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Feasibility of using isothermal microcalorimetry to evaluate the physical stability of amorphous nifedipine and phenobarbital

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Abstract

Feasibility of microcalorimetry to evaluate the physical stability of amorphous drugs was studied. Amorphous forms of nifedipine and phenobarbital were prepared by melting and subsequent cooling in a differential scanning calorimetry (DSC) sample pan, and their heats of crystallization were monitored by isothermal microcalorimetry. The time required for 10% of the amorphous drug to crystallize (t_{90}), a direct measure of the crystallization rate, could be obtained from a single microcalorimetric trace of the amorphous nifedipine or phenobarbital. The t_{90} values were also determined by conventional storage studies in which the heat of crystallization was determined by DSC. The t_{90} values obtained by microcalorimetry were consistent with those obtained by DSC, within experimental error, indicating that microcalorimetry is a useful method for evaluating the physical stability of amorphous drugs.

Keywords: Microcalorimetry; DSC; Crystallization; Stability; Amorphous; Nifedipine; Phenobarbital

1. Introduction

Isothermal microcalorimetry is a useful method studying the physical and chemical stability of pharmaceuticals. It has been used to assess changes in crystallinity induced during the processing of powders by determining the heat of crystallization [1–5], and to characterize drug powders by measuring the heat of solution or the heat of water adsorption [6–9]. Microcalorimetry has also been used to estimate the stability of pharmaceuticals during storage, including chemical and physical degradation processes [10–23].

Amorphous drugs are considered to be less stable than their crystalline forms, since they exist in a higher energy state. Loss of physical stability, as evidenced by crystallization, is an issue of concern with amorphous drugs. It is important to determine a storage temperature at which crystallization will be negligible during a drug's shelf life, and to clarify any factors that will affect the crystallization rate. The crystallization of amorphous drugs has usually been studied using differential scanning calorimetry (DSC) and X-ray diffractometry. However, isothermal microcalorimetry has an advantage over these techniques, in that it can be used to monitor the heat of crystallization continuously at a constant temperature, thus, providing information on the crystallization rate. Lehto and Laine described the crystallization of an amorphous lubricant using isothermal microcalorimetry, and compared the results with those obtained by conventional methods such as DSC and X-ray diffractometry. They concluded that isothermal microcalorimetry could rapidly give precise information about possible solid state transition mechanisms [24].

In this study, we investigated the feasibility of using isothermal microcalorimetry to evaluate the physical stability of amorphous nifedipine and phenobarbital.

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The time required for 10% of the amorphous drug to crystallize (t_{90}) was calculated from isothermal microcalorimetric traces as a measure of the crystallization rates of these drugs, and compared with the value determined by DSC.

2. Experimental

2.1. Materials

Crystalline nifedipine was purchased from Sigma Chemical Co. (St. Louis, MO). Crystalline phenobarbital was prepared from sodium phenobarbital (Wako Pure Chemical Industry, Osaka) according to the literature [25]. Amorphous nifedipine and phenobarbital were prepared from each crystalline drug by melting and subsequent cooling (−40°C/min) in a model 2920 differential scanning calorimeter (TA instrument, New Castle, DE). About 5 mg of each crystalline drug was sealed in an aluminum hermetic sample pan with a pin hole in the lid [26]. Microscopic observations of samples under polarized light were carried out in order to confirm that the sample was amorphous. The glass transition temperatures (T_g) of the amorphous nifedipine and phenobarbital samples were 49 and 45°C, respectively [26].

2.2. Measurement of heat flow of amorphous nifedipine and phenobarbital by isothermal microcalorimetry

Amorphous nifedipine or phenobarbital samples freshly prepared as described above was placed in a microcalorimetric cell made of stainless steel (2277-301, Thermometric AG, Sweden), and in order to remove moisture, space of the cell was purged by dry nitrogen before the cell was closed. The cell was placed in equilibrium position of a model 2277 microcalorimeter (Thermometric AG, Sweden) for 15 min before measurement, and then heat flow of the sample was recorded.

In order to clarify the effects of moisture on heat flow–time curves, microcalorimetric measurements were carried out in the presence of moisture. Relative humidity of the calorimetric cell was adjusted by storing the cell at 25°C for 1 h in a vessel that contains a saturated solution of potassium acetates, potassium carbonate or sodium bromide. After the cell was taken from the vessel, freshly prepared amorphous nifedipine sample was placed in the cell and the cell was closed. Heat flow of the amorphous nifedipine sample at 50°C was recorded as described above.

