Analytical Method Development for Structural Studies of
Pharmaceutical and Related Materials in Solution and the Solid State

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Analytical Method Development for Structural Studies of Pharmaceutical and Related Materials in Solution and the Solid State

An investigation of the solid forms and mechanisms of formation of cocrystal systems using vibrational spectroscopic and X-ray diffraction techniques

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Abstract

Analysis of the molecular speciation of organic compounds in solution is essential for the understanding of ionic complexation. The Raman spectroscopic technique was chosen for this purpose because it allows the identification of compounds in different states and it can give information about the molecular geometry from the analysis of the vibrational spectra. In this research the ionisation steps of relevant pharmaceutical material have been studied by means of potentiometry coupled with Raman spectroscopy; the protonation and deprotonation behaviour of the molecules were studied in different pH regions. The abundance of the different species in the Raman spectra of aqueous salicylic acid, paracetamol, citric acid and salicylaldoxime have been identified, characterised and confirmed by numerical treatment of the observed spectral data using a multiwavelength curve-fitting program. The non-destructive nature of the Raman spectroscopic technique and the success of the application of the multiwavelength curve-fitting program demonstrated in this work have offered a new dimension for the rapid identification and characterisation of pharmaceuticals in solution and have indicated the direction of further research.

The work also covers the formation of novel cocrystal systems with pharmaceutically relevant materials. The existence of new cocrystals of salicylic acid-nicotinic acid, DL-phenylalanine, 6-hydroxynicotinic acid, and 3,4-dihydroxybenzoic acid with oxalic acid have been identified from stoichiometric mixtures using combined techniques of Raman spectroscopy (dispersive and transmission TRS), X-ray powder diffraction and thermal analysis. Raman spectroscopy has been used to demonstrate a number of important aspects regarding the nature of the molecular interactions in the cocrystal. Cocrystals of
salicylic acid – benzamide, citric acid-paracetamol and citric acid -benzamide have been identified with similar analytical approaches and structurally characterised in detail with single crystal X-ray diffraction.

From these studies the high selectivity and direct micro sampling of Raman spectroscopy make it possible to identify spectral contributions from each chemical constituent by a peak wavenumber comparison of single-component spectra (API and guest individually) and the two- component sample material (API/guest), thus allowing a direct assessment of cocrystal formation to be made. Correlation of information from Raman spectra have been made to the X-ray diffraction and thermal analysis results.

Transmission Raman Spectroscopy has been applied to the study cocrystals for the first time. Identification of new phases of analysis of the low wavenumber Raman bands is demonstrated to be a key advantage of the TRS technique.

**Keywords:** in situ monitoring, acidity constants, multiwavelength spectroscopy, pH, cocrystal, Raman spectroscopy, X-Ray diffraction and DSC.
List of sections of this work have been published or reported in the following forms:

**Papers:**

- Identification of a new co-crystal of salicylic acid and benzamide of pharmaceutical relevance
  

- In situ monitoring of pH titration by Raman spectroscopy
  

  

- Raman spectroscopic Determination of the Acidity Constants of Salicylaldoxime in Aqueous Solution
  
Raman Spectroscopic Determination of Acidity Constants of Citric Acid Indicators in a Binary Ethanol-Water Mixture


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- In situ Monitoring of pH Titration by Raman Spectroscopy

2) Proceedings of the Euroanalysis XV, 6 – 10 September 2009, Innsbruck, Austria.

- Identification of a New Cocrystal of Salicylic acid and Benzamide of pharmaceutical relevance

- Raman Spectroscopy Studies of Mixtures of Salicylic Acid and Benzamide in an Aqueous Solution as a Function of pH
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- In situ monitoring of pH titration by Raman spectroscopy

M. Elbagerma, H.G.M. Edwards, M.D. Hargreaves, R.J. Telford, I.J. Scowen

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<td>Raman spectra obtained for (a) 3, 4-dihydroxybenzoic acid, (b) oxalic acid, and (c) the cocrystal.</td>
</tr>
<tr>
<td>8.3</td>
<td>Raman spectra obtained for (a) 3, 4-dihydroxybenzoic acid, (b) oxalic acid, and (c) the cocrystal. Transmission Raman spectra from 180 -40 cm(^{-1}) region obtained for (a) 3,4-dihydroxybenzoic acid, (b) oxalic acid and (c) the cocrystal.</td>
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<tr>
<td>8.4</td>
<td>Raman spectra from 500 -150 cm(^{-1}) region for (a) 3,4-dihydroxybenzoic acid, (b) oxalic acid and (c) the cocrystal.</td>
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<td>8.5</td>
<td>Powder X-ray diffraction patterns for (a) 3, 4-dihydroxybenzoic acid, (b) oxalic acid, (c) and cocrystal.</td>
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<td>Powder X-ray diffraction patterns for (a) 3, 4-dihydroxybenzoic acid, (b) oxalic acid, (c) and cocrystal.</td>
</tr>
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<td>8.7</td>
<td>Powder X-ray diffraction patterns for (a) 3, 4-dihydroxybenzoic acid, (b) oxalic acid, (c) and cocrystal.</td>
</tr>
<tr>
<td>8.8</td>
<td>DSC melting curves of cocrystal, oxalic acid and 3, 4-dihydroxybenzoic acid.</td>
</tr>
</tbody>
</table>
Chapter 1

Introduction
1.1 Introduction

Spectroscopic techniques are widely used to help understand the solid-state physical characteristics of drug substances and formulations and Raman spectroscopy is becoming an increasingly widely applied technique that potentially offers many operational advantages for providing information related to molecular structural information in organic (pharmaceutically relevant) chemistry systems.

Raman spectroscopy in combination with other methods for the solid state characterization of pharmaceutical solids (e.g. X-ray diffraction and differential scanning calorimetry) can be used to characterize pharmaceutical materials, especially active drug substances and to identify their chemical structures. In addition, Raman spectroscopy can be used to study the molecular interactions between different substances. Raman spectroscopy has been used to determine the changes in the strength of hydrogen-bonding between different pharmaceuticals [1]. Applications range from monitoring and controlling large scale manufacturing processes, to profiling the distribution of active pharmaceutical ingredients (API) and structural changes at different stages in a formulation cycle. Raman spectroscopy offers polymorph discrimination, is capable of studying aqueous and solid samples, and is particularly suited to combining with other analytical techniques given that it provides non-destructive analysis requiring little or no sample preparation. Several key research papers have demonstrated the potential of Raman spectroscopy for studies of cocrystals, particularly in investigating hydrogen bonding in pharmaceuticals [1]. Molecular speciation of organic compounds in solution is essential for understanding ionic form complexation. Determination of acidity constant has played an important part in understanding the ionic composition of many pharmaceutical active molecules. The chemical and biological activity of these substances would be expected to vary with the degree of ionization. For this reason, accurate
knowledge of the ionization constants for pharmaceutical relevant materials is a prerequisite to an understanding of their mechanism of action in both chemical and biological processes. For the evaluation of acid dissociation constants, the spectroscopic technique most widely applied is UV / Visible spectroscopy, however this technique is limited and vibrational spectroscopy (e.g. Raman spectroscopy) potentially offers detail about the specific molecular chemical changes associated with ionisation and association solution [2]. The combination of potentiometric measurements with Raman spectroscopy could present a more complete picture of the chemical changes accompanying structure changes with pH. Such an approach is relatively novel but, for example, Alia et al. [3] used Raman spectroscopy to examine hydrogen bonding in benzenesulfonic acid-acylonitrile solution.

In this thesis, Raman spectroscopy in conjunction with (with X-ray powder diffraction, single crystal X-ray diffraction, potentiometric titration and thermal analysis particularly differential scanning calorimetry (DSC) was utilised to study organic molecular species in the solid state and in solution.
1.2 Aims and objectives

Acid dissociation constants are useful physicochemical parameters describing the extent of ionization of functional groups with respect to pH. These parameters are important in research areas such as pharmaceutical drug development, where knowledge of the ionization state of a particular functional group is often crucial in order to understand processing conditions, and interpret pharmacokinetic and pharmacodynamics information. There have been several methods used for the determination of acidity constants. Spectroscopic methods are in general highly sensitive and often species specific, and as such are suitable for studying chemical equilibria in solution. When the components involved in the chemical equilibrium have distinct spectral responses, their concentrations can be measured directly, and the determination of the equilibrium constant is relatively simple. Raman spectroscopy is a very powerful tool that can give information on molecular rotations, vibrations and even electronic transitions in the case of solid and liquid samples, so is highly attractive for studying and specialising organic systems in solution.

The main objective of this project is to develop methodologies for speciation of organic complexes and applying these methods to pharmaceutically relevant cocrystal systems. This work was undertaken with a view to understanding the factors controlling the formation of cocrystals using models organic molecules containing interacting functional groups.

A systematic study has been carried out with a selection of simple acids and bases with a range of structural features. The extent to which spectroscopic properties and structures of
the starting material and those of the cocrystal products can be correlated with structural features will be explored.

To achieve these objectives, this work can be split into:

- Development of *in-situ* speciation methods in solutions with parallel measurements using Raman spectroscopy (molecular) and pH (macroscopic).
- Determination of the Raman spectroscopy limits of detection of the selected systems solution.
- Determination of the acidity constants of selective systems using multiwavelength curve fitting of Raman spectroscopic data.
- Preparation of solid state materials from stoichiometric mixtures of the selected systems and their characterisation with Raman spectroscopy, powder X-ray diffraction (PXRD), differential scanning calorimetry (DSC), and where appropriate, by single-crystal X-ray diffraction analysis.
- Correlation of data from analytical structure.
1.3 Thesis structure

This body of this thesis consists of nine chapters. The first chapter provides the context and aims of this project. Chapter two discusses methods of characterisation and identification of the solid state pharmaceuticals used in this work. Chapter three describes the role of cocrystals in the design of properties of pharmaceuticals. Chapter four describes the experimental procedures used in this work. The results and discussion chapters five to eight, are divided into two parts. Part A concerns the development of in-situ monitoring of pH titrations with Raman spectroscopy of selected drugs leading to full assignment of the spectra and computational determination of the pKa values.

Part B (chapters six to eight) presents comprehensive structural studies of Raman spectroscopic and X-Ray diffraction for the characterisation of some new cocrystals with full assignments of the Raman spectra.

Chapter nine provides the conclusions of this research and recommendations for further work.
1.4 References


Chapter 2

Characterisation methods for solid state pharmaceuticals
2.1 Introduction

A range of methods are commonly applied for the characterisation of solid state pharmaceuticals; This chapter will review several techniques used to characterise solid state pharmaceuticals including vibrational spectroscopic methods (Raman spectroscopy and IR spectroscopy); X-ray powder diffractometry (PXRD); thermal analysis (DSC) in the context of structural information, such as intermolecular interaction can be obtained. A summary of the established capabilities of each characterisation method is listed in Table 2.1

**Table 2.1 Summary of the characterisation of solid- state pharmaceuticals techniques [1-5].**

<table>
<thead>
<tr>
<th>Method</th>
<th>Time scale</th>
<th>Data measured</th>
<th>Advantages</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Molecular level properties</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raman</td>
<td>&gt; 10s</td>
<td>Intermolecular vibrations (polarisability changes)</td>
<td>-Chemical identification</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-Molecular structure information</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-Water is not active</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-Probe measurements possible</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-No sample preparation</td>
</tr>
<tr>
<td>FT-IR</td>
<td>&gt; 30s</td>
<td>Intermolecular vibrations (dipole moment changes)</td>
<td>-Chemical identification</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-Molecular structure information</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-Information about solvents</td>
</tr>
<tr>
<td>Near-infrared (NIR)</td>
<td>&gt; 10s</td>
<td>Overtones and combinations of molecular vibrations (dipole moment changes)</td>
<td>-Chemical /physical information</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-No sample preparation, rapid measurements</td>
</tr>
<tr>
<td>Solid-state NMR</td>
<td>0.5-10h</td>
<td>Magnetic resonance</td>
<td>-Chemical information</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-Phase characterisation, crystal structure determination</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-Minimal sample preparation</td>
</tr>
<tr>
<td>Practical level properties</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------------------</td>
<td></td>
<td></td>
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<tr>
<td><strong>- X-ray powder diffraction PXRD</strong></td>
<td>10-60min</td>
<td>Diffraction from powder sample</td>
<td></td>
</tr>
<tr>
<td><strong>- Single crystal X-ray diffraction,</strong></td>
<td>24 and 72 hours</td>
<td>Diffraction from single crystal</td>
<td></td>
</tr>
<tr>
<td><strong>Crystal structure, phase identification Crystalline measurements</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>DSC</strong></td>
<td>10-30min</td>
<td>Heat flow versus temperature</td>
<td></td>
</tr>
<tr>
<td><strong>TGA</strong></td>
<td>10-30min</td>
<td>Change of mass versus temperature</td>
<td></td>
</tr>
<tr>
<td><strong>Fast, very sensitive, automation</strong></td>
<td><strong>Best thermodynamic information</strong></td>
<td><strong>Glass transition temperature determination</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Fast, sensitive, automatic</strong></td>
<td><strong>Study solvates/hydrates, phase transitions</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hot-stage microscopy/ scanning electronic microscopy</strong></td>
<td>-</td>
<td>Microscopy under the influence of light or electron radiation</td>
<td></td>
</tr>
<tr>
<td><strong>Surface examination</strong></td>
<td><strong>Melting, transition and eutectic point determinations, crystallisation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Bulk level properties</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Micro-calorimetry</strong></td>
<td>&lt; 30 min</td>
<td>Heat flow versus time</td>
<td></td>
</tr>
<tr>
<td><strong>Quantification of amorphous phase formation and loss of hydrates</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Solution calorimetry</strong></td>
<td>-</td>
<td>Heat flow During dissolution</td>
<td></td>
</tr>
<tr>
<td><strong>Detection and quantisation of polymorphs and amorphous phase, Sensitive to small energy differences</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2.2 Spectroscopic Identification

2.2.1 Vibrational spectroscopy

Vibrational spectroscopic techniques, for instance, Raman, infrared, near-infrared and, more recently, terahertz pulsed spectroscopy, have become popular for solid-state analysis because they are fast and non-destructive and allow solid-state changes to be probed at the molecular level [6]. Raman and near-infrared spectroscopy, are now often coupled to fibre-optic probes and are able to characterise solid-state conversion process in-line.

