How cocrystals of weakly basic drugs and acidic coformers might modulate solubility and stability

G. Kuminek, N. Rodríguez-Hornedo, S. Siedler, H. V. A. Rocha, S. L. Cuffini and S. G. Cardoso

Supplementary information

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1. General information

Maleic acid (MLE) and salicylic acid (SLC) were purchased from Sigma-Aldrich (St. Louis, MO) and saccharin (SAC) was purchased from Acros (Geel, Belgium). Anhydrous nevirapine (NVP) was manufactured by Nortec Quimica (Xerem, Brazil). Milli-Q water was used, and all chemicals and solvents were used as received. NVP hemihydrate (NVPHH) was prepared from ethanol–water as described by Stieger et al.[1]

2. Preparation of cocrystals

NVP−MLE and NVP−SLC cocrystals were prepared using the reaction crystallization method[2] in chloroform while NVP−SAC was prepared in 1-pentanol by the same method. NVP was added to nearly saturated solutions of the coformers and stirred at room temperature for 24 hours. 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 solubilities were measured in water, pH 1.2 potassium chloride buffer, and pH 4.5 sodium acetate buffer at the eutectic point, where drug and cocrystal solid phases are in equilibrium with solution.[2] The eutectic point between cocrystal and drug was reached by suspending 250 mg of cocrystal and 50 mg of drug in 3 mL of media. Suspensions were maintained in continuous stirring at 25 ± 0.1 °C until equilibrium was reached (72−96 h). At 24 h intervals, 0.30 mL aliquots of suspension were collected and pH was measured, before filtration through a 0.45 µm pore membrane. Solid phases were also collected at 24 h intervals to ensure the sample was at the eutectic point (confirmed by presence of both drug and cocrystal solid phases and constant coformer and drug solution concentrations). After dilution of filtered solutions with mobile phase, drug and coformer concentrations were analyzed by HPLC. The equilibrium solid phases were characterized by XRPD and DSC.

The cocrystal stoichiometric solubility was calculated from measured total eutectic concentrations of drug and coformer ($[\text{drug}]_{T,eu}$ and $[\text{coformer}]_{T,eu}$) (Table S1) according to the following equations for 1:1 and 2:1 cocrystals:

$$S^{1:1}_{\text{cocrystal}} = \sqrt{[\text{drug}]_{T,eu}[\text{coformer}]_{T,eu}}$$

$$S^{2:1}_{\text{cocrystal}} = 2\left(\sqrt[3]{\frac{[\text{drug}]^2_{T,eu}[\text{coformer}]_{T,eu}}{4}}\right)$$

where $S_{cocrystal}$ is 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 15 mA 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 analysis were 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)

Solutions concentrations of drug and coformer were analyzed by Waters HPLC (Milford, MA) equipped with a Photo Diode Array (PDA) detector set at 240 nm for all compounds except for MLE that was set at 225 nm. A reversed phase Waters® Atlantis C18 column (250 mm × 4.6 mm i.d.; 5 µm) maintained at 25 ± 1 °C was employed. The mobile phase consisted of 10 mM phosphate buffer:methanol adjusted to pH 2.5 with phosphoric acid was eluted at a flow rate of 1 mL/min in different proportions for which cocrystal. The elution for NVP−SLC cocrystal was isocratic in 50:50 proportion (phosphate buffer:methanol). For NVP−SAC and NVP−MLE the elution was gradient from 70:30 to 50:50 (phosphate buffer:methanol) proportion. The injection volume was 20 µL. The peak areas were integrated using Empower™ software program.

7. Equilibrium reactions and solubility of NVP cocrystals.

7.1. NVP cocrystal with diprotic acidic coformer (1:1)

The following equilibrium reaction represents cocrystal dissociation or dissolution (left to right) and precipitation (right to left):

$$NVP-H_2A_{cocrystal} \rightleftarrows NVP_{aq} + H_2A_{aq}$$

$$K_{sp} = [NVP]_{aq}[H_2A]_{aq}$$

Since cocrystal components are ionizable, their respective equilibrium reactions are considered.

