Thermodynamic stability relationship of ternary and binary cocrystals of isoniazid: why pH and coformer concentration matter

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Supplementary information

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1. General information

1.1 Materials

INH and coformers (fumaric acid and nicotinamide) were purchased from Sigma-Aldrich and Pharma Nostra, respectively. High-performance liquid chromatography (HPLC)-grade acetonitrile and all chemicals and reagents of analytical grade were used as received. Water was obtained from the Milli-Q system (Millipore).

2. Preparation of cocrystals

Cocrystals were prepared by reaction crystallization method (RCM) by dissolving equimolar amounts of each cocrystal component in methanol.[1] The slurry was stirred for 24 h at room temperature and then filtered using a vacuum filtration apparatus. The phase purity of all solids obtained was verified by differential scanning calorimetry (DSC) and powder X−ray diffraction (PXRD).

3. Cocrystal solubility measurement

Cocrystal equilibrium solubility was determined in water (pH 6.3), pH 1.2 (HCl 0.08M) and PBS pH 7.4 (phosphate buffered saline) at the eutectic point, where two solid are in equilibrium with solution.[2] The eutectic point was achieved by adding excess cocrystal and coformer (FUM) in each solution media. At intervals of 6, 24, and 48 h, 1 mL aliquots were collected and filtered through a 0.45 µm polyamide membrane, and the pH values were measured. Drug and coformer concentrations were analyzed by HPLC. Solid phases were analyzed by PXRD to confirm that the samples were at the eutectic point, which was verified by the presence of both coformer and cocrystal solid phases. Cocrystal solubility were obtained from total concentrations of drug and coformer measured at the eutectic point ($[drug]_{T,eu}$ and $[coformer]_{T,eu}$), and the solubility product (K_{sp}) for cocrystals were obtained from nonionized concentrations of drug and coformer according to the following equations:

For 2:1 cocrystal

$$K_{sp} = [drug]_{aq}^{2}[coformer]_{aq} \quad (1)$$

For 1:1:1 cocrystal

$$K_{sp} = [drug]_{aq}[coformer\ 1]_{aq}[coformer\ 2]_{aq} \quad (2)$$

The cocrystal stoichiometric solubility was calculated from measured total eutectic concentrations of drug and coformer ($[drug]_{T,eu}$ and $[coformer]_{T,eu}$) (Table S1) according to the following equations:

For 2:1 cocrystal

$$S_{cc}^{2:1} = 2\left(\sqrt[3]{\frac{(INH)_{T,eu}^{2}\,(FUM)_{T,eu}}{4}}\right) \quad (3)$$

For 1:1:1 cocrystal

$$S_{cc}^{1:1:1} = \sqrt[3]{[INH]_T\,[NIC]_T\,[FUM]_T} \quad (4)$$

where S_{cc} is cocrystal solubility in terms of drug molarity.

4. X-ray Powder Diffraction (XRPD)

Diffractograms were obtained using a Rigaku MiniFlex diffractometer (The Woodlands, TX) equipped with Kα copper radiation (λ = 1.5418 Å), operating with a 15mA current and 30 kV voltage. The measurements were performed at room temperature, at a scan rate of 2.5°/min over a 2θ range of 2° to 40°.

5. Differential Scanning Calorimetry (DSC)

DSC was carried out in a TA Instrument (Newark, DE) operating in a temperature range of 25–300 °C. Samples weighing approximately 2 mg were heated at a rate of 10 °C/min and under nitrogen air atmosphere (50 mL/min). Standard aluminum sample pans were used for all measurements.

6. High-performance liquid chromatography (HPLC)

INH, NIC and FUM concentrations were assessed by a Shimadzu LC-10A HPLC system (Kyoto, Japan) equipped with an ultraviolet detector, pump (LC-20ADXR), autosampler (Jasco AS-2055 Plus), solvent degasser (DGU-20A3), and column heater (CTO-20AC). Chromatographic separation was performed at room temperature using a reverse phase C8 analytical column (Phenomenex Luna® C8, 5µm x 4.6 mm x 250 mm column, Phenomenex®, Torrance, EUA). A flow rate of 1 mL/min and an injection volume of 20 µL were considered in the analysis. The UV detection of INH, NIC and FUM was carried out at the wavelengths of 260 nm, 302 nm and 260 nm, respectively. The mobile phase consisted of methanol and acetate buffer 25mM (pH 2.5) in a ratio of 10/90 (v/v).