2.2.1.1 Raman spectroscopy

Raman spectroscopy is a light scattering technique. It provides qualitative and quantitative evaluation of the structure and transformation of materials, at the molecular level, through interrogation of molecular bond vibrations. Although it has only recently been developed for analytical purposes, the effect has been demonstrated for more than half century. As a vibrational spectroscopic technique it provides detailed spectral fingerprints.

Raman spectroscopy can be used to determine the molecular structure, characterise between different solid-state forms, determine the hydration states and solid-state phase transitions and it can be applied for both qualitative and quantitative analysis of pharmaceuticals [7]. Rapid, non-destructive and non-invasive measurements allow the implementation of Raman spectroscopy in the pharmaceutical industry as a routine testing technique.

Several authors have applied Raman spectroscopy for the analysis of solid-state characteristics and behaviour of drugs [8-13].
2.2.1.1.1 The Raman Effect

When monochromatic light (for instance from a laser) is passed through a material, a small portion of it is scattered by the molecules [14]. There are two types of scattering: elastic or Rayleigh scattering, where the light is scattered elastically in all directions, occurs at the same frequency as the incoming laser radiation, whereas inelastic or Raman scattering Figs 2.1 and 2.2, causes the light to be scattered with shifted frequency. Raman scattering is weaker than Rayleigh scattering but carries specific information about the rotational and vibrational energy levels of the molecule[15].

Fig. 2.1. Energy level diagram showing transitions for Raman and Rayleigh scattering
When the electromagnetic (EM) wave interacts with the system of atoms in a molecule, the interaction distorts the electron cloud of the molecules and induces an electric dipole moment. This induced dipole then radiates scattered light, with or without exchanging energy with vibrations in the molecules. The light scattering between the incident EM wave and the molecular system can be represented as:

![Diagram showing light scattering creating an induced dipole moment due to an incident EM wave](image)

Fig. 2.2. Light scattering creating an induced dipole moment due to an incident EM wave [16].

For a molecule to be active in Raman scattering there must be a change in the polarisability ($\alpha$) during a molecular vibration [17] such that,

$$\frac{\partial \alpha}{\partial Q} \neq 0 \quad (2.1)$$

where $Q$ is the normal coordinate of vibration. The polarisability of a molecule is related to the induced dipole moment, $\mu$, by the following equation;

$$\mu = \alpha E \quad (2.2)$$

The molecular polarisability is related to properties of the molecule which depend on its structure, the relative nuclear positions in the molecule and on the nature and direction of its chemical bonds[18].

13
E, the electric field strength at time t, is given by;

\[ E = E_0 \cos 2\pi \nu t \]  (2.3)

where \( E_0 \) is the electric field amplitude and \( \nu \) is the frequency of radiation. The frequency \( \nu \) can be expressed in terms of the speed of light, \( c \) (\( 3 \times 10^8 \) m s\(^{-1}\)) and the wavelength \( \lambda \). Another term commonly used in vibrational spectroscopy, the wavenumber, \( \nu \), may then be introduced which gives,

\[ \nu = \frac{c}{\lambda} \]  (2.4)

\[ \nu = \frac{1}{c} \left( \frac{1}{\lambda} \right) \text{ (cm}\text{-}^{-1}) \]  (2.5)

This is further extended by considering the transfer of energy from an electric field to a molecule, which occurs when the two interact to give an expression in terms of the energy difference, \( \Delta E \),

\[ \Delta E = h \nu = h \frac{c}{\lambda} = h\nu \]  (2.6)

where \( h \) is Planck’s constant (\( 6.0626 \times 10^{-34} \) J s). The magnitude of \( \Delta E \) depends on the vibrational transition, which typically occurs between \( 3 \times 10^3 \) and \( 10^2 \) cm\(^{-1}\) in Raman spectra.

The polarisability of electrons in the molecule will be modulated by the molecular vibration (\( \nu_m \))[22].

\[ \alpha = \alpha_0 + \sin \nu_m \]  (2.7)
2.2.1.1.2 Raman Intensity

The Raman band intensity is related to a number of factors, including

\[ I = \nu^4 \sigma_v IC \]  (2.8)

where \( \nu \) is the frequency of the incident radiation, \( \sigma_v \) is the Raman cross-section, \( I \) is the irradiance (W m\(^{-2}\)) and \( C \) is the sample concentration. Raman intensities are usually directly proportional to the concentration of the active species. The intensity of Raman scattering, therefore, depends on several factors such as the laser power and the wavelength or frequency of the incident laser radiation as well as the polarisability of the molecule[17, 20-22].

<table>
<thead>
<tr>
<th>Raman Technique</th>
<th>Typical Laser Wavelengths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dispersive/ visible</td>
<td>Visible laser: 488, 514, 633 nm</td>
</tr>
<tr>
<td>Dispersive/ Near Infra red (NIR)</td>
<td>NIR Lasers: 785, 830 nm</td>
</tr>
<tr>
<td>SERS</td>
<td>Any visible or NIR Lasers</td>
</tr>
<tr>
<td>FT- Raman</td>
<td>Nd: YAG Laser: 1064nm</td>
</tr>
<tr>
<td>UVRR</td>
<td>UV Lasers: 230, 206.5 nm</td>
</tr>
</tbody>
</table>

Table 2.2. Technological advances in Raman spectroscopy
2.2.1.3 Remote Raman measurement: Use of Fibre Optical probes

Through the use of fibre optic probes, Raman measurements can be undertaken recently using a spectrometer. Raman spectroscopy therefore applications offers for on-line, in-process control monitoring, for quality control and identification testing of raw materials, or for identity testing of packaged products, environmental monitoring, and reaction monitoring. The value of fibre optic Raman spectroscopy has been recognized by the pharmaceutical industry and polymer industry; it has been shown to be a valuable analysis technique in crystallization processes for monitoring and control of active pharmaceutical ingredients and in monitoring of cure processes of polyester and vinyl ester resins [23-27]. In biomedical and bioanalytical applications, fibre optic Raman spectroscopy is also used for determination of enzyme activity measurements.

Raman spectroscopy in conjunction with other analytical methods has been used to examine inclusion complexes between drugs and cyclodextrins, used as host molecules to improve the bioavailability of poorly water soluble drugs [28].

2.2.1.4 Selected Applications of Raman Spectroscopy in pharmaceutical materials

A wide range of applications of Raman spectroscopy for the quantitative analysis and monitoring of pharmaceutical materials have been reported during the last few years. The most significant applications are reviewed in the following: O’Brien et al.[29] used Raman spectroscopy to monitor the isothermal polymorphic transformation of Carbamazepine at elevated temperatures, from form III to form I. The use of in-line Raman spectroscopy to monitoring the mixing process of diltiazem hydrochloride pellets and paraffinic wax beads was reported by Vergote et al.[30] De Beer et al.[31] have reported
a study on the monitoring of homogenization of ibuprofen aqueous suspensions. Wikstrom et al.[32] investigate the process induced transformation of theophylline, from the anhydrous to the monohydrate state, during high shear rate wet granulation process. Okumura and Matsuoka [33] reported the measurement of the degree of crystallinity of intact indomethacin /mannitol tablets. The monitoring of anti-solvent crystallization of progesterone was performed by Falcon and Berglund.[34]

Ono et al [35] monitored the polymorphic composition of L-glutamic acid in suspension during solvent mediated phase transitions experiments. Hu et al [36] applied Raman spectroscopy to monitor the enantiotropic phase transition of flufenamic acid during seeded crystallization from Form III to Form I. Both the solute concentration and solid phase composition were measured in situ.

2.2.1.5 Advantages and disadvantages of Raman Spectroscopy

Raman spectroscopy is a non-destructive technique and requires very little sample, where typically reasonable quality spectra can be obtained from a few milligrams of material. This means that if only a small amount of sample is available than it can be reused for more analysis after a Raman spectrum has been obtained. Raman spectra can also be obtained non-invasively, which means that bulk and final products can be tested directly in their packaging, such as glass bottles, plastics and blister packs. Raman analysis time is short. Raman experiments can be carried out easily, so that the work can be done by minimally trained personnel. Since water is a weak Raman scatterer, Raman has minimal sensitivity towards interference by water and it is possible to analyse aqueous solutions and therefore in this context, an in-situ investigation of the solution chemistry of
pharmaceutical materials was feasible. Sampling for Raman spectroscopy is further eased through the use of fibre optics.

Raman spectroscopy also has drawbacks which may be summarized as follows: a major problem for Raman measurements lies in the high levels of fluorescence overlying Raman bands. Coloured samples can undergo thermal or photochemical decomposition.

2.2.1.2 Infrared Spectroscopy

Infrared (IR) spectroscopy is a technique based on the absorption of the electromagnetic radiation in the infrared region (Fig.2.3). The IR spectrum can be divided into three regions, explicitly near IR (12500 – 4000 cm\(^{-1}\)), mid-IR (4000 – 400 cm\(^{-1}\)) and far IR (400 – 10 cm\(^{-1}\)). The energy of the IR photon is not high enough to excite electronic transitions, but it excites the molecular vibrations of covalently bonded atoms and groups. A molecule is usually in its ground state at room temperature. Absorption of energy occurs to excite the molecules to a particular higher vibrational state. Also, the change of dipole moment of the molecules during a vibration is essential to incur the absorption process.
The IR absorption spectrum of sample is obtained by passing the IR radiation through the sample and the fraction of radiation absorbed at each frequency is determined (Fig. 2.4.). For a fundamental vibrational mode to be IR active, a change in the molecular dipole moment must take place during the molecular vibration. The frequency at any peak in the spectrum corresponds to the frequency of a normal mode of bond vibration in the molecule [38].
Vibrations can involve either a change in bond length (stretching) or bond angle (bending) (Figure 2.5). Some bonds can stretch in-phase (symmetrical stretching) or out-of-phase (asymmetric stretching), as shown in (Figure 2.6).

![Figure 2.5. Stretching and bending vibrations](image)

**Fig.2.5. Stretching and bending vibrations**

![Symmetry and asymmetric stretching vibrations](image)

**Fig.2.6. Symmetric and asymmetric stretching vibrations**

Bending vibrations also contribute to infrared spectra and these are summarized in Figure 2.7.

![Different types of bending vibrations](image)

**Fig.2.7 Different types of bending vibrations**
The absorbance of a solution is directly proportional to the thickness and the concentration of the sample, as follows (Beer–Lambert law):

\[ A = \varepsilon cl \quad (2.9) \]

The absorbance is equal to the difference between the logarithms of the intensity of the light entering the sample \( (I_0) \) and the intensity of the light transmitted \( (I) \) by the sample:

\[ A = \log I_0 - \log I = \log \left( \frac{I_0}{I} \right) \quad (2.10) \]

Transmittance is defined as follows:

\[ T = \frac{I}{I_0} \quad (2.11) \]

The percentage transmittance as:

\[ \%T = 100 \times T \quad (2.12) \]

and

\[ A = -\log \left( \frac{I}{I_0} \right) = -\log T \quad (2.13) \]

2.2.2 Powder X-Ray Diffractometry (PXRD)

X-ray diffraction (XRD) is an analytical technique for crystal structure determination. (XRD) was discovered in 1912 by Max Von Laue; PXRD with counter detection was developed about 1940 by William Parrish [39]. X-rays lie in the electromagnetic spectrum between UV and Gamma radiation and have approximate wavelengths of 0.1 – 100 Å. X-rays are reflected from crystals only when the angle between the X-ray and the planes in the crystal obey Bragg's Law [40]:

\[ n\lambda = 2d \sin \theta \quad (2.14) \]
where $d$ is the perpendicular spacing between the lattice planes, $\theta$ is the complement of the angle of incidence of the X-ray beam (Bragg angle), and $n$ is the order of diffraction. If $n=1$, the difference is one wavelength and the diffraction is said to be first order. If $n=2$, the difference is two wavelengths and the diffraction is second order (Fig.2.8).

