SIMULTANEOUS MEASUREMENTS OF X-RAY DIFFRACTION (XRD) AND DIFFERENTIAL SCANNING CALORIMETRY (DSC) DATA UNDER CONTROLLED HUMIDITY CONDITION: INSTRUMENTATION AND APPLICATION TO STUDIES ON HYDRATION, DEHYDRATION, AND RE-HYDRATION PROCESSES OF PHARMACEUTICAL COMPOUNDS

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ABSTRACT

An instrument for simultaneous measurement of X-ray powder diffraction (XRD) data and differential scanning calorimetry (DSC) data under controlled humidity condition has been developed in order to achieve a design concept of reliable and simple in operation. Examples of application to studies on hydration, dehydration and re-hydration processes of pharmaceutical compounds under various humidity conditions are presented. Usefulness of the present XRD-DSC system will be demonstrated.

INTRODUCTION

Hydration and dehydration reaction processes of many organic and inorganic compounds accompany phase changes, and X-ray diffraction (XRD) is a very powerful technique for studying the reaction processes. Thermal analysis is another important technique often used to obtain information needed for understanding the reaction processes. Commercially available instruments for XRD and thermal analyses had been developed separately, and an XRD instrument is commonly used only for XRD analysis and a TG-DTA (thermogravimetry- differential thermal analysis) instrument for thermal analysis. A comparison between XRD data and thermal analysis data will provide a deep understanding of the hydration and dehydration reaction processes. However, these reaction processes are generally kinematical, depending on their thermal history and environmental factor such as humidity, as will be shown later in the results and discussion section [1,2]. Therefore, it is of fundamental importance to measure XRD data and thermal analysis data at the same time and under the same environmental conditions, in particular, when the humidity is involved as one of the environmental factors in the reaction processes.

Fawcett et al. reported a construction of an instrument for simultaneous measurements of XRD data and differential scanning calorimetry (DSC) data under the same temperature and atmospheric conditions. The instrument was equipped with a position sensitive proportional counter (PSPC) for rapid scanning of whole patterns during elevation or falling of the temperature, and it was used for studying performances of polymers and catalysis [1,3]. Other instruments, developed for XRD-DSC studies, were equipped with a rotating anode generator and a scintillation counter [4] or a combined system of synchrotron radiation with PSPC [5].

Recently, we developed a new XRD-DSC instrument, as a commercially available laboratory system, by assembling a DSC unit on a standard XRD goniometer in order to achieve a design concept of reliable and simple in operation [6,7]. A humidity control unit could also be equipped with the present system, and it can be used for studying hydration and dehydration processes of organic and inorganic compounds.
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materials such as pharmaceutical compounds, some of which are unstable under high humidity conditions. In this paper, special features of the instrument are described, and examples on studying hydration, dehydration and re-hydration processes of trehalose dihydrate under various humidity conditions are presented.

INSTRUMENTATION

XRD system

Figure 1 shows the Rigaku/XRD-DSC system, consisting of (A) a sealed X-ray tube, (B) a goniometer system, (C) a scintillation counter for XRD measurement, (D) a heat-flux DSC unit at the sample position, (E) a DSC control circuit and (F) a humidity control unit. In order to keep the specimen surface horizontal during a DSC measurement, one of the Rigaku θ-θ goniometer systems (ex., ULTIMA, ULTIMA III and TTRAX) is used. The goniometer system can use either the Bragg-Brentano focusing or a parallel-beam geometry. The parallel-beam optics, consisting of a parabolic multiplayer mirror for collimating the incident beam and a parallel-slit analyzer on the diffracted beam side, is free from specimen-displacement error, and therefore, it has an advantage over the para-focusing system [8]. This advantage of the parallel-beam optics is of great importance when peak positions are significantly displaced by the thermal expansion of the specimen material at high temperatures.

A rotating-anode (18kW) can be attached, instead of a sealed-tube (3kW), onto the θ-arm on the incident-beam side, when the goniometer system TTRAX is chosen. A measurable 2θ-ranges is from 2 to 55° when the DSC unit is mounted, and typical scan rates are 0.5° to 5°/min and 10° to 40°/min for slow and fast scanning, respectively. The diffractometer system can be used also as a conventional XRD system if the DSC unit is replaced with a normal specimen attachment.
**DSC unit**

A schematic diagram of a DSC unit is shown in Figure 2. Two aluminum specimen containers with dimensions of $7 \times 7 \times 0.25\text{mm}^3$ are set side by side: one is used for the XRD measurement and the other one is used as a reference material for the DSC measurement. These containers are surrounded with cylindrical-shaped furnace made of silver. Two windows are opened for incident and diffracted X-rays, and they are covered with thin aluminum foils to prevent heat convection and heat loss. The furnace is placed inside a ceramic thermal insulator with two openings, and it is further surrounded with an aluminum container (Figure 1). Two window of the outermost container are also covered with aluminum thin foils.