Microcalorimetric measurements for amorphous nifedipine samples stored at various temperatures were also conducted in order to clarify the effects of storage on the heat flow–time curve of amorphous nifedipine. Freshly prepared amorphous nifedipine samples were placed in tubes containing P_2O_5 and stored in a thermostatic chamber (5 and 25°C) or a freezer (about −25°C). Moisture in the microcalorimetric cell was removed by purging the cell by dry nitrogen and heat flow of the amorphous nifedipine sample at 50°C was recorded as described above.

2.3. Determination of the crystallization heat of amorphous nifedipine and phenobarbital by DSC

Amorphous nifedipine or phenobarbital samples freshly prepared as described in the material section were put in tubes containing P_2O_5 and stored in thermostatic chambers at various temperatures. At appropriate time intervals, the samples were withdrawn and their thermograms were recorded at a heating rate of 20°C/min using a model 2920 differential scanning calorimeter (TA instrument, New Castle, DE). The heat of crystallization (ΔH_c) of nifedipine and phenobarbital was estimated from the thermograms.

3. Results and discussion

3.1. Heat flow–time curves of amorphous nifedipine and phenobarbital measured by isothermal microcalorimetry

Figs. 1 and 2 show typical microcalorimetric traces for amorphous nifedipine and phenobarbital, respectively, measured under dry condition. The traces for amorphous nifedipine each showed a single peak, and the time taken to reach this peak decreased as the temperature increased. The traces for amorphous phenobarbital exhibited multiple peaks.

Since crystallization from amorphous state is considered to proceed via nucleation followed by its

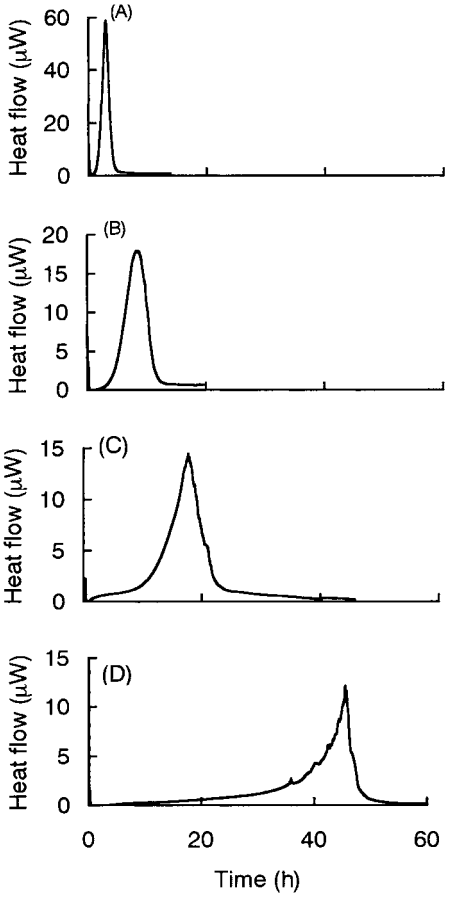

Fig. 1. Typical heat flow–time curves for amorphous nifedipine at various temperatures. Temperature: (A) 65°C; (B) 60°C; (C) 55°C; (D) 50°C. In order to remove moisture, the microcalorimetric cell was purged by dry nitrogen before the cell was closed.

