CHAPTER FOUR

NITROFURANTOIN–p-AMINOBENZOIC ACID COCRYSTAL

Activity of p-Aminobenzoic acid (PABA) on the hydration of Nitrofurantoin (NF) in aqueous medium. PABA controls hydration of NF as an additive (from 5 to 50% weight of NF) and renders NF stable to hydration as a coformer (57.6% of NF) by forming a 1:1 NF–PABA cocrystal.

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4.1 Introduction

Nitrofurantoin (abbreviated as NF; Figure 4.1) is an anti-bacterial drug used in the treatment of urinary tract infections\(^1\) and is on the WHO Model List of Essential Medicines.\(^2\) Polymorphism and pseudopolymorphism of NF are reported in the literature.\(^3\) Caira et al. reported two polymorphs of anhydrous Nitrofurantoin (\(\alpha\) and \(\beta\)) and two polymorphs of Nitrofurantoin monohydrate (Form I and Form II).\(^4\) NF \(\beta\) polymorph and monohydrate II are the stable anhydrate and monohydrate forms and exist in the marketed formulations.\(^4a,5\) The former has higher dissolution rate than the latter\(^3b,4a\) which is in accordance to the general observation that anhydrates tend to have higher dissolution rates than hydrates.\(^6\) The transformation of NF anhydrates to monohydrate II was observed during dissolution,\(^3b,4a\) pelletization\(^7\) and granulation.\(^8\) The transformation of anhydrate \(\beta\) polymorph is slower compared to \(\alpha\) polymorph with the latter showing comparable dissolution rate to that of the monohydrate II.\(^4a\) NF is a Class IV drug according to the Biopharmaceutics Classification System i.e. it has low solubility and low permeability (and bioavailability).\(^9\) A side effect of NF is nausea and emesis upon oral administration and is due to its high absorption rate.\(^10\) The same study\(^10\) reported that larger crystals (150 \(\mu\)m mesh size) of lower surface area and slower absorption reduced emesis but still conferred optimal therapeutic effect. The rapid initial dissolution rate of the stable \(\beta\) polymorph has been linked to the side effects of the drug by Caira et al.\(^4a\) Though it appears unlikely that a stable polymorph has a higher dissolution rate, the slower transformation of \(\beta\) polymorph to monohydrate II compared to metastable \(\alpha\) polymorph (which transforms faster to slow dissolving monohydrate II) renders it unimpeded during dissolution, thus leading to faster dissolution.\(^4a\) Considering these transformations, the drug is marketed as two different formulations to control fast dissolution/absorption and for sustained release purposes: macrocrystalline NF (brand name ‘Macrodantin’)\(^1,11\) and combination of macrocrystalline NF and NF monohydrate (brand name ‘Macrobid’).\(^12\)

Dehydration kinetics of NF monohydrate II\(^13\) and solution behavior of anhydrate \(\beta\) polymorph and monohydrate II has been studied.\(^14\) Several NF salts such as sodium and potassium,\(^15\) and L-arginine, L-lysine, L-histidine, L-ornithine and glycine\(^16\) were reported in the patent literature, but there is no study on the control of hydration and dissolution of the drug. A control over the dissolution rate can influence the absorption
rate which in turn may regulate the side effects of the drug. With the intent of controlling the hydration and dissolution behavior of Nitrofurantoin, the cocrystallization approach was undertaken in this study. Based on the potential heterosynthons possible with coformers containing complementary functional groups to those of NF, cocrystallization with \( p \)-aminobenzoic acid (PABA), urea and L-arginine was executed (Figure 4.1). Two 1:1 cocrystals (NF–PABA and NF–urea), a 1:1:1 salt hydrate (NF–L-arginine–H\(_2\)O) and a 1:1 methanol solvate (NF–MeOH) were obtained and their crystal structures were determined in this work. The hydration stability and dissolution rate of the adducts were compared with that of NF \( \beta \) polymorph and monohydrate II. NF–PABA cocrystal was found to be superior among the adducts in terms of minimal transformation to NF hydrate and comparable dissolution rate to the reference drug. Pseudopolymorphs and cocrystals of Nitrofurantoin were recently reported by other groups as well.

\[ \text{Nitrofurantoin (NF)} \quad \text{4-Aminobenzoic acid (PABA)} \quad \text{Urea} \quad \text{L-Arginine} \]

\[ \text{imide dimer} \quad \text{nitro furyl dimer} \quad \text{acid dimer} \]

\[ \text{nitro-amine} \quad \text{aminophenyl-nitro} \quad \text{acid-imide} \quad \text{urea-nitro} \quad \text{nitro-guanidium} \]

**Figure 4.1** (a) Molecular structures of Nitrofurantoin and coformers used in this study. (b) Some possible homosynthons and (c) heterosynthons between the molecular components in the adducts.

