

CHAPTER IV

Solid State Characterization and Interconversion of Norfloxacin Hydrates

4.1 INTRODUCTION

Norfoxacin (NF) is a 4-fluoroquinolone antibacterial agent that has been widely used to treat various infectious diseases such as urinary tract infections, upper respiratory tract infections, bone and joint infections and sexually transmitted diseases. It has a powerful activity against aerobic gram-negative bacteria with less potential to kill gram-positive bacteria (Turel, 2002). Several research publications have indicated that NF has a potential to form various solid state forms especially hydrates. Two forms of anhydrous state, Form A and B, were discovered and recently found to be enantiotropic (Šuštar, Bukovec and Bukovec, 1993; Barbas et al., 2006). Meanwhile, amorphous state was also generated and characterized (Šuštar et al., 1993). Moreover, at least six hydrate forms of NF were reported but not characterized for its complete interconversion i.e., hemihydrate (The Merck Index, 2001), sesquihydrate and dihydrate (Katdare et al., 1986; Florence et al., 2000), trihydrate (Puechagut, Bianchotti and Chiale, 1998), hemipentahydrate and pentahydrate (Mazuel, 1991; Yuasa et al., 1982). Variable-temperature Fourier Transform Infrared Spectroscopy (FT-IR) is used to monitor the transformation between anhydrous and hydrate forms (Hu et al., 2002). The IR spectra of anhydrous form show a shift of the carboxylate anion (unprotonated form) peak to the protonated carboxylic peak after heating. In addition, the NH_3^+ peak is also shifted to NH_2 peak position synchronously. This is due to the zwitterionic property of NF molecules at the carboxylic functional group and the piperazine ring. Thus, the hydrate form may result in a more highly charged molecule and could be more easily hydrated with water than the uncharged anhydrous NF form. This may in turn increase dissolution rate of solid dosage forms. Yuasa et al. (1982) reported that hemipentahydrate NF had a dissolution rate similar to pentahydrate form which was significantly higher than

anhydrous NF. However, these three different hydrate forms did not show any significant difference in bioavailability (Yuasa et al., 1982).

Various hydrate forms often have different physicochemical properties and enormously affect the pharmaceutical manufacturing processes including dosage form performances (Khankari and Grant, 1995; Byrn et al., 1999; Byrn, Xu and Newman, 2001). As mentioned earlier, the path of conversion must be fully understood to stabilize the desired solid structure of pharmaceuticals and to ensure optimal therapeutic efficacy.

In the case of NF, previous studies have shown that anhydrous NF converted to undefined NF hydrate due to exposure to moisture contact during tablet manufacturing and storage (Katdare, 1984; Katdare and Bavitz, 1984). Due to the fact that NF was able to exist as more than one hydrate form, it was very important to investigate the complete paths of conversion among possible hydrates although previously there were evidences on the paths of conversion of NF but not fully evaluated (Yuasa et al., 1982).

The main objective of this second part was to intensively explore and fully gathered information about the preparation of various NF hydrates produced and also the interconversion pathway of anhydrous NF and hydrates produced. Selected NF hydrates will be used as model hydrates in the future study on particle size reduction by thermal dehydration shown in the fifth chapter of the study.

4.2 EXPERIMENTAL

4.2.1 CHEMICALS

- Norfloxacin (anhydrous) Form A (Sigma Aldrich, USA)
- Isopropanol (IPA) (Mallinkrodt Chemicals, USA)
- Acetone (Mallinkrodt Chemicals, USA)
- Dichloromethane (Mallinkrodt Chemicals, USA)
- Ammonium hydroxide (J.T. Baker, USA)
- Hydrogen peroxide, 30% w/v (PanReac, Spain)
- Ortho-phosphoric acid (Univar, Australia)
- Lithium chloride, magnesium chloride, potassium carbonate, sodium bromide, sodium chloride, potassium bromide, potassium chloride, dextrose monohydrate, and potassium nitrate (Unilab, Australia)
- Anhydrous calcium sulfate (Drierite®, USA)
- Double distilled water

4.2.2 INSTRUMENTS

- Differential Scanning Calorimeter (822[°], Mettler Toledo, Switzerland)
- Thermogravimetric Analyzer (TGA/SDTA851[°], Mettler Toledo)
- Hot Stage (FP90, Mettler Toledo, Switzerland) equipped with optical microscope (Eclipse E2000, Nikon, Japan)
- Karl Fischer (720 KFS Titrino and 703 Ti Stand, Metrohm, Switzerland) with heating oven (KF 707, Metrohm, Switzerland)
- High Performance Liquid Chromatography (LC 10-ADvP, Shimadzu, Japan)
- X-ray Powder Diffractometer (D5000, Siemens, Germany)
- Scanning Electron Microscope (JSM-5410 LV, Jeol, Japan)
- Diffused ATR-Fourier Transformed Infrared Spectrophotometer (Spectrum One®, Perkin Elmer, USA)
- Symmetrical Gravimetric Analyzer (SGA-100, VTI Corporation, Hialeah FL., USA).

4.2.3 METHODS

Preparation of NF hydrates

Dihydrate NF

Anhydrous NF Form A was dissolved in a mixture of IPA and water (0.915 mole fraction of IPA) at 60 °C in a light resistant container. The final NF concentration was equal to 1.5 mg/ml. The clear solution was allowed to cool down and left undisturbed at ambient condition to facilitate recrystallization. The resultant crystalline powder was harvested and kept in a tight and light-resistant container.

Trihydrate NF

Preparation of trihydrate NF was modified from the method reported by Puechagut et al. (1998). Anhydrous NF Form A was dissolved in 20% w/v aqueous ammonia solution to give a final clear solution at a concentration of 17.5 mg/ml. Antisolvent was obtained by mixing 564 ml of acetone and 156 ml of dichloromethane. The aqueous ammonia NF solution of 68.5 ml was gradually poured into antisolvent with continuous agitation. White and fluffy precipitates were developed and harvested. Dichloromethane was used to wash the resultant precipitates. The product was then placed in the drying oven at 50 °C for approximately 1 hour to remove residual solvents.

Hemipentahydrate and Pentahydrate NF

Hemipentahydrate NF and pentahydrate NF were prepared by hydration of anhydrous NF Form A at specified % RH level. Anhydrous NF Form A was placed under 75% RH and 100% RH at ambient temperature for 1 week to yield hemipentahydrate NF and pentahydrate NF, respectively (Katdare et al., 1986; Yuasa et al., 1982). Additionally, pentahydrate NF was also prepared by suspending anhydrous NF Form A in excess amount of double distilled water with continuous stirring. Dispersed solid was filtered and dried at ambient condition.

Solid state characterization of NF hydrates

Thermal analysis

The thermal properties of NF crystalline hydrates were evaluated using DSC with STAR^c software. Samples (5 mg) in aluminum pan with one pinhole were evaluated from 30-230 °C at a scanning rate of 10 °C/min under nitrogen purge at 60 ml/min. TGA/SDTA was employed to investigate the liberation of volatile substance. The TGA operating conditions were the same as those used in the DSC study.

Hot stage microscopy (HSM)

HSM equipped with optical microscope was employed to evaluate solvates or hydrates (Vitez et al., 1998). Heating rate and temperature range were 10 °C/min and 30-240 °C, respectively. A small amount of sample was initially suspended in mineral oil and placed on a glass slide before being fixed on to the heating station. The liberation of gas bubbles at specified temperature was observed and recorded.

Karl Fischer titrimetry (KF)

The water contents of NF hydrates were monitored. Due to low solubility of NF hydrates in methanol, heating oven was selected as an additional attachment. Approximately 50 mg of the sample was inserted into the heating oven. The oven temperature of 160 °C was gradually increased to initiate the evaporation of water molecules. Water vapor was carried by dried nitrogen gas to react with KF reagents in the titration vessel where water contents were finally quantified.

X-ray Powder Diffraction (XRPD)

X-ray diffractometry was done with CuK α radiation at 40 kV and 20 mA. Samples were measured at a step size of 0.04 °2 θ with a scan speed 5 °2 θ /min from 5° to 35 °2 θ .

Fourier Transformed Infrared Spectroscopy (FT-IR)

ATR FT-IR was employed to observe the changes in peak position between anhydrous NF and NF hydrates. The samples were triturated and gently ground with dried potassium bromide in an agate mortar. The spectra were recorded

as percent transmittance (%T) with respect to wave number (ν) in the range of 450 to 4000 cm^{-1} .

Stability Indicating High Performance Liquid Chromatography (SI-HPLC)

SI-HPLC method was modified from the method used by Cordoba-Borrego et al.(1999). HPLC equipped with Hypersil BDS-C18 column in conjunction with C18-guard column was used. The mobile phase comprised of 0.1% v/v aqueous o-phosphoric acid: acetonitrile at volume ratio of 70:30. The flow rate was equal to 1 ml/min. UV detection was carried out at 278 nm. Degradation product of NF was prepared by dispersing anhydrous NF in 30%w/v hydrogen peroxide in clear glass vial and was exposed to light and heat (80 °C) in an oven up to 8 hours. In addition, forced degradation in basic environment condition was evaluated according to the method used to prepare trihydrate NF. Small amount of anhydrous NF was added to 20% w/v aqueous ammonia solution and heated at 80 °C to initiate degradation.

Scanning Electron Microscopy (SEM)

The morphology of sample was recorded with a SEM at 15kV. The sample was carefully attached on the metal stub. It was then coated with gold by Sputter coater for 3 minutes at 0.05 mbar, 15mA with a working distance of 5 cm.

Solid State Interconversion of NF Hydrate

In an attempt to explore the interconversion pathways among NF hydrates and the anhydrous form, specific conditions were identified. Temperature and surrounding % RH were of main interest.

Effect of Relative Humidity on the Conversion of Anhydrous NF and NF Hydrates

The effect of relative humidity on the conversion of anhydrous NF Form A was evaluated. Preliminary study on sorption and desorption behaviors of anhydrous phase was investigated by dynamic vapor sorption (DVS) using symmetrical gravimetric analyzer. Fifteen milligrams of anhydrous NF Form A was dried in a vacuum at 25 °C for 6 hours to minimize traces of surface associated water.

Isothermic equilibrium condition of the cycle was 0.01% w/w within 15 min with a maximum step time of 75 min. The step change of % RH in both sorption and desorption phase were 5% RH/step. The change in sample weight against % RH was recorded.

Due to limited amounts of the samples obtained by DVS experiments, the sample at each equilibrium % RH was not sufficient to be collected in order to monitor for their solid state characteristics by XRPD. Thus, larger amounts of anhydrous NF Form A were exposed to specific moisture levels. The generation of various % RH in an air tight and light resistant container was made by using saturated solutions of lithium chloride (11.3% RH), magnesium chloride (32.8% RH), potassium carbonate (43% RH), sodium bromide (57.5% RH), sodium chloride (75% RH), potassium bromide (81% RH), potassium chloride (84% RH), dextrose monohydrate (87% RH), potassium nitrate (93.7% RH) and purified water (100% RH) at 25 °C (Nyquist, 1983; Kotny and Connors, 2002). The sample powders were exposed to each relative humidity for 7 days before being characterized.

The preliminary results obtained by DVS and relative humidity exposures, indicated that phase transformation of anhydrous NF Form A to various stoichiometric hydrates occurred. Thus, every stoichiometric NF hydrate produced was subjected to an extreme moisture level of 100% RH and an extremely dry environment of 0% RH (Drierite®) and monitored for further transformation. The samples were stored for 7 days and then characterized by XRPD compared to the corresponding references. Additional storage time was needed in some cases where 7 days was insufficient to induce any changes in solid state transformation of NF hydrates.

Effect of Temperature on the Conversion of NF Hydrates

The temperature effect, particularly heating, was aimed to investigate dehydration of NF hydrates. A moderate temperature of 60 °C was selected in an attempt to avoid chemical degradation. NF hydrates were placed in the drying oven at 60 °C for 48 hours before being characterized by XRPD. However, additional exposure time up to one month was needed for some NF hydrates to confirm the solid state transformation.

4.3 RESULTS AND DISCUSSION

Solid State Characterization of NF Hydrate

Anhydrous NF starting material was characterized by XRPD, DSC and TGA. TGA revealed negligible mass loss of less than 1% w/w which was in accordance with USP and BP specifications of anhydrous NF (USP 27; BP 2002). DSC and TGA thermograms of various NF forms are shown in Figure 4.1. DSC confirmed a single sharp endotherm at a temperature range of approximately 220 to 225 °C for anhydrous NF (Figure 4.1A). XRPD of anhydrous NF (Figure 4.3A) showed characteristic peak positions identical to those reported for NF Form A (Barbas et al., 2006; Katdare et al., 1986; Yuasa et al., 1982). It was hence concluded that the anhydrous NF in our experiment was polymorphic anhydrous NF Form A.

