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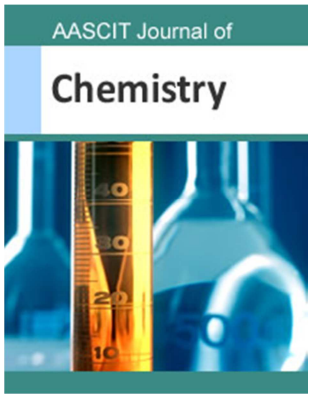

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Synthesis, Raman Spectroscopy, X-Ray and DSC Studies of Salicylic Acid Cocrystals Prepared by Slow Evaporation

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Abstract
Cocrystals have been increasingly recognized as an alternative delivery form for solid drug products. In this work Raman spectroscopy, X-ray powder diffraction/X-ray crystallography, and differential scanning calorimetry have been used to study the phenomenon of cocrystal formation in stoichiometric mixture of salicylic acid – DL-phenylalanine. Raman spectroscopy was particularly useful for characterization of the products and was used to determine the nature of the interaction in the cocrystals. Several new vibrational bands were identified in the cocrystal which were not manifest in the raw material and could be used as diagnostic features of cocrystal formation. An understanding of the effects of cocrystal formation on the vibrational mode was obtained by complete assignment of the spectra of the starting material and of cocrystal component. Single crystal X-ray studies confirmed the formation of a cocrystal with a 1:1 stoichiometry between salicylic acid and DL-phenylalanine. The asymmetric unit of the cocrystal contains four molecules: two molecules of salicylic acid and both R- and S- enantiomers of phenylalanine. Each of the phenylalanine molecules is in the zwitterion form.

1. Introduction

The number of publications and reviews detailing the topics of crystal engineering and supramolecular synthesis of API-based cocrystals is extensive and continuing to grow. [1, 2] More specifically, researchers usually highlight themes pertaining to functional group compatibility (synthons), for example, acid/N-heterocycle, acid/amide, phenol/N-heterocycle, and/or cocrystal growth strategies such as evaporation, [3] solid-state grinding, [4] sonication, [5] and melting. [6] Slow evaporation and grinding are the most commonly used techniques for producing cocrystals, expressing some 63% from the overall total [7].

Hydrogen bonds are the basis of molecular recognition phenomena in pharmaceutical systems. Moreover, they are key elements in the design of molecular assemblies and supermolecules in the solid states. In the crystalline state, hydrogen bonds are accountable for the creation of families of molecular networks with the same molecular components or with different molecular components (multiple component crystals or cocrystals) [8-18]

Gonnade et al. [19] determined the Observation of Efficient Preferential Enrichment Phenomenon for a Cocrystal of (dl)-Phenylalanine and Fumaric Acid under Nonequilibrium Crystallization Conditions. Also Elnagerma et al. [20] studied a new co-crystal of salicylic acid and benzamide of pharmaceutical relevance by using Raman spectroscopy, X-ray powder diffraction/ X-ray crystallography and differential scanning calorimetry.

Single crystal X-ray diffraction is the preferred characterization technique in determining whether a cocrystalline material has been generated;

Salicylic acid is the ortho form of monohydroxybenzoic acid. Since ancient times, it is known for the ability of pain and fever relief. Now it is best known for the use in anti-acne treatments. No polymorphs of salicylic acid have been reported so far. Similar to other carboxylic acids, the –COOH group makes it a good cocrystal coformer to theophylline. The chemical structures of the molecules in this work are shown in Figure 1.

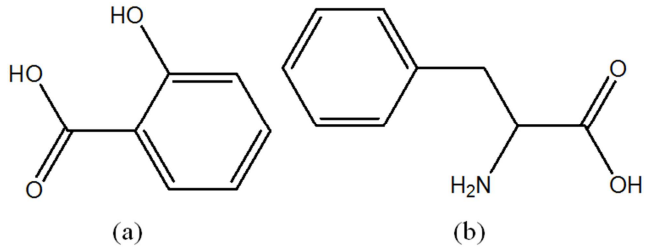


Figure 1. *Molecular structure of (a) salicylic acid and (b) DL-phenylalanine.*

2. Experimental Section

2.1. Materials

Salicylic acid and DL-phenylalanine were purchased from Sigma Aldrich at > 98%. These materials were used as received. The solvent (ethanol) was HPLC grade and obtained from Reidel de Haen or Fisher scientific.

Cocrystal formation was identified initially using Raman spectroscopy and the difference in melting points between the pure components and the product; the co-crystalline structures were confirmed by X-ray powder diffraction and single crystal X-Ray Diffraction.

2.2. Cocrystallization Via Slow Evaporation

Salicylic acid (28.2mg, 0.204mmol) was mixed with DL-phenylalanine (33.73mg, 0.204mmol) in stoichiometric ratio (1:1) was dissolved in 3 ml ethanol with slight warming until dissolution was complete. The solution was then allowed to slowly evaporate at room temperature (22-23°C). Then the solid phase was harvested by vacuum filtration and dried at room temperature under reduced pressure (25 mmHg) on Whatman 50 filter paper (Maidstone, England) for 30 minutes to remove loosely bound solvent. The solid phases were confirmed to be CIT: PA cocrystal by x-ray powder diffraction, FT-IR spectroscopy, and differential scanning calorimetry.

2.3. Raman Spectroscopy

Raman spectra of the co-crystal samples and those of the single components were obtained using an Via Raman microscope (Renishaw plc.) with 785 nm stabilized diode laser excitation. The laser power at the sample was approximately 25 mW. A 50 x objective lens was used giving a laser spot diameter (footprint) of about 2 μm at the sample. Spectra were obtained for a 10 s exposure of the CCD detector in the wavenumber region 3600-50 cm-1 using the extended scanning mode of the instrument.