### 4.2 Crystallization of the Adducts

Commercial NF (Alfa Aesar) matches with \( \beta \) polymorphic modification (PXRD profile match in Figure 4.2) and the material was used for all experiments of this study. NF monohydrate form II was obtained upon grinding the material by adding few drops of
water (PXRD profile match in Figure 4.3a). Evaporative crystallization of NF with PABA, urea and L-arginine respectively in equimolar stoichiometry resulted in single crystals of the corresponding adducts (detailed in Experimental Section). A methanol solvate of NF was crystallized from methanol. X-ray crystallographic parameters are shown in Table 4.1 and hydrogen bonds in Table 4.2. Macroscopic amounts of the adducts were obtained upon solid state grinding (also called neat grinding)\textsuperscript{20} in case of cocrystals (NF–PABA and NF–urea) and water-assisted grinding for the L-arginine salt hydrate (PXRD profile match in Figure 4.3b-d).

**Figure 4.2** Overlay of the calculated lines from X-ray crystal structure (red) of NF \(\beta\) polymorph and the experimental PXRD pattern (black) of the commercial material shows peak-to-peak match.

![Overlay of the calculated lines from X-ray crystal structure](image)

(a) (b)

**Figure 4.3** Overlay of the calculated lines from X-ray crystal structures (red) and experimental PXRD patterns (black) show peak-to-peak match. (a) NF monohydrate form II obtained upon water-assisted grinding\textsuperscript{20} of NF. (b) NF–PABA and (c) NF–urea cocrystals and (d) NF–L-Arg–H\(_2\)O salt hydrate obtained upon neat grinding and water-assisted grinding of the respective components in equimolar stoichiometry.
Table 4.1 Crystallographic parameters.

<table>
<thead>
<tr>
<th>Molecular adduct</th>
<th>NF–PABA</th>
<th>NF–Urea</th>
<th>NF–L-Arg–H₂O</th>
<th>NF–MeOH</th>
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</thead>
<tbody>
<tr>
<td>empirical formula</td>
<td>C₈H₆N₄O₅⁻</td>
<td>C₈H₇NO₂⁻</td>
<td>C₈H₆N₄O₅⁻</td>
<td>C₆H₁₅N₄O₂⁻</td>
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<td>formula weight</td>
<td>375.30</td>
<td>298.23</td>
<td>430.40</td>
<td>270.21</td>
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<td>crystal system</td>
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<td>monoclinic</td>
<td>orthorhombic</td>
<td>monoclinic</td>
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<td>P̅1</td>
<td>P 2₁/n</td>
<td>P 2₁2₁2₁</td>
<td>P 2₁/c</td>
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<tr>
<td>Z</td>
<td>4</td>
<td>8</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>T/K</td>
<td>298(2)</td>
<td>100(2)</td>
<td>100(2)</td>
<td>298(2)</td>
</tr>
<tr>
<td>a/Å</td>
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<td>6.681(3)</td>
<td>5.7872(6)</td>
<td>6.4279(17)</td>
</tr>
<tr>
<td>b/Å</td>
<td>7.5460(12)</td>
<td>13.653(6)</td>
<td>15.3731(16)</td>
<td>6.7824(18)</td>
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<tr>
<td>c/Å</td>
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<td>13.189(6)</td>
<td>21.173(2)</td>
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<tr>
<td>α/°</td>
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<td>90</td>
<td>90</td>
<td>90</td>
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<tr>
<td>β/°</td>
<td>92.538(2)</td>
<td>97.460(7)</td>
<td>90</td>
<td>92.149(4)</td>
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<td>γ/°</td>
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<td>90</td>
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<td>90</td>
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<td>1192.9(9)</td>
<td>1883.7(3)</td>
<td>1169.4(5)</td>
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<td>1.661</td>
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<td>observed reflns.</td>
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<td>I&gt;2σ(I)]</td>
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<td>0.0539</td>
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<td>wR₂[all]</td>
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a Z = Z'' (no. of crystallographically non-equivalent molecules of any type in the asymmetric unit)²¹ × no. of independent general positions of the space group; b Ref. 18.

Table 4.2 Hydrogen bonds in crystal structures of the adducts.*

<table>
<thead>
<tr>
<th>Interaction</th>
<th>H···A/Å</th>
<th>D···A/Å</th>
<th>ZD–H···A/°</th>
<th>Symmetry code</th>
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<tr>
<td>NF–PABA</td>
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<td></td>
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<tr>
<td>N4–H4···O5</td>
<td>1.83</td>
<td>2.827(2)</td>
<td>165.3</td>
<td>–x, 1–y, 1–z</td>
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<tr>
<td>N5–H5A···O4</td>
<td>2.44</td>
<td>3.204(2)</td>
<td>131.5</td>
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<tr>
<td>N5–H5B···O3</td>
<td>2.25</td>
<td>3.265(2)</td>
<td>176.3</td>
<td>1–x, 2–y, 2–z</td>
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<tr>
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<td>2.589(2)</td>
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<td>2.50</td>
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<td>N4–H4···O6</td>
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<td>2.49</td>
<td>3.308(3)</td>
<td>137.1</td>
<td>x, y, 1+z</td>
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99
<table>
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<th>Symmetry</th>
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<tr>
<td>N6–H6A···O6</td>
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<td>C3–H3···O5</td>
<td>2.35</td>
<td>152.9</td>
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<td>2.70</td>
<td>146.1</td>
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**NF–L-Arg–H₂O**