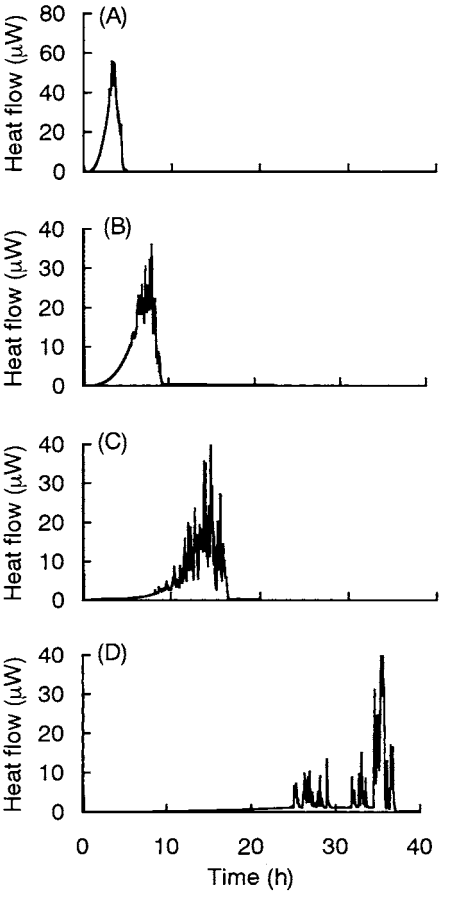

Fig. 2. Typical heat flow–time curves for amorphous phenobarbital at various temperatures. Temperature: (A) 65°C; (B) 60°C; (C) 55°C; (D) 50°C. In order to remove moisture, the microcalorimetric cell was purged by dry nitrogen before the cell was closed.

growth reaction [27], microcalorimetric trace for crystallization is expected to exhibit a peak [12,18,28,29]. Similar trace patterns to those shown in Fig. 1 have been reported for crystallization of amorphous lactose [1,3,5] and a amorphous hydrophobic drug (L-365,260) [4].

After the microcalorimetric measurement, the amorphous nifedipine and phenobarbital samples were found to be crystalline by microscopic observations under polarized light. DSC thermograms of the samples exhibited the same melting peaks as the stable crystalline forms and neither glass transition at around 45°C nor crystallization at around 120°C was observed, as shown in Fig. 3. The heat of crystallization determined by DSC for amorphous nifedipine (56–58 J/g) and phenobarbital (67–70 J/g) prior to storage were similar to the integrated heat of amorphous nifedipine (57–63 J/g) and phenobarbital (58–62 J/g) determined by microcalorimetry. These results suggest that the heat production of amorphous nifedipine and phenobarbital observed by microcalorimetry may be due mainly to the crystallization of amorphous nifedipine and phenobarbital.

3.2. Factors affecting heat flow–time curve of amorphous nifedipine

In order to clarify the effect of relative humidity in microcalorimetric cells on microcalorimetric traces, heat flow of amorphous nifedipine was measured

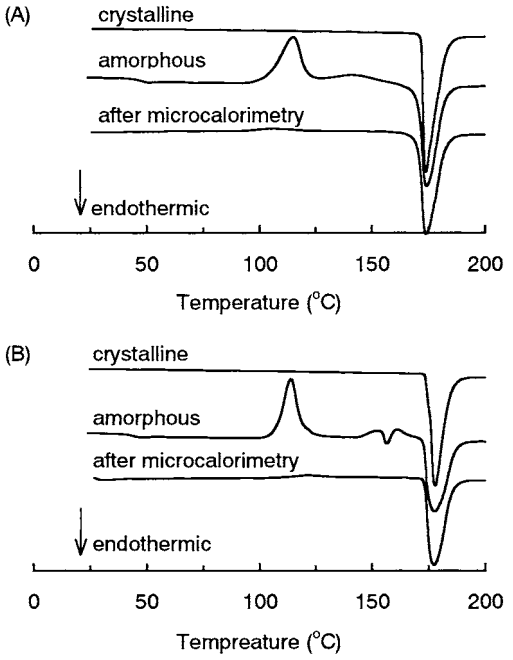

Fig. 3. DSC thermograms for nifedipine (A) and phenobarbital (B).

under various humidity conditions as shown in Fig. 4. The time taken to reach peak of the trace decreased as the humidity increased, indicating that the crystallization of amorphous nifedipine was enhanced by moisture in the cell. This suggests that the control of the relative humidity of the cell is necessary in order to get reproducible results.