7. Equilibrium reactions and predictive solubility equations

7.1 Derivation of 1:1:1 INH-NIC-FUM solubility as a function of pH

INH, a basic drug, exhibits two pK_a values (pK_{a1} = 3.53 e pK_{a2} = 11.14). Only pK_{a1} was considered for these calculations,

as pK_{a2} is irrelevant for the pH range studied. The total INH concentration in aqueous phase can be described by the sum of its ionized and nonionized species in solution as

$$[INH]_T=[B]_{aq}+[BH^+]_{aq} \quad (5)$$

where and BH^+ and represent INH nonionized and protonated species, respectively. Subscript T represents the concentrations of all species in solution and subscript aq represents the aqueous phase. The nonionized INH aqueous concentration $[B]_{aq}$ is the intrinsic INH solubility, also expressed as $S_{INH,0.}$ Experimental $S_{INH,0}$ is 1.298 (± 0.002) M.

The equilibrium reactions and constants of INH in solution are

$$BH^+_{aq} \overset{K_{a,\,INH}}{\leftrightarrow} B_{aq} + H^+_{aq} \quad (6)$$

$$K_{a,\,INH} = \frac{[H^+]_{aq}[B]_{aq}}{[BH^+]_{aq}} \quad (7)$$

Substituting appropriate equilibrium constants into mass balance equation (Equation 5), total drug concentration can be derived as

$$[INH]_T = [INH]_0\left(1 + \frac{[H^+]_{aq}}{K_{a,INH}}\right) \quad (8)$$

Equation 8 can also be expressed in terms of pH, pK_a and INH solubility as

$$S_{INH,\,T} = S_{INH,0}(1 + 10^{pK_{a,INH} - pH}) \quad (9)$$

Similarly, nicotinamide (NIC) is a basic molecule and is expressed as B, and then ionization and solubility are described by the same equations used for INH (eq 5 to 9).

FUM, a diprotic acid is expressed as H_2A. The total fumaric acid (FUM) concentration in aqueous phase can be described by the sum of its ionized and nonionized species in solution as

$$[FUM]_T = [H_2A]_{aq} + [HA]^-{}_{aq} + [A]^{2-}{}_{aq} \quad (10)$$

Where H_2A, HA^- and A^{2-} represent FUM nonionized and first and second ionized species, respectively. Subscript T represents the concentrations of all species in solution and subscript aq represents the aqueous phase.

$$H_2A_{aq} \overset{K_{a1,H_2A}}{\leftrightarrow} HA^-_{aq} + H^+_{aq} \quad (11)$$

$$K_{a1,H_2A} = \frac{[HA^-]_{aq}[H^+]_{aq}}{[H_2A]_{aq}} \quad (12)$$

$$HA^- \overset{K_{a2,H_2A}}{\leftrightarrow} A^{2-}_{aq} + H^+_{aq} \quad (13)$$

$$K_{a2,H_2A} = \frac{[A^{2-}]_{aq}[H^+]_{aq}}{[HA^-]_{aq}} \quad (14)$$

Substituting appropriate equilibrium constants into mass balance equation (Equation 10), total coformer concentration can be derived as

$$[FUM]_T = [FUM]_0\left(1 + \frac{K_{a1,H_2A}}{[H^+]} + \frac{K_{a1,H_2A}\,K_{a2,H_2A}}{[H^+]^2}\right) \quad (15)$$

Equation 15 can also be expressed in terms of pH, pK_a and FUM solubility as

$$S_{FUM,T} = S_{FUM,0}\left(1 + 10^{pH - pK_{a1,H_2A}} + 10^{2pH - pK_{a1,H_2A} - pK_{a2,H_2A}}\right) \quad (16)$$

A 1:1:1 cocrystal of a basic drug, a basic coformer and a diprotic acid coformer dissociates in solution according to its solubility product (K_{sp}), as

$$INH - B - H_2A_{cocrystal} \overset{K_{sp}}{\leftrightarrow} INH_{aq} + B_{aq} + H_2A_{aq} \quad (17)$$

The above equilibrium represents cocrystal dissociation or dissolution (left to right) and precipitation (right to left).

$$K_{sp} = [INH]_{aq}[B]_{aq}[H_2A]_{aq} \quad (18)$$

Where INH, B and H_2A represent the nonionized species of INH, NIC and FUM.

By combining equations 5 and 10, and substituting appropriate equilibrium constants, an expression for total drug concentration as a function of total coformer concentrations can be derived as

$$[INH]_T = \frac{K_{sp}}{[NIC]_T[FUM]_T}\left(1+\frac{[H^+]_{aq}}{K_{a,INH}}\right)\left(1+\frac{[H^+]_{aq}}{K_{a,B}}\right)\left(1+\frac{K_{a1,H2A}}{[H^+]}+\frac{K_{a1,H_2A}\,K_{a2,H2A}}{[H^+]^2}\right) \quad (19)$$

For a 1:1:1 cocrystal, the cocrystal solubility under stoichiometric conditions is described as

$$S_{cc} = [INH]_T = [B]_T = [H_2A]_T \quad (20)$$

Therefore, eq (19) can be rewritten as

$$S_{cc}^{1:1:1} = \sqrt[3]{K_{sp}\left(1+\frac{[H^+]_{aq}}{K_{a,INH}}\right)\left(1+\frac{[H^+]_{aq}}{K_{a,B}}\right)\left(1+\frac{K_{a1,H_2A}}{[H^+]_{aq}}+\frac{K_{a1,H_2A}\,K_{a2,H_2A}}{[H^+]_{aq}{}^2}\right)} \quad (21)$$