![Diagram of Bragg theory of X-ray diffraction](image)

Fig.2.8. Diagram of Bragg theory of X-ray diffraction

There are many diffraction planes possible through the crystal lattice, but only a limited number that give reflections within the range: $2 < \theta/\text{degrees} < 180$. 
2.2.3 X-ray crystallography

X-ray crystallography is the principal method by which the 3-dimensional structures of molecules have been determined. It is a technique in which the pattern produced by diffraction of X-rays through the closely spaced lattice of atoms in a crystal is recorded and then analyzed to reveal the nature of the lattice. This generally leads to an understanding of the material and molecular structure of a substance. The data collected from a diffraction experiment is a reciprocal space representation of the crystal lattice. The position of each diffraction (spot) is governed by size and shape of the unit cell and the inherent symmetry within the crystal. The intensity of each diffracted ray is recorded and electron density map is obtained. This electron density map is the starting point of building a 3-D model of the structure. The main aim of a crystallographic study is to determine the 3-D structure of a solid (Fig.2.9.)

![Fig.2.9. Schematic diagram of X-ray crystallography](image-url)
2.3 Thermal Analysis

Thermal analysis includes a group of techniques in which the physical properties of a substance are measured as a function of temperature whilst the substance is subjected to a controlled temperature programme. These methods frequently cannot be used for identification themselves without complementary data.

2.3.1 Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) is a technique for measuring the energy necessary to establish a nearly zero temperature difference between a substance and an inert reference material, as the two specimens are subjected to identical temperature regimes in an environment heated or cooled at a controlled rate. It is a useful tool for identifying the polymorphs of organic compounds. There are two types of DSC systems in common use; heat flux (Fig 2.10) and power compensated.

1- Heat-flux DSC

In heat-flux DSC, the sample and reference are heated by the same source and the ΔT (temperature difference) is measured. So, the heat flow path between the sample and the reference is provided by a metal strip, whose thermal conductivity is high and well defined [40-43]. The equation for heat flow from the furnace to each crucible is then given by

\[ \frac{dQ}{dt} = \frac{\Delta T}{R} \] (2.14)

where \( Q \) = heat, \( t \) = time, \( \Delta T \) = temperature difference between the furnace and the crucible, and \( R \) = thermal resistance of the heat path between the furnace and the crucible. From Equation 2.14, the temperature difference between the sample and reference is a measure of the difference in heat flow due to the presence of the sample in one of the
crucibles, provided that the furnace and heat paths are truly symmetrical. The $\Delta T$ signal requires calibration to provide a heat flow as a function of temperature, and this is usually carried out by the use of standards that are usually pure metals with known enthalpies of melting and materials with known heat capacities [44, 45].

![Diagram](image)

**Fig.2.10.** heat flex DSC (S, sample pan and R reference pan)

### 2.4 Conclusion

It is clear that an array of solid-state molecular techniques is crucial to a multidisciplinary approach in the characterization of pharmaceutical solids. Equally clear, each technique has its limitations and these limitations must be clearly recognized and understood, so that data are not misinterpreted.
2.5 References


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Chapter 3

Crystallization and Design Properties of Pharmaceutical Materials
3.1 Introduction

Engineers and chemists in the pharmaceutical industry usually seek to deliver crystalline forms of their active compounds, mainly due to the inherent stability of crystalline materials and the entrenched impact of crystallization processes on purification and isolation of chemical substances [1]. Active pharmaceutical ingredients (APIs) can be produced in a variety of distinct solid forms, including polymorphs, solvates, hydrates, salts, co-crystals and amorphous solids (Fig.3.1). Each form shows unique physicochemical properties that can influence the bioavailability, purification, stability and other characteristics of the drugs of which they form a part [2, 3].

Properties of different API solid forms are a consequence of differences in molecular interactions, structure and composition that result from different energetics [4].

![Fig.3.1. Classification of active pharmaceutical ingredient](image-url)
A pharmaceutical cocrystal is a single crystalline homogenous phase consisting of a drug and excipient or another drug. The components in a cocrystal exist in a definite stoichiometric ratio, and assemble via non-covalent interactions such as hydrogen bonds, ionic bonds, π-π or van der Waals interactions. Cocrystals thus possess different composition and structure when compared to the crystals of parent components [4-8]. The first application of crystal engineering to the generation of pharmaceutical cocrystals was a series of studies reported by Zerkowski et al.[9].

3.2 Design of cocrystals

The rational design of cocrystals has attracted the attention of crystal engineers due to their implications in pharmaceutical industries, and is based on a fundamental understanding of inter-molecular interactions that govern the assembly of molecules into a network superstructure [10, 11]. The molecules in the system are held together by synthons that are basic structural units formed from non-covalent interactions, for example, from van der Waal’s interactions, π – π interactions, and hydrogen bonds between the functional groups in the molecules [12-15]. Fig.3.2 shows some examples of synthons in the crystal structures of single and multiple-component materials. Crystal structures are the result of intermolecular interactions; hydrogen bonding is the most important interaction type in crystal engineering because it combines strength with directionality [6,7 16-21]. From studying the packing motifs and hydrogen-bond patterns in the crystal structures of great number of organic compounds, Etter and co-workers have proposed the rules to facilitate the deliberate design of hydrogen-bonded solids [18, 22-24]:
- All acidic hydrogens available in a molecule will be used in hydrogen bonding in the crystal structure of that compound.
- All good acceptors will be used in hydrogen bonding when there are available hydrogen bond donors.
- The best hydrogen bond donor and best acceptor will preferentially form hydrogen bonds to one another.

From these rules, hydrogen bond and synthon formation between the functionalities of different molecules can be predicted, and the strategies for cocrystal design can be derived [25, 26].

Fig. 3.2. Common hydrogen bonded synthons used in crystal engineering [14, 8, 27-29].
Supramolecular synthons based on intermolecular interactions can either be *homomeric* or *heteromeric* motifs. There are instances when homomeric motifs are formed from two identical centrosymmetrically related hydrogen bonds, such as the carboxylic acid dimer motif. Other synthons that contain self-complementary hydrogen bonds are the carboxamide dimer motifs (3.3.1). Heteromeric supramolecular synthons constitute intermolecular interactions formed from two different chemical moieties.

Heteromeric motifs are not only limited to strong interactions; they can also arise from a combination of strong and weak interactions. These types of synthons can be formed using heterocycles that contain weakly acidic hydrogens on carbon atoms ortho- to the hydrogen-bond acceptor atom (3.3.2); the carboxylic acid-pyridine synthon is formed from a combination of strong O–H···N and weak C–H···O hydrogen bonds. The *heteromeric* structure is more stable than the *homomeric* analogue [28, 30]. Fig. 3.3. show example of hydrogen bonding X-ray patterns.
3.3 Hydrogen bonded synthon selectivity

Supermolecular synthons that can occur in common functional groups in order to design new cocrystals and certain functional groups such as carboxylic acids, amides and alcohols are particularly amenable to the formation of supermolecular heterosynthon [31]. Some examples are:
3.3.1 Carboxylic acid and primary amide

3.3.2 Carboxylic acid and pyridine

3.4 Methods of Preparation of Co Crystals

Cocrystals can be prepared from two molecules of any size having complementary hydrogen bond functionalities. The ability of an API to form a cocrystal is dependent on a large number of variables, including the solvents, API co-former ratio, type of co-former, the temperature and the crystallization technique. Design strategies for cocrystal formation are still being researched and their mechanism of formation is far from understood [32].
Synthesis /preparation of cocrystals can be processed by a number of methods [33], including:

- Slow solvent evaporation crystallization from solution.
- Solvent reduction (slurring and solvent drop grinding).
- Solvent free (grinding and melting [hot stage microscopy]).

### 3.4.1 Solution cocrystallization

Crystallization from solution has wide use in the pharmaceutical industry. This process is a typical phase transition which occurs in a condensed system. There are a number of reasons for the popularity of this solution-based approach. Solution crystallisation can yield large, well-formed single crystals, from which one may easily evaluate crystal habit and surface features. Analysis of the diffraction pattern of a single crystal is typically the best means of obtaining an absolute crystal structure determination. Additionally, solution crystallisation is an established and effective purification step.

The interactions of the solute and the solvent molecules govern the mechanisms of crystallization from solution. In solution, the crystal growth process is affected by the diffusion of the solute to the surface separating the solid and the liquid phase.

For cocrystallization from solution, the two components must have similar solubility; otherwise the least soluble component will precipitate out exclusively. Though similar solubility alone will not guarantee the successful formation of a cocrystal. It may be useful here to consider polymorphic compounds, which exist in more than one crystalline form, as cocrystallising components. If a molecular compound exists in several polymorphic forms it has demonstrated a structural flexibility and is not locked into a single type of crystalline lattice or packing mode, although the chance of bringing such a
molecule into a different packing arrangement in coexistence with another molecule is increased [34].

3.4.1.1 Solubility

Crystallization can take place over the concentration range limited by the equilibrium composition of the system under specified conditions. This equilibrium is dependent on solute-solvent energetics competing against the energy difference between those of solute-solute and solvent-solvent interactions themselves. Thermodynamic equilibrium refers to the solutions saturated with respect to the solute; where the rates of dissolution and crystallization are equal under these conditions.

A solution with solute concentration below the saturation limit (shown in Fig. 3.4 by a blue line) is termed undersaturated and existing crystals will dissolve. In order for crystallization to occur, the system must be brought into a non-equilibrium state where the concentration of the solute exceeds its equilibrium concentration. The driving force for crystallization is therefore the degree of supersaturation, expressed as the difference in concentration between the supersaturated and saturated solutions. The solution temperature was cooled down from A to B. After that, it entered into the metastable region; the metastable zone width depends on the purity of the system, the thermal history of the solution and the density of foreign particles present in the solution and is of practical importance since it defines the working area for designed crystallizations [35].
Fig. 3.4. Schematic solubility and supersolubility diagram [36].

3.4.1.2 Nucleation

In the pharmaceutical industry, the understanding and control of nucleation is of fundamental importance. Nucleation occurs at C in Fig. 3.4.

This nucleation process can be divided into two main groups [37]:

- Primary nucleation, where no crystals are originally present in the solution.
- Secondary nucleation, where crystals of the solute are already present.
Nucleation can be classified into three mechanisms:

1- Primary homogeneous, which occurs in systems that contain only pure solution (not often occurring in large volumes, because most solutions contain random impurities which may induce nucleation) [38, 39].

2- Primary heterogeneous, which occurs at the interfaces or crystal surfaces and may be induced by foreign particles.

3- Secondary nucleation, generated on the surface of crystals present in a supersaturated system.

### 3.4.1.2.1 Nucleation Theory

The classical theory of homogeneous nucleation can be considered as follows

\[
\Delta G_{\text{total}} = \Delta G_s + \Delta G_v = 4\pi r^2 \gamma + \frac{4}{3} \pi r^3 \Delta G_v
\]

(3.1)

where is the

- \( \Delta G \) : free energy change for the formation of the nucleus.
- \( \Delta G_s \) : free energy change for the formation of the nuclear surface.
- \( \Delta G_v \) : free energy change for the phase transformation.
- \( \Delta G_v \) : free energy change of the transformation per unit volume.

- \( r \) : radius of the particle

- \( \gamma \) : interfacial tension between the developing crystalline surface and the supersaturated solution.
Fig. 3.5. Free energy diagram for nucleation explaining the existence of a “critical nucleus” [40]

Therefore, supersaturation is required to overcome the free energy barrier to nucleation. After this stage, the cluster then becomes viable and is termed a nucleus, which eventually grows into a crystal. For a polymorphic system, the polymorph that nucleates first is thought to come from the cluster that exhibits the fastest growth rate as a result of its lowest free energy barrier to nucleation. However, the nature of the polymorph that eventually crystallizes is determined by the combination of the relative nucleation rates and the relative crystal growth rates [41]. The evidence suggests that the classical nucleation theory may not be qualitatively correct [42-45] and recent studies suggest that nucleation of solutes from solution is a two-step process [46-47]: the creation of a droplet of a dense liquid, metastable with respect to the crystalline state, followed by ordering within this droplet to form a three-dimensional lattice structure.
3.4.1.2.2 Crystal Growth

After the formation of the stable nuclei in the system, they then begin to grow into crystals of various sizes. Compared to nucleation, the real growth process is much faster since the crystals contain dislocations which provide the necessary growth points. Growth then spreads outwards from the nucleating site and the solute molecules add to the growing crystal in a prearranged system, where started in crystal nucleation. Crystal growth is a multi-step process and includes (Fig. 3.6):

- transport of a growth unit from or through the bulk solution to an impingement place, which is not necessarily the final growth site (i.e. the site of incorporation into the crystal)
- Adsorption of the growth unit at the impingement place.
- Diffusion of the growth units from the impingement place to a growth place.
- Incorporation into the crystal lattice.