Fig. 5 shows typical heat flow–time curves of amorphous nifedipine stored under various temperature conditions. The peak of the trace for the samples stored at 25°C for 2 days (Fig. 5(D)) and at 5°C for 40 days (Fig. 5(C)) was observed in shorter time compared to that for freshly prepared samples (Fig. 5(A)). The heat of crystallization of these samples determined by DSC was same as that prior to storage, indicating that nifedipine undergoes little or no crystallization under the condition studied. These results

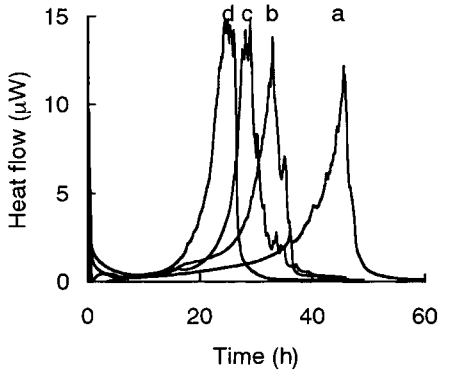

Fig. 4. Typical heat flow–time curves for amorphous nifedipine in the presence of moisture. The microcalorimetric cell was stored for 1 h in a vessel that contains saturated solution of potassium acetate (b), potassium carbonate (c) or sodium bromide (d). The cell was purged by dry nitrogen before the cell was closed (a). Microcalorimetric measurements were carried out at 50°C.

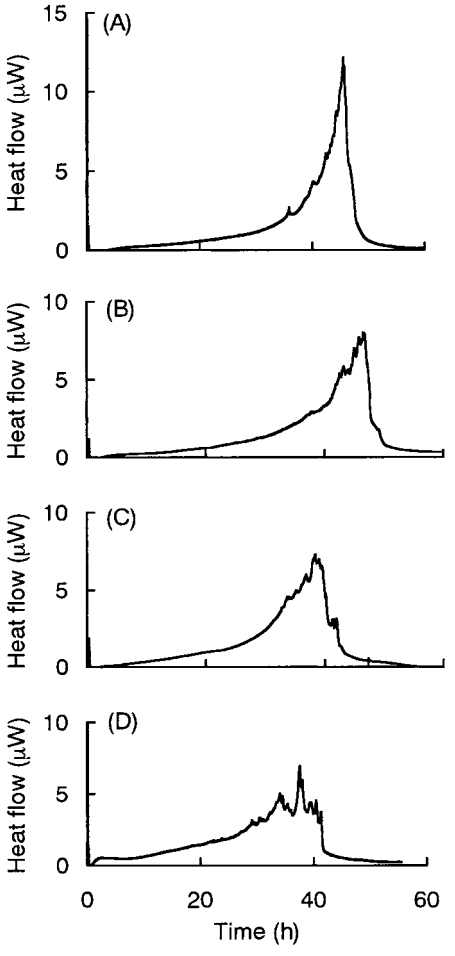

Fig. 5. Typical heat flow–time curves for amorphous nifedipine stored at various temperature condition: (A) initial; (B) −25°C for 17 months; (C) 5°C for 40 days; (D) 25°C for 2 days. Microcalorimetric measurements were carried out at 50°C.

suggest that changes in amorphous nifedipine matrices such as nucleation may occur during storage. This change was not observed at −25°C (Fig. 5(B)).

3.3. Determination of crystallization rate of amorphous nifedipine and phenobarbital by isothermal microcalorimetry

The amount of amorphous nifedipine and phenobarbital remaining were estimated by both isothermal microcalorimetry and DSC. The total area under the heat flow–time curve (q) and the area under the heat flow–time curve from time 0 to a defined time (q_t) were calculated from the time profiles of heat production detected by microcalorimetry. The time profiles of the amount of amorphous nifedipine or phenobarbital remaining (R_{amr}) were calculated from these values using the equation assuming that crystallization proceeds completely:

$$R_{amr} = \left(1 - \frac{q_t}{q}\right) \times 100$$

and the results are shown in Fig. 6.

