Changes in Solid-State Structure of Cyclophosphamide Monohydrate Induced by Mechanical Treatment and Storage

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The effects of mechanical treatment and various storage conditions on the structure of cyclophosphamide monohydrate were evaluated by thermal and X-ray analyses and molecular modeling. The monohydrate form of cyclophosphamide was found to convert to the anhydrous form through a metastable phase. Metastable forms were produced by mechanical treatment and by desiccation. These forms could be detected in differential scanning calometric thermograms as endothermic peaks, at approximately 39°C, and X-ray powder diffractometric analysis, e.g.; by a characteristic reflection at 15.3° (2θ). Molecular modeling was used to study molecular interactions and putative metastable structures. The dehydration enthalpies of the cyclophosphamide monohydrate obtained from quantum chemical calculations and DSC analysis were 51.6 and 36.1 J/g, respectively. In a unit cell of the stable monohydrate, a water molecule is held by O(7) of the cyclophosphamide molecule and N(6)H of a neighboring cyclophosphamide molecule, with hydrogen bonds enabling existence of a water tunnel. The metastable form of cyclophosphamide is detected when a sterically formed block in the possible tunnel is removed, and the water molecules are allowed to leave the system one by one.

KEY WORDS: cyclophosphamide monohydrate-anhydrate transition; metastable form; thermal analysis; X-ray diffractometric analysis; molecular modeling; quantum mechanics.

INTRODUCTION

Normally in the investigations dealing with the monohydrate-anhydrate transitions of drug substances (e.g.; 1,2), only conditions of humidity and temperature are employed to induce change in the structure of the studied substance via dehydration or rehydration. Surprisingly few studies have (e.g.; 3 - 5) investigated the mechanical treatments used which can induce phase transitions within the matrix of a drug substance, even though many mechanical effects occur during the manufacture of solid dosage forms, e.g.; grinding and tableting. If the enthalpies of phase transitions are relatively low or if the formation of metastable forms are possible for a given drug substance, then the application of mechanical treatments can be especially important.

Cyclophosphamide monohydrate is a commonly used antineoplastic agent. Cyclophosphamide therapy usually begins with injectable formulations and continues later with oral administration in the form of tablets. Because of its low melting range (48 - 52°C), the tableting of cyclophosphamide monohydrate typically proceeds at cool temperatures (8 - 12°C) to prevent sticking and gelation of the cyclophosphamide. The monohydrate form has been found to, especially on the surfaces of powder compacts, form a gel during compression at normal room temperatures (6). This gelation of the tablet surface causes sticking and results in a decrease in the rate of release and bioavailability for the drug. Gelation is thought to be due to the formation of intermediate metastable forms of cyclophosphamide.

The unit cell of a cyclophosphamide monohydrate has been reported to consist of two cyclophosphamide molecules per unit cell, where a water molecule is held by O(7) from one of the cyclophosphamide molecules and by N(6)H from the other (7). It has also been reported (1) that cyclophosphamide monohydrate is stable if the relative humidity is higher than 70% and the temperature is lower than 30°C. In the same study it was suggested that the monohydrate-anhydrate transition is reversible and that metastable forms exist. Further, it has been suggested (2) that the mechanisms of dehydration and rehydration for cyclophosphamide are different, and that anhydrous and monohydrate forms can form eutectic mixtures.

The aim of this study was to evaluate the effects of mechanical treatment and various storage conditions on the structure and stability of cyclophosphamide by thermal and X-ray analyses and molecular modeling. In addition, the location of water and the necessary energies for breaking the water-cyclophosphamide interactions were also investigated.

MATERIALS AND METHODS

Material

The material studied was commercial cyclophosphamide monohydrate USP (M.W. 279.10; Orion-Farmos Pharmaceuticals, Turku, Finland), crystallized from toluene. Anhydrous cyclophosphamide (M.W. 261.09) was prepared by storing the monohydrate for four months at room temperature in a vacuum desiccator containing silica gel.