2.4. Powder X-Ray Diffraction

Powder diffraction patterns of solid phases were recorded with Bruker D8 diffractometer in Bragg–Brentano θ-θ geometry with Cu Kα1,2 radiation (1.5418 Å) using a secondary curved graphite monochromator. The X-ray tube was operated at 40 kV, 30 mA. Samples were scanned in a vertical Bragg–Brentano (θ/2θ) geometry (reflection mode) from 5° to 40° (2θ) using a 0.005° step width and a 1.5s count time at each step. The receiving slit was 1° and the scatter slit 0.2°. The solid phase was analyzed by x-ray powder diffraction and results were compared to the diffraction patterns of each pure phase.

2.5. Differential Scanning Calorimetry (DSC)

The thermal behavior of the solid phases was studied using DSC; the DSC profiles were generated in the range of (-50 to 160°C) using a TA Q2000 DSC instrument with an RGS90 cooling unit. Temperature calibration was performed using an indium metal standard supplied with the instrument at the appropriate heating rate of 10°C min^{-1}. Accurately weighed samples (1-2 mg) were placed in Tzero aluminium pans using a similar empty pan as reference. The data were collected in triplicate for each sample and were analyzed using a TA Instruments Universal Analysis 2000 version 4.3A software.

2.6. Single-Crystal X-Ray Diffraction

Single crystal data were collected on a Bruker Apex II CCD diffractometer with Mo Kα radiation (0.71073 Å). The structure was solved by direct methods with SHELXS-97 and refined by a full-matrix least squares analysis on F2 with anisotropic displacement parameters for non-H atoms in SHELXL-97.

3. Characterization of the Cocrystals

3.1. X-Ray Powder Diffraction (PXRD)

PXRD patterns obtained for salicylic acid, DL-phenylalanine and the stoichiometric 1:1 salicylic acid, DL-phenylalanine component are shown in Figures 2 and 3. The diffraction patterns of the three materials were found to be very different, with the product exhibiting characteristic

peaks at 5.5, 15.7, 11.17, 19.34 and 24.23 degrees 2θ, and salicylic acid exhibits characteristic peaks at 11.00 and 17.1 degrees 2θ, whereas DL- phenylalanine exhibits characteristic peaks at 5.6 and 22.65 degrees 2θ.

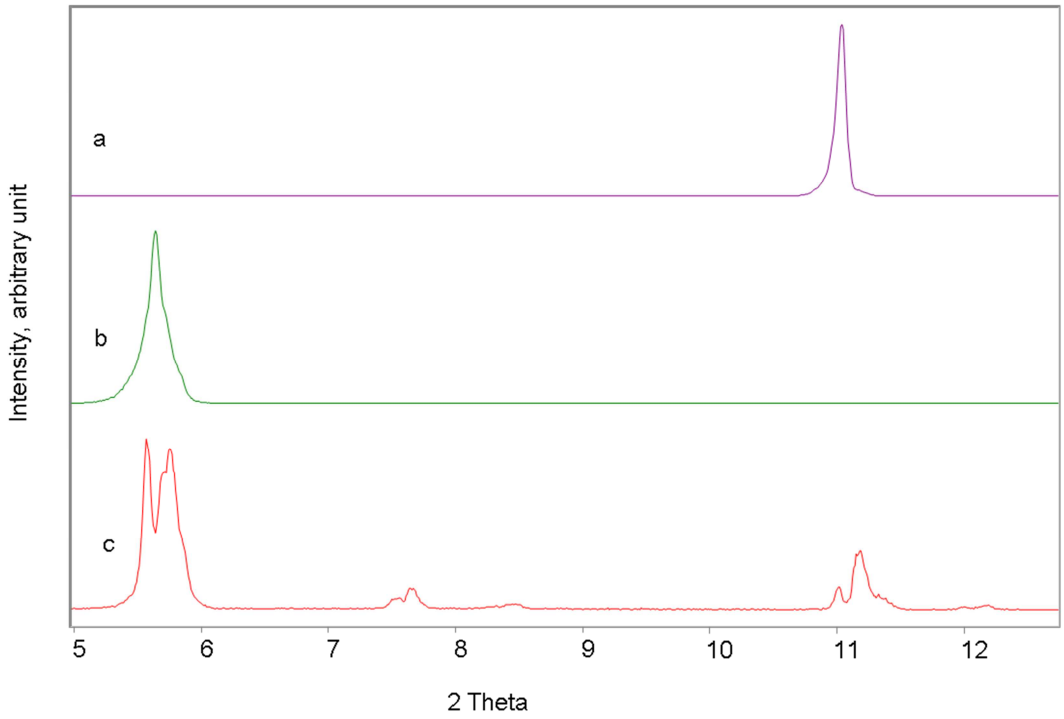


Figure 2. *Powder X-ray diffraction patterns for (a) salicylic acid, (b) DL- phenylalanine and (c) the cocrystal from 5 2θ to 12 2θ.*

