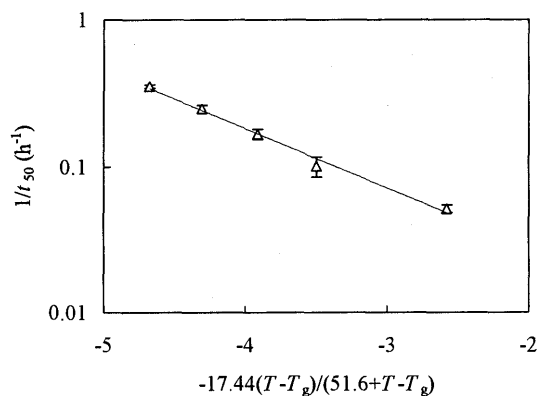
Fig. 4. WLF Plot of Crystallization Rate for Fused Nifedipine

The error bar in the figure represents the standard deviation of three experiments.

Fig. 5. Time Profiles of Amorphous Nifedipine Remaining (○) and Water Content (△) for Fused Nifedipine at 50 °C and 37% RH

The error bar in the figure represents the standard deviation of three experiments.

suggests that the crystallization rate of amorphous nifedipine could be analyzed using the WLF equation, and that it correlated with the matrix viscosity.

Effect of Humidity on the Crystallization Rate The effects of humidity on heat production by fused nifedipine were investigated. The RH was controlled with appropriate salt solutions and heat production was measured at 50 °C. Figure 5 shows the time profiles of amorphous nifedipine remaining and the water content of fused nifedipine samples measured at 50 °C and 37% RH. The total heat production was similar to that observed in the absence of moisture. The water content increased rapidly within 1 h and then gradually declined as the amount of amorphous nifedipine remaining decreased. The time profile of the water content changes suggests that water absorption by amorphous nifedipine matrices was rapid, and that water was absorbed by the amorphous matrices rather than by the crystalline regions. Once the matrices were crystallized, water which had been absorbed by the amorphous regions was desorbed by the matrices, resulting in a decreased water content.

Figure 6 shows the relationship between RH and $1/t_{50}$. The crystallization rate, represented by $1/t_{50}$, increased as the RH increased, indicating that moisture accelerated the crystallization of amorphous nifedipine. The T_g of fused nifedipine stored at 50 °C and the indicated RH values for 1 h decreased as the RH increased (Fig. 6). Therefore, the increased crystallization rates were accompanied by decreases in the T_g. Figure 7 shows $1/t_{50}$ plotted, according to the WLF equation, as a function of the T_g of the nifedipine matrices. The temperature dependence of $1/t_{50}$ measured in the absence of moisture shown in Fig. 4 is also presented for comparison. A similar regression line could be applied to the crystallization rates in the presence and absence of moisture, which suggests that the effects of moisture on the crystallization rate may be ascribed to decreased nifedipine matrix viscosity.

It has been pointed out that the T_g of amorphous matrices affects the chemical and physical stability of drugs in such matrices.[11] The results described in this paper also suggest that the T_g is one of the most important physicochemical properties of amorphous matrices and that it provides useful information for assessing the stability of amorphous drug preparations.
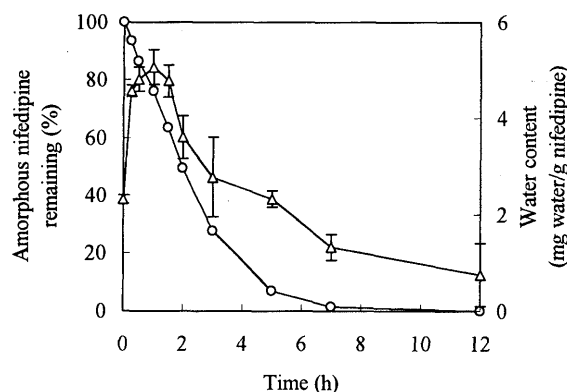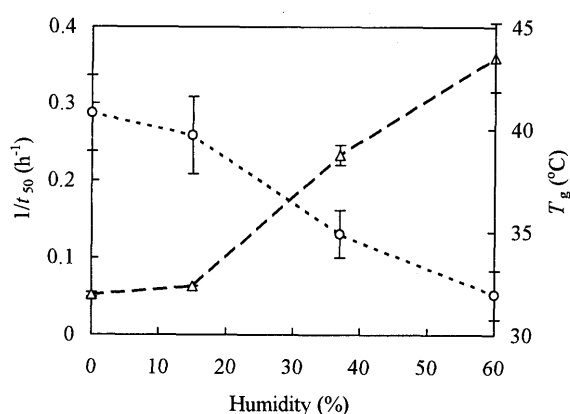

Fig. 6. The Effect of Humidity on the Crystallization Rate (△) and the T_g (○) of Fused Nifedipine

The T_g of fused nifedipine was measured after storage at 50 °C and various relative humidity for 1 h. The error bar in the figure represents the standard deviation of three experiments.
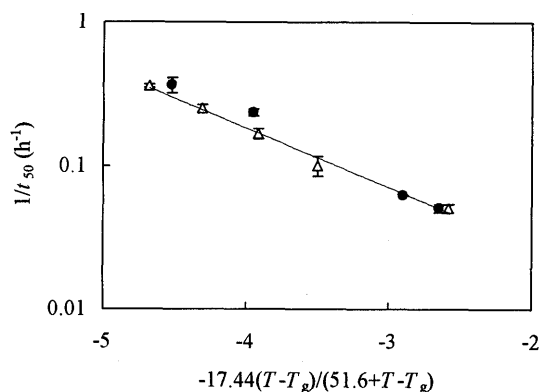

Fig. 7. WLF-Type Plot of Crystallization Rate of Fused Nifedipine with Varying T_g (●)

△ represents data shown in Fig. 4. The error bar in the figure represents the standard deviation of three experiments.

In conclusion, isothermal microcalorimetry can be applied to the evaluation of the physical stability of amorphous nifedipine. The heat production due to the crystallization of amorphous nifedipine and the transformation of its metastable crystalline form into a stable crystalline one was detected at a temperature above its

glass transition temperature. The crystallization rate increased as the temperature and humidity of the atmosphere increased. A linear WLF plot of the crystallization rate suggested that its crystallization rate correlated with matrix viscosity, which is related to the molecular motion of nifedipine.

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