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<td>164.5</td>
<td>1/2+x, 1/2–y, 1–z</td>
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<tr>
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<td>124.8</td>
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**NF–MeOH**

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<tr>
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<th>Distance</th>
<th>Angle</th>
<th>Symmetry</th>
</tr>
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<tr>
<td>N4–H4···O6</td>
<td>1.77</td>
<td>172.7</td>
<td>–x, 1/2+y, 3/2–z</td>
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<td>O6–H6···O5</td>
<td>1.83</td>
<td>174.0</td>
<td>1–x, –1/2+y, 3/2–z</td>
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<td>C2–H2···O3</td>
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<td>137.1</td>
<td>1+x, y, z</td>
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* O–H, N–H and C–H distances are neutron-normalized to 0.983, 1.009 and 1.083 Å respectively.

### 4.2.1 Cocrystal of NF and PABA

The crystal structure solved in the space group $P\overline{1}$ and contains each of the NF and PABA molecules in the asymmetric unit. NF molecules are hydrogen-bonded via the imide dimer and PABA molecules are connected via the acid dimer homosynthons (Figure 4.4). Such dimeric units are connected via the N–H···O + C–H···O two-point
aminophenyl–nitro motif into zigzag tapes which extend into 2D sheets through N–H⋯O and bifurcated C–H⋯O interactions (Figure 4.4). The 2D sheets are sustained by auxiliary C–H⋯O interactions and lie at an interplanar separation of 3.6 Å. An alternative crystal structure (polymorph) of this cocrystal was anticipated with acid–imide heterosynthons (Figure 4.1) between NF and PABA, but such a structure was not realized experimentally. Solid state grinding of the components in equimolar stoichiometry resulted in the same cocrystal. This cocrystal was found to be stable to slurry crystallization in water suggesting that it is a stable form (discussed later).

![Figure 4.4 NF and PABA molecules are hydrogen-bonded via imide dimer and acid dimer homosynthons, which are in turn connected by aminophenyl-nitro N–H⋯O and C–H⋯O bonds to form zigzag tapes that extend into a sheet structure through N–H⋯O and bifurcated C–H⋯O interactions.](image)

**4.2.2 Cocrystal of NF and Urea**

The crystal structure solved in the space group $P2_1/n$ and contains each of the NF and urea molecules in the asymmetric unit. There is no two-point urea-nitro synthon (Figure 4.1) in this crystal structure. A linear tape of nitrofuryl C–H⋯O dimers of NF molecules is connected through N–H⋯O hydrogen bonds to the urea dimer (Figure 4.5a). Such parallel tapes form offset stacks along the $a$-axis through C–H⋯O interactions (Figure 4.5a). The non-planar consecutive tapes along the $c$-axis make an angle of 28.2° with each other and are connected through auxiliary interactions in 3D (Figure 4.5b).
Figure 4.5 (a) Linear tapes of nitrofuryl C–H···O dimers of NF molecules are connected through N–H···O bonds to the urea dimer. The parallel offset tapes are connected via C–H···O interactions. (b) Adjacent tapes (shown as ball-stick and capped-stick models for clarity) along c-axis make an angle of 28.2° with each other.

4.2.3 Hydrate of NF and L-Arginine Salt

A salt of Nitrofurantoin and L-arginine with a solvent of crystallization was reported in a patent but without any X-ray crystal structure details.\textsuperscript{16} When an equimolar mixture of NF and L-arginine was crystallized in acetonitrile–isopropanol solvent mixture (in 1:1 v/v), a 1:1:1 NF–L-Arg–H\textsubscript{2}O salt crystal was obtained.\textsuperscript{18} It solved in the space group \textit{P}2\textsubscript{1}2\textsubscript{1}2\textsubscript{1} and contains each of the NF, L-arginine and water molecules in the asymmetric unit. The imine proton of NF is transferred to the \(\alpha\)-amino group of L-arginine. There is no two-point nitro-guanidium synthon (Figure 4.1) between NF and L-arginine molecules, instead N–H···O and N–H···N hydrogen bonds between imide and guanidium groups connect the molecules and form a tape along [106] axis (Figure 4.6a). The tapes are arranged in a herringbone T-motif which makes channels for water inclusion along the \(a\)-axis (Figure 4.6b).
Solid state grinding of the components (to avoid contact with water) did not afford an anhydrous salt as monitored by PXRD. Attempts to dehydrate the salt hydrate did not succeed as the material decomposed abruptly around 180 °C as monitored by DSC (exotherm) and TGA (weight loss) (Figure 4.7). The measured weight loss of 27% in TGA corresponds to the theoretical loss of four volatile components from the salt hydrate – CO₂, H₂O, NH₃ and NO₂ (calculated 29.04%). The elemental analysis of the decomposed material showed C 51.12%, H 5.82%, N 27.35%, and O 15.71% (remaining), which matches with the empirical formula C₁₃H₁₈N₆O₃ that can be obtained by the loss of the above volatile components. This shows that the molecular adduct is stable with a water of crystallization in the lattice.
4.2.4 NF methanolate