Fig. 3.6. Crystal surface showing three types of growth places.
3.4.1.2.3 Crystal habit

In the drug development process, it is important to control crystal habits, because these could affect the processing properties of materials, for example, washing, drying, compression behaviour, tabletting behaviour and packing density [48, 49]. Crystal habit is used to describe the shape and size of crystals and is decided by the relative growth rate of solutes on different crystallographic faces. The crystallization driving force is supersaturation, which is thought to be the major factor affecting the crystal habit. The supersaturation parameter is mainly controlled by the initial solution concentration and by environmental/physical parameters such as temperature, relative humidity, surface area and roughness of the substrate. However, the re-crystallization from different solvents and processes might also change the crystal habit [50, 51].

3.4.2 Grinding

In spite of its simplicity, the solid-state grinding approach remains less familiar than cocrystal formation from solution. The solid-state grinding approach to preparing cocrystals is an alternative to the traditional method of solution crystallisation, and possesses several distinct advantages, in particular for poorly soluble compounds. Up to now, very little research has been directed at understanding the mechanism of organic cocrystal formation by solid-state grinding. One great attraction of preparing cocrystals by solid-state grinding is that, in certain cases, cocrystals unobtainable by other methods may be prepared.

This mechanochemical technique was first reported in the late 19th century and was revisited in detail by Etter [19, 52]. It has been reported that particular cocrystals could be prepared both by solid-state grinding and by solution growth, some could be prepared only by solid-state grinding, while others could be prepared only by solution growth [22,
The new technique of adding small mounts of solvent during the grinding process has been shown to enhance the kinetics and facilitate cocrystal formation and has led to increased interest in solid-state grinding as a method for cocrystal preparation [57-59]. Cocrystals that could not be identified by the slow evaporation of organic solvents or by dry grinding were identified by cogrinding reactants with small quantities of solvent, with the outcome being dependent on the nature of the solvent used.

### 3.4.3 Anti-solvent addition

This is one of the methods used for precipitation of the co-crystal former and active pharmaceutical ingredient. In anti-solvent crystallization, supersaturation is generated by addition of another solvent in which the solute has limited solubility. Anti-solvent crystallization is normally carried out at room temperature, whereby high yields of crystals can be expected.

### 3.5 Applications of cocrystals

Cocrystals have advantages such as stable crystalline forms, where there is no need to make or break covalent bonds can also be advantageous alternatives for drug discovery (new molecule synthesis) and drug delivery (solubility, bioavailability). Experts are of the opinion that the pharmaceutical intellectual property landscape may benefit through cocrystallization [8, 60-62].
3.6 Conclusion

Pharmaceutical cocrystals represent an advantageous class of crystal form in the context of pharmaceuticals. Cocrystals of drugs and drug candidates represent a new type of material for pharmaceutical development. Cocrystals are relatively new to the pharmaceutical industry and pharmaceutical cocrystals have given a new direction to deal with problems of poorly soluble drugs. Cocrystals have the potential to be much more useful in pharmaceutical products than solvates or hydrates. Cocrystals – High Throughput gives vital information on relationship between formation and chemical structure of the API and co-former and the screening of API’s with a library of cocrystal formers requires further investigation to include all possible conformers.
3.7 References


Chapter 4
Experimental
4.0 Experimental Procedure

This chapter provides experimental details of the work undertaken in the development of methods for in-situ Raman measurement of titrations and in the preparation and characterisation of selective cocrystal systems.

4.1 Reagents

All reagents in this study were used as supplied and without further purification. Origins and purities of all reagents used are summarised below:

Table 4.1 Origin of Reagents used in the experimental studies

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Origins</th>
<th>Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzamide</td>
<td>Aldrich</td>
<td>99%</td>
</tr>
<tr>
<td>Citric acid</td>
<td>Sigma-Aldrich</td>
<td>ACS reagent, ≥99.5%</td>
</tr>
<tr>
<td>DL-Phenylalanine</td>
<td>Sigma</td>
<td>≥98% (TLC)</td>
</tr>
<tr>
<td>3,4-Dihydroxybenzoic acid</td>
<td>Alfa Aesar</td>
<td>purum, ≥97.0%</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Fischer Scientific, UK</td>
<td>Absolute</td>
</tr>
<tr>
<td>6-Hydroxy Nicotinic acid</td>
<td>Aldrich</td>
<td>97%</td>
</tr>
<tr>
<td>Methanol</td>
<td>Fischer Scientific, UK</td>
<td>≥99.5%</td>
</tr>
<tr>
<td>Chemical</td>
<td>Supplier</td>
<td>Purity/Concentration</td>
</tr>
<tr>
<td>--------------------------</td>
<td>-------------------</td>
<td>----------------------------</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>Sigma</td>
<td>≥99.5% (HPLC)</td>
</tr>
<tr>
<td>Nitric acid</td>
<td>Sigma</td>
<td>Convol, 0.1M</td>
</tr>
<tr>
<td>Nitric acid</td>
<td>BDH</td>
<td>Conc.</td>
</tr>
<tr>
<td>Oxalic acid</td>
<td>Sigma-Aldrich</td>
<td>≥99.0% (RT)</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>Sigma-Aldrich</td>
<td>BioXtra, ≥99.0%</td>
</tr>
<tr>
<td>Potassium nitrate</td>
<td>Sigma-Aldrich</td>
<td>BioXtra, ≥99.0%</td>
</tr>
<tr>
<td>Potassium hydroxide</td>
<td>Sigma-Aldrich</td>
<td>reagent grade, ≥90%</td>
</tr>
<tr>
<td>Potassium hydrogen phthalate</td>
<td>Sigma-Aldrich</td>
<td>BioXtra, ≥99.95%</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>Sigma-Aldrich</td>
<td>ACS reagent, ≥99.0%</td>
</tr>
<tr>
<td>Salicylaldoxime</td>
<td>Aldrich</td>
<td>purum, ≥98.0% (NT)</td>
</tr>
</tbody>
</table>

### 4.2 Preparation of new cocrystal systems

#### 4.2.1 Preparation of salicylic acid and pharmaceutical relevant cocrystals

The method of preparation is exemplified as follows:

Salicylic acid (28.2 mg) and nicotinic acid (25.14 mg) were dissolved in 3 ml ethanol. The mixture was warmed on a hot plate and the solution was allowed to cool to room temperature. Slow evaporation of the solution resulted in the formation of a solid product which was isolated by filtration and air-dried.

Similarly, for the other cocrystals the preparation details are given in table 4.2.
<table>
<thead>
<tr>
<th>Compound (A)</th>
<th>Wt/ mg</th>
<th>Compound (B)</th>
<th>Wt/ mg</th>
<th>Ratio</th>
<th>Solvent (ethanol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>salicylic acid</td>
<td>100 (0.724 mmol)</td>
<td>benzamide</td>
<td>87.70 (0.724 mmol)</td>
<td>1:1</td>
<td>15 ml</td>
</tr>
<tr>
<td>salicylic acid</td>
<td>28.2 (0.204 mmol)</td>
<td>DL-phenylalanine</td>
<td>33.73 (0.204 mmol)</td>
<td>1:1</td>
<td>3 ml</td>
</tr>
<tr>
<td>salicylic acid</td>
<td>100 (0.724 mmol)</td>
<td>6-hydroxynicotinic acid</td>
<td>100.71 (0.724 mmol)</td>
<td>1:1</td>
<td>4 ml</td>
</tr>
<tr>
<td>citric acid</td>
<td>100 (0.520 mmol)</td>
<td>paracetamol</td>
<td>78.68 (0.520 mmol)</td>
<td>1:1</td>
<td>10 ml</td>
</tr>
<tr>
<td>citric acid</td>
<td>158.5 (0.825 mmol)</td>
<td>benzamide</td>
<td>100 (0.825 mmol)</td>
<td>1:1</td>
<td>5 ml</td>
</tr>
<tr>
<td>3,4-dihydroxybenzoic acid</td>
<td>171.19 (1.110 mmol)</td>
<td>oxalic acid</td>
<td>100 (1.110 mmol)</td>
<td>1:1</td>
<td>5 ml</td>
</tr>
</tbody>
</table>
4.3 Raman spectroscopy

4.3.1 Instrumentation

Three types of dispersive Raman spectrometers were used in this study; a Renishaw In Via confocal Raman microscope, a Renishaw portable Raman analyzer (RX210), and a cobalt systems transmission Raman spectrometer.

4.3.1.1 Renishaw In Via confocal Raman microscope

The Renishaw In Via confocal Raman microscope (Figs. 4.1 and 4.2) can be used with laser excitation ranging from UV to the near IR region of spectrum, depending upon the installed grating and filters. The excitation sources used during the course of this research were an argon- ion (Ar⁺) laser (488 and 515 nm), helium neon (He-Ne) laser (633 nm) and a gallium-aluminium-arsenide (GaAlAs) diode laser (785 nm).

The alignment and frequency scale calibration of the instrument were checked before the experiment as a routine set up by obtaining a spectrum of a silicon wafer (reference band at 520 cm⁻¹ ± 1 cm⁻¹). Samples on glass or steel slides were placed on the microscope stage and the area of interest was focused with an objective lens. Raman spectra were generally acquired at 2 cm⁻¹ spectral resolution and recorded in the range 3200-100 cm⁻¹.
The required parameters such as spectral frequency range, exposure time of the charged coupled device (CCD) detector and the number of spectra to be accumulated were selected using the proprietary instrument software, Renishaw WiRE™ 2.0. The software switches the spectrometer to the required excitation wavelength. The filter wheel is rotated if saturation of the CCD detector occurred to reduce the laser intensity at the sample. The use of a confocal microscope can increase the spatial resolution up to 1μm (micron) which is beneficial for the analysis of a small spot within the sample.
4.3.1.2 Renishaw Portable Raman Analyser RX210 ‘RIAS’

The Renishaw portable Raman analyser (RX210) (Figs 4.3 and 4.4) gives a rapid, non-destructive analysis of Raman spectroscopy in a compact portable unit. This instrument is well-known as the prototype RIAS or ‘Raman In A Suitcase’. The RIAS is equipped with a diode laser emitting at 785 nm and a thermoelectrically cooled charged coupled device (CCD) detector. An attached fibre-optic probe was equipped with a 75 mm focal distance. The diffraction grating (1000 lines/mm) limits the spectral range to ~2100-100 cm\(^{-1}\) with a spectral resolution of 10 cm\(^{-1}\). The maximal output power of the diode laser at the source is 500 mW and ~ 50mW at the sample. Daily calibration of the wavenumber axis is required and is achieved by recording the Raman spectrum of silicon (1 accumulation, 10 seconds) for static mode. If necessary, an offset correction was
performed to ensure that the position of the silicon band is 520.50±0.10 cm$^{-1}$. Spectra were generally recorded with the conditions: accumulation 4 scan, 10s exposure and full laser power. Data manipulation was subsequently undertaken off line. The instrumental control software, Renishaw Wire 2™ service packs 8.

Fig. 4.3 Renishaw portable Raman analyser (RX210)
Fig. 4.4 A schematic diagram of Renishaw Portable Raman Analyser (RX 210)

(Source: Renishaw Plc.)
4.3.1.3 Transmission Raman spectroscopy

The TRS100 (Fig 4.5) enables a rapid non-destructive Raman spectroscopic analysis to be carried out; the scattered radiation is collected from the opposite side of the sample to that illuminated. The advantage of TRS is that it samples the entire volume so giving an average of the whole object and removes the sub-sampling effects of conventional Raman spectroscopy. Transmission Raman spectroscopy can be applied throughout the pharmaceutical production chain on all types of material up to ca. 10 mm thickness.

Fig. 4.5 A schematic diagram of Transmission Raman spectroscopy.

(Source: Cobalt Light Systems)
4.3.2 Methods of routine analysis

4.3.2.1 Methods for solid state experimental analysis

4.3.2.1.1 Renishaw In Via confocal Raman microscope

Raman spectra were collected using a Renishaw In Via confocal Raman microscope. The Raman scattering was excited with a 785 nm and a 50x objective lens giving a laser spot diameter of 5 μm. Spectra were obtained for a 10s exposure of the CCD detector in the wavenumber region 100-3200 cm\(^{-1}\) using the extended scanning mode of the instrument, with 100% laser power and 4 accumulations. Spectral acquisition, presentation, and analysis were performed with Raman Renishaw WIRE (service pack 9) and GRAM© AI version 8.