Drug ionization:

$$NVPH^+ \rightleftarrows NVP + H^+$$

$$K_{a,NVPH^+} = \frac{[NVPH^+]}{[NVP][H^+]}$$

Coformer ionization:

$$H_2A \rightleftarrows HA^- + H^+$$

$$K_{a1,H_2A} = \frac{[HA^-][H^+]}{[H_2A]} \qquad HA^- \rightleftarrows A^{-2} + H^+$$

$$K_{a2,H_2A} = \frac{[A^{-2}][H^+]}{[HA^-]}$$

Mass balance on coformer:

$$[A]_T = [H_2A] + [HA^-] + [A^{-2}]$$

$$[A]_T = \frac{K_{sp}}{[NVP]}\left(1 + \frac{K_{a1,H_2A}}{[H^+]} + \frac{K_{a1,H_2A}K_{a2,H_2A}}{[H^+]^2}\right)$$

Mass balance on drug:

$$[NVP]_T = [NVP] + [NVPH^+]$$

$$[NVP]_T = [NVP]\left(1 + \frac{[H^+]}{K_{a,NVPH^+}}\right)$$

$$[NVP] = \frac{[NVP]_T}{\left(1 + \frac{[H^+]}{K_{a,NVPH^+}}\right)}$$

The solubility of a 1:1 cocrystal is:

$S_{cocrystal} = [A]_T = [NVP]_T$.

Combining equations above gives:

$$[A]_T = \frac{K_{sp}}{[NVP]_T}\left(1 + \frac{[H^+]}{K_{a,NVPH^+}}\right)\left(1 + \frac{K_{a1,H_2A}}{[H^+]} + \frac{K_{a1,H_2A}K_{a2,H_2A}}{[H^+]^2}\right)$$

and therefore,

$$S_{cocrystal} = \sqrt{K_{sp}\left(1 + \frac{[H^+]}{K_{a,NVPH^+}}\right)\left(1 + \frac{K_{a1,H_2A}}{[H^+]} + \frac{K_{a1,H_2A}K_{a2,H_2A}}{[H^+]^2}\right)} \text{ or}$$

$$S_{cocrystal} = \sqrt{K_{sp}(1 + 10^{pK_{a,drug} - pH})(1 + 10^{pH - pK_{a1,coformer}} + 10^{2pH - pK_{a1,coformer} - pK_{a2,coformer}})}$$

7.2. NVP cocrystal with monoprotic acidic coformer (2:1)

The following equilibrium reaction represents cocrystal dissociation or dissolution (left to right) and precipitation (right to left), characterized by a solubility product, Ksp:

$$NVP_2 - HA_{cocrystal} \rightleftarrows 2NVP_{aq} + HA_{aq}$$

$$K_{sp} = [NVP]^2_{aq}[HA]_{aq}$$

Since cocrystal components are ionizable, their respective equilibrium reactions are considered.

Drug ionization:

$$NVPH^+ \rightleftarrows NVP + H^+$$

$$K_{a,NVPH^+} = \frac{[NVPH^+]}{[NVP][H^+]}$$

Coformer ionization:

$$HA \rightleftarrows A^- + H^+$$

$$K_{a,HA} = \frac{[A^-][H^+]}{[HA]}$$

The subscript aq denotes species in the aqueous phase. K_a is the ionization constant for cocrystal components. Activities are replaced by concentrations as a first approximation applicable to dilute solutions.