Slow recrystallization of NF solution in IPA:water mixture resulted in dihydrate NF. Thermal properties and water content of this hydrate are shown in Figure 4.1C. DSC and TGA thermograms showed endothermic peaks along with weight loss at the same temperature range of 80 to 140 °C. HSM also showed water vapor bubbles within the same temperature range (Figure 4.4F). Water content obtained by KF titration agreed well with the weight change obtained by TGA (Table 4.1) which indicated a stoichiometric dihydrate formation. XRPD pattern of the dihydrate NF was not reported in any previous publications for reference. Thus, a single crystal X-ray diffraction (SC-XRD) data from crystals obtained by the above recrystallization method was compared to NF dihydrate single crystal X-ray diffraction data reported by Florence et al. (2000) and were found to be identical. Therefore, the experimental XRPD pattern of NF dihydrate (Figure 4.3C) was confirmed by the calculated powder diffraction pattern generated from SC-XRD data by MERCURY[®] software and served as reference XRPD pattern for dihydrate NF in future experiments. However, this recrystallization process was time-consuming and chemical degradation of NF is of great concern. The results obtained from SI-HPLC of the recrystallized NF dihydrate did not show degradation (Figure 4.2). Thus, the quality of NF dihydrate produced was essentially free from degraded compounds and was acceptable to be used as the reference for future studies.

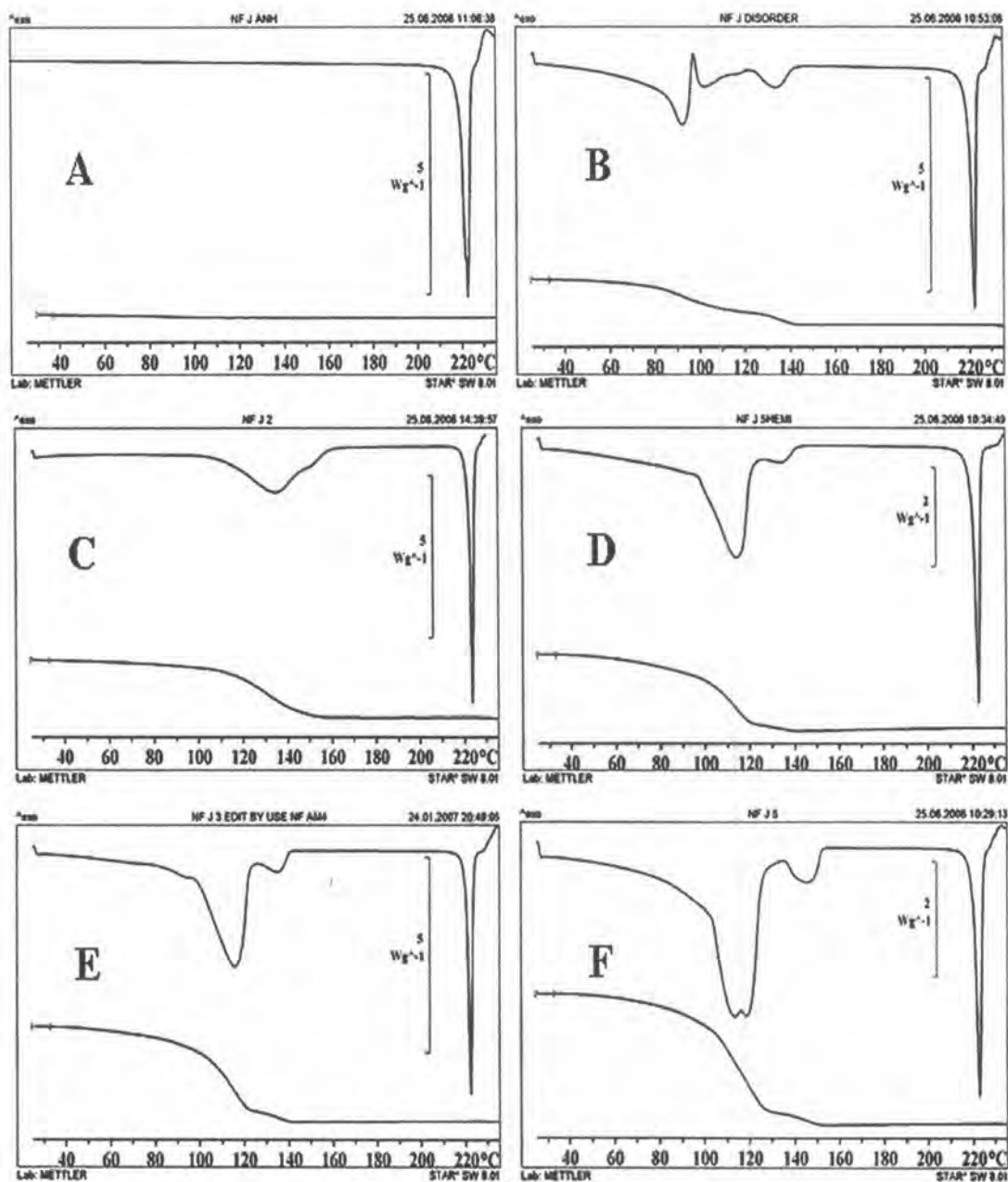


Figure 4.1 DSC and TGA thermograms of anhydrous NF Form A (A), disordered NF state (B), dihydrate NF (C), hemipentahydrate NF (D), trihydrate NF (E) and pentahydrate NF (F)

Table 4.1 Water content (KF), percent weight loss (TGA) and stoichiometry between NF and water molecules

Method of preparation	KF water content (%)	TGA % weight loss	Stoichiometry (NF:water molecule)
Desiccation NF pentahydrate	5.55(0.561)	6.24(0.372)	---
Recrystallization from IPA:water mixture	10.10 (0.080)	9.34 (0.136)	1:2
Exposure to 75% RH	11.55 (0.611)	12.12 (0.039)	1:2.5
Precipitate from aqueous ammonia solution	14.49 (0.342)	14.81 (0.046)	1:3.0
Exposure to 100%RH	20.55 (0.367)	20.87 (0.153)	1:5.0

SD shown in parentheses.

Trihydrate NF generated from antisolvent precipitation was characterized. The results from HSM confirmed the existence of solvate or hydrate as seen from the evolution of vapor bubbles during heating. DSC yielded a large endotherm immediately followed by another minor endotherm at the temperature range of 80 to 130 °C (Figure 4.1E). Total weight loss obtained by TGA was 14.81% w/w and occurred at the same temperature range as that of the DSC endotherm (Figure 4.1E). Meanwhile, KF confirmed the trihydrate stoichiometry of the crystalline precipitate (Table 4.1). XRPD pattern shown in Figure 4.3E was used as reference XRPD pattern of trihydrate due to the fact that no reference XRPD pattern was available in any previous works. In addition, SI-HPLC did not detect any NF degradation after trihydrate NF was generated (Figure 4.2).

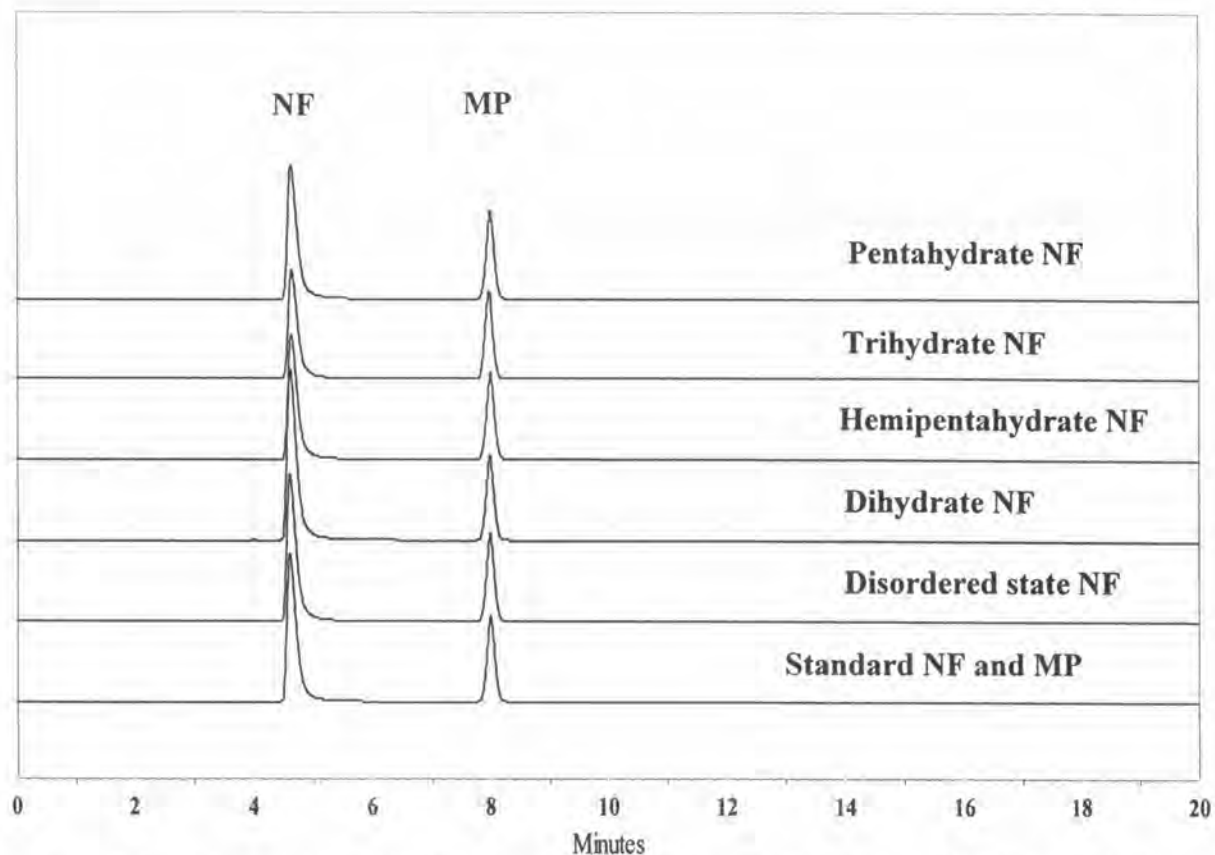


Figure 4.2 Comparative HPLC chromatograms of various hydrates NF with standard NF in conjunction with the use of methyl paraben (MP) as internal standard

DSC analysis of the hemipentahydrate NF (Figure 4.1D) and the pentahydrate NF (Figure 4.1F) which were produced from direct exposure to moisture, showed large endotherm followed by a smaller endotherm at approximately 120 °C and 140 °C, respectively. TGA showed a two step weight loss at the same temperature as achieved by DSC. The total weight loss from TGA and the water content obtained from KF were in good agreement confirming the stoichiometry of the hemipentahydrate NF and the pentahydrate NF (Table 4.1). HSM showed continuous liberation of vapor bubbles during the temperature ranges corresponding to their DSC and TGA dehydration endotherms (Figure 4.4A to 4.4D). XRPD of both hydrates are illustrated in Figure 4.3D and 4.3F and the XRPD patterns were essentially the same as XRPD of the hemipentahydrate NF and the pentahydrate NF reported by Yuasa et al (1982).