4.3.2.1.2 Transmission Raman spectroscopy

Transmission Raman spectra were collected using a Cobalt Light Systems TRS100 dispersive Raman spectrometer. The sample vial was held in place (at an angle ~ 45°) and the excitation laser directed onto the underside of the sample. Light was collected at ~ 180° from the direction of the excitation laser light, for the other side of the vial. A NIR laser 830 nm was used, with a power at the sample of approximately 1 W and a beam diameter of 3-4 nm. The imaging zone on the vial is determined by the collection optics. All samples were measured for a 1s exposure time, with 10 accumulations, giving a total scan time of 10 seconds. Transmission data were collected at a resolution of ~ 5-6 cm\(^{-1}\) diameter over the full dispersive range of the instrument ~ 45-2500 cm\(^{-1}\).
4.3.2.2 Method for solution speciation

4.3.2.2.1 Experimental procedure for laser power stability experiment

An experiment to study the stability of the laser on the Portable Raman Analyser (RX 210) spectrometer at 785 nm was carried out by repeatedly measuring the solutions (0.6 mol dm$^{-3}$ of salicylic acid and paracetamol. Also, 1.0 mol dm$^{-3}$ of citric acid and salicylaldoxime) were recorded at 100% laser power, over 4 accumulations and with a 10 second laser exposure with the cosmic ray filter device enabled. The experiment was carried out over two days, taking spectra under the same conditions.

4.3.2.2.2 Calibration of Raman Renishaw portable Raman analyser ‘RIAS’

The solutions of 0.4 mol dm$^{-3}$ salicylic acid, 0.6 mol dm$^{-3}$ paracetamol and 1.0 mol dm$^{-3}$ salicylaldoxime were used for calibration purposes. They were placed in a glass Raman cell (Figure 4.6) and spectra were acquired with the 785 nm fibre optic probe attached to the Renishaw ‘RIAS’ Raman spectrometer. Spectra were obtained with 10s exposure and 100 % laser power for 4 accumulations of data. The results of these experiments are discussed in Section 5.

![Schematic diagram of experimental setup](image-url)
4.3.2.2.3 Experimental procedure for limits of detection experiment

Samples of paracetamol and salicylic acid (see Table 4.3) at concentrations of 0.6, 0.5, 0.4, 0.3, 0.2, 0.1, 0.09, 0.08, 0.06, 0.04, 0.03 and 0.02 mol dm\(^{-3}\) were analysed. The spectra were acquired on the Renishaw portable Raman analyser ‘RIAS’ at 782 nm over spectral range of \(\sim2100–100\) cm\(^{-1}\), using 100 % laser power, over 4 accumulations and 10 second laser exposure with the cosmic ray filter device enabled.

Samples of citric acid and salicylaldoxime (Table 4.3) at concentrations 1.0, 0.8, 0.6, 0.4, 0.2, 0.1, 0.08, 0.06, 0.05, 0.04 and 0.03 mol dm\(^{-3}\) were analysed and run under the same conditions. The procedure described here was used in the experiments discussed in section 5.

4.3.2.2.4 Experimental procedure for potentiometric/Raman experiment

The experimental procedure consists of taking pharmaceutical model solutions of concentrations specified in (Table 4.3.) and placed in a glass Raman cell with the temperature maintained at 30 ±0.1 °C, as shown in (Fig 4.6). The cell contains three probes namely, the pH probe, a temperature probe combination electrode for use with the pH probe and finally the Dosimat inlet for delivery of the potassium hydroxide solution. All titrations were performed by using either an automatic titrator (665 Dosimat) with magnetic stirrer stand rod and an electrode holder was also incorporated into the titration. The fibre optic Raman probe is a non-contact probe and sits outside the cell. The solution was left to stir for two minutes after potassium hydroxide solution additions to give the system time to equilibrate before spectra were acquired and measurements were taken. For pH only measurements, the readings were not taken until the system stabilised.
Typically this interval was ca.10 seconds. [The pH data was noted manually into a laboratory notebook and subsequently tabulated using Microsoft Excel].

The calibration of the pH meter was carried out in the usual way using two buffer solutions in aqueous media. The pH values in ethanol/water solvent mixtures were corrected using the equation of Douheret: $\text{pH}^* = \text{pH}(R) - \delta$. [2,3] Where $\text{pH}^*$ is the corrected reading and $\text{pH}(R)$ is the meter reading obtained in partially aqueous organic solvent mixtures. The values of $\delta$ for an aqueous solution containing varying proportions of each of the organic solvents were determined by Douheret. For ethanol/water mixed solvent, 60 wt% of ethanol, the value is small (<0.20). Douheret reported 0.02–0.03 deviations in $pK_a$ values as a result of using this method for pH correction [2, 3].

Raman spectra were also collected from pharmaceutical model solutions using a Renishaw portable Raman analyser ‘RIAS’ (Wotton-under-Edge, UK) equipped with a diode laser emitting at 785 nm and a thermoelectrically cooled charged coupled device (CCD) detector. An attached fibre-optic probe was equipped with a 75 mm focal distance lens. The diffraction grating (1000 lines/mm) limits the spectra range to ~2100-100 cm\(^{-1}\) with a spectral resolution of 10 cm\(^{-1}\). Spectra were obtained for a 10s exposure over 4 accumulations with full laser power (approx 50 mW). Reference Raman spectra of the pure solid pharmaceutical models and potassium nitrate were obtained to be used as reference spectra for comparison with the spectra of the solution samples. Reference Raman spectra of the water-ethanol mixtures were presented for spectral analysis in a mini quartz cell.
Table 4.3 Pharmaceutical samples preparation and experimental conditions

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH range</th>
<th>Con. (mol dm$^{-3}$)</th>
<th>L.O.D</th>
<th>Temperature °C</th>
<th>Ionic strength (mol dm$^{-3}$)</th>
<th>Raman conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Peak area</td>
<td>Peak intensity</td>
<td>KNO$_3$</td>
<td>HNO$_3$</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>2.70 - 13.41</td>
<td>0.4</td>
<td>0.024</td>
<td>0.042</td>
<td>30 ± 0.1</td>
<td>0.96</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>2.39 – 13.95</td>
<td>0.6</td>
<td>0.039</td>
<td>0.033</td>
<td>30 ± 0.1</td>
<td>0.96</td>
</tr>
<tr>
<td>Salicylaldoxime</td>
<td>2.92 - 12.66</td>
<td>1.0</td>
<td>0.05</td>
<td>0.063</td>
<td>30 ± 0.1</td>
<td>0.096</td>
</tr>
<tr>
<td>Citric acid</td>
<td>1.23 - 13.2</td>
<td>0.8</td>
<td>0.07</td>
<td>0.078</td>
<td>30 ± 0.1</td>
<td>0.096</td>
</tr>
</tbody>
</table>
4.4 Computer hardware and software

All Raman spectra were digitised and transferred in ASCII format to a GRAMS/AI version 8 for analysis. Determination of acidity constants was performed by regression analysis of the Raman spectra using a specific program running in the MATLAB 7.2 (The MathWork Co.) environment. Calculations were performed by Dr. Gholamhassan Azimi (University of Arak) using an iterative algorithm based on non-linear least squares [4, 5]. Digitised spectra (Raman intensities) at different pHs for each titration were combined in a single file. Noise filtering was carried out based on singular value decomposition (SVD). For this purpose after decomposition of the data file matrix to two matrices (the loading and score matrices), the data matrix was reconstructed by considering the major principal component that is equal to the number of Raman active components involved in acid–base equilibria of the pharmaceuticals. Then the data were processed using the proposed chemical equilibria (model) and an initial estimate of the pKa values. The program then minimizes the following equation based on the variation of pKa in each titration and considers some restrictions such non-negativity in concentration and the spectral profiles [6].

\[
\frac{1}{n_s} \sum_{i=1}^{n_s} \sum_{k=1}^{n_w} (I_{\text{exp}} - I_{\text{calc}})_{i,k}^2 \leq 0 \quad (4.1)
\]

where \(n_s\) is the number of solution (total titration steps) and \(n_w\) is wavenumber. “exp” and “calc” are referred to recorded and calculated signals, respectively. The potentiometric data was also treated in the above manner. In this way, the pH–volume data was processed according to the following equation:

\[
\frac{1}{n_t} \sum_{i=1}^{n_t} (pH_{\text{exp}} - pH_{\text{calc}})_{i}^2 \leq 0 \quad (4.2)
\]
4.5 Data acquisition

4.5.1 Data acquisition for laser power stability experiment

The Renishaw *InVia* and Raman Portable Raman Analyser (RX 210) spectra were collected using the Renishaw Wire [7] software and the spectra have been compared using Grams software [8].

4.5.2 Data acquisition for limits of detection experiment

The Renishaw Portable Raman Analyser (RX 210) spectra were collected using the Renishaw Wire [7] software; the spectra have been compared using propriety Grams software [8] and the results tabulated using Microsoft Excel [9].

4.5.3 Data acquisition for potentiometric/Raman experiment

The pH data was noted manually into a laboratory notebook and subsequently tabulated using Microsoft Excel. All samples were run in Raman Renishaw portable ‘RIAS’ system with a laser operating at 785 nm via a fibre optic probe. The parameters were defined in section as 4.3.1.3.2.

4.5.4 Baseline Correction

Background in Raman spectra is common and arises from either a luminescence process (fluorescence), non-laser-induced emissive processes (room lights) or Raman scattering from optics or solvents. The background can be removed by employing baseline correction; the resulting background spectral trace is then subtracted from the original spectrum.
4.5.5 Normalisation

The Raman spectroscopic technique is not based on absolute intensity because different instrumental parameters for normalisation, the most intense peak in the spectrum is given a fixed, arbitrary value to which all other intensities are scaled in proportion to this peak. Min/max normalisation was used in this work to display stack-plotted spectra, whereby the relative intensities of peaks in the spectra can be compared. Normalisation results in an increased stability of the calibration procedures, compensating for small changes in experimental parameters. It can be performed by using either an internal or an external standard. An internal standard should not interact with the sample or perturb or overlap the analyte spectrum. Internal standards should be strong emitters, so that a small quantity produces a recorded signal of adequate intensity.

4.5.6 Integration of band areas and height

The band area integration is performed using the trapezoidal rules. The area under the curve is separated into trapezoids, for which the limits are equalised by the use of baseline correction. The integrated band height is obtained by determining the distance of the peak maximum from the baseline at the X coordinate (wavenumber).

4.6 Powder diffraction analysis

Powder diffraction was carried out on all samples using a Bruker D-8 diffractometer in Bragg -Brentano 0-0 geometry with Cu Ka1,2 radiation (1.5418 Å) using a secondary curved graphite monochromator. The samples were ground to a powder and then placed in sample holders and mounted on the diffractometer. Data were collected from each sample in the 2θ from 5 to 40° 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 samples were analysed using diffract + Eva software and compared with the ICSD database for identification [10].

4.7 Single crystal X-ray diffraction experimental

X-ray intensity data were collected by Dr. Ian Scowen at the University of Bradford using a Bruker Apex II CCD diffractometer with Mo Kα radiation (0.71073 Å). Structures were solved by direct methods with SHELXS-97 and refined by a full-matrix least squares analysis on $F^2$ with anisotropic displacement parameters for non-H atoms in SHELXL-97.

Figure 4.7. Bruker X-8 at analytical centre of the University of Bradford
4.8 Differential scanning calorimetry (DSC) experimental

DSC data were collected by DSC Q2000 Fig.4.8. DSC profiles were generated in the range of 50-160 °C using a TA Q 2000 DSC instrument with an RGS 90 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. An accurately weighed sample (1-3 mg) was placed in Tzero aluminium pans using a similar empty pan as reference. The data were collected in triplicate for each sample and were analysed using a TA instruments Universal Analysis 2000 version 4.3A software.

Figure 4.8. DSC Q2000 instrument
4.9 References


7. Renishaw WIRE version 2.0.

8. Thermo Galactic Grams/AI version 8.


10. www.cds.ac.uk, 15-02-2010
Section A

Solution speciation and spectral studies
Chapter 5

In situ monitoring of pH titration by Raman spectroscopy
5.1 Introduction

Raman spectroscopy is a versatile tool in pharmaceutics and biopharmaceutics, with a wide field of applications ranging from characterization of drug formulations to elucidation of kinetic processes in drug delivery. Technological improvements in Raman spectroscopy have made this technique applicable for the quantitative and qualitative analysis of pharmaceuticals [1, 2]. Water is a very weak Raman scatterer and hence yields little significant signal in Raman spectra. As a consequence, Raman measurements in aqueous environments become most attractive[3]. The intensity of a Raman line is directly proportional to the concentration of the scattering component of a sample in a laser beam.

\[ I = \alpha C \]

where \( I \) is the intensity of Raman scattering, \( C \) is the concentration of the active ingredient and \( \alpha \) is a coefficient representing the features of the sample analysed and the spectrum conditions [4, 5].

