Water Vapor Adsorption Behavior of Sodium Deoxycholate Anhydrous Forms

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Pseudopolymorphism of sodium deoxycholate (NaDC) was investigated. Intact NaDC (dihydrate) was dried at 60 °C under reduced pressure resulting an anhydrous amorphous phase. On the other hand, intact NaDC was altered to an anhydrous crystalline form by heating at 200 °C. The water vapor adsorption and desorption isotherms of dehydrated NaDCs were determined using an automatic gravimetric water vapor adsorption analyzer. In the case of NaDC dehydrated at 60 °C, the weight was increased in rising relative humidity and it was transformed into the NaDC tetrahydrate above 60% RH, which was identified by TG/DTA and powder X-ray diffraction. During the water vapor adsorption process of the sample dehydrated at 200 °C, the NaDC dihydrate was obtained in the range of 50 to 70% RH and then transformed into the NaDC octahydrate above 85% RH. The NaDC octahydrate was characterized by TG/DTA and powder X-ray diffraction for the first time. During the desorption process, the octahydrate was changed to the tetrahydrate between 80 and 40% RH.

Key words pseudopolymorphism; sodium deoxycholate; hydrate; water vapor adsorption; thermal analysis; powder X-ray diffraction

During pharmaceutical development processes, various investigations of physicochemical properties of a drug substance are conducted in order to guarantee the efficacy and the stability of pharmaceutical products. It is well known that the physicochemical properties of drug substances are changed by influence of water vapor under high and low relative humidity conditions.[2—4)]

A compound holding stoichiometric solvent molecule incorporated within the crystal lattice is designated a solvate. In particular, when the solvent is water, the compound is termed a hydrate, which is the most common solvate of drug substances.[5)] Because the investigation of manufacturing and formulation processes are conducted with particular attention to the behavior of drug substance hydrate, the existence of the hydrate state has a major impact on the drug development process.[6)] Moreover, the reduction in bioavailability may occur as the solubility of hydrate in general is lower than that of anhydrous form.[7,8)] There are many studies in which the behavior of the crystal water molecule is influenced by relative humidity.[9,10)] A compound tends to incorporate a water molecule in the crystal structure at high relative humidities, while a hydrate may release the water molecule from the crystal at low relative humidities. It is therefore very important that the effect of relative humidity on the interconversion between the hydrate and anhydrous forms are investigated.

Studies of the process of hydration and dehydration are of significance in order to predict the stability of the drug substance. Many hydrated compounds are stabilized by formation of hydrogen bonding networks between water and the drug substance molecule. A hydrated crystal is easily broken by heating and also upon storage at low relative humidities, leading to possible transformation into the anhydrous form by crystal lattice rearrangement. As a result, the drug substance may form the metastable state.

Many hydrates of drug substances have been reported, for instance, carbamazepine,[11)] theophylline,[12)] tetracaine hydrochloride,[13)] levofloxacin,[14)] and diclofenac acid salts.[15)] Among them, the hydrated form exists in several possible hydration states. Tetracaine hydrochloride has hemi-, mono-, and tetrahydrate states, nafagrel hydrochloride has hemi- and monohydrate states, and norfloxacin has sesqui- and pentahydrate states.[16)]

Sodium deoxycholate (NaDC) containing steroid skeleton is a kind of cholic acid salts. It is known that NaDC forms a micelle,[17)] gel,[18)] and complex[19)] with drugs. It is a white powder used as a supportive solubilizing agent in pharmaceutical formulation. D'Archivio *et al.* suggested the possibility that NaDC was stabilized by the formation of a tetrahydrate helix structure of NaDC with the four water molecules held rigid by the formation of ion–ion, ion–dipole interactions and hydrogen bonding.[20)] Vold *et al.* presumed the existence of NaDC octahydrate by the investigation of the solubility curve of NaDC.[21)] However, their findings could not sufficiently obtain the concrete evidence to support the NaDC octahydrate.

Hydrate formation can be investigated using powder X-ray diffraction, thermal analysis, infrared spectroscopy, near-infrared spectroscopy, Raman spectroscopy, and solid-state nuclear magnetic resonance. Recently, an automatic gravimetric water vapor adsorption analyzer was developed and prevailed. This equipment plays an important role in the investigation of the water vapor adsorption and desorption behavior of drug substance instead of the saturated salt method.[22)]

In this study, the characterization of the hydrates of NaDC prepared by various treatment conditions were investigated by powder X-ray diffraction, TG/DTA, and automatic gravimetric water vapor adsorption analysis.