Mass balance on coformer:

$$[A]_T = [HA] + [A^-]$$

$$[A]_T = \frac{K_{sp}}{[NVP]^2}\left(1 + \frac{K_{a1,HA}}{[H^+]}\right)$$

Mass balance on drug:

$$[NVP]_T = [NVP] + [NVPH^+]$$

$$[NVP]_T = [NVP]\left(1 + \frac{[H^+]}{K_{a,NVPH^+}}\right)$$

$$[NVP] = \frac{[NVP]_T}{\left(1 + \frac{[H^+]}{K_{a,NVPH^+}}\right)}$$

Combining equations above:

$$[A]_T = \frac{K_{sp}}{[NVP]_T^{\,2}}\left(1 + \frac{[H^+]}{K_{a,NVPH^+}}\right)\left(1 + \frac{K_{a1,HA}}{[H^+]}\right)$$

The solubility of a 2:1 cocrystal is

$S_{cocrystal} = [A]_T = 1/2[NVP]_T$

and therefore,

$$S_{cocrystal} = \sqrt[3]{\frac{K_{sp}}{4}\left(1+\frac{[H^+]}{K_{a,NVPH^+}}\right)^2\left(1+\frac{K_{a1,HA}}{[H^+]}\right)} \quad \text{or}$$

$$S_{cocrystal} = \sqrt[3]{\frac{K_{sp}}{4}\left(1+10^{pK_{a,drug}-pH}\right)^2\left(1+10^{pH-pK_{a1,coformer}}\right)}$$

The above equation can be expressed in terms of moles of drug as

$$S_{cocrystal} = 2\sqrt[3]{\frac{K_{sp}}{4}\left(1+10^{pK_{a,drug}-pH}\right)^2\left(1+10^{pH-pK_{a1,coformer}}\right)}$$

8. Molar fraction speciation plots as a function of pH

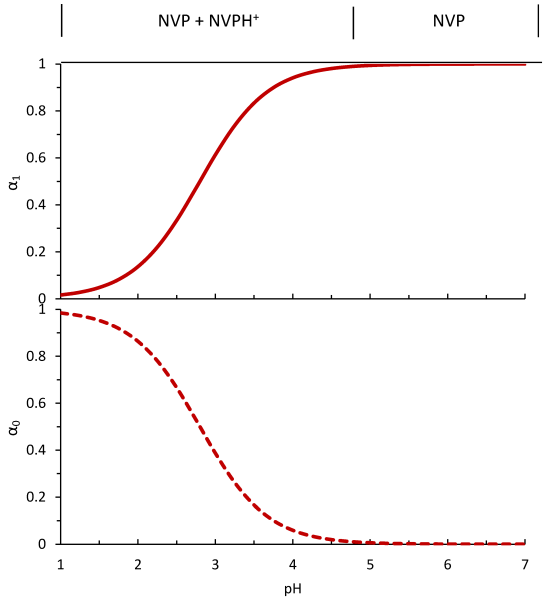

Fig. S1. Molar fraction speciation plot for NVP (pK_a = 2.8)