Mechanical Treatment

Cyclophosphamide was treated mechanically by milling with a vibration ball mill (Retsch K9MM, Retsch Mühle, Germany) and by compression with a hydraulic press. Cyclophosphamide monohydrate samples, 1.4 g in weight, were milled for 10 to 1200 seconds at a vibration speed of 60 rpm. Anhydrous cyclophosphamide was milled for 1200 seconds (20 minutes). Both unmilled anhydrous and monohydrate forms of the cyclophosphamide were compressed into tablets (13 mm in diameter) with force of 50 or 120 kN. The compression force was maintained for 3 or 10 minutes. The mean weight of the compressed tablets was 250 mg.

Storage

Untreated powder samples of cyclophosphamide mono-

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Table I. Effects of Mechanical Treatment, Storage Condition and Time on the Proportion of Monohydrate (%) Based on X-ray Diffraction Measurements, on Water Content (%), on the Enthalpy (ΔH) (J/g) and Onset Temperature (°C) Values of Monohydrate-anhydrate Transition Based on DSC Measurements. Standard Deviations in Parenthesis

Sample code	Mechanical treatment	Storage condition	Storage time	Monoh. (%)	Water content (%)	ΔH (J/g)	Onset temp. (°C)	Onset temp.* (°C)
A	**	+7°C/airtight container	6 months	100	6.5 (<0.1)	126 (3)	47.7 (0.2)	—
B	**	+20°C/97% RH	6 months	98	6.7 (0.2)	124 (2)	47.2 (0.1)	—
C	**	+7°C/0% RH	4 months	70	6.0 (<0.1)	121 (1)	46.5 (0.3)	39.4 (<0.1)
D	20 min milling	**	**	26	6.5 (<0.1)	125 (1)	46.5 (<0.1)	—
E	1 min milling	+7°C/0% RH	4 months	19	5.8 (<0.1)	120 (0)	45.6 (<0.1)	39.2 (<0.1)
F	**	+33°C/10% RH	4 months	16	3.1 (<0.1)	115 (1)	46.2 (0.5)	39.3 (<0.1)
G	20 min milling	+7°C/0% RH	4 months	12	5.3 (0.1)	113 (5)	45.1 (0.2)	38.9 (<0.1)
H	**	+20°C/0% RH	4 months	0	<0.1 (<0.1)	90 (1)	49.9 (0.6)	—

* Onset temperature of an endothermic reaction approximately at 39°C if existed.
** Was not proceeded.

hydrate were stored under the following conditions: in an airtight plastic container without any drying material at + 7°C (Table I, sample A) or in a desiccator containing a saturated $PbNO_3$ solution at room temperature (+ 20°C, 97% relative humidity) (Table I, sample B) for six months; or in an airtight glass container over silica (United Desiccants-Gates, United Catalysts Inc. Group, NJ, USA) at + 7°C (0% RH) (Table I, sample C), or in an incubator (+ 33°C, 10% RH) (Table I, sample F), or in a vacuum desiccator over silica at room temperature (+ 20°C, 0% RH) (Table I, sample H) were stored for four months. All milled samples were stored for 30 days at + 7°C in small airtight glass containers over silica (0% RH). As a reference, an untreated powder sample of the monohydrate was stored under identical conditions. Tablets were stored for 36 days at + 7°C (0% RH) after compression.