A 1:1 methanol solvate (NF–MeOH) was crystallized from methanol (detailed in Experimental Section). In the crystal structure, a tape of NF molecules assembled via C–H···O interactions connects to methanol molecules through O$_{\text{MeOH}}$–H···O$_\text{NF}$ hydrogen bond. The tapes of Nitrofurantoin molecules form a herringbone motif, which makes channels of methanol molecules parallel to the $b$-axis (Figure 4.8).

![Figure 4.8](a and b) A tape of NF molecules formed by C–H···O interactions connects the channel of solvent methanol through O$_{\text{MeOH}}$–H···O$_\text{NF}$ hydrogen bonds along the $a$-axis. (c) Screw related methanol molecules reside in channels parallel to the $b$-axis and the overall crystal structure has a herringbone motif.

The methanolate crystals turned opaque after complete evaporation of the solvent and slowly converted to NF monohydrate form II at ambient temperature and humidity as monitored by PXRD (Figure 4.9). Guest exchange of NF methanolate with water was instant as the crystals opaqued immediately when suspended in water and converted to monohydrate II. The channel structure of NF methanolate could be a reason for its low stability and facile transformation to the hydrate form. Though there are no apparent similarities between the methanolate and hydrate II crystal structures, except that the guest molecules reside in channel/cavity along the $b$-axis in the structures, presence of atmospheric moisture and the higher stability of the latter seem to favor the transformation instantly. DSC of NF methanolate showed an endotherm at 105 °C prior to melting and decomposition (270 °C) which is due to desolvation of methanol as observed from the weight loss of the material in TGA at the corresponding temperature
(experimental 11.83% and calculated 11.85% for 1 mole of MeOH) (Figure 4.10). Desolvation of solvates is known result in new polymorphs$^{23}$ and accordingly NF methanolate was subjected to controlled desolvation at 125 °C for 1 h. The resultant powdery material was found to be the stable NF $\beta$ polymorph by PXRD profile match (with reference to Figure 4.2).

**Figure 4.9** Transformation of NF methanolate to NF monohydrate II with time monitored by PXRD.

**Figure 4.10** DSC (black) and TGA (red) of NF methanolate. The weight loss in TGA is consistent with one mole of MeOH in the solvate structure.
4.3 Solubility and Dissolution study of the Adducts

Solubility and dissolution are important physico-chemical parameters that influence the bioavailability of drugs. Solubility is the concentration of the substance at equilibrium between the solution and the undissolved solid. The rate at which this equilibrium state is reached is the dissolution rate. The former is a thermodynamic parameter while the latter is a kinetic indicator. The extent of drug dissolved in a particular time period is measured by the dissolution rate. During equilibrium solubility conditions (high supersaturation, agitation, long duration, typically 24 h), a solid drug form may dissociate or transform due to polymorphic change, hydrate formation, precipitation etc. Nevertheless, the formulation is advantageous if it facilitates drug release (in solution for absorption and consequent pharmacological action) within a desirable time-frame before it is destabilized. This evaluation whether the drug is sustained in the medium for the therapeutic retention time (usually 0.5–8 h) is achieved through a dissolution study.

Nitrofurantoin is a BCS Class IV drug with low water solubility. The reported aqueous solubility values of NF are quite far apart, 80 mg/L and 190 mg/L at 25 °C. In this study, equilibrium solubility value obtained for NF (82 mg/L) at ambient temperature matched with the lower number. Caira et al. suggested that as anhydrous Nitrofurantoin (commercial NF form of this study) transforms to monohydrate II during solubility testing, the measured solubility of the drug actually corresponds to NF monohydrate II. The above transformation was confirmed by PXRD of the undissolved drug after the solubility experiment in this work. The transformation demonstrates higher stability of NF monohydrate II than the anhydrate form at ambient temperature and humidity. Similarly, equilibrium solubility experiments were done on NF–PABA, NF–urea and NF–L-Arg–H2O in water at 25 °C. Only the solubility of NF–PABA cocrystal could be determined (216 mg/L is the molar equivalent solubility of NF in the cocrystal) because the other two adducts transformed to NF monohydrate II within one hour of the solubility experiment (analyzed by PXRD of the undissolved residue). NF–PABA cocrystal did not show any transformation to any of the NF forms (polymorph, hydrate) and was quite stable even after 3 days in the equilibrium solubility conditions. The solubility of NF–PABA cocrystal follows the ‘coformer solubility rule’ i.e. high solubility coformer (PABA aqueous solubility 5.8 g/L) led to high solubility cocrystal. From a different perspective, this also means that low solubility NF resulted in even less
soluble NF–PABA cocrystal with respect to PABA. Thus, the compensation of solubility of NF (low) and PABA (high) resulted in intermediate solubility for the cocrystal.