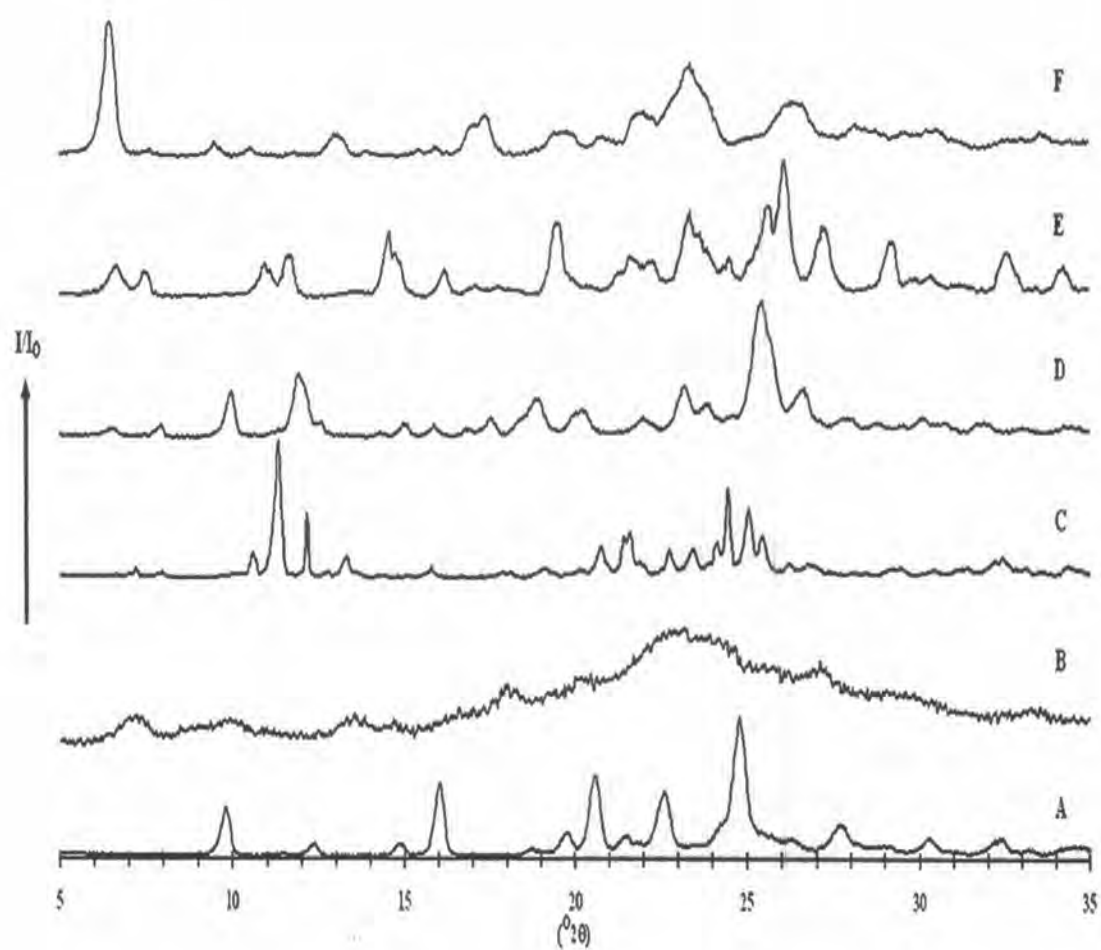


Figure 4.3 XRPD patterns of anhydrous NF Form A (A), disordered NF state (B), dihydrate NF (C), hemipentahydrate NF (D), trihydrate NF (E) and pentahydrate NF (F)

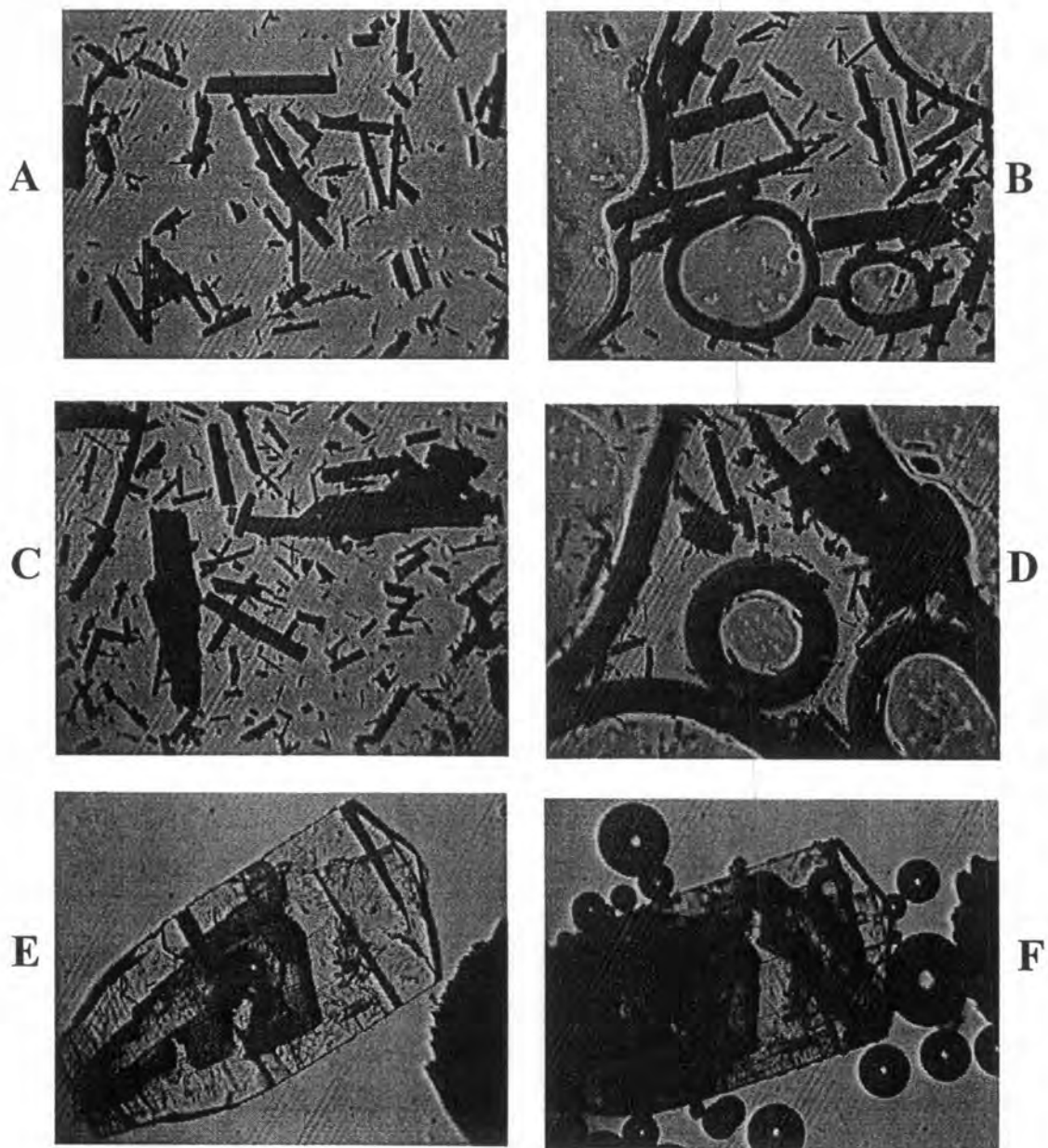


Figure 4.4 HSM photomicrographs of NF hydrates immerse in mineral oil upon heating (hemipentahydrate NF at ambient temperature (A), hemipentahydrate NF at temperature over 120°C (B), pentahydrate NF at ambient temperature (C), pentahydrate NF at temperature over 120°C (D), dihydrate NF at ambient temperature (E), dihydrate NF at temperature over 100°C (F))

Pentahydrate NF, which was obtained from an alternative method of suspending anhydrous NF Form A in water also provided the same thermal behavior and XRPD pattern as the one hydrated NF at 100% RH. However, the crystal habits of the two pentahydrate NF were different. SEM micrographs of each solid were generated. Light yellow and coarse powder of anhydrous NF Form A (Figure 4.5A) was converted to opaque white, needle-like fluffy pentahydrate NF after having directly come into contact with water (Figure 4.5C). In contrast, exposure of anhydrous NF Form A to 100% RH did not change the appearance of the original powder (Figure 4.5A) even when the structure was found to be converted to the pentahydrate NF.

A different method of preparation and level of moisture in an environment greatly impacted on the formation of hydrate NF. The direct contact between anhydrous NF Form A with water was an issue. The precipitation of dispersed anhydrous NF Form A in excess amount of water was filtered and dried at ambient condition. An intact physical appearance of anhydrous NF Form A (light yellow fine powder) was rapidly converted to fluffy and waxy solid with opaque white color. SEM photomicrograph of solid obtained was fine needle-like particles fused together forming a network (Figure 4.5). It might be due to water partially dissolved the surface of anhydrous NF crystal and recrystallized and bridged together as NF hydrates. Light microscopy was used to investigate such phenomena. Anhydrous NF Form A with a drop of water was prepared on glass slide. The result suggested that the transformation of anhydrous NF Form A to the new unknown form immediately happened after contact with water (Figure 4.6B to D). The longer the contact time the more developed the new unknown NF form. The new unknown form was characterized and found to be pentahydrate NF. Thus, different methods of preparation could provide an identical internal structure of NF hydrate with different observable habit.

The phase transformation of anhydrous NF Form A to pentahydrate after direct contact with water is a valuable data for the pharmaceutical industries. Some made NF tablets by wet granulation with aqueous binder showed the physical transformation of powder blend after binder was added. The new physical character of the powder blend was hard and waxy agglomerates. It directly impact on the mixing efficiency and working capability of mixer. In the case of low efficiency mixer, mixer

was usually broken during the mixing/granulating process. High efficiency mixer could overcome the above problem. However, the uniformity of powder blend was argued. It was suggested that a new phase in powder blend was strongly contributed from the transformation of anhydrous NF Form A to the pentahydrate NF with needle-like appearance. Non aqueous binding solution was suggested to protect the solid state transformation of anhydrous NF Form A.

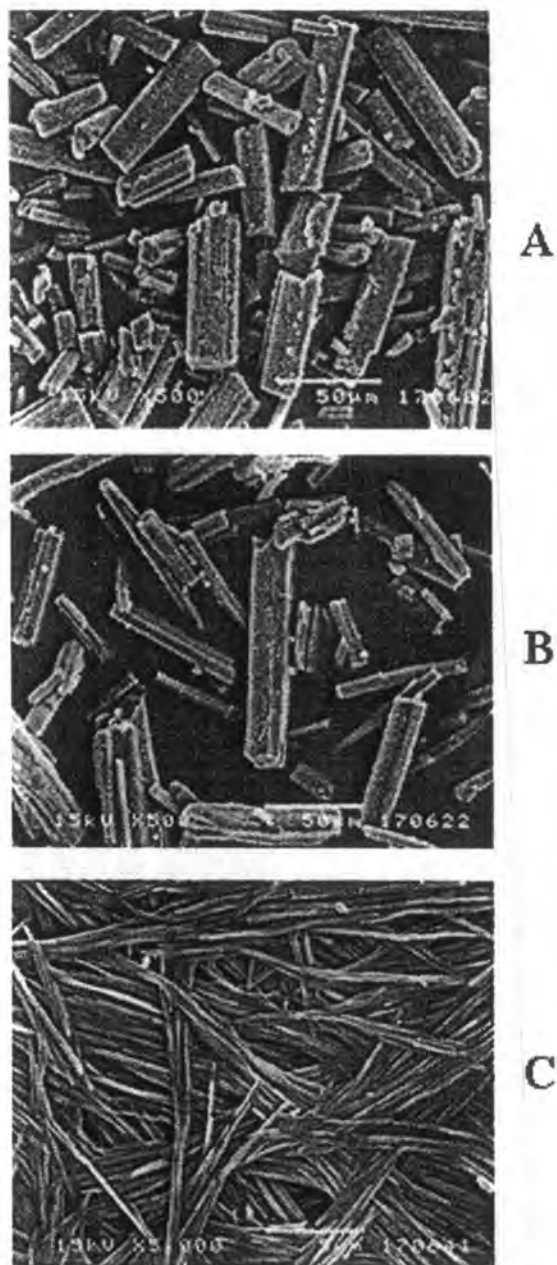


Figure 4.5 Scanning electron photomicrographs of anhydrous NF Form A (A), pentahydrate NF obtained from 100%RH vapor exposure (B) and pentahydrate NF from directly dispersed in water (C)