The specimens, kept stationary during the measurement, can be heated from room temperature (RT) to 350°C at a heating/cooling rate of 0.5 to 10°C. Any combinations of heating/holding/cooling stages can easily be programmed on a PC, and they can be cycled. The specimens can also be cooled from RT to -40°C if a low temperature attachment is used. In this case, the DSC unit can be cooled by heat conduction of cold nitrogen gas, vaporized from liquid nitrogen.

![Figure 2. Schematic diagram of DSC unit.](image)

**Humidity control unit**

In the humidity control unit, nitrogen gas, coming into the unit, is divided into two branches, and two mass-flow controllers control their flow rates. The nitrogen gas in one of the branches is introduced into a water bath to produce water vapor. The two nitrogen gases, one is dry and the other one of high humid, are joined together, and then they are introduced into the DSC unit. Signals of temperature and humidity, which are detected with sensors located near the specimen, are feed-backed to control mass-flow controllers, keeping preset values of the temperature and humidity. The humidity generator can provide wet gas in the humidity range from 5% relative humidity (RH) at 25°C to 90% RH at 60°C (from 0.16% to 17.7% H$_2$O-N$_2$). The humidity control unit is available as an optional component.

**Functions for data output**
As will be shown later, multiple XRD patterns can be plotted as a function of temperature. Integrated intensities of some selected reflections in the XRD pattern can also be plotted as the function of temperature. They can be replaced with peak height intensities or full-width at half-maximum (FWHM).

EXPERIMENTAL

Specimen

Powder of commercially available trehalose dihydrate (Sigma, Lot No.111K377) was used. The powder specimens, each of which has a weight of approximately 10mg, were mounted on aluminum specimen containers described earlier, and their surfaces were gently made flat. The specimen used in the present experiment was identified as virtually pure trehalose dihydrate by preliminary XRD measurement.

XRD-DSC measurements

The XRD-DSC system, used in this study, consists of a goniometer of Rigaku/ULTIMA, a DSC unit based on Rigaku/ThermoPlus DSC8230 and a humidity control unit of Rigaku/Hum-1A.

XRD experimental conditions were: CuKα radiation generated at 40kV and 50mA, a fixed-angle divergence slit of 0.5°, an anti-scatter slit of 0.5°, a receiving slit of 0.3mm, a graphite monochromator, and a scintillation counter. Profile intensities in the 2θ-range of 5-40° were step-scanned at scanning speed of 20-40°/min.

In order to supplement the experiment, the TG analysis was conducted with Rigaku differential thermobalance Model ThermoPlus 8210.

RESULTS AND DISCUSSION

Dehydration of trehalose dihydrate under dry nitrogen gas flow

Dehydration process of trehalose dihydrate was first observed under dry nitrogen gas flow. Heating rate was 3°C/min and XRD scan speed was 20°/min, and thus the temperature was raised by about 6°C during the observation of one XRD pattern. In Figure 3, powder diffraction patterns observed during the dehydration process are plotted as a function of temperature. Figure 4 shows variations of integrated intensities of selected X-ray reflections in Figure 3 and a variation of DSC curve, plotted on the same temperature axis as that used in Figure 3. A clear correlation between XRD and DSC data can easily been seen in Figure 4.

Structural changes of trehalose dihydrate with increasing temperature can be divided into five stages. No appreciable change was first observed in trehalose dihydrate up to the temperature of 63°C (stage I). As the temperature was further increased, an endothermic peak was observed on the DSC curve, and the intensities of XRD peaks of trehalose dihydrate gradually decreased. Along with these variations, a new intermediate phase appeared together with a small amount of amorphous phase as represented as the presence of halo in the diffraction patterns, and intensities of the new phase were increased. At the temperature of ~89°C, which is just after the observation of the endothermic peak
maximum on the DSC curve, the XRD peaks of dihydrate disappeared, whereas the intensities of the new intermediate phase reaches almost the maximum. From supplemental observation of TG curve, this event was identified as dehydration of trehalose dihydrate and formation of anhydrate (stage II). After a small endothermic DSC peak at 120-133°C, this intermediate anhydrate phase changes into an amorphous phase as revealed by the XRD pattern (stage III): Around an exothermic DSC peak region of 175-200°C this amorphous phase were further changed into a crystalline anhydrate as shown by the XRD patterns (stage IV). Finally, it melts at 205-218°C, indicated by the endothermic DSC peak and loss of crystalline peaks in the XRD pattern (stage V).
The total time for XRD + DSC measurement was about an hour.