As the NF–PABA cocrystal was found to be resistant to hydration, the effect of PABA as an additive in reducing the hydration of the drug in water was studied. With increase in the % of PABA to NF (from 5% to 60% by weight) in water slurry medium, a steady increase in the concentration of NF–PABA cocrystal and decrease in NF monohydrate II content in the residue (Figure 4.11) was observed as analyzed by PXRD (Table 4.3). This shows that PABA reacted with NF to form stable NF–PABA cocrystal in solution. Complexation between NF and PABA was observed as a visible color change of the solution from yellow to orange (NF/ NF–H₂O II is yellow, PABA is white and NF–PABA is orange), while the excess NF transformed to the monohydrate. The gradual change in the color of the residue (from yellow to orange) with increase in the concentration of NF–PABA cocrystal is shown in Figure 4.12. At 57.6% of PABA-to-NF (molar proportion of components in the 1:1 cocrystal), there was no trace of NF monohydrate II in the residue and the material was entirely NF–PABA cocrystal (with reference to PXRD pattern in Figure 4.3b). This shows that PABA was effective as a coformer in the 1:1 cocrystal composition to make NF stable to hydration and also as an additive in reducing its hydration.

Figure 4.11 Variation in the composition of NF–PABA cocrystal and NF monohydrate II in the water slurry residue with increase of PABA concentration.
Table 4.3 Activity of PABA on NF in water slurry medium.

<table>
<thead>
<tr>
<th>% of PABA to NF in water slurry</th>
<th>% of NF–PABA in residue</th>
<th>% of NF monohydrate II in residue</th>
<th>$R_p$ from PXRD</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>10</td>
<td>90</td>
<td>0.27</td>
</tr>
<tr>
<td>10</td>
<td>32</td>
<td>68</td>
<td>0.26</td>
</tr>
<tr>
<td>20</td>
<td>48</td>
<td>52</td>
<td>0.22</td>
</tr>
<tr>
<td>30</td>
<td>71</td>
<td>29</td>
<td>0.25</td>
</tr>
<tr>
<td>40</td>
<td>88</td>
<td>12</td>
<td>0.33</td>
</tr>
<tr>
<td>50</td>
<td>92</td>
<td>8</td>
<td>0.26</td>
</tr>
<tr>
<td>57.6</td>
<td>100</td>
<td>0</td>
<td>0.15</td>
</tr>
<tr>
<td>60</td>
<td>100</td>
<td>0</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Figure 4.12 Color change of water slurry residue from yellow to orange with increase in the concentration of NF–PABA cocrystal.

Nitrofurantoin is a typical drug whose rapid initial dissolution and consequent faster absorption leads to side effects of nausea and emesis after oral administration,\textsuperscript{10} though being a BCS Class IV drug (low solubility and low permeability).\textsuperscript{9} The dissolution profile of the drug is variable: after a high initial dissolution the drug is released (dissolved) slowly because it converts to the more stablehydrate in aqueous medium (anhydrate $\beta$ with faster dissolution rate $\rightarrow$ monohydrate II with slower dissolution rate).\textsuperscript{4a} Hence, the drug is administered as macrocrystalline NF\textsuperscript{11} and a combination of macrocrystalline NF and NF monohydrate\textsuperscript{12} to facilitate slower dissolution and reduce the side effects due to faster absorption. Therefore, a drug formulation which can regulate the dissolution and hydration of NF is desirable. In this context, dissolution behavior of the adducts (NF–PABA, NF–urea and NF–L-Arg–H\textsubscript{2}O) was compared with that of NF $\beta$ polymorph and NF monohydrate II, in three different media viz. pure water (pH 6.4), 0.1 N HCl (pH 1.2) and disodium hydrogen phosphate buffer (pH 6.8) at 37 °C.\textsuperscript{18} The intrinsic dissolution rates (IDRs) of the compounds were estimated based on their individual molar extinction coefficients in the respective medium (Table 4.4). The IDR of the compounds was found to be higher for the first 30 min of the dissolution experiment and the value decreased with time except in a few
cases (Table 4.5). The IDR in water followed the order: NF–L-Arg–H₂O > NF–PABA > NF β > NF hydrate II > NF–urea for the first 30 min and NF–L-Arg–H₂O > NF–PABA > NF β > NF–urea > NF hydrate II for 4 h (Table 4.5 & Figure 4.13). In 0.1 N HCl the order is NF–L-Arg–H₂O > NF–PABA > NF–urea > NF β > NF hydrate II and the same trend is observed in pH 6.8 buffer (Table 4.5 & Figure 4.13).