Physical Characterization of Cyclophosphamide

The physical structure of the cyclophosphamide sam-

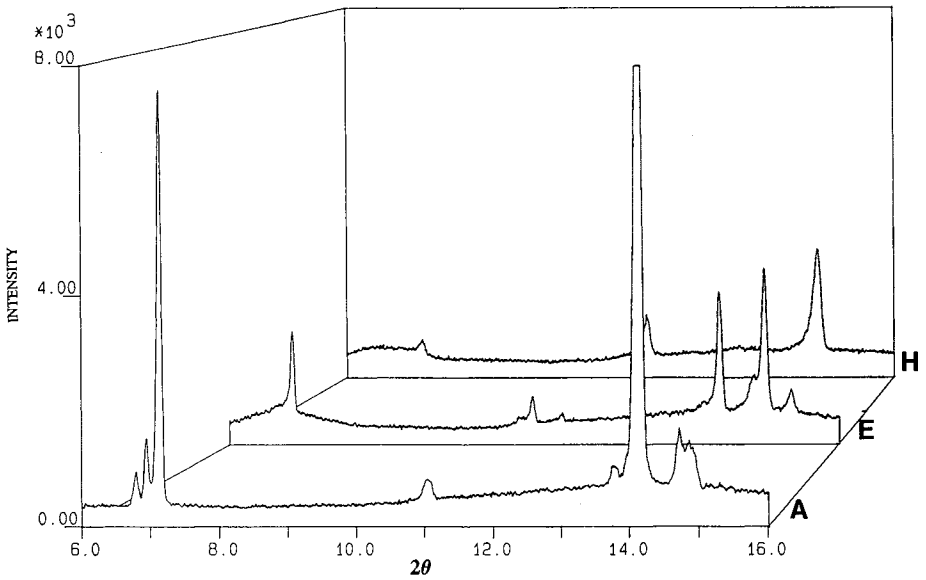

Figure 1. X-ray powder diffractograms of cyclophosphamide monohydrate (A) (characteristic reflection for monohydrate at 13.6-14.2° (2θ)) and anhydrous cyclophosphamide (H). Diffractogram E is a typical diffractogram for possible metastable cyclophosphamide sample (an extra reflection at 15.3° (2θ)). Sample codes are explained in Table I.

ples was studied with differential scanning calorimetry (DSC) and X-ray powder diffractometry. The extrapolated onset temperatures and enthalpies of transitions for each thermal event were determined by differential scanning calorimeter (Perkin-Elmer DSC7, Perkin-Elmer Co., CT, USA) for every cyclophosphamide batch and expressed as the mean of four measurements. All DSC runs were performed under an atmosphere of dry nitrogen (flow 23 ml/min) using the heating rate of 5 °C/min. Powder samples of 3 - 8 mg in weight were crimped in 50 µl aluminum pans. For the DSC analysis, compressed tablets were ground gently to a powder. An empty pan, sealed in the same way as the sample was always used as a reference. The temperature axis was calibrated with indium and gallium, having melting points of 156.60 and 29.78°C, respectively.

X-ray powder diffraction measurements for samples A-H (Table I) were made by a Phillips PW1820 diffractometer (Phillips, The Netherlands) with step scan (step size 0.020°, sample time 1 s) and proportional detection. The diffractograms were recorded under the following conditions: Ni filtered CuK_α radiation (λ = 0.15418 nm), voltage 50 kV, current 40 mA, automatic divergence slit (irradiated sample length 12.5 mm), receiving slit 0.1 mm, and scatter slit 4°. The relative proportions of the monohydrate and anhydrous forms were evaluated by direct intensity measurement (step size 0.015°, sample time 2 s) of the monohydrate's characteristic reflection at 13.6 - 14.2° (2θ), and the degree of transition was determined by comparing the background corrected intensities with the corresponding intensity of a reference sample which contained only the monohydrate of cyclophosphamide. Data were collected and analyzed with the Phillips ADP1700 program.

Water content of the cyclophosphamide samples were determined by a Mettler DL35 Karl Fischer titrimeter (Mettler-Toledo AG, Switzerland) and expressed as the mean of three measurements.

Molecular Modeling

The crystal structures of anhydrous and monohydrous cyclophosphamide were obtained from the Cambridge Structural Database (7-9). The crystal structure of the monohydrate was expanded by SYBYL molecular modeling software (10). Quantum chemical calculations were made using MOPAC 5.0 (QCPE, IN, USA) with PM3 parametrization (11). Optimization was considered complete after the energy gradient was lower than 10^{-9} kcal/mol (4.19×10^{-6} J/mol) (GNORM keyword). Results from these calculations were used to calculate the theoretical value for the enthalpy of monohydrate-anhydrate transition ($\Delta H_{dehydr.}$) by using the following equation:

$$\Delta H_{dehydr.} = \Delta H_{hf\ (mohohydr.)} - (\Delta H_{hf\ (anhydr.)} + \Delta H_{hf\ (water)}) \quad (1)$$

where ΔH_{hf} is the heat of formation.

RESULTS

The results of X-ray powder diffractometric and DSC analyses of samples A-H are summarized in Table I. The characteristic X-ray reflections of monohydrate and anhydrous cyclophosphamide are presented in Figure 1 (diffractograms of samples A and H, respectively). The DSC thermograms of samples A-H are presented in Figure 2.