The vibrational spectrum of a molecule is a fundamental spectroscopic tool in the study of molecular structure and chemical dynamics. Correct spectral assignment is the first step in the investigation of molecular structure, inter/intramolecular interactions, energy transfer mechanisms and chemical reaction dynamics, in addition to other transient phenomena. Raman spectra of certain chemical groups such as -OH, NH\(_2\), C=O, CH\(_3\) and C=O are often used as fingerprints to identify chemical species and to study the chemical dynamics for the molecules containing these groups [6].
Determination of acidity constants is highly important and lets us understand other properties. In addition, a knowledge of the ionization constant (pKₐ) of substance is very important in the pharmaceutical industry and drug design, in the chemical manufacturing industry (environmental impact compliance) and in the environmental field (environmental fate of toxic substances) [7-10]. Furthermore, for the assessment of pharmacodynamic features, compounds of potential therapeutic significance should be evaluated for properties which will contribute to their pharmacokinetic profile, such as solubility, lipophilicity, hydrogen bonding capacity and charge; these parameters can be easily estimated once the acidity dissociation constants are known. Moreover, the knowledge of pKₐ values is crucial for a better understanding of the chemical interaction between the compounds. For all these reasons, the acidity constant is a key parameter for the understanding and quantification of chemical phenomena.

The determination of the dissociation constant of an acid in a binary mixed solvent provides useful data for the theoretical understanding of the ionization process in systems where two dipolar entities, ethanol and water in our case, as well as the anions, can compete for the proton. The ionization constants of acids are influenced by the nature of the solvent and in alcohol-water mixtures vary with solvent composition in a manner which is not completely understood [11-13].

There have been several methods for the determination of acidity constants such as potentiometric titration, spectrophotometry, and conductimetry. Of these, potentiometric titration and spectrophotometric titration are widely used. Spectroscopic methods for determination of stability constants can be adopted for the determination of stability constants in solution under different experimental conditions. Overlapping of spectra of different chemical species involved in the equilibria is an important problem because it makes the determination of stability constants by classical methods difficult or even
impossible and can cause uncertainties in the results. For this purpose, recently, most systems in the literature have involved the use of both model-based and model-free chemometrics methods to estimate the number of species simultaneously present at equilibrium, their stoichiometries and their stability constants. Multiwavelength (multivariate) nonlinear least squares methods have been applied to spectroscopic data successfully. A substantial amount of work has been done in the field of pKₐ determination spectrophotometrically [14-23].

Salicylic acid (H₂Sal) and its derivatives are also biologically important ligands; a well known and widely used derivative, aspirin, reduces the risk of many diseases associated with ageing and is used in the treatment of rheumatic fever, pain, and the prevention of thrombosis in the vascular system. Salicylic acid comprises three organic functionalities; an aromatic ring, a carboxylic group and a phenolic hydroxyl group. Salicylic acid has a low pKₐ₁ (2.8) which corresponds to the ionisation of the proton of the carboxyl group and a high pKₐ₂ (ca.13.4) due to ionisation of the hydroxyl group [24]. The deprotonation of the hydroxyl group takes place at a very high pH value [25].

The high value of the dissociation constant of salicylic acid in aqueous solution is generally ascribed to the stabilisation of the (HSal⁻) by intermolecular hydrogen bonds between the phenolic (OH) groups and the oxygen atoms of the carbonyl groups [26, 27]

The vibrational spectrum of the salicylic acid molecule, based on Raman and IR measurements and normal coordination calculations, was studied by Volovsek et al [28]. Tejedor et al. [29] Suggest a model of complexation between the salicylate and iron atoms at the goethite interface, Trout et al [30] studied the interaction of soluble organic compounds (benzoic acid, salicylic acid and phthalic acid) with metal cations. Also, Trout and Kubicki [31] have used salicylic acid and phthalic acids to study the complexation of aromatic carboxylic acids with Al (III) in solution and on mineral surfaces by using UV
resonance Raman spectroscopy. The pH values of the acid solutions were based on the pKₐ values, for the different acids to examine the neutral and charged species. The assignment of vibrations of salicylic in aqueous solution was discussed by Humbret et al [32]. On the other hand, Boczar et al [33] have presented a quantitative description of infrared spectra in the O-H stretching region of hydrogen bonded salicylic acid.

4-Hydroxyacetanilide was first prepared by Morse in 1878. Under the names paracetamol, acetaminophen and panadol, it is now used as an analgesic and antipyretic drug. Paracetamol, a weak acid having pKₐ ca. 9.5, rapidly becomes absorbed and distributed after oral administration and is easily excreted in urine [34]. A continuous flow-based spectroscopic method for the determination of paracetamol [35] in pharmaceuticals has been reported by Criado et al [36].

Many IR and/or Raman studies on paracetamol are in the literature including methods of its identification [35, 37-45] and quantification [39, 40, 46, 47]. The conversion of the paracetamol into the corresponding oxyanion and dianion by means of both quantitative IR and ab initio calculations has been studied by Binev et al [48]. The hydrogen bonding in drug receptor interactions has been studied by Ghafourian et al [49]. The partial molar volume and the related volumetric parameters of paracetamol in dilute aqueous solutions at different temperatures and pressures have been investigated [50]. IR spectra of paracetamol has been measured for the powder crystal and for solution in chloroform and dimethylsulfoxide have been reported by Burgina et al [51]. Through complete neglect of differential overlap, calculations and structural spectrometric studies of paracetamol complexes have been reported by El-Shahawy et al [52]. However, the Raman spectra of anionic derivatives of paracetamol in aqueous solution are not available in the literature.
The chemistry of organic chelating agents (N–O donor ligands) such as oximes and hydroxyoximes has evoked keen interest owing to their application in the extractive hydrometallurgy industry, has been specially relating to the extraction of copper and nickel [53-58]. Lumme et al. have determined potentiometrically the acidity constant of salicylaldoxime [59-61] and some acidity constants in mixtures of water with non-aqueous solvents [62-64] have been reported. Tshuma et al. have studied the $pK_a$ of salicylaldoxime spectrophotometrically in aqueous solution at 25°C [65].

Citric acid (2-hydroxy-1,2,3-propanetricarboxylic acid), a natural constituent and common metabolite of plants and animals, is the most versatile and widely used organic acid in the field of food and pharmaceuticals. Citric acid has a distinctive sharp taste and is found in the juice of lemons and other sour fruits. Citric acid is used as a flavouring and preservative in food and beverages, especially soft drinks. Citrate salts of various metals are used to deliver those minerals in a biologically available form in many dietary supplements. The buffering properties of citrates are used to control pH in pharmaceuticals [66]. Citric acid contains three carboxylic acid functional groups (-COOH) and has a molecular formula of $H_3C_6H_5O_7$, a molecular weight of 192.124 g/mol. Citric acid is a relatively strong organic acid as indicated by the first dissociation constant, $7.4\times10^{-4}$ at 25 °C [67]. Citric acid is the strongest chelating agent of all the common food acids and is used in the binding of metal ions in food stuffs to prevent spoilage. It is an important multipotent chemical binding ligand toward some biologically relevant metal ions, such as Fe$^{3+}$, Al$^{3+}$, Ga$^{3+}$, and Bi$^{3+}$. At pH 5.2, citric acid chelates Ca$^{2+}$, Mg$^{2+}$, Mn$^{2+}$ and Fe$^{2+}$ [68-71].

Determination of high concentrations of citric acid is normally carried out by strong base titration to an endpoint pH of around 7.0, while low concentrations are currently determined by a spectrophotometric method based on a reaction with pyridine and acetic
anhydride [72]. Also, enzymatic[73] and HPLC methods[74] are in use. In recent times, other methods based on ion chromatography[75], gas chromatography[76], and fluorescence spectroscopy[77] have been proposed. Kevin et al [78] have studied the determination of the micro-acidity constants for citric acid; furthermore, the corresponding pKₐ dissociation constant of the citric acid and/or of solutes of comparable structures have been determined in different mixtures of solvent; various methods were employed, most of them involving potentiometric titration or UV spectroscopy [79-83]. Vaclav et al [84] reported determination of dissociation constant of citric acid by capillary isotachophoresis. Several papers have been published on the IR and Raman spectra of citric acid in aqueous solution [85-89]. Nevertheless, to the best of our knowledge, there has been no report hitherto concerning the Raman spectroscopic determination of the equilibrium constants of salicylic acid, paracetamol salicylaldoxime and citric acid in a water-ethanol mixture under the same conditions.

In the present work, a quantitative determination method by Raman spectroscopy of an aqueous pharmaceutical namely salicylic acid, paracetamol, citric acid and salicylaldoxime was developed. This research concerned the conformational dependence of the pKₐ values and a vibrational spectroscopic analysis.
5.2 Potentiometric method for analysis of equilibrium\[90\]

The potential $E$ of an electrochemical cell is related to the activity $a$ of an ion taking part in a reversible electrode can be explained as the equation:

$$E = E_o + (RT/nF) \ln a + E_j \quad (5.1)$$

where $F$ is the Faraday, $n$ is the number of electrons participating in the cell reaction, $E_o$ is a constant for the cell, $R$ the gas constant, $T$ is the temperature and $E_j$ is the term representing a liquid-liquid junction potential.

If the activity of an ionic species undergoes a change as a consequence of complexation equilibrium, and if an electrode is available that is reversible with respect to this ion the equation (5.1) applies.

5.3 Computational methods for determination of equilibrium constants

Multivariate curve resolutions are a group of chemometrics methods dealing with extraction concentration profiles and pure spectra of the species coexist in the reaction system [91-95]. The application of these methods for studying acid–base equilibria has now become popular in chemistry [96-99]. There are available some commercial programs such as SQUAD [100-102], SPECFIT [103], DATAN 2.1 [98, 104], HYPERQUAD [105], DALSFEC [106] and EQUISPEC [107] that can be used to calculate acidity constants.
5.3.1 Model-Based Analyses

The goal of model-based analytical methods is to facilitate the 'translation' from original data to useful chemical information. The result of a model-based analysis is a set of values for the parameters that quantitatively describe the measurement, ideally within the limits of experimental noise. The most important prerequisite is the model, the physical-chemical, or other, description of the process under investigation. An example helps clarify these statement. The measurement is a series of absorption spectra of a reaction solution; the spectra are recorded as a function of time. The model is a second order reaction $A+B\rightarrow C$. The parameter of interest is the rate constant of the reaction. The purpose of model-based analyses is the determination of the 'best' set of parameters for a particular given model and one or a collection of measurements. In other words, we fit the parameter(s) to the measurement(s).

It cannot be over-stressed that the task of finding the 'best' model for the measurement is a much more difficult undertaking. A crucial difference between finding the optimal parameters for a given model and finding the optimal model lies in the fact that the parameters of a model form a continuous space, while models are discrete entities. Model-based parameter fitting relies on the continuous relationship between the quality of the fit and the parameters. There are no equivalent continuous transitions from one trial model to the next and thus, all the powerful fitting algorithms are useless. A lot of chemical intuition, experience, knowledge etc. is involved in the process of establishing the correct model. The usual procedure is to chose a selection of reasonable models and fit them all, and subsequently make a decision on the 'best' or 'correct' one by analysing
the individual results of these analyses. Some data-fitting algorithms provide statistical information that allows an estimation of the quality of the fit and thus about the suitability of the model.

The model defines a mathematical function, either explicitly (e.g. first order kinetics) or implicitly (e.g. complex equilibria), which in turn is quantitatively described by one or several parameters. In many instances, the function is based on such a physical model, e.g. the law of mass action. In other instances an empirical function is chosen because it is convenient (e.g. polynomials of any degree) or because it is a reasonable approximation (e.g. Gaussian functions and their linear combinations are used to represent spectral peaks).

A crucial point is the question about the meaning of the expression 'best' parameters. Intuitively, it seems to be clear; they are the parameters for which the calculated data match the measured data as closely as possible. Almost invariably, the sum of the squares of the differences between the measured data and the calculated model function is minimized and is the measure for the quality of the fit.

5.3.2 Non-Linear Regression

Non-linear regression calculations are extensively used in most sciences. The goals are very similar to the ones used for Linear Regression. Now, however, the function describing the measured data is non-linear and as a consequence, instead of an explicit equation for the computation of the best parameters, we have to develop iterative procedures. Starting from initial guesses for the parameters, these are iteratively improved or 'fitted', i.e. those parameters are determined that result in the optimal 'fit', or, in other words, that result in the minimal sum of squares of the residuals.
There are a multitude of methods for this task. Those that are conceptually simple usually are computationally intensive and slow, while the fast algorithms have a more complex mathematical background. We have used the Newton-Gauss-Levenberg/Marquardt algorithm, not because it is the simplest but because it is the most powerful and fastest method. The following Figure shows the Newton-Gauss-Levenberg/Marquardt algorithm strategy.

Fig. 5.0. The Newton-Gauss/Marquardt algorithm
As expected in an iterative algorithm, it starts from an initial guess for the parameters. This parameter vector is subsequently improved by the addition of an appropriate parameter shift vector $\delta p$, resulting in a better, but probably still not perfect, fit. From this new parameter vector the process is repeated until the optimum is reached.

Depending on the change of the sum of squares the Marquardt parameter, $mp$, is adjusted; the parameter is reduced upon convergence and increased otherwise. There are no general rules on how exactly this should be done in detail; it depends on the specific case. Usually convergence occurs with no Marquardt parameter at all; it is thus initialized as zero. The complexity of the flow diagram shown might be surprising. A few remarks are appropriate: it is possible that the Marquardt parameter reaches a high value and these results in a very small shift vector. Consequently, the change in ssq gets very small too and the algorithm decides prematurely that the minimum has been reached. To prevent this sequence, one last iteration is done without a Marquardt parameter ($mp=0$) if the termination criterion is satisfied but $mp$ is not yet zero.