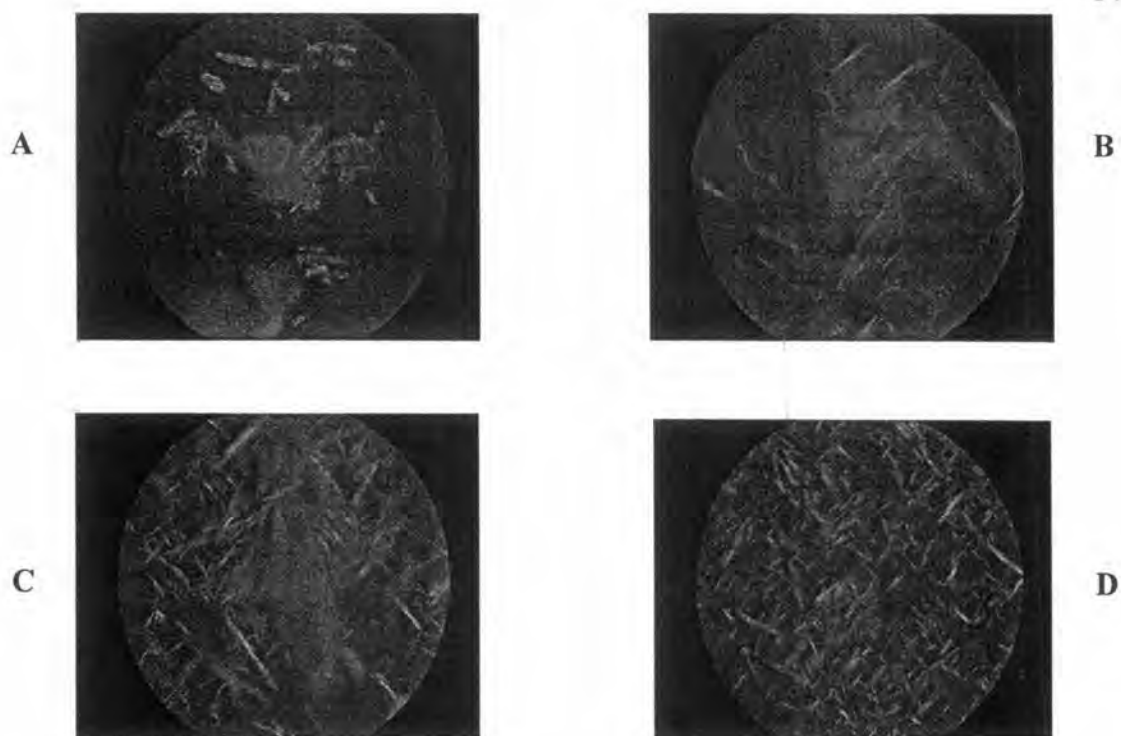


Figure 4.6 The photomicrographs of anhydrous NF Form A after dispersed in water at various contact time at the magnification of 400. (A. initial, B. 15 minutes, C. 60 minutes and D.180 minutes)

Another NF hydrate form found in this study, the disordered NF state, has not been previously reported elsewhere. Dehydration of pentahydrate NF via desiccation over time produced a so-called disordered NF state. DSC and TGA of the disordered NF state are shown in Figure 4.1B where a complex dehydration behavior was observed. Dehydration was detected during the first broad endotherm (100 °C) and immediately followed by a sharp exotherm (115 °C) and another broad endotherm. Mass loss of disordered NF state also took place over the same temperature range as found in the DSC. The sharp exotherm was possibly due to the rearrangement of NF molecules after water molecules were partially removed. Disordered NF provided an XRPD pattern similar to the amorphous material (Figure 4.3B). However, minor peak intensity in certain regions could still be observed. In comparison with amorphous NF (Šuštar et al., 1993), the DSC of disordered NF showed large endotherm with rapidly changing exotherm at the range of approximately 80 °C to 110 °C while amorphous NF exhibited an endotherm immediately followed by an exotherm from 80 to 150 °C. In addition, disordered NF showed two broad endothermic peaks while this thermal

behavior was not seen in an amorphous NF upon heating. Thus, the disordered NF was believed not to be a true amorphous but only microcrystalline disorder state. The mild condition used to remove water molecules from pentahydrate NF might perturbed the crystal structure and led to a new arrangement or disordered state. Thus, short range order of crystal lattice was still preserved. Amorphous or disordered state generally occurred with anhydrous materials. However, some amorphous materials absorbed water molecules in the structure. For example, raffinose pentahydrate could become amorphous with water content equivalent to stoichiometric monohydrate (Hogan and Buckton, 2001).

In order to characterize the complex thermal behavior of the disordered NF, XRPD was utilized to monitor the molecular rearrangement of intact and heated disordered NF at predetermined times by using DSC. One sample was heated from 25 to 120 °C (D-I) and the other sample was heated from 25 to 160 °C (D-II). The DSC thermograms of heated sample of D-I and D-II are illustrated in Figure 4.7. DSC thermogram of D-I showed a complete disappearance of the initial complex thermal behavior comprised of large endotherm with followed by rapid exotherm. Subsequently, D-II exhibited the thermogram consisted of only second endotherm after the initial thermal event was removed. It indicated that two complex thermal

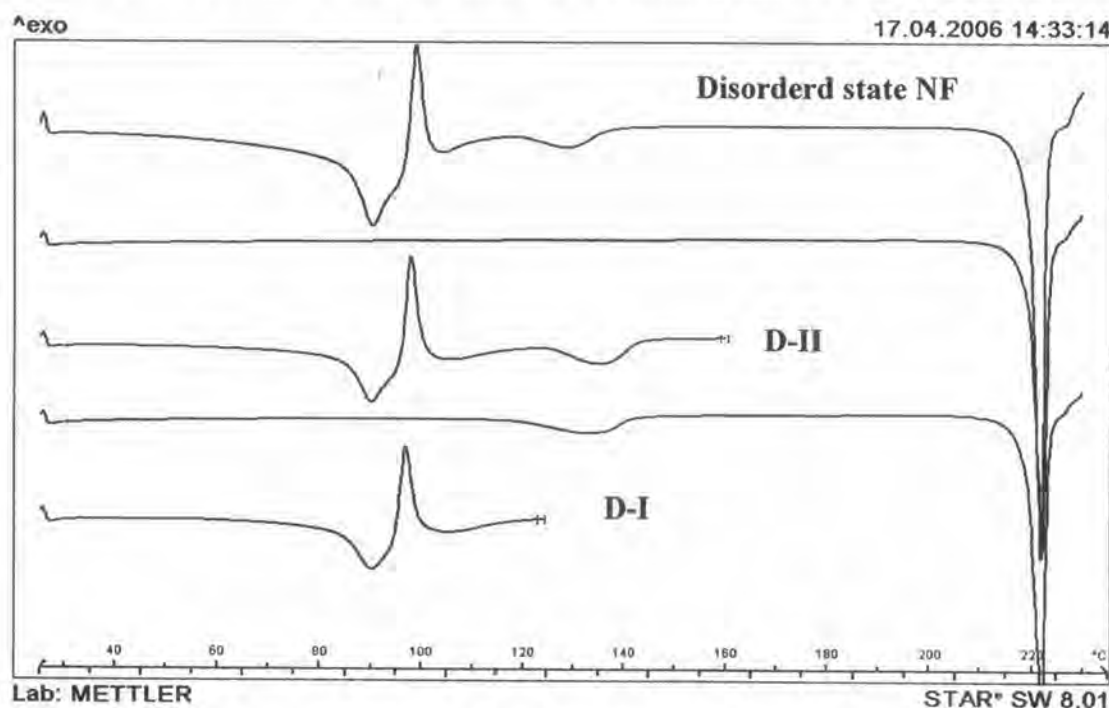


Figure 4.7 DSC thermograms of disordered NF with respect to different heating temperature programs

events of disordered NF did not have any interaction together. After the first two events were removed, DSC thermogram displayed only one sharp endotherm correlated to the melting endotherm of anhydrous NF Form A. It could be initially concluded that two locations of water molecules of disordered NF were separated in different lattice space. Moreover, the XRPD pattern of D-I is displayed in Figure 4.8B. It showed increased in crystallinity compared to the initial disordered NF. Initial disordered NF was also heated from and its XRPD pattern is illustrated in Figure 4.8C. The solid obtained after D-II exhibited higher order than that of initial disordered NF and after D-I treatment. The XRPD pattern was shown to be identical to that of the anhydrous NF Form A. TGA confirmed that the solid obtained after D-II treatment showed no weight loss. It could, hence, be concluded that solid collected after D-II treatment is an anhydrous Form A. The total weight change from D-I to D-II was approximately 1.64% which was higher than the value allowed for anhydrous NF in the official monographs (less than 1%) (USP 27; BP 2002). Thus, the solid resulted from D-I treatment was a hydrated transitional phase which, in turn, would convert to the anhydrous Form A upon further heating. In addition, the water content of hydrated transitional phase obtained from TGA was in the range of 2.5 to 3.5 %w/w that was in the range of hemipentahydrate NF form. It should be indicated the hydrated transitional phase was the one hydrate form of NF.

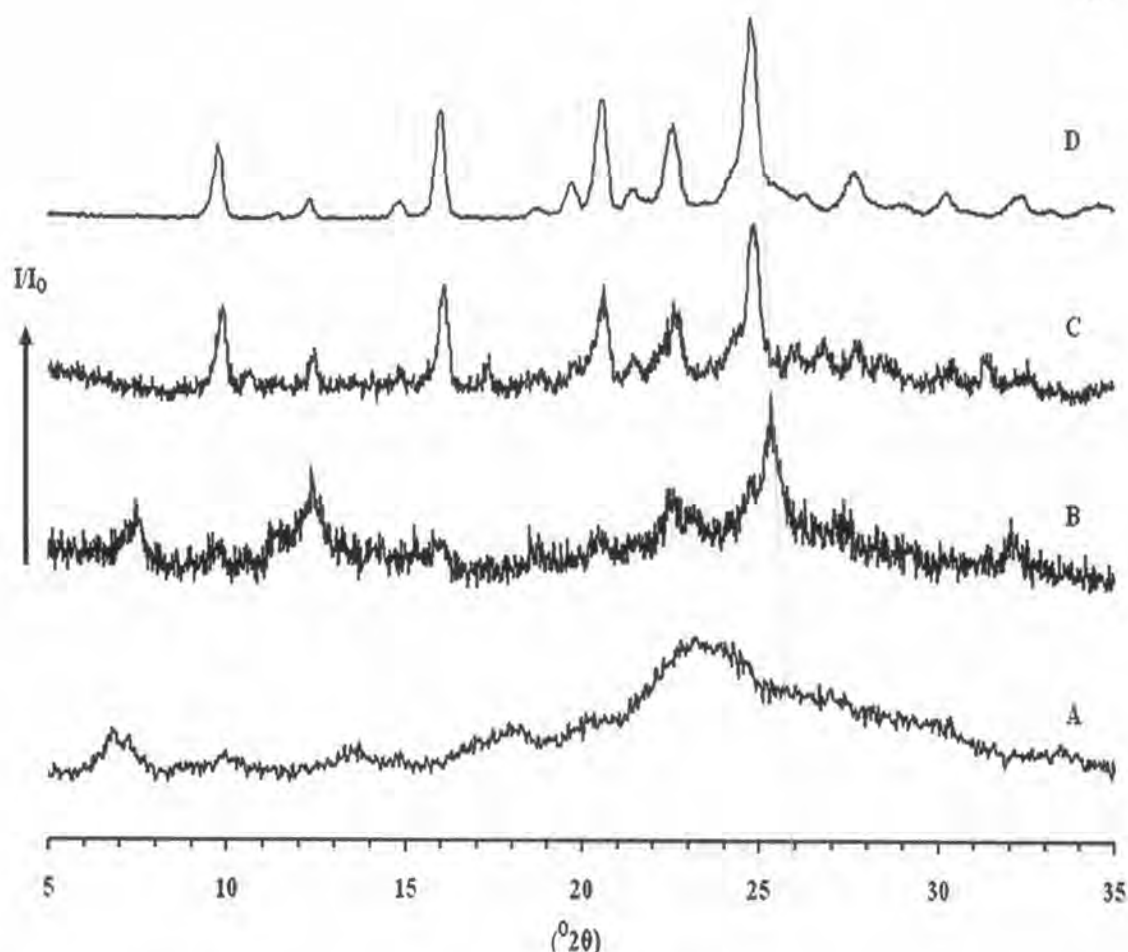


Figure 4.8 XRPD patterns of disordered NF state (A), after D-I (B), after D-II (C) and anhydrous NF Form A (D)

In general, materials of disordered molecular arrangement are more sensitive to moisture than the ordered crystalline phase. Consequently, the moisture sensitivity of the disordered NF was a critical issue. The disordered NF was thus exposed to various humidity levels for 7 days and the XRPD patterns were recorded (Figure 4.9). The transformation of the disordered NF to the crystalline pentahydrate NF form was completed when at least 57% RH was used. At 32.8% RH, partial transformation to the pentahydrate was seen according to the presence of peaks at 6.40, 13.00, 17.28, 23.36 and 26.20 °2θ. On the other hand, the disordered NF state was stable under 11.3% RH for at least 2 months similar to the XRPD pattern after 7 days exposure to 11.3% RH. Thus, exposing the disordered NF to more than 32.8% RH would eventually generate the crystalline pentahydrate NF. However, at humidity of 11.3% RH or below, the disordered NF structure was retained.