When stored under high relative humidity (97%), even after a relatively long period, the physical structure of the monohydrate did not change (Table I, samples A and B). This is in good agreement with the results of an earlier study (1). When the monohydrate was stored under dry conditions, either at + 33°C (10% RH) or especially under vacuum desiccation (+ 20°C, 0% RH), loss of water occurred (Table II). Within four months, the original monohydrate sample could be considered as the anhydrous form of cyclophosphamide when stored at room temperature under dry conditions (0% RH) (Table I, sample H). Anhydrous cyclophosphamide had

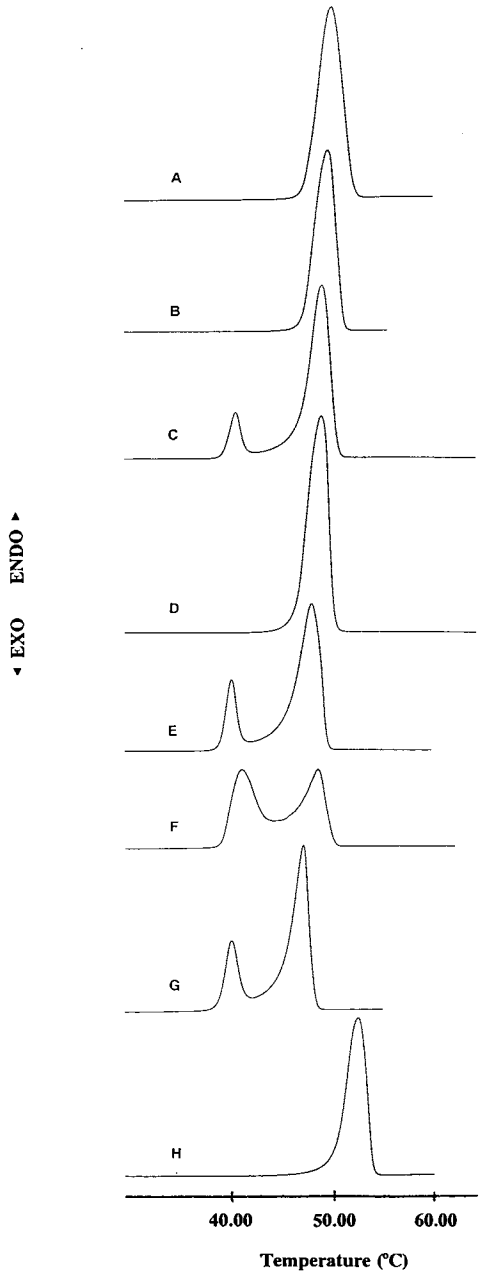

Figure 2. DSC thermograms of samples A - H described in Table I. A is the thermogram of the monohydrate form and H is the thermogram of the anhydrous cyclophosphamide.

Table II. Effect of Drying Condition and Storage Time (d) on Enthalpy of Transition (ΔH) (J/g), Onset Temperature (°C) (Both Based on DSC Measurements) and Water Content (%) Values of Cyclophosphamide Monohydrate. Standard Deviations in Parenthesis

	+33°C/10% RH			+20°C/0% RH		
Storage time (d)	ΔH (J/g)	Onset temp. (°C)	Water content (%)	ΔH (J/g)	Onset temp. (°C)	Water content (%)
0	126 (3)	47.7 (0.2)	6.5 (<0.1)	126 (3)	47.7 (0.2)	6.5 (<0.1)
5	125 (1)	47.1 (<0.1)	6.5 (<0.1)	102 (6)	40.4 (0.1)	3.2 (0.1)
9	126 (1)	47.2 (0.1)	—*	99 (1)	40.6 (0.1)	—*
15	126 (1)	47.4 (0.1)	6.4 (0.1)	96 (1)	40.9 (0.1)	1.6 (<0.1)
30	110 (<1)	39.8 (0.1)	3.6 (0.1)	92 (1)	42.2 (2.8)	0.6 (<0.1)

* — Was not determined.

a higher melting point (onset temperature = 49.9°C) and a lower enthalpy of fusion (ΔH = 89.5 J/g) than the monohydrate (Table I). Differences in characteristic reflections between these two forms were observed in X-ray diffractograms (Fig. 1, samples A and H), e.g.; when comparing the intensities of the reflections at approximately 7.0, 11.0, and 14.0° (2θ).