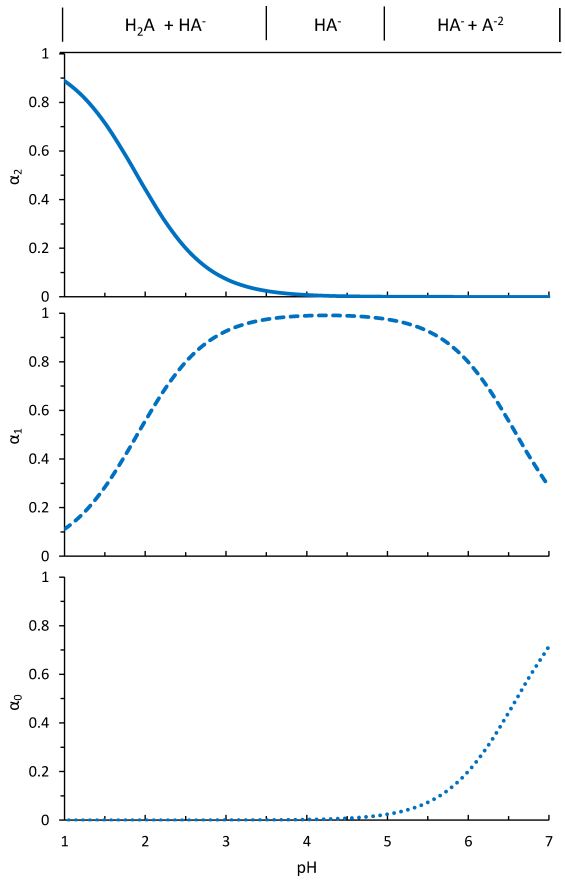

Fig. S2. Molar fraction speciation plot for MLE (pK_{a1}=1.9 and pK_{a2}=6.6)

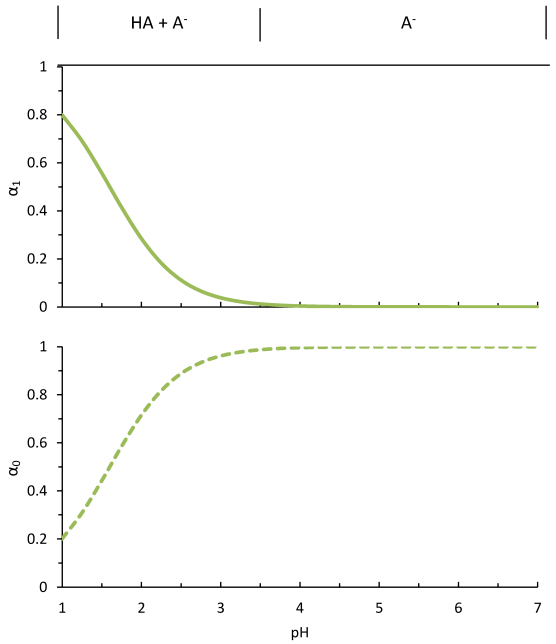

Fig. S3. Molar fraction speciation plot for SAC (pK_a=1.6)

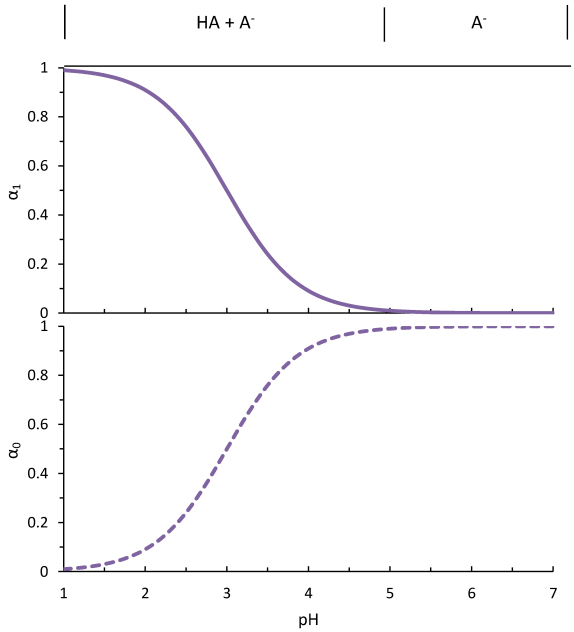

Fig. S4. Molar fraction speciation plot for SLC (pK_a=3.0)

9. Cocrystal and drug eutectic measurements

Table S1. Characterization of cocrystal/drug eutectic points in water and buffers: Solution pH, eutectic concentrations of drug and coformer, and solid phases.