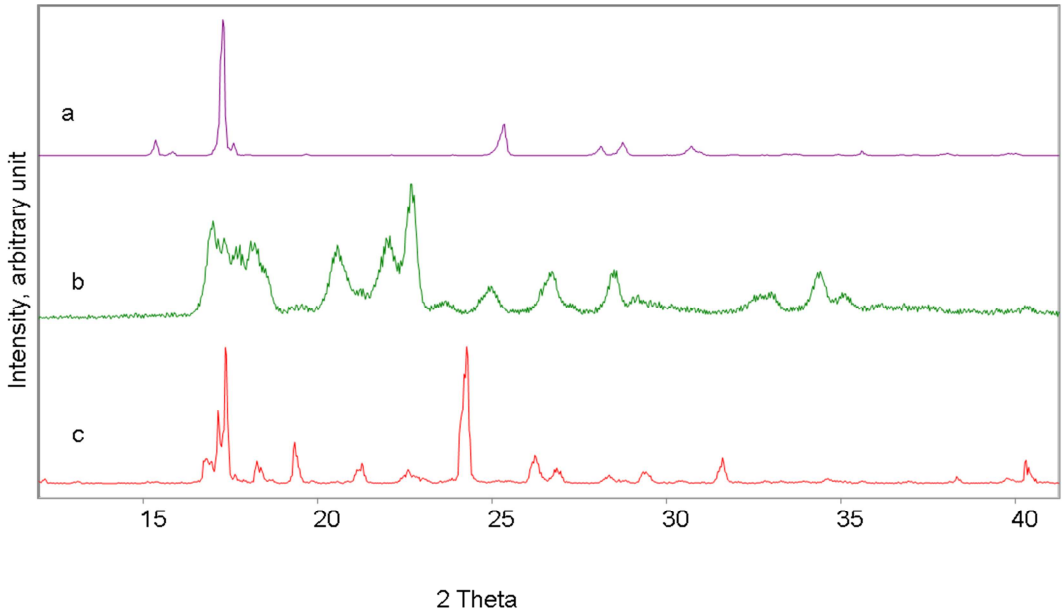


Figure 3. *Powder X-ray diffraction patterns for (a) salicylic acid, (b) DL-phenylalanine and (c) the 1:1 cocrystal in the range 2θ 15 - 40°.*

3.2. Differential Scanning Calorimetry (DSC)

The DSC thermograms of benzamide, salicylic acid and their 1:1 cocrystal product are presented in (Figure 4) showing the thermogram of salicylic acid (melting endotherm maximum at 159.83°C) and the thermogram of DL- phenylalanine (melting endotherm maximum at 276.53°C). The DSC thermogram of the 1:1 stoichiometric product proved that the benzamide-salicylic acid cocrystal had been formed and exhibits a melting endotherm maximum at 118°C followed by a broad entotherm, assigned to decomposition of the mixed product.

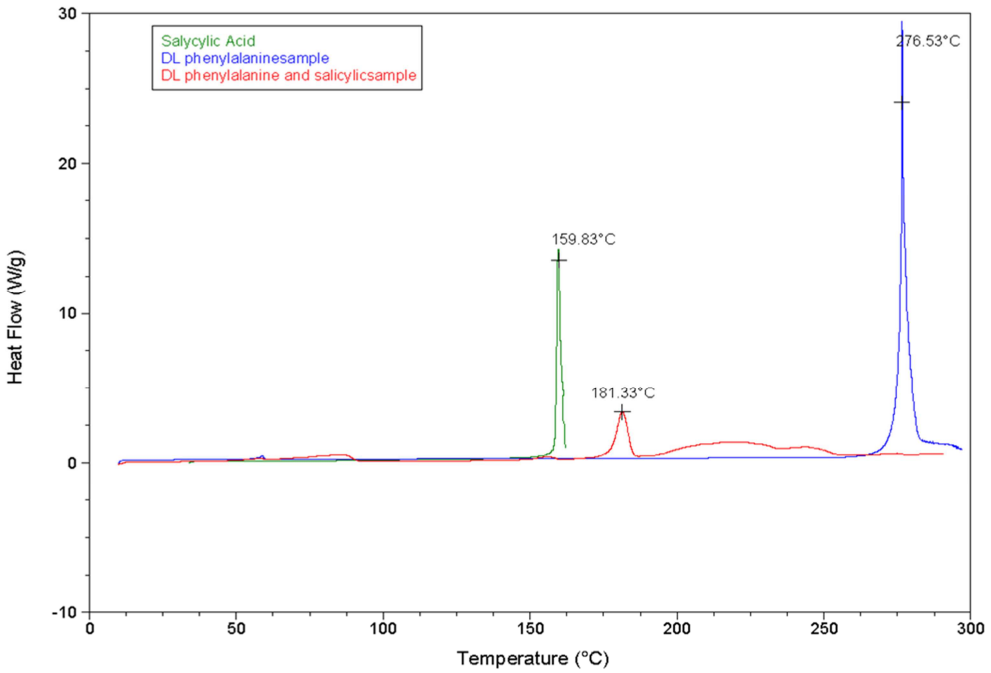


Figure 4. *DSC melting curves of DL- phenylalanine (——), cocrystal (——) and salicylic acid (—).*

3.3. Raman Spectroscopic Characterisation

The Raman spectra in the regions of 1800 to 1100, 1100 to 650 and 600 to 200 cm^{-1} for salicylic acid, DL-phenylalanine, and the salicylic acid - DL- phenylalanine crystal are presented in Figures (5-7). The assignments for the most characteristic vibrational bands are listed in Table 1. During the formation of the salicylic acid – DL – phenylalanine crystal the OH and C=O bands of the salicylic acid are shifted to lower or higher wavenumber by 5 to 21cm^{-1}, which suggests that changes in hydrogen bonding patterns has occurred, consistent with formation of salicylic acid - DL- phenylalanine cocrystal [11]. The Raman spectrum for pure salicylic acid in the starting material has strong bands at 1632 cm^{-1}, 1245 cm^{-1} and a medium intensity peak at 1383 cm^{-1} corresponding to C=O stretching, $((C\text{-}O)_h$ stretching (vibrational of hydroxylic group)) and $((O—H)_h$ in-plane bending, respectively. During the cocrystallization, the band at 1632 cm^{-1} disappears and the bands at 1383 cm^{-1} and 1245 cm^{-1} shift to 1363 cm^{-1}, respectively. The disappearance of the C=O stretching mode and decrease in the $((C\text{-}O)_h$ str, $(O—H)_h)$ wavenumber of salicylic acid indicate that the carboxylic group is participating in strong hydrogen bonding. The peaks in the spectrum of DL- phenylalanine occurring at 1346 cm^{-1} ((O-H) in-plane deformation), 1337 cm^{-1}, 1322 cm^{-1} and 1309 cm^{-1} (CH_2 wagging) and the peak of salicylic acid at 1321 cm^{-1} ((C—O)c stretching (vibration of the carboxylic group)), appear as a band doublet at 1325 and 1317 cm^{-1}.