According to the DSC thermograms, a transition from monohydrate to anhydrate occurred during drying. Before dehydration was fully complete there existed hydrated forms with lower melting points than those of the monohydrate or anhydrate (Fig. 2, Tables I and II). Some of these samples had a weak endothermic reaction at approximately 39°C. Also, some reflections were observed in the X-ray powder diffraction analysis from these samples (e.g.; at approximately 15.3°) which were characteristic of neither the monohydrate nor the anhydrous forms of cyclophosphamide (Fig. 1, sample E). Such low temperature forms could even be induced by an effective mechanical treatment, e.g.; by milling or compression (Fig. 3, Table III). This implies that the interaction forces which are holding water in the crystal lattice, are rather weak and easy to break, and that the coordinated water may become less coordinated, thus creating possibilities for the formation of metastable forms. This suggestion is supported by the fact that the total water content of the milled monohydrate did not change even after a relatively effective mechanical treatment. However, according to the X-ray analysis, the proportion of monohydrate in the sample decreased significantly (Table I, sample D). The metastable form became observable after the milled sample was stored under dry conditions (Table I, sample G). Storage of the monohydrate in an airtight glass container (+7°C, 0% RH) decreased the total enthalpies of transition, and with relatively gentle mechanical treatment, this decrease was enhanced during storage (Fig. 3A). Furthermore, the enthalpy of the new endotherm at 39°C was more pronounced with milled samples (Fig. 3B).

According to the DSC analysis, milling did not have any

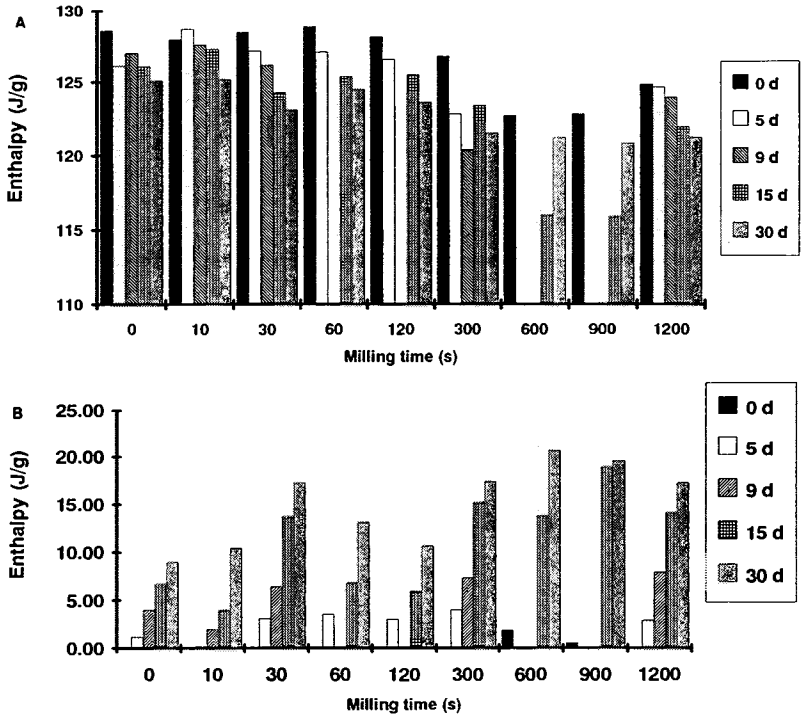

Figure 3. Effect of milling time (s) and storage time (d) on total enthalpy (J/g) of endothermic reactions (A) and on enthalpy of a weak endothermic reaction (extrapolated onset temperature at approximately 39 °C) (B) based on DSC measurements. All samples were stored in an airtight glass container at + 7 °C (0 % RH). 5d and 9d samples milled for 600 and 900 seconds were not measured.