	Initial pH	**Final pH**	**Time (h)**	**Drug (mM)**	**Coformer (mM)**	**Initial solid phase(s)[a]**	**Final solid phase(s)[a]**
NVP-MLE	1.2	1.0	72	10.4±0.2	180.9±0.1	NVP-MLE and NVPHH	NVP-MLE and NVPHH
	Water	1.3	72	3.6±0.3	180.6±1.2	NVP-MLE and NVPHH	NVP-MLE and NVPHH
	4.5	1.5	72	2.35±0.06	195.7±0.3	NVP-MLE and NVPHH	NVP-MLE and NVPHH
	1.2	1.1	72	14.5±0.1	135.6±1.4	NVP-MLE	NVP-MLE and NVPHH
	4.5	1.6	3	6.05±0.01	166.6±0.4	NVP-MLE	NVP-MLE and NVP HH
NVP-SAC	1.2	1.2	72	8.06±0.01	2.9±0.1	NVP-SAC and NVPHH	NVP-SAC and NVPHH
	Water	2.4	72	0.91±0.01	7.4±0.3	NVP-SAC and NVP	NVP-SAC and NVP
	4.5	2.7	72	0.46±0.01	46.5±0.3	NVP-SAC and NVPHH	NVP-SAC and NVPHH
	1.2	1.2	72	5.63±0.01	6.87±0.01	NVP-SAC	NVP-SAC
	4.5	2.3	3	0.76±0.01	44.1±0.1	NVP-SAC	NVP-SAC
NVP-SLC	1.2	1.2	72	7.44±0.05	1.84±0.06	NVP-SLC and NVPHH	NVP-SLC and NVPHH
	Water	3.2	72	0.31±0.02	1.81±0.04	NVP-SLC and NVP	NVP-SLC and NVP
	4.5	4.0	72	0.10±0.01	24.8±0.7	NVP-SLC and NVPHH	NVP-SLC and NVPHH
	1.2	1.2	72	4.06±0.01	10.32±0.02	NVP-SLC	NVP-SLC
	4.5	4.2	3	0.303±0.002	13.08±0.04	NVP-SLC	NVP-SLC

[a]NVP = nevirapine anhydrous, NVPHH = nevirapine hemihydrate

10. S_{cc}/S_{drug} example calculation for NVP-MLE cocrystal at pH 1 and at 25°C:

$$S_{drug} = S_{drug,0}\left(1 + 10^{pK_{a,drug} - pH}\right)$$

$$S_{drug} = 0.172\ (1 + 10^{2.8-1})$$

$$S_{drug} = 11.22\ mM$$

$$S_{cc}^{1:1} = (K_{sp}\left(1 + 10^{(pK_{a,drug} - pH)}\right)\left(\left(1 + 10^{(pH - pK_{a1,coformer})}\right)\left(10^{(2pH - pK_{a1,coformer} - pK_{a2,coformer})}\right)\right))^{1/2}$$

$$S_{cc}^{1:1} = ((1.96\times10^{-5}\left(1 + 10^{(2.8-1)}\right)\left(\left(1 + 10^{(1-1.9)}\right)\left(10^{(2\times1-1.9-6.6)}\right)\right))^{1/2}$$

$$S_{cc}^{1:1} = 3.758\times10^{-2}\ M = 37.58\ mM$$

$$\frac{S_{cc}}{S_{drug}} = \frac{37.58}{11.22} = 3.4$$

$S_{drug,0}$ represents the solubility of NVP hydrate under nonionizing conditions, 0.172mM. K_{sp} is the NVP-MLE cocrystal solubility product $1.96\text{x}10^{-5}$ M^2. K_a represents the ionization constant of cocrystal components, $pK_{a,NVPH+}$ = 2.8[3], $pK_{a1,MLE}$ =1.9 and $pK_{a2,MLE}$= 6.6.[4]

1 N. Stieger, M. R. Caira, W. Liebenberg, L. R. Tiedt, J. C. Wessels and M. M. De Villiers, *Cryst Growth Des*, 2010, **10**, 3859-3868.

2 N. Rodríguez-Hornedo, S. J. Nehm, K. F. Seefeldt, Y. Pagán-Torres and C. J. Falkiewicz, *Mol Pharm*, 2006, **3**, 362-367.

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