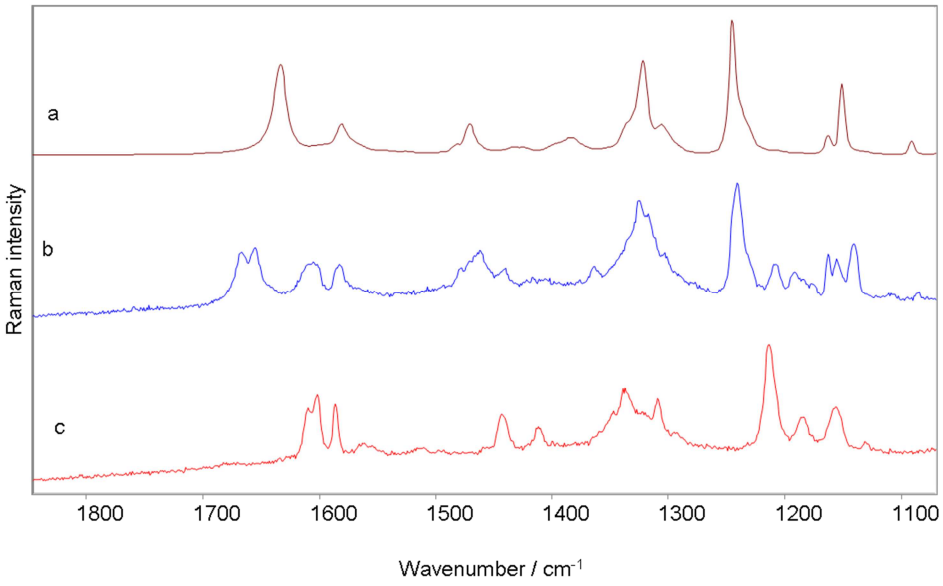


Figure 5. *Raman spectra obtained for (a) salicylic acid, (b) the cocrystal and (c) DL-phenylalanine.*

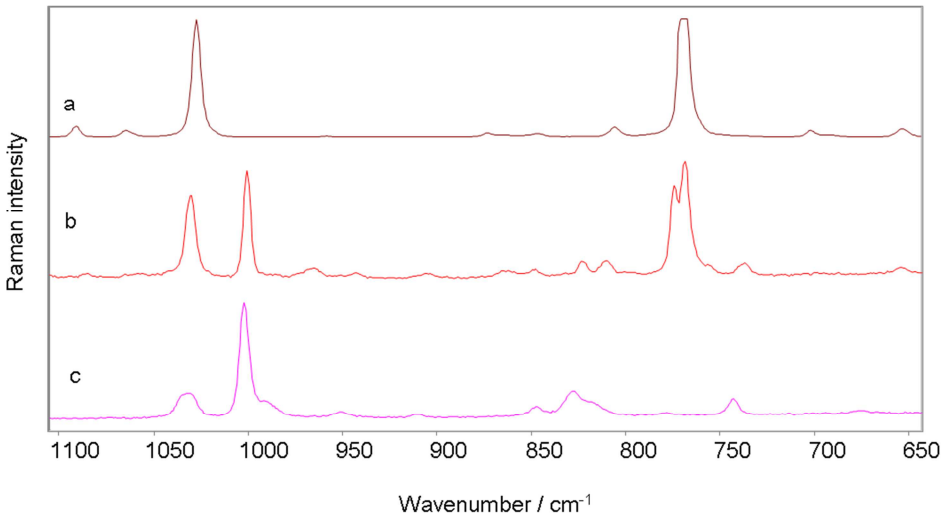


Figure 6. *Raman spectra obtained for (a) salicylic acid, (b) the cocrystal and (c) DL-phenylalanine.*

The spectra of pure DL- phenylalanine has strong bands at 1609 cm^{-1} corresponding to ($[NH_3]^+$ asym deformation phenyl ring quadrant. ring str (ν8a)) and 1602 cm^{-1} appear as broad bands centred at 1582 cm^{-1} with decreased intensity. Also, the bands at 1411 cm^{-1}, 1213 cm^{-1} and 1185 cm^{-1} corresponding to (COO sym. str.), phenyl ring CH out of plane deformation (chain); CH_2 twist and phenyl ring in plane CH def (ν9a); C-O (H) str respectively. In the cocrystal material, the band at 1411 cm^{-1} in phenylalanine weakens and appears at 1410 cm^{-1} as a broad very weak intensity band, the peak at 1213 cm^{-1} was shifted to 1207 cm^{-1} with decreased intensity and the peak at 1185 cm^{-1} appeared as a weak broad peak at 1192, 1183 and 1175 cm^{-1}. These observations are consistent with strong hydrogen bonding involving both carboxylic acid and amide functions.

On the other hand, pure DL- phenylalanine has medium peaks at 602, 518 and 468 cm^{-1} attributed to O-C=O in plane deformation, (C-C=O) in plane deformation, phenyl in-plane ring deformation (ν_{6a}) and COO^- rocking, respectively. Moreover, new bands at 1666, 1655, 965, 940 and 478 cm^{-1}, are not present in the reference spectra of the pure material (Figures 5 - 7).