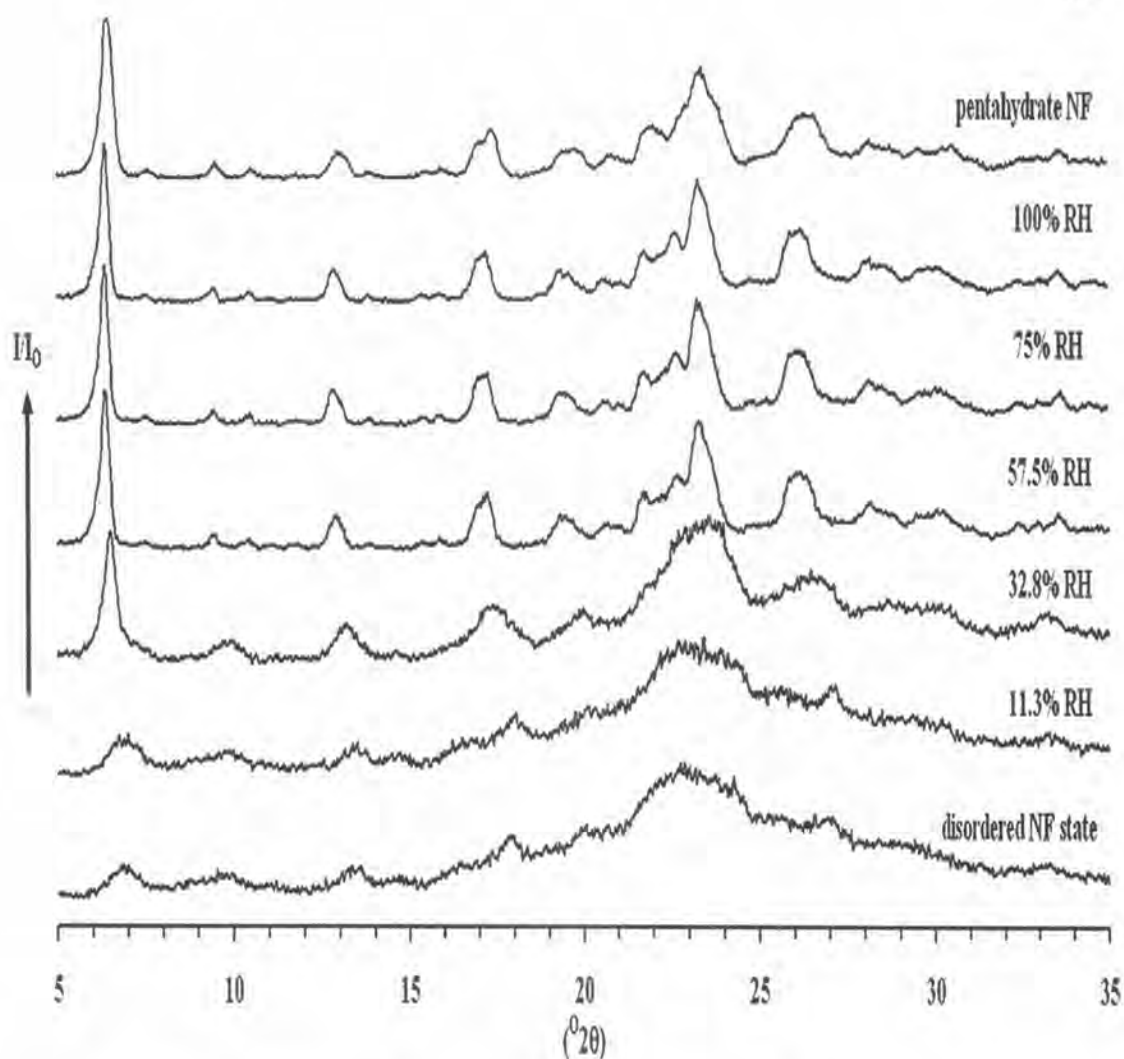


Figure 4.9 XRPD patterns of disordered NF state exposed to different relative humidities for 7 days

Chemical interaction between water of crystallization and active moiety of every NF hydrate was investigated by spectroscopic FT-IR. The spectra of every NF hydrate form were recorded and compared (Figure 4.10). The signal at specific wavenumber can be interpreted in terms of the functional group of the material. The IR spectrum of anhydrous NF Form A exhibited main absorption peaks at 1732 and 1253 cm^{-1} indicating C=O and C-O bond stretching of carboxylic group, respectively. When water molecules are incorporated into the crystal structure, the response of C=O and C-O are found to gradually decrease as a function of increased number of water of crystallization (Mazuel, 1991). Meanwhile, the responses at 1584 and 1340 cm^{-1} of carboxylate anion are markedly increased. The above results suggested that

structures of the carboxylic group in these hydrates are the carboxylate anion (Hu et al., 2002). In addition, the responses in the regions of 3700 to 3250 cm^{-1} owing to OH stretching were clearly present in all NF hydrates, signifying hydrogen bonding between carboxylic group and water molecules in the crystal structure (Byrn et al., 1999). The FT-IR spectrum of the disordered NF was also investigated. The presence of peaks at 1581 and 1334 cm^{-1} confirmed the occurrence of carboxylate anion identical to other hydrates and the lack of responses at 1732 and 1253 cm^{-1} confirmed that C=O and C-O stretching of carboxylic group was disturbed by water molecule as well. It can be concluded that water molecules in disordered NF formed structural hydrogen bonds with NF molecules similar to those of other stoichiometric hydrates. Thus, it is believed that the disordered NF form was not a true amorphous state but a metastable phase with short range ordered structure.

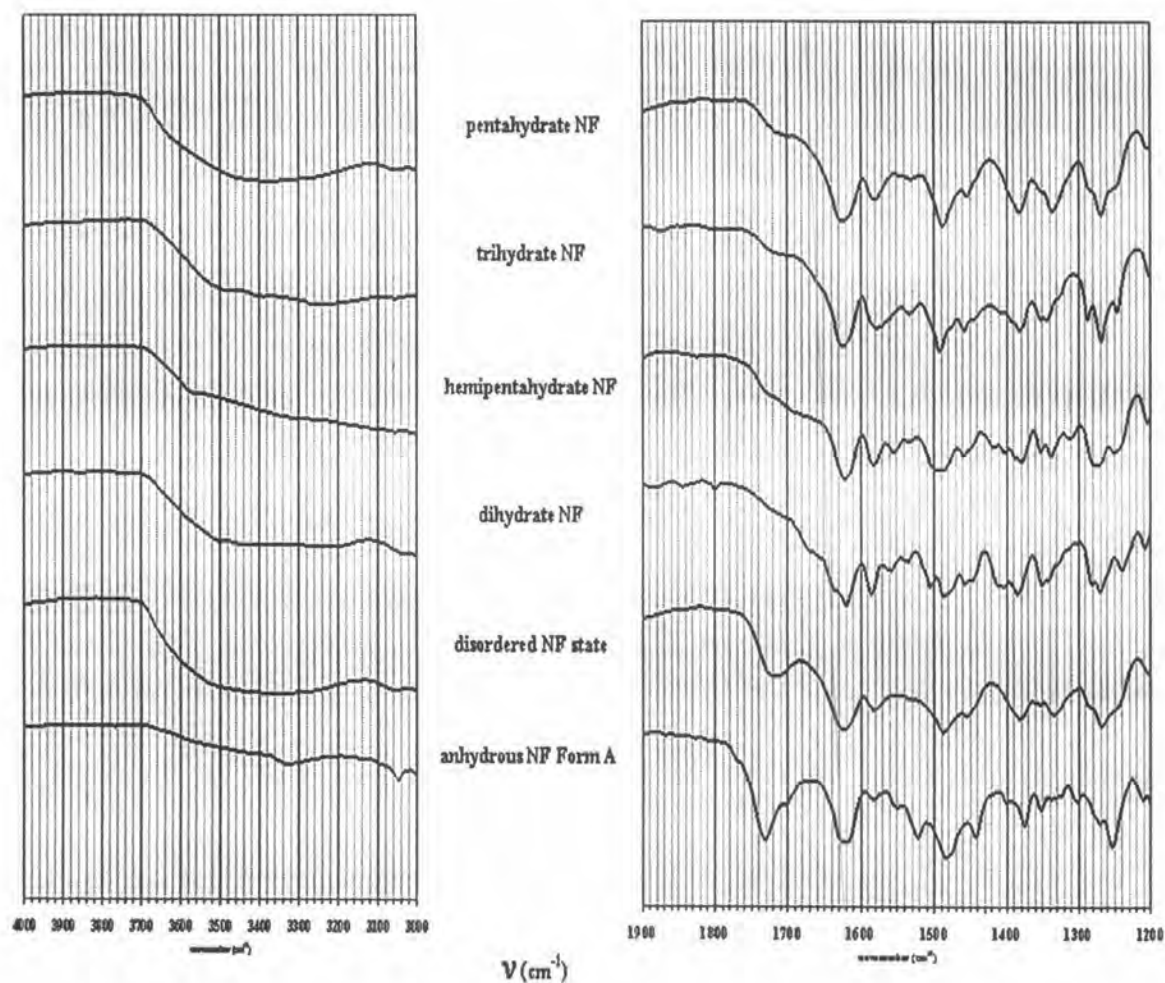


Figure 4.10 FT-IR spectra of anhydrous NF Form A, disordered NF state and other stoichiometric hydrates of NF

Solid State Interconversion of NF Hydrate

XRPD patterns of NF hydrates (Figure 4.3) were used as reference patterns to show specific characteristics of each form and were used to identify the solid state transformation. The following studies gathered evidences on the solid state transformation of NF hydrates under different environmental i.e. relative humidity and temperature. It should be noted that the observed trends are based on visual inspection of the diffraction patterns and are not intended to be quantitative.

Effect of Relative Humidity on Solid State Transformation of NF Hydrates

Moisture content in the environment usually plays the most pivotal part in hydrate formation of many organic compounds (Zhu et al., 1996a; Zhu and Grant, 1996b). The anhydrous NF Form A placed under different % RH were found to form varying stoichiometric NF hydrates (Katdare et al., 1986; Yuasa et al., 1982). The moisture sorption study was used as a rough evaluation on the hydrate formation behavior due to moisture. Moisture vapor sorption data of the anhydrous NF Form A obtained by DVS showed that under 60% RH, the anhydrous structure was retained (Figure 4.11). On the other hand, at moisture levels higher than 60% RH, anhydrous NF Form A showed a marked mass increase. The higher the relative humidity of the environment above 60% RH the higher the weight gain. The final solid structure formed at the end of the sorption phase was later found to be the pentahydrate NF by XRPD.

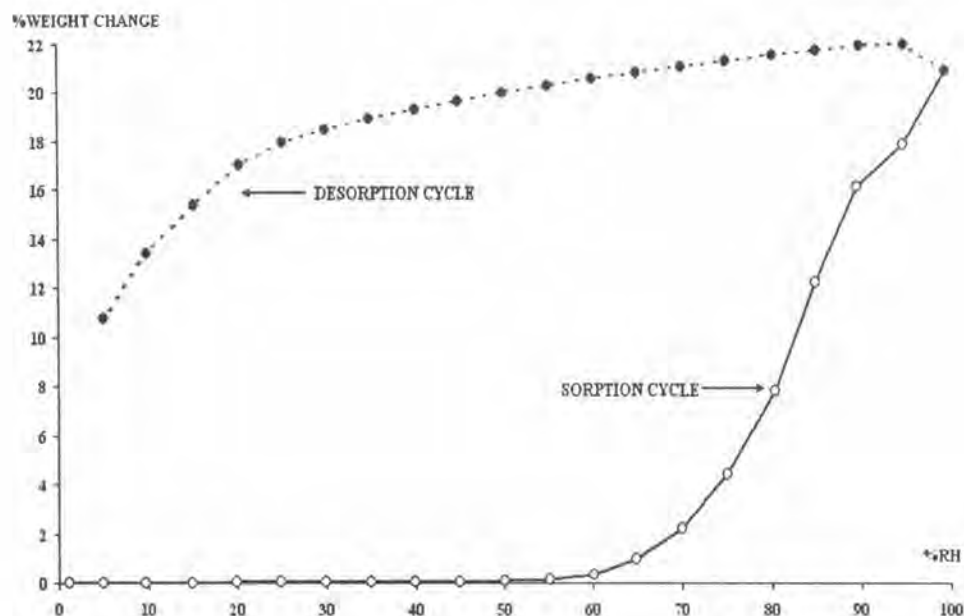


Figure 4.11 Dynamic water vapor moisture sorption and desorption isotherms of anhydrous NF Form A at 25°C

Desorption phase of the induced the pentahydrate NF showed that the pentahydrate NF was very stable even at below 30% RH. However, when the environment reached very low humidity of below 20% RH, significant weight loss occurred. The result suggested that for dehydration of the pentahydrate NF to occur the environment must reach very low relative humidity. These data could be used to determine a suitable storage condition of NF raw material. The suggested storage condition for the anhydrous NF Form A should be in an environment where moisture level is not more than 60% RH at room temperature. The pentahydrate NF form should not be stored in areas where relative humidity is below 20% RH to prevent dehydration.