Dehydration of trehalose dihydrate under highly humid nitrogen gas flow

Dehydration behavior of trehalose dihydrate has dramatically been changed when the environmental atmosphere was switched from dry nitrogen gas to highly humid nitrogen gas. In this experiment, the nitrogen gas had a partial water vapor pressure of 12.0kPa (equivalent to 77% RH at 55°C), and its temperature was raised at a heating rate of 2°C/min. XRD patterns were recorded at a scan speed of 40°/min, corresponding to the elevation of temperature by 2°C during the observation of one XRD pattern.

Figures 5 and 6 show XRD and DSC patterns, presented as those shown in Figures 3 and 4. As demonstrated in these diagrams, dehydration began at 80°C, and it was followed by the formation of well-crystallized anhydrate phase at about 95°C (stage II). In contrast to the dehydration process in the case of dry nitrogen gas flow, the well-crystallized anhydrate trehalose, which is identical to that appeared above 170°C in Figure 3, was formed even at the temperature as low as 100°C. Two small endothermic peaks appeared on the DSC curve at 115-128°C, but no significant change was observed in XRD patterns. A broad DSC profile in the temperature range below melting point suggested a possible progress of decomposition of anhydrate phase as observed as the decrease in XRD intensities. Variations of the DSC curve

![Figure 5](image_url)  
Fig. 5. Powder diffraction patterns observed during the dehydration process of trehalose dihydrate plotted as a function of temperature. Numbers represent I) dihydrate, II) anhydrate and III) liquid.
and the XRD intensities in Figure 6 show that well crystallized anhydrate phase was formed in the process of dehydration and decomposed near the melting temperature.

It may be very interesting to note that the dehydration of trehalose dihydrate in highly humid atmosphere derived well-crystallized anhydrate phases at lower temperatures than those in dry or low humid atmosphere.

**Dehydration and re-hydration of trehalose dihydrate**

In this experiment, trehalose dihydrate was first heated up to 101°C, and then cooled down near to RT under the dry nitrogen gas flow. In the succeeding stage, the specimen was kept at 36°C and low humid nitrogen gas, having initially a partial water vapor pressure of 1.7kPa (29% RH at 36°C), was introduced. After 180 min from start of the experiment, the humidity was slightly increased to 2.2kPa.

As shown in Figure 7, XRD results showed that trehalose dihydrate was first dehydrated under the dry nitrogen gas flow at the elevated temperature. Anhydrate phase of trehalose (stage II), thus produced, was, however, re-hydrated followed the introduction of low humid nitrogen gas (stage III). On the DSC curve in Figure 8, two exothermic peaks were observed, and they appeared when 1) the low humid nitrogen gas was first introduced and 2) the humidity was then elevated from 1.7 to 2.2kPa. Correspondingly to these exothermic peaks, XRD intensities of dihydrate phase were rapidly increased, indicating that the increase of humidity accelerates the formation of crystalline dihydrate.
Figure 7. Powder diffraction patterns observed during the dehydration process of trehalose dehydrate plotted as a function of temperature. Numbers represent I) dihydrate, II) anhydrate, III) dihydrate (initial state) and III') dihydrate (final state).

Figure 8. Variations of integrated intensities of some selected reflections in Figure 7 and DSC curve plotted as a function of temperature.

SUMMARY

An instrument for simultaneous measurement of XRD and DSC data under controlled humidity condition has been developed as a commercially available system. It was designed in order to achieve both reliable and simple in operation. Experimental results of dehydration and re-hydration process of trehalose dihydrate are presented as examples of application of the present XRD-DSC system. Phase changes of the trehalose dihydrate are kinematical, greatly depending on the environmental conditions of temperature and humidity, and usefulness of the XRD-DSC system has been
demonstrated by the present experiments. Some characteristic features of the present XRD-DSC system are summarized as follows:

1) XRD and thermal data can simultaneously be measured under the same environmental conditions of temperature and humidity,
2) XRD and DSC data can complement each other for understanding reaction processes,
3) reaction processes can be observed under controlled humidity conditions, and it is especially very useful for the studies on hydration and dehydration processes,
4) specimens in very small amount of < 10mg can be measured within 1 or 2 hours during one run under temperature and humidity variations.

REFERENCES