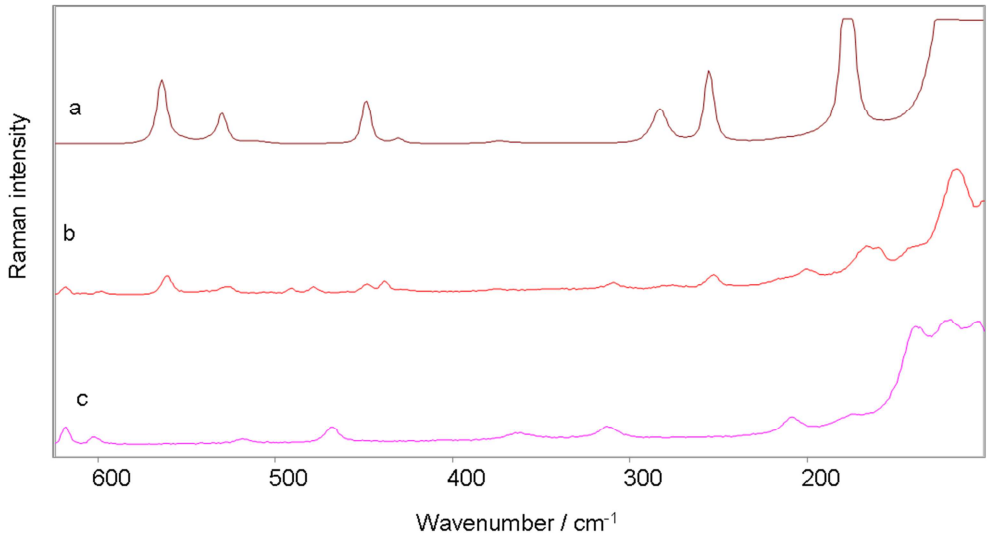


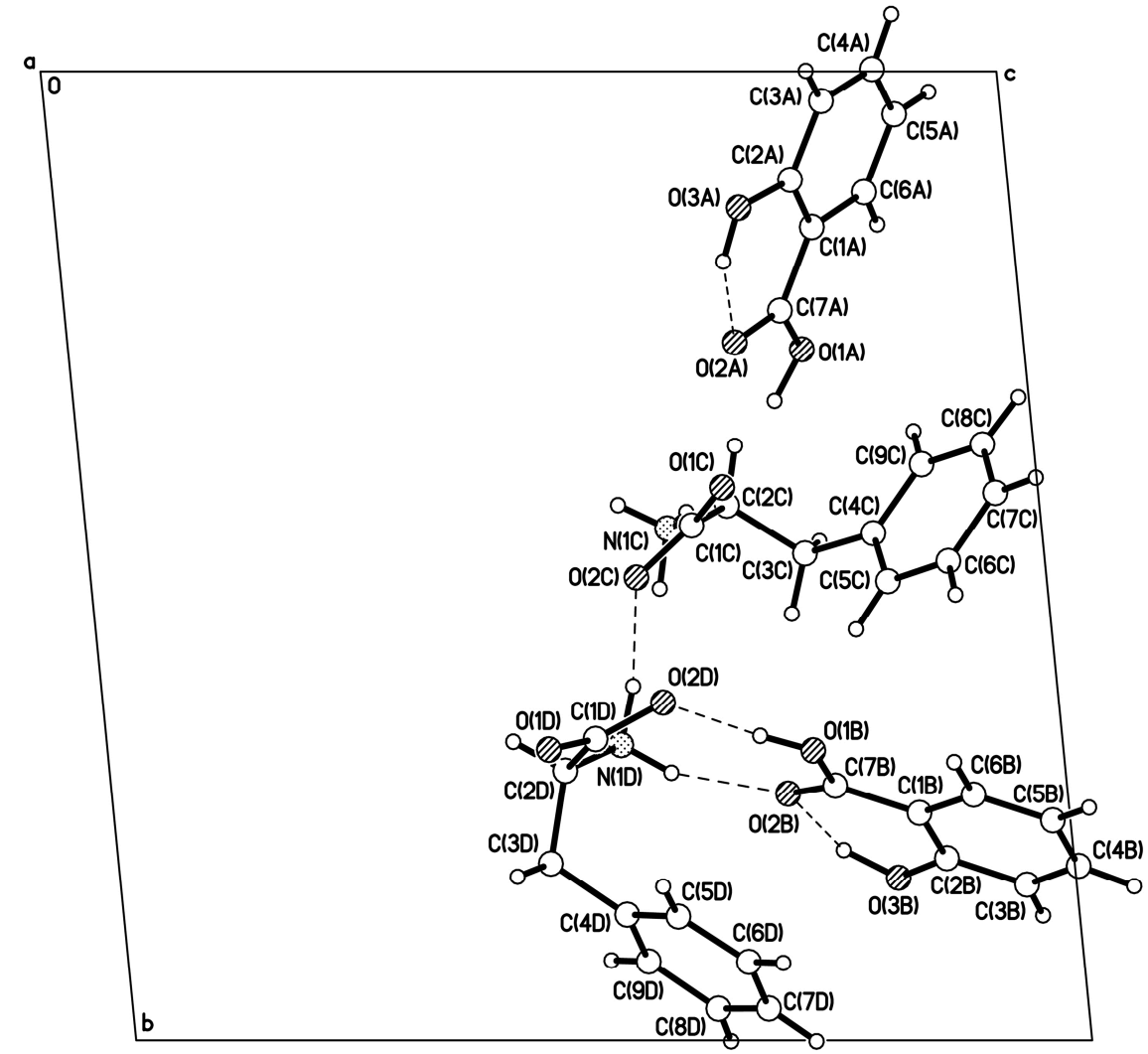
Figure 7. *Raman spectra obtained for (a) salicylic acid, (b) the cocrystal and (c) DL-phenylalanine.*