The degree of hydration of anhydrous NF Form A with respect to relative humidity was investigated and characterized by XRPD (Figure 4.12). The hemipentahydrate NF was achieved when anhydrous NF Form A was exposed to 75%RH as mentioned in the previous section. XRPD patterns of the anhydrous NF Form A which were stored between 81% RH to 87% RH, however, showed mixed characteristics at $6.48^{\circ}2\theta$ and $25.48^{\circ}2\theta$ of the pentahydrate NF and the

hemipentahydrate NF, respectively. Increasing the moisture level was found to accentuate the intensity of the peak at $6.48^\circ 2\theta$. Meanwhile the intensity at $25.48^\circ 2\theta$ was reduced. When anhydrous NF Form A was exposed to humidity higher than 93.7% RH, pure pentahydrate NF was found. In addition, exposure of the anhydrous NF Form A at very high humidity did not generate any degradation products as confirmed by SI-HPLC.

NF hydrates were placed under 100%RH for 7 days after which XRPD patterns were recorded. The XRPD results revealed that every sample converted to the pentahydrate NF, except the dihydrate NF. The dihydrate NF exposed to 100% RH showed mixed characteristics of both dihydrate NF and pentahydrate NF (Figure 4.13). It could be inferred that the pentahydrate NF was the most stable form in extremely high moisture environments.

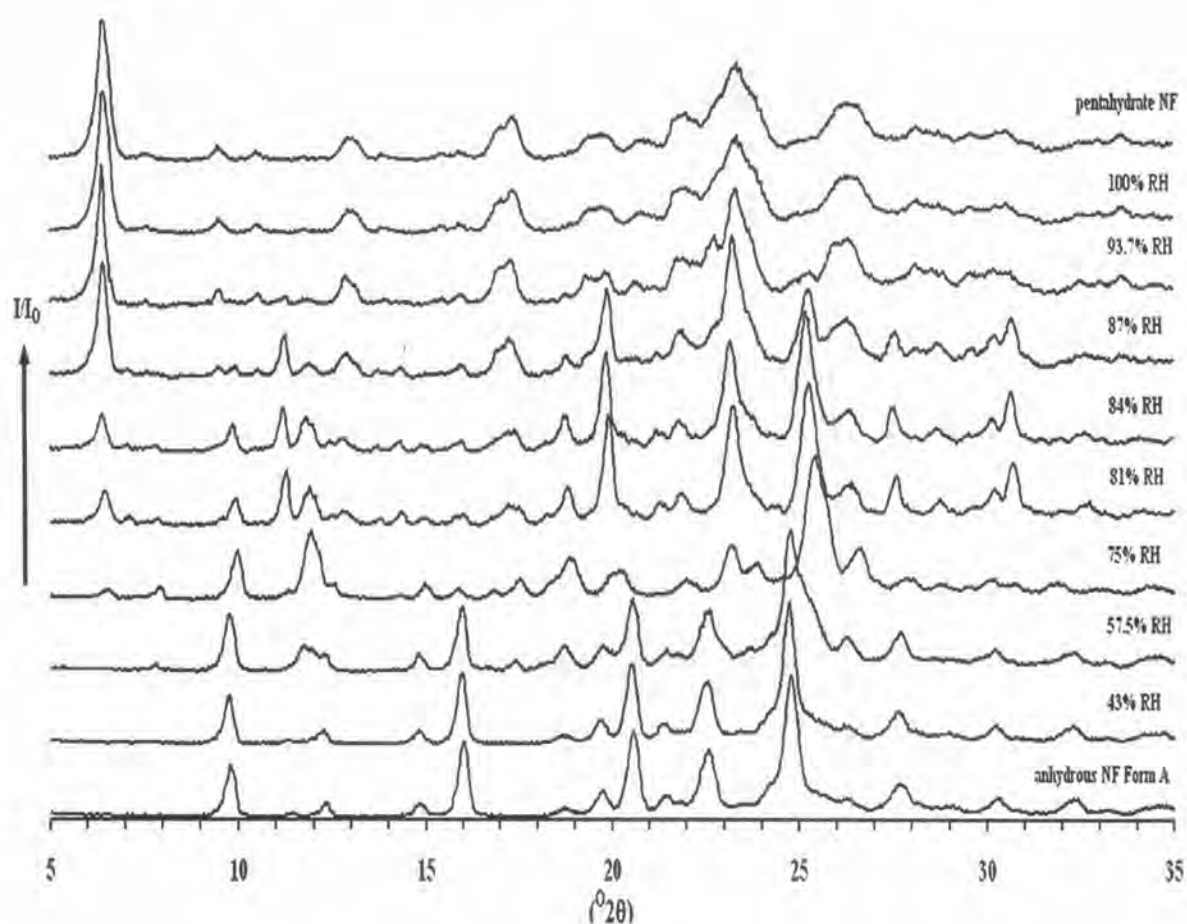


Figure 4.12 XRPD patterns of anhydrous NF Form A under different relative humidities for 7 days

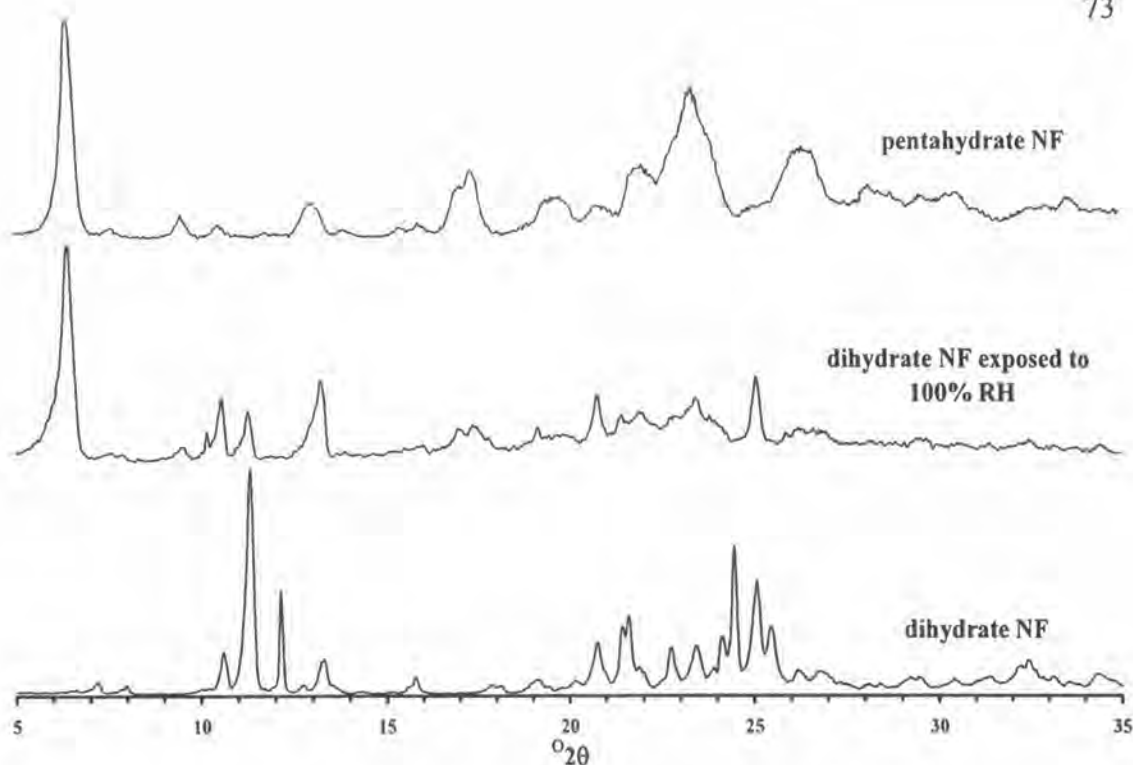


Figure 4.13 XRPD patterns of dihydrate NF under 100% RH for 7 days

On the other hand, the reduction to near 0% RH was also investigated. The pentahydrate NF was transformed to the disordered NF state as discussed earlier. The XRPD pattern of the hemipentahydrate NF at 0% RH is illustrated in Figure 4.14. The characteristic peak at $25.48^\circ 2\theta$ was slightly shifted to lower angle of $24.84^\circ 2\theta$ which corresponded to the anhydrous NF Form A. Meanwhile, the intensity at $26.68^\circ 2\theta$ gradually decreased as a function of exposure time. The longer contact time to dry environment led to the formation of a mixture of the two forms. The trihydrate NF showed the same phenomenon on the conversion to the anhydrous NF Form A during exposure to 0% RH condition. The XRPD patterns of the trihydrate NF during dehydration are shown in Figure 4.15. After 7 days of dehydration, peak responses at 9.84 , 20.52 and $24.84^\circ 2\theta$ of the sample were found to be of the anhydrous NF Form A. Peak positions at 7.52 and $25.40^\circ 2\theta$ were also apparent and related to the hydrated transitional phase similar to the heat treated (D-I) of the disordered NF state (Figure 4.8B). Meanwhile, other strong and characteristic trihydrate peaks still existed. In summary, dehydration by reduction of environmental moisture was not an effective method to convert neither the hemipentahydrate NF nor the trihydrate NF to the pure anhydrous NF Form A even after 90 days exposure. Therefore, the dihydrate NF was not further evaluated due to lack of dehydration efficiency by this approach.

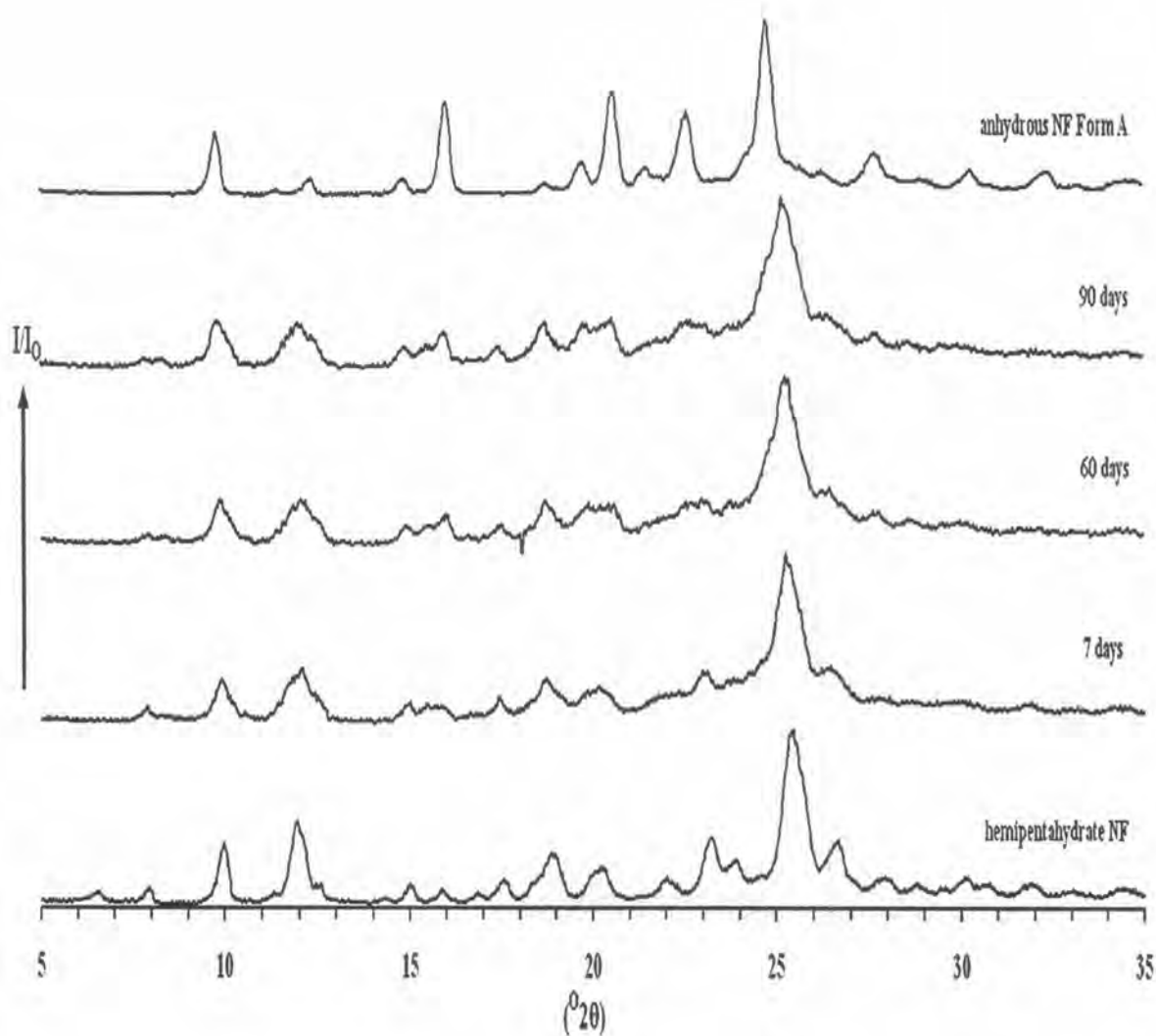


Figure 4.14 XRPD patterns of hemipentahydrate NF under desiccant (0% RH) as a function of exposure time