Which terms of pH and pK_a becomes

$$S_{CC}^{1:1:1} = \sqrt[3]{K_{sp}\left(1+10^{pK_{a,INH}-pH}\right)\left(1+10^{pK_{a,B}-pH}\right)\left(1+10^{pH-pK_{a1,H_2A}}+10^{2pH-pK_{a1,H_2A}-pK_{a2,H_2A}}\right)} \quad (22)$$

7.2 Derivation of 2:1 INH-FUM solubility as a function of pH

A 2:1 cocrystal of a basic drug and a diprotic acid coformer dissociates in solution according to its solubility product (K_{sp}), as

$$INH-H_2A_{cocrystal} \overset{K_{sp}}{\leftrightarrow} 2INH_{aq}+H_2A_{aq} \quad (23)$$

$$K_{sp} = [INH]_{aq}^2[H_2A]_{aq} \quad (24)$$

Where INH and H_2A represent the nonionized species of INH and FUM.

INH and FUM equilibrium reactions were previously described from equation (5) to (16).

By combining equations 5 and 10, and substituting appropriate equilibrium constants, an expression for total drug concentration as a function of total coformer concentrations can be derived as

$$[INH]_T^2 = \frac{K_{sp}}{[FUM]_T}\left(1+\frac{[H^+]_{aq}}{K_{a,INH}}\right)^2\left(1+\frac{K_{a1,H2A}}{[H^+]}+\frac{K_{a1,H_2A}\,K_{a2,H2A}}{[H^+]^2}\right) \quad (25)$$

Therefore, for a 2:1 cocrystal, the cocrystal solubility under stoichiometric conditions is described as

$S_{cc\ T} = ½[INH]_T = [H_2A]$ (26)

Therefore, eq (25) can be rewritten as

$$S_{cc}^{2:1} = \sqrt[3]{\frac{K_{sp}}{4}\left(1+\frac{[H^+]_{aq}}{K_{a,INH}}\right)^2\left(1+\frac{K_{a1,H_2A}}{[H^+]_{aq}}+\frac{K_{a1,H_2A}\,K_{a2,H_2A}}{[H^+]_{aq}^{\,2}}\right)} \quad (27)$$

$S_{cc,T}$ in terms of moles of drug:

$$S_{cc}^{2:1} = 2\sqrt[3]{\frac{K_{sp}}{4}\left(1+\frac{[H^+]_{aq}}{K_{a,INH}}\right)^2\left(1+\frac{K_{a1,H_2A}}{[H^+]_{aq}}+\frac{K_{a1,H_2A}\,K_{a2,H_2A}}{[H^+]_{aq}^{\,2}}\right)} \quad (28)$$

In terms of pH and pK_a, eq (27) becomes

$$S_{CC}^{2:1} = 2\sqrt[3]{\frac{K_{sp}}{4}(1+10^{pK_{a,INH}-pH})^2(1+10^{pH-pK_{a1,H_2A}}+10^{2pH-pK_{a1,H_2A}-pK_{a2,H_2A}})} \quad (29)$$

8. Cocrystal and drug eutectic measurements

Table S1. Eutectic measurements in three different pH conditions. Solution pH, eutectic concentrations of cocrystal components, and solid phases at equilibrium.

Cocrystal	Initial pH	final pH[a]	INH (mM)	FUM (mM)	NIC (mM)	Solid phases at equilibrium
INH-FUM	1.20	3.26	351.12 ± 8.43	503.63 ± 5.32	-	INH-FUM + FUM
	6.70	3.34	306.22 ± 10.98	549.84 ± 12.82	-	INH-FUM + FUM
	7.40	3.36	307.25 ± 7.55	571.43 ± 9.37	-	INH-FUM + FUM
INH-NIC-FUM	1.20	3.03	121.41 ± 7.32	168.09 ± 1.42	134.91 ± 1.23	INH-NIC-FUM + FUM
	6.70	3.17	115.17 ± 9.11	153.65 ± 7.44	136.73 ± 2.07	INH-NIC-FUM + FUM
	7.40	3.21	116.75 ± 3.82	168.10 ± 2.51	144.55 ± 1.72	INH-NIC-FUM + FUM

[a] equilibrium pH (24-48h)

References

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pharmaceutical molecular complexes. *Mol. Pharm.* **3**, 362–367 (2006).

2. Good, D. J. & Rodríguez-Hornedo, N. Solubility advantage of pharmaceutical cocrystals. *Cryst. Growth Des.* **9**, 2253–2264 (2009).