Experimental

Material Sodium deoxycholate (NaDC, lot MON8679, Nacalai Tesque, Japan) of reagent grade was used without further purification.

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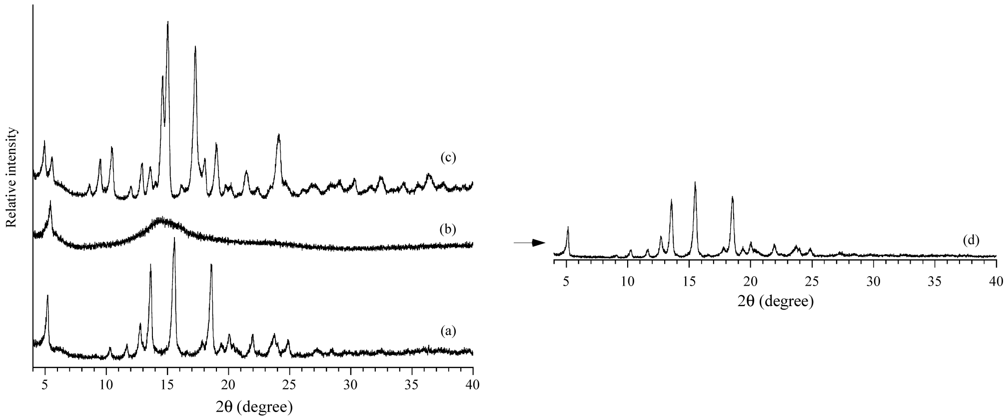

Fig. 1. Comparison of Powder X-ray Diffraction Patterns of Intact NaDC and NaDC Obtained by Various Treatments

(a) Intact NaDC, (b) dehydrated at 60 °C, (c) dehydrated at 200 °C, (d) after storage of sample (b) at 83% RH and 25 °C.

Preparation of Anhydrous Forms Dehydrated NaDC(I) was obtained by drying intact NaDC at 60 °C under reduced pressure with silica gel for 2 h using a vacuum sample drying apparatus, VSD-95 (Ishii, Japan). Dehydrated NaDC(II) was prepared by storing intact NaDC at 200 °C under atmospheric pressure with silica gel for 2 h.

Thermogravimetric Analysis (TG) and Differential Thermal Analysis (DTA) Thermogravimetric and differential thermal analyses (TG/DTA) were performed on the TG/DTA 2000 instrument (Seiko Instruments Inc., Japan). The operating conditions were as follows: sample weight, 5—15 mg; pan, open-aluminum pan; heating rate, 5 °C/min; and nitrogen gas flow rate, 60 ml/min.

Powder X-Ray Diffraction Powder X-ray diffraction patterns of the samples were obtained using an X'Pert-MPD PW 3050 diffractometer (Philips, Netherlands). Powder samples were pressed in the glass holder cavity. The operating conditions were as follows: target, Cu; filter, Ni; voltage, 35 kV; current, 20 mA; scanning speed, 4° 2θ/min; and scanning angle, 4—40°.

Water Vapor Adsorption and Desorption Behavior The water vapor adsorption and desorption data were obtained using an automatic gravimetric water vapor adsorption analyzer, SGA-100 (VTI, U.S.A.). About 20 mg of sample was placed in a glass holder, and the holder suspended in an isothermal chamber held constant at 25.0 °C. The relative humidity (RH) was controlled by mixing dry and wet nitrogen gas and detected using a dew point hygrometer (Edge Tech, U.S.A.). The sample was exposed to a stepwise increase in RH from 5 to 95%. After reaching maximum RH, the hydrated sample was subjected to similar step-wise RH decrements to 5% RH. As a pre-treatment, dry nitrogen gas was flowed into the sample chamber at 60 °C for 2 h. The weight of the sample was measured every 2 min by the microbalance D-200 (Cahn, U.S.A.) and the data recorded using computer controlled software. At each relative humidity, the sample was allowed to equilibrate until the mass was stable within 0.03% over a 10 min period before the relative humidity was changed. Complete water vapor adsorption and desorption isotherms were collected using the equilibrium end points. Alternatively, constant relative humidities were prepared by using saturated-salt aqueous solutions of lithium chloride, potassium bromide, and potassium nitrate. The individual desiccators were maintained at the relative humidities (RHs) of 11, 83, and 93% at 25 °C, respectively.