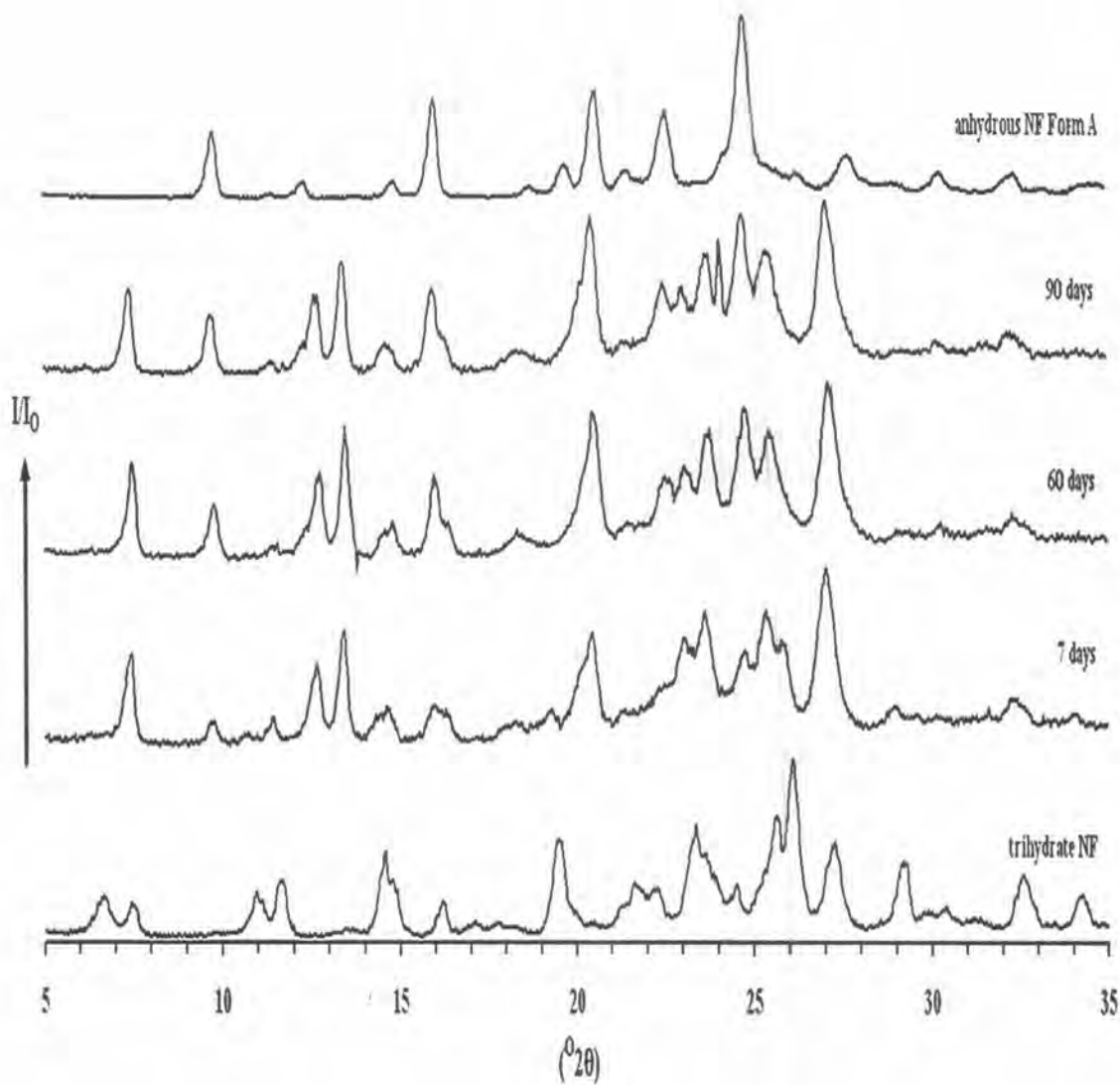


Figure 4.15 XRPD patterns of trihydrate NF under desiccant (0% RH) as a function of exposure time

Effect of Elevated Temperature on Solid State Transformation of NF Hydrates

Thermal dehydration is the most common way to prepare anhydrous materials in the pharmaceutical industry. There are many publications reported the polymorphic transformation or occurrence of desolvation upon thermal treatment (Lin and Chien, 2003; Willart et al., 2002; Landgraf et al., 1998; Hakanen and Laine, 1995). Hence, the conversion of NF hydrates using selected elevated temperature was performed. In this study, a moderate temperature of 60 °C was selected to minimize potential chemical degradation associated with higher temperatures.

The disordered NF was heated at 60 °C for 48 hours. XRPD showed that the anhydrous NF Form A was transformed from the disordered NF after heating (Figure 4.16B). The residual water content of the heated samples was investigated using KF. The water contents were 1.02, 0.60 and 0.46 for heated samples of the disordered NF, the hemipentahydrate NF and the pentahydrate NF, respectively. The results revealed that all heated samples were essentially anhydrous because the water content was approximately at or below the maximum limit (1%) for NF anhydrous specified in the monograph (USP 27; BP 2002). In the case of the heated hemipentahydrate NF, the XRPD pattern was similar to that of the disordered NF (Figure 4.16C). Note that the heated pentahydrate NF resulted in a similar XRPD pattern to that of the anhydrous Form A but with two additional peaks at 7.52 and 25.40 °2 θ (Figure 4.16D). These two peaks were assumed to be the residual of the hydrated transitional phase (Figure 4.8B) found during D-I treatment of the disordered NF state.

The results from the heated dihydrate NF and the heated trihydrate NF are shown in Figures 4.17 and 4.18, respectively. The XRPD of the dehydrated dihydrate NF revealed that a partial anhydrous phase was generated after thermal dehydration for 48 hours. However, peaks at 10.60, 11.32 and 13.16 and 25.00 °2 θ corresponding to the dihydrate NF were still present. Extended heating time of up to 1 month gave material with an identical pattern to that of the 48-hour treated sample. Thus, the longer heating time did not fully convert the dihydrate NF to the anhydrous NF Form A. The trihydrate NF also behaved in the same way upon thermal dehydration. XRPD of the treated trihydrate NF showed the anhydrous Form A peaks at 9.80, 16.04, 22.68 and 24.84 °2 θ . Additional peak position at 7.52 and 25.40 °2 θ were also noticeable and related to the hydrated transitional phase while the trihydrate NF peak at 23.36

$^{\circ}2\theta$ remained pronounced indicating a mixture of the three forms. Extended thermal dehydration of the trihydrate NF at 60°C of up to 1 month did not generate the pure anhydrous NF Form A from.

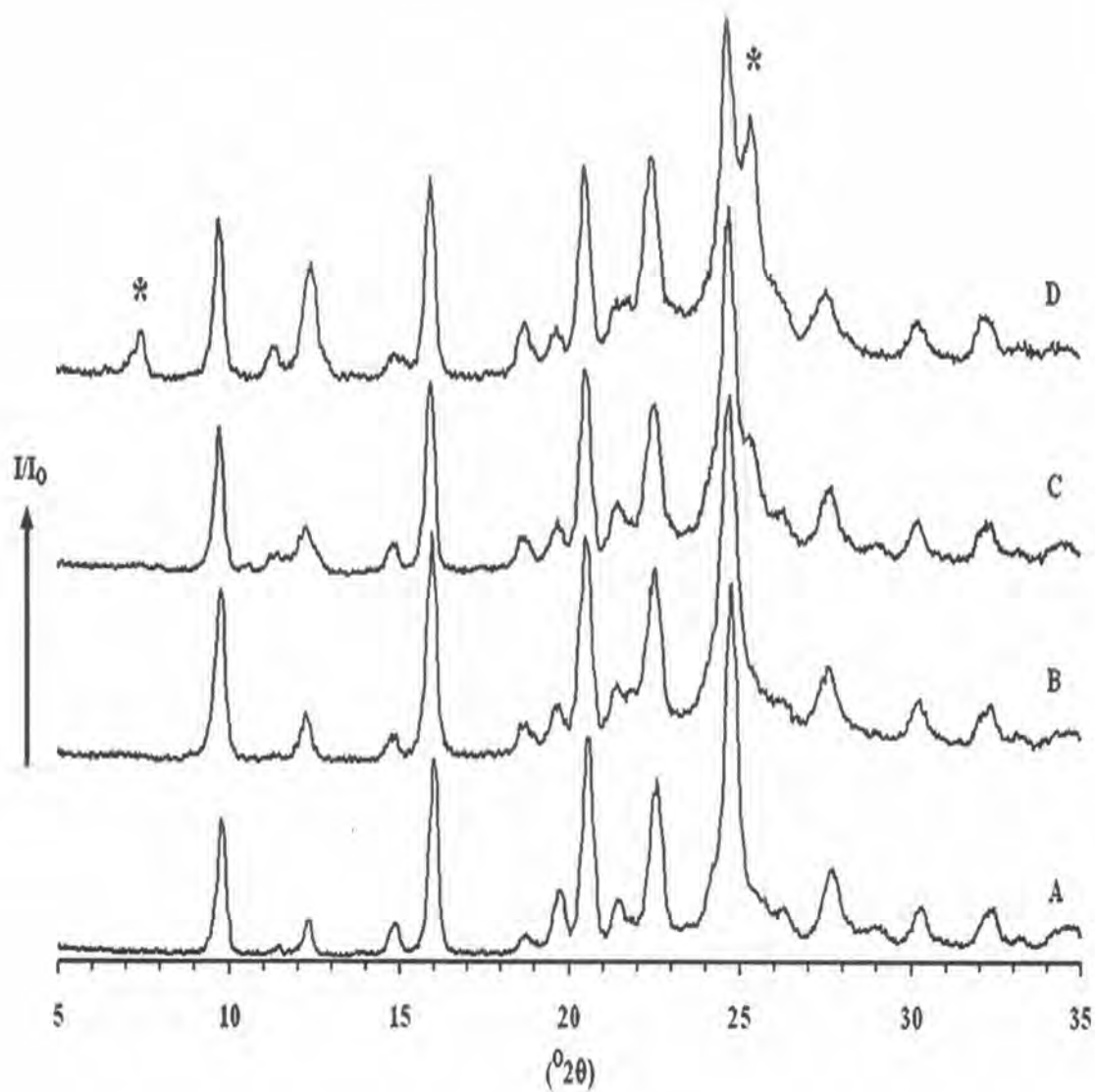


Figure 4.16 XRPD patterns of anhydrous NF Form A (A), disordered state NF (B), hemipentahydrate NF (C) and pentahydrate NF (D) after heated at 60°C for 48 hours