3.4. Single–Crystal X-Ray Diffraction

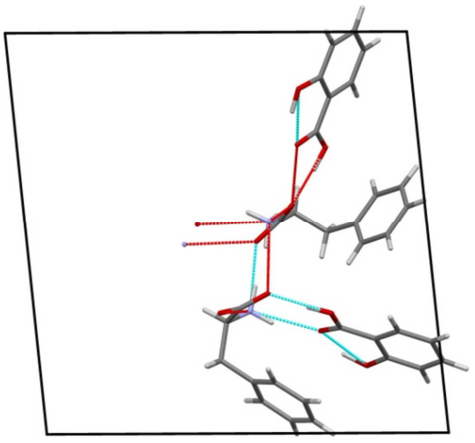
Single crystal X-ray studies confirmed the formation of a cocrystal with a 1:1 stoichiometry between salicylic acid and DL-phenylalanine. The asymmetric unit of the cocrystal (Figure 8a and 8b) contains four molecules: two molecules of salicylic acid and both R- and S- enantiomers of phenylalanine. Each of the phenylalanine molecules is in the zwitterion form. Hydrogens on the amine are located in tetrahedral structure, with the resulting charge interactions appearing to form the basis of complex H-bonded network (Tables 2 and 3). The network may best be described as comprising two distinct phenylalanine- phenylalanine ladder motifs (labelled *l*1 and *l*2) which are based on H-bonded ring structures between amide and carboxylate functions of the zwitterions (Figures 9 and 10). The ladder form *l*2 essentially lies perpendicular to the central *l*1 motif and this forms a central ladder, Z-shaped in cross section that runs parallel to

the a- axis of the unit cell.

Further hydrogen bonding attaches the salicylic acid molecule to these Z-shaped ladders. The hydrogen bonding of salicylic acid to the phenylalanine ladders again shows two discrete forms: a phenylalanine-salicylic acid ring motif (r1) and a further phenylalanine- salicylic acid H. bonded pair (labelled c1) (Figures 11-12).



(a)



(b)

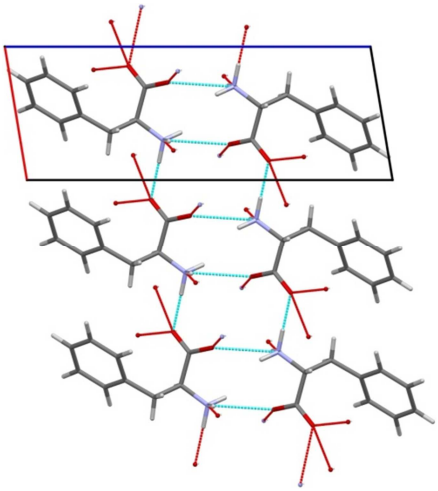


Figure 8. *The asymmetric unit of (salicylic acid)$_2$ and (DL- phenylalanine). Viewed down the a. axis of the unit cell and showing the numbering scheme adopted.*

Figure 9. *The centrosymmetric ladder motif l1 formed from R-phenylalanine molecular of the asymmetric unit (O1C- H32C) viewed down the b- axis of the unit cell.*

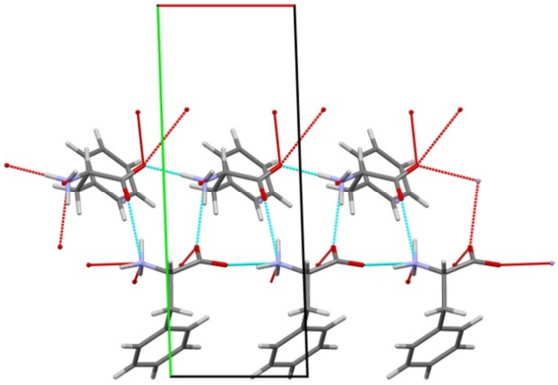


Figure 10. *The ladder motif formed l2 by R- and S- phenylalanine molecules in the asymmetric unit viewed down the c- axis of the motif cell.*

It is notable that each of these phenylalanine ladder motifs is formed by ring structures derived from phenylalanine molecules of opposite chirality *l*1 axis based on a crystallographic centrosymmetric dimer and *l*2 is formed from opposite enantiomers in the asymmetric unit.