5.3.3 Determination of Protonation Constants Based on Potentiometric and Spectrophotometric Titration Data Using Model-Based Analyses.

The calculation of protonation constants using model-based analysis mainly contain three steps, preparation of data (preprocessing and noise filtering), assign a proper chemical model (i.e., define the number of protonation / deprotonation equilibria), and starting the programs using a set of initial parameters (e.g. estimate of the protonation constants and/or initial concentration). The final selection of a model is based on some statistic parameters such as the sum of squares of residuals (ssq) and standard deviation on fitted parameters as well as the extent of knowledge about the selected model (i.e. all fitted
parameters should have a physical meaning). In new and as yet unknown chemical systems with fitting problems involving several parameters, it is sometimes difficult to come up with reasonable initial guesses for all parameters. If some of them are seriously wrong, even the Marquardt algorithm collapses and there is no result.

In the case of potentiometric data, the iterative algorithm starts with a set of initial estimates of parameters (main_EqAHn.m) and based on these values the concentration of all species calculated using a Newton Raphson algorithm (NewtonRaphson.m). The calculation in this function is based on the mass balance equations for the acid and proton. The sum of squares error between the calculated and experimental values of pH is calculated in the next step (Rcalc_EqAHn.m). If the computed ssq is smaller than a threshold value, the calculation is terminated, otherwise the parameters will shifted to new values using the Marquardt algorithm (nglm3.m) and the process will start with new values as a set of estimates. The iterative process is continued until the ssq reach threshold criteria for ssq or the maximum number of iterations.

In case of spectrophotometric data the same iterative procedure is used except that the residuals of sum of squares is computed based on the differences between the calculated and experimental values of absorbance, Raman and or fluorescence intensities (Rcalc_Eqfix.m).
5.4 Results and discussion

- **Study to test the Raman laser power stability**

The preparation of solutions used in this study are discussed in section 4.4 (Experimental procedure for laser power stability experiment) and the experimental procedure associated with these studies outlined in section 4.3.2.2.1

The table 5.0 displays the changes in the salicylic acid bands wavenumber positions in this experiment. It is evident that at ± 0.06 % and 0.13 errors for the peaks 1457 cm\(^{-1}\) and 773 cm\(^{-1}\) respectively, the reproducibility of the data is very good. Therefore, in subsequent experiments, observed small changes in band wavenumber position will be significant.

Table 5.0 Raman bands wavenumber position of aqueous salicylic acid solutions. Data collection Renishaw portable Raman analyser.

<table>
<thead>
<tr>
<th></th>
<th>1457 cm(^{-1})</th>
<th>773 cm(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>1457.167</td>
<td>772.8333</td>
</tr>
<tr>
<td>High</td>
<td>1458</td>
<td>774</td>
</tr>
<tr>
<td>Low</td>
<td>1456</td>
<td>772</td>
</tr>
<tr>
<td>error %</td>
<td>0.06</td>
<td>0.13</td>
</tr>
</tbody>
</table>
5.4.1 Salicylic acid

5.4.1.1 Determination of limit of detection (LOD)

As a preliminary study to the pH titration, a formal Raman limits of detection (LOD) (Figs. 5.1 and 5.2) study for salicylic acid in aqueous-ethanol (50% wt/wt) was undertaken. Spectra were collected from salicylic acid solutions in the range 0.6 mol dm$^{-3}$ to 0.01 mol dm$^{-3}$ and at constant pH value; the spectra from the concentration range 0.6 mol dm$^{-3}$ to 0.03 mol dm$^{-3}$, show no change in peak positions. Each solution was measured in triplicate. Using GRAMS software the spectra were peak fitted to calculate the area of the peak at 773 cm$^{-1}$; the peak areas where than averaged to give the mean peak area of each measurement. Calibration curves were then plotted of the mean peak area against concentration and the standard deviation (s) was calculated by using the replicate of the blank solution. The theoretical limit of detection LOD was than calculated using the equation below; Statistically, LOD = 3s/S where s is the standard deviation of the blank solution and S is the gradient of the calibration curve. Also, the peak intensity was used to determine the LOD; the Limit of Detection as determined by the peak area and peak intensity of the band at 773 cm$^{-1}$ are 0.024 mol dm$^{-3}$ and 0.042 mol dm$^{-3}$, respectively. The use of the peak area to estimate the LOD is therefore considered more preferable than the use of the peak intensity.
Fig. 5.1. Limit of detection of H$_2$Sal by using intensity

Fig. 5.2. Limit of detection of H$_2$Sal by using peak area
5.4.1.2 Potentiometric data

The potentiometric titrations of salicylic acid solution (0.4 mol dm\(^{-3}\), 50% wt/wt ethanol/water) in the presence of 1.0 mol dm\(^{-3}\) KNO\(_3\) started with a base solution (KOH, 1.2 mol dm\(^{-3}\)) at pH ca 2.77 and continued to pH ca. 13.41. A potentiometric titration is shown in Fig. 5.3. The deprotonation equilibria of salicylic acid based on its chemical structure can be written as follows:

\[
C_6H_4 (OH) COOH \rightarrow C_6H_4 (OH) COO^- + H^+ \quad pK_a = 4.22 \quad (1)
\]

\[
C_6H_4 (OH) COO^- \rightarrow C_6H_4 (O^-)COO^- + H^+ \quad pK_a = 14.0 \quad (2)
\]

The carboxyl groups can lose protons in an acidic medium, but it was proved by detailed potentiometric and spectrophotometric measurement that the dissociation of the phenolic OH can be detected and determined (but with a rather large experimental error) to the strong intramolecular hydrogen bond between the COO\(^-\) and OH group \([108-110]\).

The potentiometric data were fitted to a diprotic acid (H\(_2\)L) model as described in the software section. It can be seen that good agreement is observed between the experimental values and calculated values of pH. The calculated pK\(_a\) values are given in Table 5.1. As can be seen, the results are in good agreement with those calculated from spectroscopic data and also with the literature within the reported errors.

<table>
<thead>
<tr>
<th>Equilibrium</th>
<th>Spectrophotometric</th>
<th>Potentiometric</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA(^-) + H(^+) \rightarrow H_2A</td>
<td>pK(_{a1}) = 3.91±0.24</td>
<td>pK(_{a1}) = 4.22±0.30</td>
</tr>
<tr>
<td>A(^2-) + H(^+) \rightarrow HA</td>
<td>pK(_{a2}) = 14.08±0.3</td>
<td>pK(_{a2}) = 14.0±0.21</td>
</tr>
<tr>
<td>Sig r</td>
<td>0.00436</td>
<td>0.10502</td>
</tr>
</tbody>
</table>

The sig(r) or (\(\sigma_r\)) is standard deviation of the measurement error on the signal.
Fig. 5.3. Potentiometric titration curve of SA in an 1.0 mol dm$^{-3}$ KNO$_3$ and HNO$_3$ ionic medium at 30\textdegree C.

5.4.1.3 Spectral changes and assignment

Raman spectra of salicylic acid solutions were recorded in the pH range 2.70 - 3.57 (Fig. 5.4). The results of Raman spectroscopic analysis with band assignments are given in Table 5.2. The spectra in the range 1500-1700 cm$^{-1}$, show there are three bands observed at about 1664, 1620 and 1592 cm$^{-1}$. The highest frequency is characteristic of the C=O of the carboxylic group, the band at 1620 cm$^{-1}$ was attributed to $\nu$ 8a + $\nu$(C=O) – $\nu$ (C\Phi – OH) + $\delta$ (C\Phi – OH) a, b, and the band at 1592 cm$^{-1}$ accredited to $\nu$8b + $\nu$(C=O) + $\delta$ (C\Phi –OH)c. The peak at 1325 cm$^{-1}$ was assigned to a $\nu$ (C\Phi –OH) carboxylic function. The peak at 1247 cm$^{-1}$ was attributed to $\nu$ (C\Phi –OH) $\nu$ (CO–COOH). However, the band 773 cm$^{-1}$ was attributed to $\nu$( C\Phi—COOH ) + $\nu$( C\Phi—OH) + $\delta$(COOH) [111].
The normal modes of a benzene ring C—C stretching vibration are usually characterised by the 8a, 19 band 14 modes of benzene according to the Wilson’s numbering sequence; for benzene the 8, 19 and 14 modes are, respectively, at 1596, 1486 and 1310 cm\(^{-1}\). They shift to 1620 (8a), 1592 (8b), 1457 (19a), 1484 (19b) and 1312 (14) cm\(^{-1}\). In the above pH range no peak at 814 cm\(^{-1}\) was observed as shown in the Fig. 5.4.

Raman spectra of salicylic acid have measured in the pH range 3.81 to pH 4.16. In the range 770 to 820 cm\(^{-1}\) two bands were observed at about 814 cm\(^{-1}\) and at 773 cm\(^{-1}\), the first band characteristic of the \(\nu(\text{C}\Phi—\text{COO}^-) + \delta(\text{C}\Phi—\text{OH}) + \delta_s(\text{COO}^-)\) for the salicylate monoanion (H\(_2\)Sal\(^-\)). The second band was attributed to the \(\nu(\text{C}\Phi—\text{COOH}) + \nu(\text{C}\Phi—\text{OH}) + \delta(\text{COOH})\) as shown in Fig. 5.4.

![Raman spectra of salicylic acid at different pHs](image-url)
The peak at 814 cm$^{-1}$ appears as only a weak peak at pH 3.81 and becomes a medium peak at higher pH and the peak at 773 cm$^{-1}$ starts as a strong peak and disappears with higher pH (Figs. 5.4 and 5.5). Also, in this range of pH we observed that the band at 67 cm$^{-1}$ could be assigned to the 1 mode (Wilson's numbering). This band decreases at pH < 3.81. For these observations, in this pH range there are two forms of salicylic acid, namely form (H$_2$Sal) and the salicylate monoanion (HSal$^-$), Fig. 5.6a and 5.6b.

Fig. 5.5. Relationship between the 773 and 814 cm$^{-1}$ peak area with pH.

Fig. 5.6. Structural formulae of salicylic acid and its ionic derivatives
The peak at 1664 cm\(^{-1}\) disappeared at pH 5.11. Also, at the same pH the peak of the \(\nu(COH)\) band at 1325 cm\(^{-1}\) disappears and the peak of the \(\nu(C\Phi—OH) + \nu(C\Phi—COOH)\) band at 1249 cm\(^{-1}\) was shifted to a lower wavenumber position at 1228 cm\(^{-1}\); this peak was attributed to \(\nu(C\Phi—OH) - \nu(C\Phi—COO-) + \delta(C\Phi—OH)\), although alternatively one might argue that this is a (C—O) stretching coupled with the in-plane deformation of the phenolic group. The slight wavenumber shift observed with an increasing pH may be due to the intramolecular hydrogen bond, which is stronger in the anion than in the acid.

The peak at 773 cm\(^{-1}\) decreases significantly in intensity whatever is appropriate pH 5.11 as illustrated in Fig. 5.5, and has disappeared at pH 5.37. In this range of pH all the peaks have unchanged wavenumber positions. The band assignment of the \(CH_3 CH_2 OH\) and KNO\(_3\) solution are given in Table 5.3.

### 5.4.1.4 Acid Dissociation Constants

The calculated dissociation constants of the salicylic acid by potentiometric and Raman spectroscopy are tabulated in Table 5.1.

The good agreement between to within one standard deviation of Raman spectroscopy and potentiometric titration measurements indicates that Raman spectroscopy in principle is an excellent technique to monitor a titration, provided that some identifiable spectroscopic features vary with the titration. We compare the literature values of pKa that were found for salicylic acid, our calculated pKa values are significantly higher than the corresponding pKa values of Laurih et al (pK\(_{a1}\), 2.8 and pK\(_{a2}\), 13.4) [25, 112]. These discrepancies could be related to differences in the ionic media and other working conditions since our ionic medium was 1.0 mol dm\(^{-3}\) (NO\(_3\)). The decrease of ionization on increasing the alcohol content in solution may be attributed to changes in the \(\pi\) electron density of the C=O groups by solvent molecules [113]. The species distribution
curves (given in Fig.5.7) show that the major species in the acidic pH range in the system are H2SALI (aq) and HSALI; but in the high basic pH range HSALI\(^-\) loses a proton and becomes SALI\(^-\) type species. These curves were calculated using the in-house MATLAB program.

Fig. 5.7. Distribution of the species present in a 0.4 ± 0.01 mol dm\(^{-3}\) aqueous solution of salicylic acid as a function of pH at 30 ± 0.1 °C in an ionic medium of I = 1.0 mol dm\(^{-3}\) (NO\(_3^\)\(^-\)).
### Table 5.2 Salicylic acid band assignments at different pHs.