Table III. Effect of Compression Force (kN), Compression Time (min) and Storage Time (d) on Enthalpy of Transition ΔH (J/g) Values (Based on DSC Measurements) of Cyclophosphamide in Tablets. Standard Deviations in Parenthesis

	Monohydrate form				Anhydrous form			
Compression force (kN)	50	120	50	120	50	120	50	120
Compression time (min)	3	3	10	10	3	3	10	10
Storage time 0 d								
ΔH (J/g)	124*	120*	125	121	89	90	89	89
	(1)	(3)	(1)	(2)	(1)	(1)	(<1)	(2)
Storage time 36 d								
ΔH (J/g)	117*	119*	115*	120*	90	89	91	91
	(1)	(2)	(<1)	(<1)	(<1)	(3)	(1)	(<1)

* Weak endothermic peak approximately at 39°C (onset temperature).

effect on the structure of anhydrous cyclophosphamide, the melting point and enthalpy of fusion being 49.5°C and 89.4 J/g, respectively.

When the DSC thermograms of the tablets made from the monohydrate were evaluated, it was noticed that a compression time of three minutes, regardless of the compression force, produced a weak endothermic reaction at approximately 39°C (Table III). This weak endothermic reaction always had an enthalpy of transition less than 2.5 J/g and became more evident after a storage time of 36 days for all monohydrate tablets, including those compressed for 10 minutes. This effect was caused apparently by dehydration, and was also observed as a decrease in the total enthalpy of transition. When the anhydrous form was compressed, no changes in enthalpy were observed.

DISCUSSION

Both DSC and X-ray diffractometric analyses indicated that the transition of cyclophosphamide monohydrate to the anhydrate, under dry conditions, proceeds through a metastable form. The input of mechanical energy or mechanical treatment, e.g.; milling or compression, seems to enhance this transition. Process factors, like milling time and compression force, as well as storage conditions, affect the rate of phase transition extensively.

Because the structure of the observed metastable form of cyclophosphamide had not been investigated, molecular modeling was used to characterize the intermolecular interactions and putative metastable structures. Figure 4 shows a model consisting of four cyclophosphamide molecules and two water molecules, both water molecules being hydrogen bound to two cyclophosphamide molecules. In this model, the molecular orientation makes the existence of 'water tunnels' possible, as posited in a theory presented by Shiozawa (2). The dehydration enthalpy of the system was 51.6 J/g (Fig. 4), as calculated with MOPAC. This is rather close to the value obtained by DSC analysis (36.1 J/g), considering that all these calculated enthalpy values are theoretical and do not give any information as to how high an activation energy is needed to start the dehydration process. Normally the strength of a hydrogen bond is about 8 - 62 kJ/mol (12), and with cyclophosphamide monohydrate this energy should be between 28 - 222 J/g for a single hydrogen bond. It is likely that hydrogen bonding is the main factor which stabilizes the monohydrate structure of cyclophosphamide, since the dehydration enthalpy is remarkably close to the energy of a weak hydrogen bond. Thus, if the sterical block can be opened, then water molecules will begin to escape from the monohydrate matrix (Fig. 4). If the opening does not disrupt the entire crystal lattice, as in the temporary thermal movement of atoms caused by mechanical treatments or drying, then water molecules can leave the system slowly one by one, and metastable forms of cyclophosphamide are formed.

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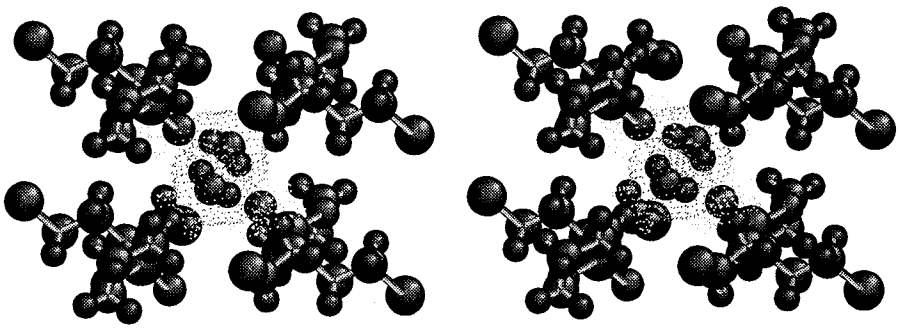

Figure 4. Stereo image of a crystal packing consisting of four cyclophosphamide molecules and two water molecules. The van der Waals radii of water molecules and connecting hydrogen bound atoms are shown as dot surfaces.

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