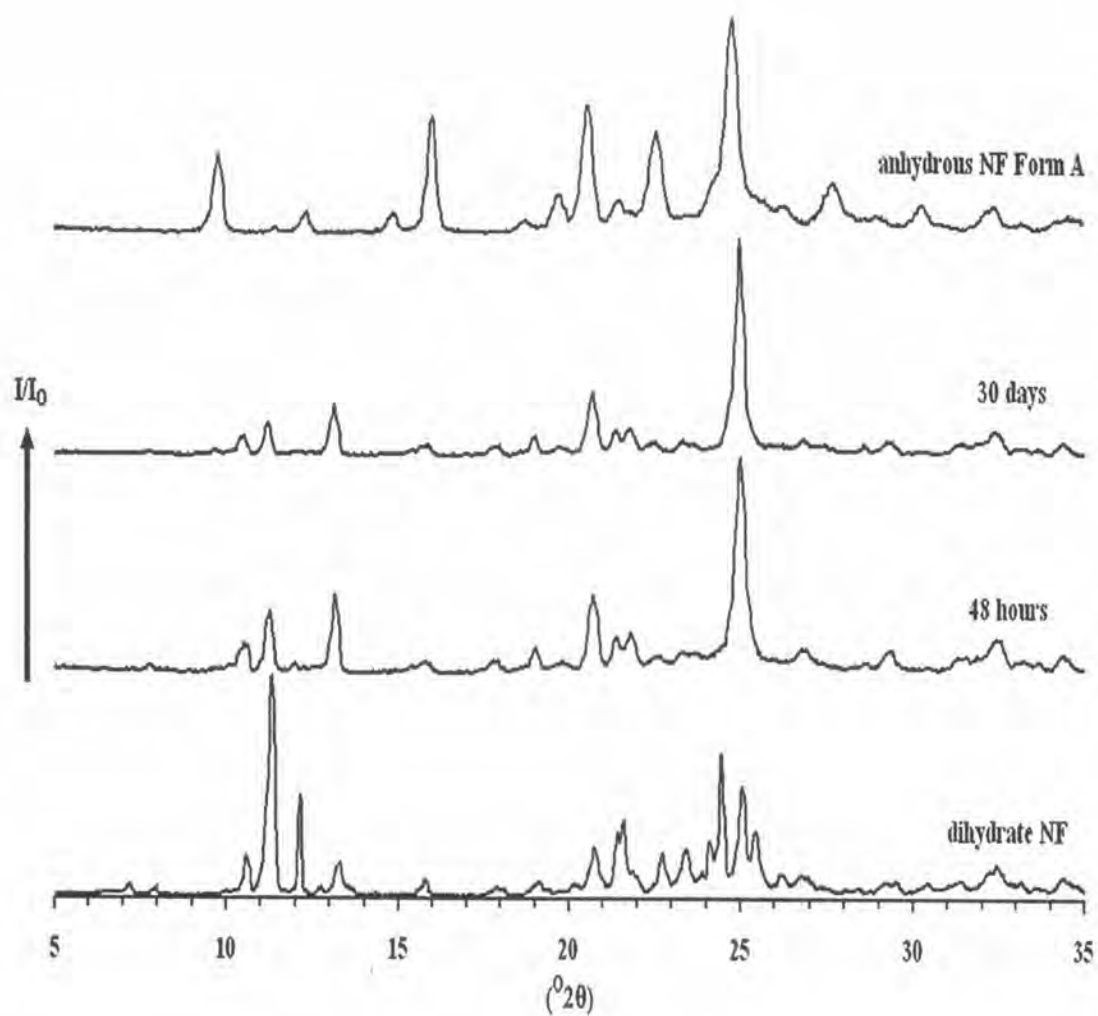


Figure 4.17 XRPD patterns of dihydrate NF after heated at 60°C for various time period

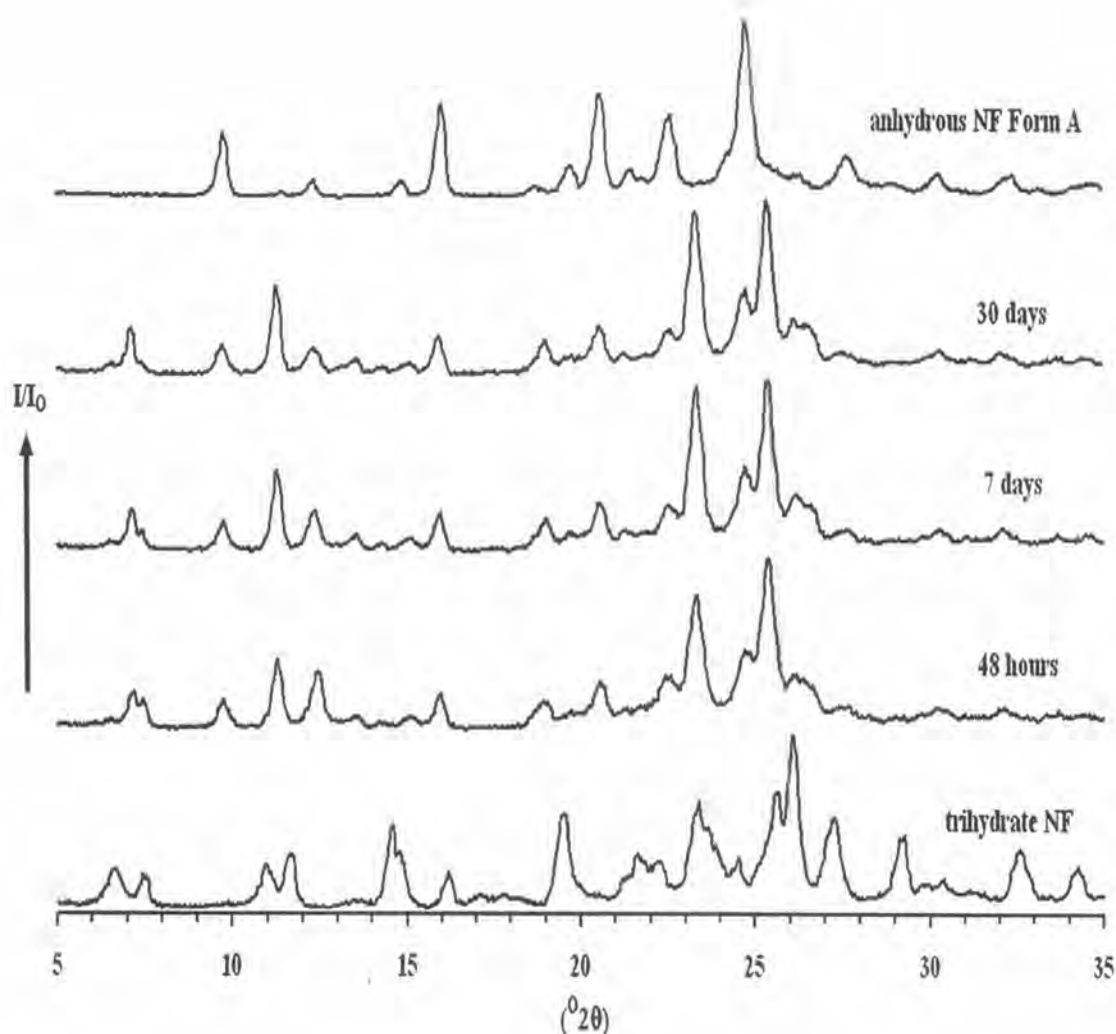


Figure 4.18 XRPD patterns of trihydrate NF after heated at 60°C for various time period

The solid state interconversion of NF hydrates is summarized in Figure 4.19. The anhydrous NF Form A and the other hydrate forms transformed to the pentahydrate NF when exposed to saturated water vapor. Meanwhile, the anhydrous NF Form A could be produced from thermal dehydration of the disordered NF state and hemipentahydrate NF. On the contrary, dihydrate NF, trihydrate NF and pentahydrate NF were not fully converted to the anhydrous NF Form A upon heating. Dehydration of NF hydrates with the aid of desiccant did not provide pure anhydrous NF Form A. Instead, it generated the disordered NF state from the pentahydrate NF.

The disordered NF state had specific rehydration behavior and instability against humidity such that it could easily be transformed to the pentahydrate NF starting at very low moisture of 32.8% RH compared to the anhydrous NF Form A where it needs 93.7% RH to convert to the pentahydrate NF.

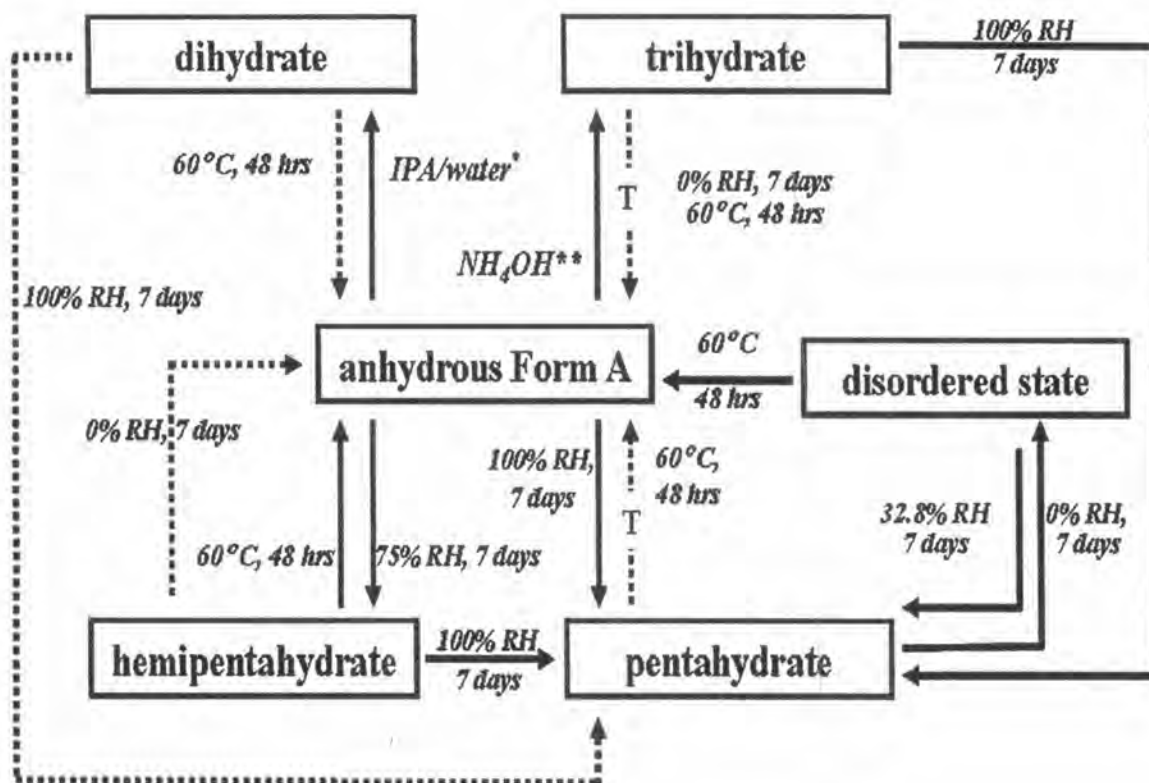


Figure 4.19 Summary of the solid state interconversion of anhydrous NF Form A and its hydrates (— = complete transformation, --- = incomplete transformation, T = hydrated transitional phase or hemipentahydrate NF, * = the dihydrate NF derived from recrystallization in the mixture of IPA and water, ** = the trihydrate NF generated by antisolvent precipitation from aqueous ammonia NF solution)

4.4 CONCLUSIONS

NF hydrates could be generated by various approaches. The approaches used in this study include slow recrystallization from mixture of IPA and water, direct exposure of the anhydrous Form A under 75% RH, precipitation from basic ammoniated solution with antisolvent mixture to produce the dihydrate NF, the hemipentahydrate NF and the trihydrate NF, respectively. In addition, direct exposure of the anhydrous NF Form A to 100% RH and dispersing the anhydrous NF Form A in water could also produced the pentahydrate NF. The pentahydrate NF formed from different methods of preparation possessed different crystal habits while the internal structures were identical. Interestingly, dehydration of the pentahydrate NF via reduction in moisture resulted in the disordered NF. The specific site of water of crystallization in NF hydrates was defined using single crystal analysis and hydrogen bonding between carboxylic groups could be detected using IR spectroscopy. The levels of environmental moisture greatly affected the transformation of not only the anhydrous NF Form A but also other stoichiometric hydrates. Extremely dry environment, 0% RH, was ineffective in withdrawing the internal water molecules from NF hydrates. On the other hand, water of crystallization was removed by moderate temperature elevation. However, the water of crystallization in the dihydrate and the trihydrate NF was partially removed by thermal energy and, thus, resulted in the mixture of the anhydrous Form A and their original hydrates. In conclusion, the information on the solid state characterization and interconversion of NF hydrates appraised in this study is crucial basic understanding for the generation and further introduction into the next part of study concerning the particle size reduction by thermal dehydration with the emphasis on the molecular level.