Results and Discussion

Characterization of Dehydration Behavior The powder X-ray diffraction patterns of intact NaDC, dehydrated NaDC(I), and dehydrated NaDC(II) are shown in Fig. 1. The pattern of intact NaDC (Fig. 1a) showed many X-ray diffraction peaks showing the crystalline property, while the diffraction pattern of dehydrated NaDC(I) (Fig. 1b) was almost halo suggesting the crystallographic change from the crystalline to amorphous state by heating at 60 °C. Moreover, the pattern of dehydrated NaDC(II) (Fig. 1c) showed high crystallinity which was different from that of intact NaDC and that of dehydrated NaDC(I).

The TG/DTA curves of intact, dehydrated NaDC(I), and dehydrated NaDC(II) are shown in Figs. 2 and 3. The intact NaDC showed two broad endothermic peaks on the DTA curve and a weight loss of 8.0% on the TG curve in the temperature range of 50—120 °C. The weight fraction of water calculated for the dihydrate of NaDC was 8.00% and this value was in good agreement with the value obtained from the TG analysis for the intact NaDC.

The DTA trace for dehydrated NaDC(I) showed a weak broad endotherm at around 50 °C and the TG curve yielding a weight loss of 2.6% from 40 to 120 °C. This weight loss corresponded to 0.6 molecules of water per molecule of NaDC. As the halo pattern was observed in the powder X-ray diffraction pattern, the observed water content was considered to be due to surfaced adsorbed water. The dehydrated NaDC(II), on the other hand, showed a small endotherm on the DTA and negligible weight loss on the TG curves from 25 to 100 °C. Above results suggest that the dehydrated NaDC(II) was anhydrous crystal of NaDC.

Both DTA curves of intact and dehydrated NaDC(I) showed an exothermic peak at 190 °C. In contrast, no exothermic peak was observed in the DTA curve of dehydrated NaDC(II). From the comparison of powder X-ray diffraction patterns of dehydrated NaDC(I) and dehydrated NaDC(II), it is suggested that the exothermic peak at 190 °C shown on the DTA curve was due to crystallization of the amorphous anhydrous state to the anhydrous crystalline state. For intact NaDC (dihydrate), the amorphous anhydrous form was produced during the dehydration process, then the resulted amorphous should crystallize at 190 °C. This was confirmed from the agreement of powder X-ray diffraction patterns of dehydrated NaDC(II) and 200 °C heat-treated sample of dehydrated NaDC(I) (data not shown).

Water Vapor Adsorption and Desorption Behavior of Intact NaDC The water vapor adsorption and desorption isotherms of intact NaDC in the range of 0 to 95% RH at 25 °C are shown in Fig. 4. After the drying process at 60 °C for 2 h as a pre-treatment, the state of starting material was changed to dehydrated NaDC(I) as described above. The weight of dehydrated NaDC(I) increased with increasing relative humidity until 60% RH and attained a constant value of 16—17%, suggesting the formation of hydrated compound

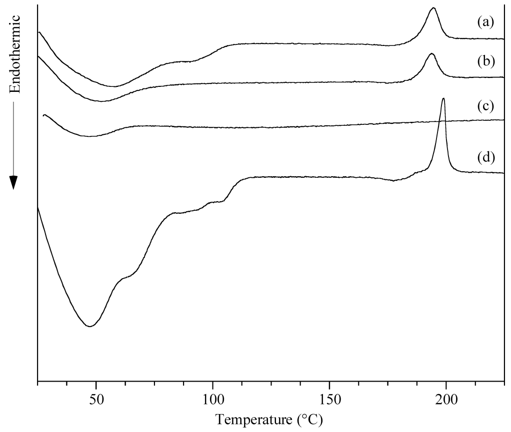

Fig. 2. DTA Curves of Intact NaDC and NaDC Obtained by Various Treatments

(a) Intact NaDC, (b) dehydrated at 60 °C, (c) dehydrated at 200 °C, (d) after storage sample (b) at 83% RH and 25 °C.

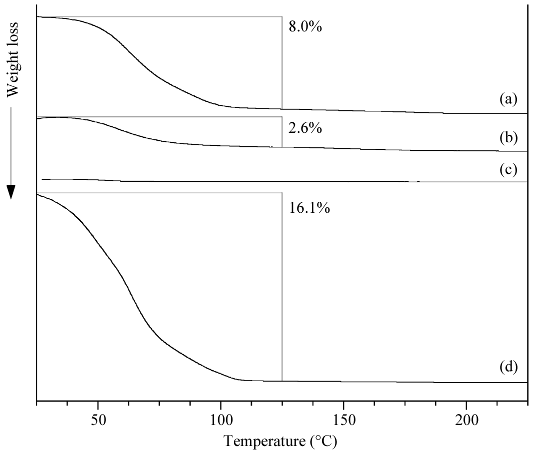

Fig. 3. TG Curves of Intact NaDC and NaDC Obtained by Various Treatments

(a) Intact NaDC, (b) dehydrated at 60 °C, (c) dehydrated at 200 °C, (d) after storage sample (b) at 83% RH and 25 °C.