Table 4.4 Molar extinction coefficients of the compounds in different media.

<table>
<thead>
<tr>
<th>Molar extinction coefficient, mL mg⁻¹ cm⁻¹</th>
<th>NF β</th>
<th>NF monohydrate II</th>
<th>NF–Urea</th>
<th>NF–PABA</th>
<th>NF–L-Arg–H₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>73.69</td>
<td>68.31</td>
<td>58.41</td>
<td>46.10</td>
<td>43.56</td>
</tr>
<tr>
<td>0.1 N HCl</td>
<td>68.73</td>
<td>61.04</td>
<td>51.02</td>
<td>43.91</td>
<td>37.04</td>
</tr>
<tr>
<td>pH 6.8 buffer</td>
<td>71.61</td>
<td>66.70</td>
<td>57.24</td>
<td>44.80</td>
<td>40.85</td>
</tr>
</tbody>
</table>

Table 4.5 IDR s of the compounds in different media.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Water</th>
<th>0.1 N HCl</th>
<th>pH 6.8 buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDR in µg cm⁻² min⁻¹ (for 30 min)</td>
<td>IDR in µg cm⁻² min⁻¹ (for 30 min)</td>
<td>IDR in µg cm⁻² min⁻¹ (for 30 min)</td>
<td></td>
</tr>
<tr>
<td>IDR in µg cm⁻² min⁻¹ (for 4 h)</td>
<td>IDR in µg cm⁻² min⁻¹ (for 4 h)</td>
<td>IDR in µg cm⁻² min⁻¹ (for 4 h)</td>
<td></td>
</tr>
<tr>
<td>NF β</td>
<td>44.2</td>
<td>37.1</td>
<td>39.7</td>
</tr>
<tr>
<td>NF monohydrate II</td>
<td>37.8</td>
<td>33.9</td>
<td>27.3</td>
</tr>
<tr>
<td>NF–Urea</td>
<td>32.5</td>
<td>36.5</td>
<td>39.9</td>
</tr>
<tr>
<td>NF–PABA</td>
<td>58.5</td>
<td>54.4</td>
<td>50.3</td>
</tr>
<tr>
<td>NF–L-Arg–H₂O</td>
<td>119.6</td>
<td>107.1</td>
<td>75.9</td>
</tr>
</tbody>
</table>
The IDR of NF solid forms was higher in phosphate buffer compared to pure water and least in acidic medium (Table 4.5), indicating the role of pH in drug dissolution. The higher dissolution rate of NF $\beta$ polymorph than NF monohydrate II is consistent with previous reports.$^{3b,4a}$ The IDR of NF–urea cocrystal is closer to both NF $\beta$ polymorph and NF monohydrate II in the three media (Table 4.5). The lower IDR of NF–urea cocrystal is attributed to its faster dissociation and consequent transformation of unbound NF on the exposed surface of the dissolution tablet (also called disk or pellet) to NF monohydrate II. Earlier, Caira et al.$^{4a}$ reasoned the closeness of dissolution rates of NF $\alpha$ polymorph and NF monohydrate II as due to the transformation of the former to the latter such that the dissolution rate actually measured was that of the growing layer of
NF monohydrate II on the tablet surface exposed to aqueous medium, and confirmed it through DSC analysis of tablet surface. Similar to the case, the PXRD analysis of undissolved material of the NF–urea cocrystal (recovered after dissolution experiment) revealed NF monohydrate II content (about 25% in 0.1 N HCl and 30% in pH 6.8 buffer media) demonstrating partial transformation of the former to the latter (Figure 4.14). The NF–PABA cocrystal which is stable to hydration in water (in equilibrium solubility conditions) was also stable in water as the dissolution medium, but partially converted to NF monohydrate II in 0.1 N HCl (22% NF monohydrate II content by PXRD analysis, Figure 4.15) and pH 6.8 buffer media (14% NF monohydrate II content). This variation in the physicochemical behavior of NF–PABA cocrystal can be due to change in the ionization state of PABA (having two ionizable groups – carboxylic acid and amine) at different acidity levels, resulting in cocrystal dissociation and causing unbound NF to hydrate. The higher IDR of NF–PABA cocrystal compared to that of the NF–urea (Table 4.5) is attributed to its slower transformation to NF monohydrate II as observed from its higher content in the undissolved material at the end of dissolution experiments. Thus, the dissolution behavior of NF–PABA cocrystal is similar to that of NF $\beta$ polymorph, which was known to exhibit higher IDR and slower transformation.\textsuperscript{4a}

![Overlay of the calculated X-ray crystal structures of NF–urea (red) and NF monohydrate II (blue) on the PXRD pattern of NF–urea cocrystal subjected to dissolution in pH 6.8 buffer medium (black) shows 70:30 composition by Rietveld refinement ($R_p = 0.15$).](image)