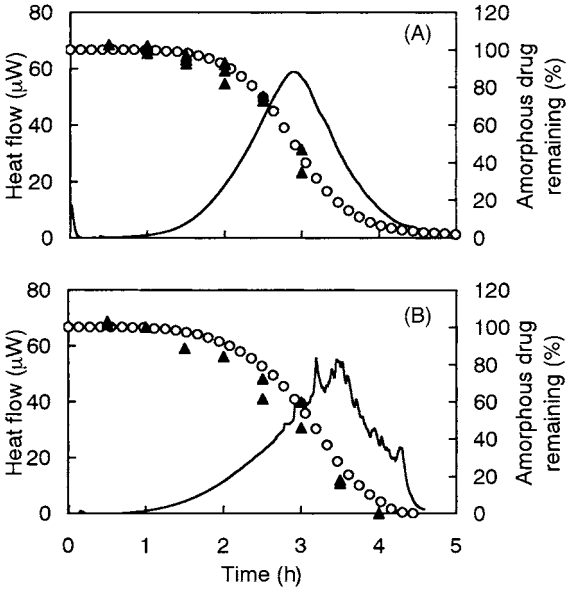

Fig. 6. Heat flow–time curves for amorphous nifedipine (A) and phenobarbital (B) at 65°C, and the amounts of amorphous drug remaining. Amount of amorphous drug remaining determined by microcalorimetry (○); amount of amorphous drug remaining calculated from the heat of crystallization determined by DSC (▲).

The R_{amr} values were also calculated from the heat of crystallization determined by DSC, as shown in Fig. 6. Amorphous nifedipine and phenobarbital both showed a crystallization peak at around 120°C (Fig. 3). If the heat of crystallization detected by DSC is assumed to be proportional to the amount of amorphous drug present, the R_{amr} value can be calculated from the equation

$$R_{amr} = \frac{\Delta H_t}{\Delta H_0} \times 100$$

where ΔH_0 and ΔH_t are the heats of crystallization observed for the initial sample and for the sample after storage at a constant temperature for time t, respectively. The R_{amr} values obtained by isothermal microcalorimetry showed similar time profiles to those obtained by DSC (Fig. 6).

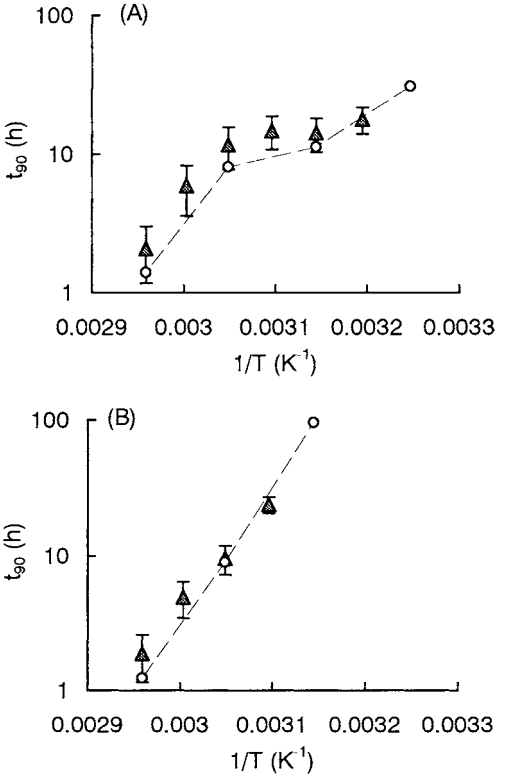

Fig. 7. Temperature dependence of t_{90} determined by microcalorimetry (solid symbols) and DSC (open symbols) for amorphous nifedipine (A) and phenobarbital (B). Error bars in the figure represent standard deviation ($n = 3$).

The time required for the R_{amr} value to fall to 90 (t_{90}) was determined from the R_{amr}–time curve as a measure of the crystallization rate. The temperature dependence of the t_{90} value is shown in Fig. 7. The t_{90} obtained by microcalorimetry was consistent with that obtained by DSC within experimental error. This indicates that microcalorimetry is a useful method for estimating the t_{90} of crystallization of amorphous drugs at temperatures around their glass transition temperatures (T_g). The crystallization rate at room temperature can also be estimated by the extrapolation of t_{90} data obtained by microcalorimetry, if the temperature dependence of t_{90} data at temperatures around room temperature is known. The temperature dependence of t_{90} of amorphous nifedipine and phenobarbital can be estimated from the temperature dependence of their mean relaxation time that is calculated from the heating rate dependence of T_g according to the Adam–Gibbs–Vogel equation as reported in the previous paper [30].

4. Conclusion

The t_{90} value (a measure of the crystallization rate) could be directly obtained from a single microcalorimetric trace for amorphous nifedipine or phenobarbital. This value was consistent with that obtained by conventional storage studies in which the heat of crystallization was determined by DSC, within experimental error. These findings indicate that microcalorimetry is a useful method for evaluating the physical stability of amorphous drugs.

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