(a). r1- down b-axis

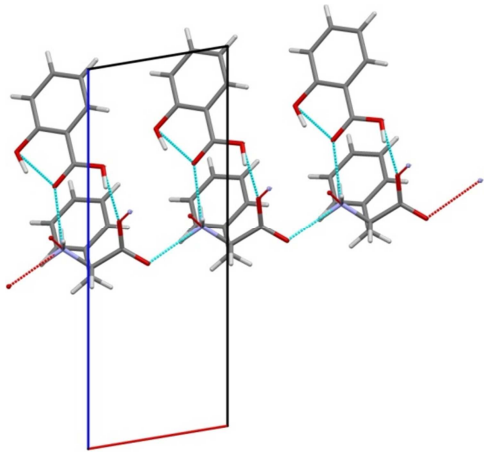
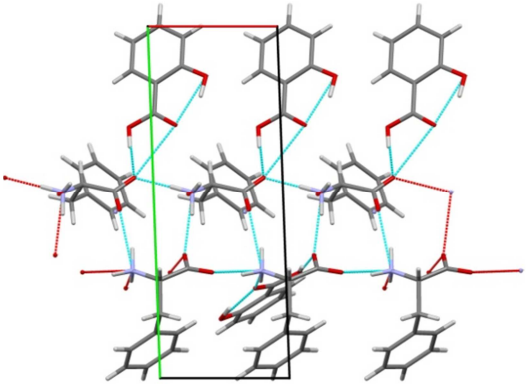
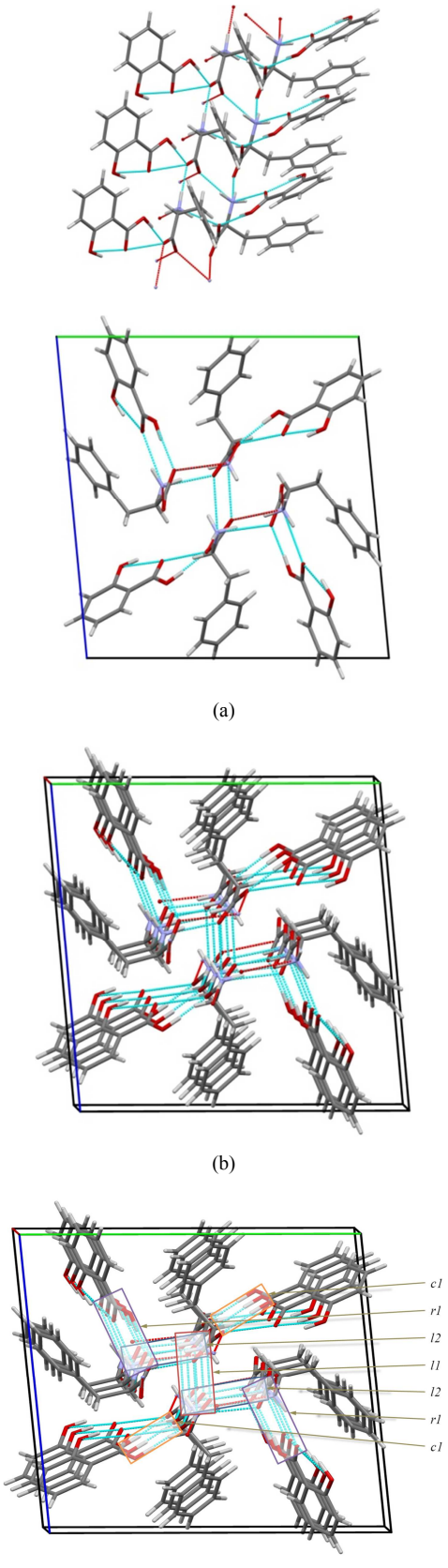


Figure 11. *Hydrogen bonding between salicylic acid molecules and the phenylalanine Z-motif in the cocrystal of salicylic acid –p phenylalanine 8-membered (a) ring structures (labelled r1) viewed down b-axes of the unit cell. (b) C-OH…O=C structure (labelled c1) viewed down c-axis of unit cell.*

(b). c1 – down c-axis (also r2 visible)



r2 r3 c1



(a)

(b)

(c)

Figure 12. *Crystal packing of (salicylic acid) (phenylalanine) viewed (a) down the a axis of the unit cell in orthographic projection (b) The same packing viewed with slight rotate to highlight the ladder and ring motifs of the structure. (c) a view labelling the structural motifs of the packing.*

Table 1. *Major bands of transmission Raman Spectroscopy (TRS) of salicylic acid, DL-phenylalanine and their 1:1 cocrystal products.*

Salicylic acid	DL-Phenylalanine	Cocrystal	Assignment [21-24]
-	-	1670m	-
-	-	1659m	-
1632s	-	-	C=O str
-	1611w	1606 w broad	$[NH_3]^+$ asym def; ϕ quad. ring str (ν_{8a})
-	1580w	1582m	ϕ quad. ring str (ν_{8b}); $[NH3]^+$sym def
1583m	-		8a
	1561w	-	COO^- asym. str.
	1510w	-	C-N asym str
1473m	-	1465m broad	19a
	1443w	1441w	-
	1411w	1410 vw abroad	COO^- sym. str.
1385w	-	1365vw	$(O-H)_h$ i.p.bend
	1346m		(O-H) i.p. def
	1337s	Double broad peaks	-
	1322m	1325m, 1317m	ϕ sextant ring str. (ν_{14})
1321s	-		$(C—O)_c$ str
	1214m	1208w	ϕ CH o.p. def (chain); CH_2 twist
	1193w	1185w	ϕ i.p. CH def (ν9a); C -O (H) str
1163m	-	1162w	-
	1158m	1156m	ϕ i.p. CH def (ν_{15})
1152s	-	1142m	15
1028vs	-	1031s	18b
-	1028s		C-C str
-	1003vs	1000s	ϕ i.p. ring. def (ν_{12})
-	827w	-	C-C str
770w	-	770w	-
-	217w	-	-
188w	-	177w broad	-
-	149m	-	
132m	-	127m	
114w	114w	-	
-	-	95m	
83m	-	-	Lattice modes
-	66s	-	
54m	-	57w	
-	49w sh	48 vwsh	

Table 2. *Crystal data and structure refinement for the co-crystal of salicylic acid and DL-phenylalanine.*

Identification code	me_salphenala_0m	
Empirical formula	$C_{16}\ H_{17}\ N\ O_5$	
Formula weight	303.31	
Temperature	173(2) K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group	P-1	
Unit cell dimensions	a = 5.8872(3) Å	α= 83.822(2)°.
	b = 15.8284(9) Å	ß= 80.281(2)°.
	c = 16.0469(9) Å	γ = 86.986(2)°.
Volume	1464.40(14) Å3	
Z	4	
Density (calculated)	1.376 Mg/m3	
Absorption coefficient	0.103 mm-1	
F(000)	640	
Theta range for data collection	2.59 to 25.18°.	
Index ranges	-6<=h<=7, -16<=k<=18, -18<=l<=16	
Reflections collected	40528	
Independent reflections	4228 [R(int) = 0.0616]	
Completeness to theta = 25.18°	80.6%	
Absorption correction	None	
Refinement method	Full-matrix least-squares on F2	
Data / restraints / parameters	4228 / 0 / 533	
Goodness-of-fit on F2	1.067	
Final R indices [I>2 sigma(I)]	R1 = 0.0394, wR2 = 0.0671	
R indices (all data)	R1 = 0.0837, wR2 = 0.0801	
Largest diff. peak and hole	0.159 and -0.218 e.Å-3	