**Figure 4.14** Overlay of the calculated X-ray crystal structures of NF–urea (red) and NF monohydrate II (blue) on the PXRD pattern of NF–urea cocrystal subjected to dissolution in pH 6.8 buffer medium (black) shows 70:30 composition by Rietveld refinement ($R_p = 0.15$).
In contrast, NF–L-Arg–H₂O salt exhibited highest IDR of all compounds (Table 4.5) even though it completely transformed to NF monohydrate II in all the three media (analyzed by PXRD with reference to Figure 4.3a). The dissolution behavior of this salt conforms to Nangia’s model of cocrystal solubility.²⁹ According to the model, a cocrystal (or salt) containing a high soluble coformer (or salt former) can facilitate faster dissolution of a low soluble component. This happens via the fast release of high soluble coformer into aqueous medium (because of its higher affinity to the latter) that results in the dissociation of cocrystal, thereby leaving behind the low soluble component in an amorphous/randomized state, which understandably leads to an increase in the solubility/dissolution of the low soluble component.²⁹ In this case, before the unbound NF (formed upon release of L-arginine from the lattice) hydrates, it is believed that much of it actually dissolves in the medium thus giving rise to higher IDR values. The same behavior (high IDR despite transformation to slow dissolving monohydrate II) was not observed for the NF–urea cocrystal which showed low IDR values close to that of the monohydrate II upon transformation (Table 4.5). This anomalous behavior of NF–urea cocrystal, despite greater aqueous solubility of urea (1.21 g/mL)³⁰ than L-arginine (0.18
g/mL), could be due to relative differences between solute-solute (NF and urea/L-arginine) interactions and solute-solvent interactions which govern dissolution kinetics. In all, both NF–PABA and NF–urea cocrystals are more stable and exhibited desirable low IDRs compared to NF–L-Arg–H2O salt in the aqueous dissolution experiments. The significance and take home point of these findings is that it runs contrary to the popular belief that salts are more preferable to cocrystals for drug formulation.

4.4 Conclusions

Cocrystallization with a few coformers was evaluated as a pharmaceutical development methodology to control the hydration and dissolution behavior of Nitrofurantoin. All the adducts except NF methanolate were found to be stable at ambient temperature and humidity conditions. Crystal structure analysis of the cocrystals NF–PABA and NF–urea suggest a possibility of polymorphs for the cocrystals based on the lack of expected supramolecular synthons in the manifested structures. Both the cocrystals come under the category of pharmaceutical cocrystals because of the GRAS (Generally Recognized as Safe) status of the coformers. The two cocrystals showed desirable physicochemical properties viz. hydration stability (NF–PABA) and lower dissolution rate (NF–urea) compared to NF–L-Arg–H2O salt. Among the three adducts, NF–PABA cocrystal is least susceptible to transformation to NF monohydrate II in the three media of different pHs. PABA was found to control hydration of NF both as an additive (from 5 to 50% of NF) and a coformer (57.6% of NF) by forming NF–PABA cocrystal. Thus, NF–PABA combination can be useful as a novel/alternate formulation that can control hydration and dissolution and consequently the absorption rate of the drug. The amount of actual drug absorbed can be adjusted by modifying PABA content in the NF drug formulation. This limited study suggests that, in some cases, the relatively new cocrystals methodology can be advantageous than the conventional salt forms for controlling the physicochemical properties of drugs.

4.5 Experimental Section

Materials and Methods: Commercially available Nitrofurantoin (Alfa Aesar) was used without further purification. All other chemicals were of analytical or chromatographic grade. Water filtered through a double deionized purification system (Milli Q Plus Water System from Millipore Co., USA) was used for experiments.
Crystallization of the molecular adducts

NF–PABA: NF (23.8 mg, 0.1 mmol) and PABA (13.7 mg, 0.1 mmol) were dissolved in 5 mL hot acetonitrile and left for slow evaporation at room temperature. Brownish red crystals were formed after a few days upon solvent evaporation. The cocrystal has no definite melting point and started to decompose from 210 °C (m.p. of NF 263 °C, m.p. of PABA 186 °C).

NF–Urea: A powdered mixture of NF (46.8 mg, 0.2 mmol) and urea (12 mg, 0.2 mmol) was dissolved in 4 mL hot DMF–dioxane solvent mixture (1:1 v/v) and left for slow evaporation at room temperature. Yellow crystals were formed after few days upon solvent evaporation. The cocrystal has no definite melting point and started to decompose at 160 °C (m.p. of urea 133 °C).

NF–L-Arg–H2O: A powdered mixture of NF (23.8 mg, 0.1 mmol) and L-arginine (17.4 mg, 0.1 mmol) was dissolved in 6 mL of hot 1:1 acetonitrile–isopropanol solvent mixture (1:1 v/v). Brown crystals of 1:1:1 NF–L-Arg–H2O were formed after a few days upon solvent evaporation at room temperature. The salt has no definite melting point and started to decompose at 170 °C (m.p. of L-arginine 222 °C).