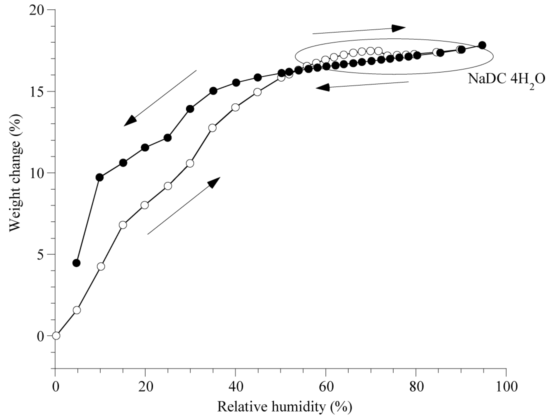

Fig. 4. Water Vapor Adsorption and Desorption Isotherms of Intact NaDC at 25 °C

Key, ○, adsorption; ●, desorption.

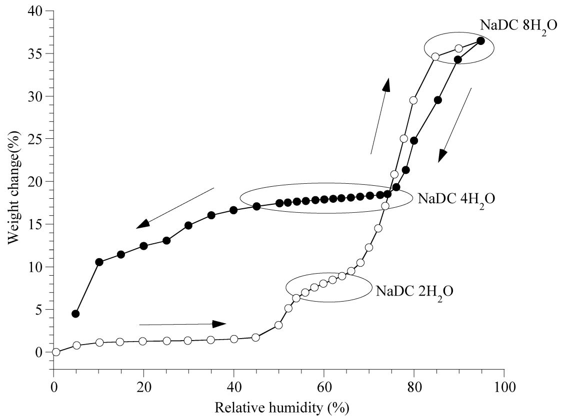

Fig. 5. Water Vapor Adsorption and Desorption Isotherms at 25 °C of Dehydrated NaDC(II) at 200 °C

Key, ○, adsorption; ●, desorption.

between 60 and 95% RH. Dehydrated NaDC(I) was alternatively stored at 83% RH and 25 °C for 24 h, and then the powder X-ray diffraction pattern of the stored sample was obtained as shown in Fig. 1d. The positions of the diffraction peaks obtained for the stored NaDC were obviously different from those of the intact NaDC (dihydrate). The DTA and TG traces of the stored sample are shown in Figs. 2d and 3d, respectively. The weight loss of 16.1% observed on the TG curve corresponded to the theoretical water content of tetrahydrate, 14.81%. From these results, it was concluded that intact NaDC was transformed to the dehydrated NaDC(I) under the drying condition employed, and subsequently transformed to the tetrahydrate by adsorption of water vapor at high RH condition.

Water Vapor Adsorption and Desorption Behavior of Dehydrated NaDC(II) The water vapor adsorption and desorption isotherms of dehydrated NaDC(II) between 0 and 95% RH at 25 °C are shown in Fig. 5. The isotherms were quite different from those of dehydrated NaDC(I). A small weight gain (1st step) was detected in the range of 0 to 50% RH. The stepwise weight change up to 8% (2nd step) was observed between 55 and 70% RH, which was estimated to the result of the pseudopolymorphic transition from the anhydrous to dihydrate form. The weight further increased with increasing relative humidity with almost constant weight being achieved between 85 and 95% RH (where the water content was about 35% (3rd step), suggesting the existence of NaDC octahydrate). During the desorption process, a drastic weight loss of the sample was observed with decreasing relative humidity until 80% RH. The weight change was held constant at approximately 18% between 80 and 40% RH, which corresponded to the tetrahydrate. Below 40% RH, the weight again decreased with decreasing relative humidity. A significant decrease was seen around 10% RH on the desorption process in Fig. 5. In order to examine this state, NaDC tetrahydrate was stored at 11% RH and analyzed by powder X-ray diffraction and TG/DTA. These patterns were in accordance with those of the intact NaDC, suggesting the formation of the dihydrate. From the water vapor adsorption and desorption isotherms of dehydrated NaDC(II), it was found that the dehydrated NaDC(II) transformed to three hydration states, dihydrate, tetrahydrate and octahydrate, depending upon the relative humidity and the process of sorption (adsorption or desorption).