Table 3. *Hydrogen bond dimensions (d /Å; ∠/°) in the co-crystal of salicylic acid and DL-phenylalanine.*

D-H	*d*(*D*-H)	*d*(H…*A*)	∠(*D*-H…*A*)	*d*(*D*…*A*)	*A*
O1A-H1OA	0.955	1.649	175.24	2.602	O1C[a]
O3A-H3OA	0.944	1.742	148.58	2.594	O2A
O1B-H1OB	0.911	1.715	168.29	2.613	O2D
O3B-H3OB	1.034	1.659	149.14	2.601	O2B
N1C-H1NC	0.987	1.744	177.56	2.730	O1C[a]
N1C-H2NC	0.915	2.234	129.84	2.907	O2C[b]
N1C-H2NC	0.915	2.630	129.55	3.289	O1D[c]
N1C-H3NC	0.988	1.887	167.84	2.861	O2D[a]
N1D-H1ND	0.968	1.865	158.30	2.788	O2C
N1D-H2ND	0.947	1.961	161.57	2.875	O2B
N1D-H3ND	1.043	1.733	168.00	2.761	O1D[a]

[a] [*x*-1, *y*, *z*]
[b] [-*x*+1, -*y*+1, -*z*+1]
[c] [-*x*+2, -*y*+1, -*z*+1]

4. Conclusions

Cocrystals of salicylic acid with DL- phenylalanine has been characterised by PXRD and substantiated by Raman spectroscopy, DSC and single crystal structure analysis.

Raman spectroscopy has been used to demonstrate a number of important aspects regarding the nature of interactions in the cocrystal. In general, the formation of a cocrystal system based on a carboxylic acid H-bonded motif causes broadening of the carbonyl bands and this can be indicative of the existence of the cocrystals. Alterations in the energies of bands associated with the carbonyl vibrations of the carboxyl group clearly corresponded to structure changes in the solids. The other main interaction point is the (-OH group) as it becomes deformed in the cocrystal.

References

[1] Blagden, N.; de Matas, M.; Gavan, P. T.; York, P. *Ad*V. *Drug. Deli* V*ery Re* V. 2007, 59. p. 617-630.

[2] Rodriguez-Spong, B.; Price, C. P.; Jayasankar, A.; Matzger, A. J.; Rodrı´guez-Hornedo, N. AdV. Drug Deli Very Re V. 2004, 56. p. 241-274.

[3] One general example: Weyna, D. R.; Shattock, T.; Vishweshwar, P.; Zaworotko, M. J. Cryst. Growth Des 2009, 9. p. 1106-1123.

[4] One general example: Fris˘c˘ic´, T.; Jones, W. Cryst. Growth Des 2009, 9. p. 1621-1637.

[5] Friscic, T.; Childs, S. L.; Rizvi, S. A. A.; Jones, W. CrystEngComm 2009, 11. p. 418-426.

[6] Blagden, N.; Berry, D. J.; Parkin, A.; Javed, H.; Ibrahim, A.; Gavan, P. T.; De Matos, L. L.; Seaton, C. C. New J. Chem. 2008, 32. p. 1659-1672.

[7] Sheikh A. Y., Abd Rahim S., Hammond R. B., Roberts K. J., CrystEngComm., 2009. 11. p. 501-509.

[8] Aakeroy C. B., Salmon D. J., CrystEngComm., 2005. 7. p. 439-448.

[9] Bettinetti G., Caira M., Callegari A., Merli M., Sorrenti M., Tadini C., J. Pharm. Sci., 2000. 89. p. 478-488.

[10] M. R. Caira., J. Cryst. Spectrosc., 1992. 22. p. 193-200.

[11] Caira M. R., Nassimbeni L. R., Wildervanck A. F., J. Chem. Soc. Perkin Trans., 1995. 2. p. 2213-2216.

[12] Etter M. C., Frankenbach G. M., Chem. Mater., 1989. 1. p. 10-12.

[13] Etter M. C., Frankenbach G. M., Adsmond A D., Mol. Cryst. Liq. Cryst., 1990. 187. p. 25-39.

[14] Etter M. C., Reutzel S. M., J. Am. Chem. Soc., 1991. 113. p. 2586-2598.

[15] Etter M. C., J. Phys. Chem., 1991. 95. p. 4601-4610.

[16] Nangia A., Desiraju G. R., Acta Crystallogr., 1998. A54. p. 934-944.

[17] Rodriguez-Spong B., Price C. P., Jayasankar A., Matzger A. J., Rodríguez-Hornedo N., Adv. Drug. Del. Rev., 2004. 56. p. 241-274.

[18] Desiraju G. R., Acc. Chem. Res., 2002. 35. p. 565-573.

[19] Rajesh G., Gonnade, Sekai Iwama, Yuko Mori, Hiroki Takahashi, Hirohito Tsue, and Rui Tamura, Cryst. Growth Des., 2011, 11 (2). p 607-615.

[20] Elbagerma M. A., Edwards H. G. M., Munshi T., Scowen I. J., Anal. Bioanal. Chem., 2010. 397. p 137-146.

[21] Ravikumar B. R., Rajaram K., Ramakrishnan V., J. Raman Spectrosc., 2006. 37. p. 597-605.

[22] Rajkumar B. J. M., Ramakrishnan V., Spectrochim. Acta Part A., 2002. 58. p. 1923-1934.

[23] Ramachandran E., Natarajan N., Cryst. Res. Technol., 2007. 42. p. 617-620.

[24] Mary M. B., Sasirekha V., Ramakrishnan V., Spectrochim. Acta Part A., 2005. 62. p. 446-452.