NF–MeOH: 50 mg NF was dissolved in 15 mL hot methanol and left for slow evaporation at room temperature. Yellow crystals in equilibrium with the mother liquor formed after one day were filtered and used for characterization and experiments.

Grinding: The cocrystals were prepared in bulk quantity by neat grinding and the hydrates (salt hydrate and NF monohydrate II) by water-assisted grinding. About 200 mg of the components, combined together as per the stoichiometric ratio in the crystal structure, was ground for 15-20 min using a mortar-pestle. 8-10 drops water was added during grinding in case of water-assisted grinding. PXRD and melting point of the ground material was recorded to confirm complete reaction of starting materials and formation of a new crystalline phase.

X-ray Crystallography: X-ray reflections were collected on Bruker SMART-APEX CCD diffractometer equipped with a graphite monochromator and Mo-Kα (λ = 0.71073 Å) fine-focus sealed tube. Data reduction was performed using Bruker SAINT software. Intensities were corrected for absorption using SADABS. Structures were solved and refined using SHELX-97 with anisotropic displacement parameters for non-H atoms. Hydrogen atoms on O and N were experimentally located in difference electron density
maps. All C–H atoms were fixed geometrically using HFIX command in SHELX-TL. The final CIF files and hydrogen bond geometries were validated in PLATON. X-Seed was used to prepare packing diagrams.

**Powder X-ray Diffraction:** Powder X-ray diffraction of the samples were recorded on Bruker D8 Advance diffractometer using Cu-Kα X-radiation ($\lambda = 1.5406$ Å) at 40 kV and 30 mA. Diffraction patterns were collected over 2θ range of 5-50° at scan rate of 1° min⁻¹. Powder Cell 2.4 was used for Rietveld refinement.

**Thermal Analysis:** DSC was performed on a Mettler Toledo DSC 822e module and TGA on a Mettler Toledo TGA/SDTA 851e module. The typical sample size is 3-5 mg for DSC and 8-12 mg for TGA. Samples were placed in sealed pin-pricked aluminum pans for DSC experiments and alumina pans for TGA experiments. A heating rate of 5 °C min⁻¹ in the temperature range 30-300 °C was applied. Samples were purged by a stream of dry nitrogen flowing at 80 mL min⁻¹ for DSC and 50 mL min⁻¹ for TGA.

**CHN Analysis:** Microanalysis was performed on ThermoFinnigan/EA 1112 CHNS analyzer on a 5 mg sample.

**Equilibrium solubility and Intrinsic dissolution measurements:** Prior to solubility and dissolution measurements, calibration curves of each of the compounds in all the three media (water, 0.1 N HCl and pH 6.8 buffer) were obtained and their molar extinction coefficients were determined spectrophotometrically (Table 4.5) on a Thermo Scientific Evolution 300 UV-Vis spectrometer based on the absorbance at 368 nm ($\lambda_{\text{max}}$ of Nitrofurantoin devoid of interference from other compounds). The respective molar extinction coefficients of the compounds were used to estimate solubility and dissolution values. Equilibrium solubility was determined in water using the shake-flask method. 100 mg of powdered compound was added to 5 mL water and the resulting suspension was stirred at 25 °C for 24 h. The suspension was equilibrated for one hour and then filtered through 2.5 µm Whatman filter paper. The concentration of the solution thus obtained was determined spectrophotometrically after appropriate dilution using the molar extinction coefficients of the respective compounds. IDR experiments in water, 0.1 N HCl and pH 6.8 buffer were carried on a USP certified Electrolab TDT-08L Dissolution Tester for 4 hours by the disk intrinsic dissolution rate (DIDR) method. The pH 6.8 buffer was prepared as per the International Pharmacopoeia (3rd edition, 2003). For IDR testing, 100 mg of the compound was taken in the intrinsic attachment
and compressed to a 0.5 cm$^2$ disk using a hydraulic press at a pressure of 2.5 ton inch$^{-2}$ for 5 min. The intrinsic attachment was placed in a jar of 500 mL medium preheated to 37 °C and rotated at 150 rpm. Aliquots of 5 mL were collected at specific time intervals and concentration of the aliquots was determined spectrophotometrically using the molar extinction coefficients of the respective compounds. The linear region of the dissolution profile (regression $>0.99$) was used to determine the IDR of the compound as (slope of the amount dissolved ÷ surface area of the disk) per unit time. The identity of the undissolved materials after solubility and dissolution experiments was established through PXRD. There is no transformation of the compounds upon compression.

4.6 References


33. GRAS/EAFUS (Everything Added to Food in the United States) substances list: [http://www.fda.gov/Food/FoodIngredientsPackaging/ucm115326.htm](http://www.fda.gov/Food/FoodIngredientsPackaging/ucm115326.htm).