Characterization of NaDC Octahydrate In order to investigate the formation of NaDC octahydrate, dehydrated NaDC(II) was stored at 93% RH for 2 h. The powder X-ray

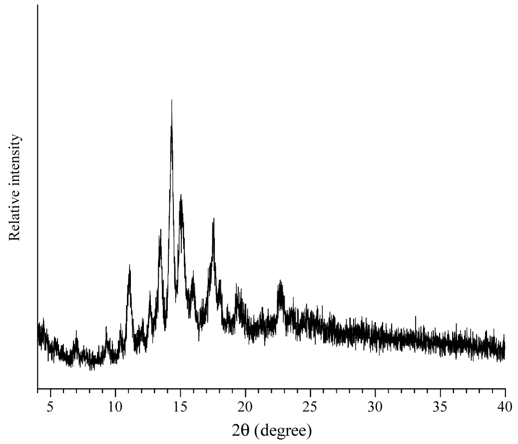

Fig. 6. Powder X-ray Diffraction Pattern of Dehydrated NaDC(II) after the Storage at 93% RH, 25 °C

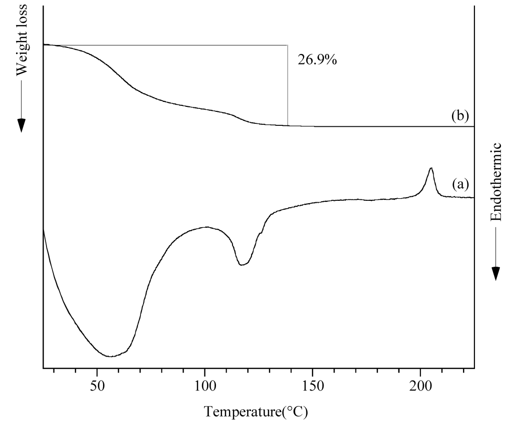

Fig. 7. TG and DTA Curves of Dehydrated NaDC(II) after the Storage at 93% RH, 25 °C

(a) DTA curve, (b) TG curve.

diffraction pattern for the stored sample is shown in Fig. 6. New diffraction peaks were observed which were different from other NaDC crystal forms. Curves (a) and (b) in Fig. 7 are TG and DTA curves of the sample stored at 93% RH. The TG curve showed the weight loss of 26.9% from 25 to 140 °C and the DTA curve showed endothermic peaks at 60 and 120 °C due to dehydration. This weight loss agrees with the theoretical water content value of the octahydrate, which is 25.80%. The formation of NaDC octahydrate was only predicted from the solubility curve by Vold *et al.*[21] It was confirmed for the first time that NaDC octahydrate was formed from dehydrated NaDC(II) by water vapor adsorption.

Conclusions

The hydrate states of NaDC were investigated using automatic gravimetric water vapor adsorption analyzer, powder X-ray diffraction and thermal analysis (TG/DTA). Intact NaDC (dihydrate) converted to the amorphous anhydrous form (dehydrated NaDC(I)) by drying at 60 °C. Dehydrated NaDC(I) transformed to the tetrahydrate by moisture uptake of relative humidities greater than 60%. The anhydrous crystalline form (dehydrated NaDC(II)) was obtained by heating intact NaDC at 200 °C. During the water vapor adsorption process, dehydrated NaDC(II) converted to the dihydrate between 55 and 70% RH, and then to octahydrate above 85% RH. The NaDC octahydrate was confirmed for the first time by powder X-ray diffraction and TG/DTA. The octahydrate transformed to the lesser hydrated state during the dehydration process, at first to the tetrahydrate between 80 and 40% RH and the dihydrate at low RH condition. The phase interconversion of various NaDC hydrates is shown in Chart 1. Various hydration states of NaDC were distinguished through effective use of the automatic gravimetric water vapor adsorption analyzer which was able to measure small amounts of sample.

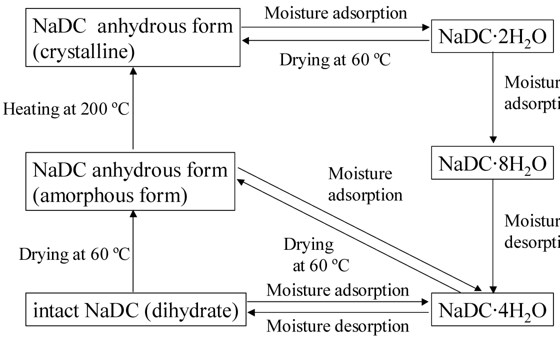

Chart 1. Phase Interconversion of NaDC Hydrates

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