<table>
<thead>
<tr>
<th>Salicylic acid at pH 2.77 to 3.57</th>
<th>Salicylic acid at pH 3.81 to 4.30</th>
<th>Salicylic acid at pH 5.11 to 5.35</th>
<th>Salicylic acid at pH 5.98 to 13.41</th>
<th>Peak assignments[32]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1664 w</td>
<td>1662 w</td>
<td>-</td>
<td>-</td>
<td>ν (C=O) trans</td>
</tr>
<tr>
<td>1638 w</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>1620 vw</td>
<td>1618 w</td>
<td>1621 w</td>
<td>1620 w</td>
<td>ν 8a + ν (C=O) - ν(C=O-OH) + δ(C=O-OH)² ³</td>
</tr>
<tr>
<td>1592 vw</td>
<td>1592 w</td>
<td>1593vwsh</td>
<td>1593vwsh</td>
<td>ν 8b + ν (C=O) + δ(C=O-OH)⁴ ⁵</td>
</tr>
<tr>
<td>1485 vw</td>
<td>1485 w</td>
<td>1487 w</td>
<td>1484 w</td>
<td>ν 19b</td>
</tr>
<tr>
<td>1457 s</td>
<td>1458 s</td>
<td>1459 s</td>
<td>1459 s</td>
<td>ν 19a</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>1390 m</td>
<td>1389m</td>
<td>ν14 + ν₆ (COO⁻) + δ(C=O-OH) + ν(C=O-OH)</td>
</tr>
<tr>
<td>1325 m</td>
<td>1325 m</td>
<td>-</td>
<td>-</td>
<td>ν(C=O) carboxylic function</td>
</tr>
<tr>
<td>1247 m</td>
<td>1250 w</td>
<td>-</td>
<td>-</td>
<td>ν(C=O-OH) + ν(C=O-COOH)</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>1228 w</td>
<td>1228 w</td>
<td>ν(C=O-OH) - ν(C=O-COO⁻) + δ(C=O-OH)</td>
</tr>
<tr>
<td>1156 w</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ν 9a</td>
</tr>
<tr>
<td>1147 vw</td>
<td>1147 w</td>
<td>1146 w</td>
<td>1146 w</td>
<td>ν 9b</td>
</tr>
<tr>
<td>1090 m</td>
<td>1089 m</td>
<td>1089 m</td>
<td>1090 m</td>
<td>ν 15</td>
</tr>
<tr>
<td>1075 w</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ν 12</td>
</tr>
<tr>
<td>-</td>
<td>814 w</td>
<td>814m</td>
<td>815m</td>
<td>ν(C=O-COO⁻) + C₂(C=O-OH) + δ(COO⁻)</td>
</tr>
<tr>
<td>773 s</td>
<td>777 m</td>
<td>-</td>
<td>-</td>
<td>ν(C=O-COOH) + ν(C=O-OH) + δ(COOH)</td>
</tr>
<tr>
<td>568 m</td>
<td>569 w</td>
<td>570 w</td>
<td>570 w</td>
<td>ν 6a</td>
</tr>
<tr>
<td>545 w</td>
<td>545 w</td>
<td>547 w</td>
<td>548 w</td>
<td>ν 6b</td>
</tr>
<tr>
<td>518 w</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>471 w</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>414 m</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>396 w</td>
<td>396 vw</td>
<td>396vw</td>
<td>397vw</td>
<td></td>
</tr>
<tr>
<td>370 w</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Cₐ = carbon of the aromatic ring.

+ = in-phase movement.

- = out-of-phase movement.

w = weak.

vw = very weak.

s = strong.

vs = very strong.

m = medium
Table 5.3: Raman spectra of CH$_3$CH$_2$OH and KNO$_3$ [114-116]

<table>
<thead>
<tr>
<th>Wavenumbers (cm$^{-1}$) for ethanol</th>
<th>assignment</th>
<th>Wavenumbers (cm$^{-1}$) for KNO$_3$</th>
<th>assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>solid</td>
<td>solution in H$_2$O/ethanol</td>
</tr>
<tr>
<td>2973w</td>
<td>$v_1$ (CH3)</td>
<td>2935vw</td>
<td>-</td>
</tr>
<tr>
<td>2930m</td>
<td>$v_2$(CH3)</td>
<td>2596vw</td>
<td>-</td>
</tr>
<tr>
<td>2878m</td>
<td>$v_1$(CH2)</td>
<td>2580w</td>
<td>2580vw</td>
</tr>
<tr>
<td>2719vw</td>
<td>-</td>
<td>1688vw</td>
<td>-</td>
</tr>
<tr>
<td>2578vs</td>
<td>-</td>
<td>1478vw</td>
<td>-</td>
</tr>
<tr>
<td>1478w</td>
<td>$\delta$ (CH$_2$)</td>
<td>1359vw</td>
<td>-</td>
</tr>
<tr>
<td>1454s</td>
<td>$\delta_1$ (CH$_3$)</td>
<td>1048vs</td>
<td>1048vs</td>
</tr>
<tr>
<td>1279m</td>
<td>t(CH2) + $\delta$(COH)</td>
<td>715m</td>
<td>715w</td>
</tr>
<tr>
<td>1098m</td>
<td>$v$(CO) + r(CH3) + $\delta$(COH)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1054m</td>
<td>$v_2$ (CCO)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>881vs</td>
<td>$v_3$(CCO)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>796vw</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>598w</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>434w</td>
<td>$\delta$ (CCO)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
5.4.2 Paracetamol

5.4.2.1 Determination of the limit of detection (LOD)

As a preliminary study to the pH titration, a formal Raman limit of detection study for paracetamol in aqueous-ethanol (50% wt/wt) was undertaken (Figs 5.8 and 5.9). Spectra were collected from paracetamol solutions in the range 0.6 mol dm$^{-3}$ to 0.01 mol dm$^{-3}$ and at a constant pH value; the spectra in the concentration range 0.04 mol dm$^{-3}$ to 0.6 mol dm$^{-3}$ show no change in the peak wavenumber positions. Each solution was measured in triplicate. Using GRAMS software the spectra were peak fitted to calculate the area of the peak at 796 cm$^{-1}$ and the peak areas were then averaged to give the mean peak area of each measurement. Calibration curves were then plotted of the mean peak area against concentration. Also the peak intensity was used to determine the LOD. The limit of detection as determined by the peak area and peak intensity of the band at 796 cm$^{-1}$ are 0.039 mol dm$^{-3}$ and 0.033 mol dm$^{-3}$, respectively. Clearly, the results obtained from both methods are comparable.
Fig. 5.8. Limit of detection of paracetamol using peak intensity.

\[ y = 4703x \]

\[ R^2 = 0.9999 \]

Fig. 5.9. Limit of detection of paracetamol using peak area.

\[ y = 6021.4x \]

\[ R^2 = 0.9999 \]
5.4.2.2 Potentiometric data

The potentiometric titrations of the paracetamol solution (0.6 mol dm$^{-3}$ (50% wt/wt) ethanol / water) in the presence of 1.0 mol dm$^{-3}$ KNO$_3$ started with a base solution at pH ca 2.39 and continued up to pH ca 13.95. The potentiometric titration results verified our suppositions from this calculation (Fig.5.10) Table 5.4. From Fig. 5.10 it can be seen that good agreement is observed between the experimental values and theoretical values.

![Fig. 5.10. Potentiometric titration curve of paracetamol in an 1.0 mol dm$^{-3}$ KNO$_3$ and HNO$_3$ ionic medium at 30 ± 0.1°C.](image-url)
5.4.2.3 Spectral changes and assignment

In the range 1650 cm\(^{-1}\) to 300 cm\(^{-1}\), the spectra of solutions at different pHs differ mainly in intensity and position (Table 5.5). The peak at 1649 cm\(^{-1}\) appearing as a shoulder in the solution at 1661 cm\(^{-1}\) at pH 2.39 was attributed to the amide (I) and (C=O) stretch. This is ascribed to the emergence of the paracetamol oxyanion, (\(-\text{O-C}_6\text{H}_4\text{-NH-COCH}_3\)) in the solution. The peak at 1618 cm\(^{-1}\) attributed to (vCC, \(\sigma\text{CCC}, \sigma\text{ipHNC}\)) is shifted to 1605 cm\(^{-1}\) at pH 11.02 and becomes more intense with increasing pH as shown in Fig. 5.11. The peak at 1565 cm\(^{-1}\) for the amide III (N-H in plane deformation) disappears at pH 11.02 and a new peak appears at 1545 cm\(^{-1}\) becoming more intense with increasing pH. The peak at 1543 cm\(^{-1}\) appears with weak intensity at high pH and becomes more intense with increasing pH. This peak can be ascribed to the (vC-N, vC=O, vCC) for the paracetamol dianion (\(-\text{O-C}_6\text{H}_4\text{-N-COCH}_3\)). The peak at 1517 cm\(^{-1}\) at pH 2.39 due to the \(\sigma\) in-plane (HCC, vCCC, \ vCC) mode disappears at pH 12.03 as shown in Figs. 5.11, 5.12 and 5.13. The peak at 1236 cm\(^{-1}\) at pH 2.39 is shifted to 1230 cm\(^{-1}\) at pH 11.02 and becomes more intense with increasing pH. The peak at 798 cm\(^{-1}\), attributed to aromatic ring stretching shifts to 811 cm\(^{-1}\) at pH 12.03, and the peak at 713 cm\(^{-1}\) appears at 721 cm\(^{-1}\) at pH 11.50. The peak at 330 cm\(^{-1}\) tentatively assigned to intramolecular hydrogen bond (IMHB) stretching disappears at pH 12.03 Fig. 5.11. These changes are illustrated in Fig. 5.11 and are correlated with structural changes in the paracetamol with pH, Fig. 5.14.
Fig. 5.11. Raman spectra of paracetamol at different pHs.

Fig. 5.12. Relationship between 1618 and 1605 cm\(^{-1}\) Raman peak intensities with pH for paracetamol.
Fig. 5.13. Relationship between 1543, 1517 and 330 cm\(^{-1}\) Raman peak intensities with pH for paracetamol.

Fig. 5.14. Structural formulae of the paracetamol, oxyanion and dianion.
5.4.2.4 Acid dissociation constants

The calculated equilibrium constants (logK) of the paracetamol obtained from the potentiometric and Raman spectroscopic data are tabulated in Table 5.4.

The deprotonation equilibria of paracetamol based on its chemical structure can be written as follows:

\[
\text{C}_8\text{H}_9\text{NO}_2 \rightarrow \text{O-}\text{C}_6\text{H}_4\text{-NH-COCH}_3 \quad \text{pK}_a = 9.8
\]

A shift in pKa values from 9.5 in water at 25 °C is expected. The HA form of paracetamol is more stable in a solvent with higher lipophilicity such as ethanol while its conjugated base (A⁻) is more stable in water. Therefore, the dissociation of a large acid such paracetamol decreases in the presence of ethanol. These discrepancies can be attributed to differences in the ionic media and other working conditions since our ionic medium was 1.0 mol dm⁻³ (NO₃⁻), compared with the analysis value of 0.1 mol dm⁻³ in the literature.

The species distribution curves (given in Fig.5.15) show that the major species in the acidic pH range in the system are C₈H₉NO₂ (aq) and O-C₆H₄-NH-COCH₃ in base media.

From Fig. 5.15 it can be seen that a good agreement is observed between the experimental values and theoretical values; these dissociation constants represent the first derivation from experimental Raman spectra.
Fig. 5.15. Distribution of the species present in a 0.6 ± 0.01 mol dm$^{-3}$ aqueous solution of paracetamol as a function of pH at 30 ± 0.1 °C in ionic medium of I = 1.0 mol dm$^{-3}$ (NO$_3^-$)

Table. 5.4 Equilibrium constants of paracetamol

<table>
<thead>
<tr>
<th>Equilibrium</th>
<th>Spectrophotometric</th>
<th>Potentiometric</th>
</tr>
</thead>
<tbody>
<tr>
<td>$HA^- + H^+ ===== H_2A$</td>
<td>$pK_a = 11.08 ± 0.31$</td>
<td>$pK_a = 10.96 ± 0.03$</td>
</tr>
</tbody>
</table>
Table.5.5 Paracetamol band assignment (cm$^{-1}$) in the solid state and in solution at different pHs

<table>
<thead>
<tr>
<th>Paracetamol solid</th>
<th>Paracetamol solution from pH 2.39 to 10.33</th>
<th>Paracetamol solution from pH 10.42 to 10.69</th>
<th>Paracetamol solution from pH 10.82 to 11.02</th>
<th>Paracetamol solution from pH 11.10 to 11.30</th>
<th>Paracetamol solution from pH 11.50 to 13.90</th>
<th>Peak assignments [52, 117]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>Amide I band (C=O stretch)</td>
</tr>
<tr>
<td>1649vs</td>
<td>1621m</td>
<td>1621m</td>
<td>1607m</td>
<td>1607s</td>
<td>1607s</td>
<td>$\nu_{CC}, \sigma_{CCC}, \sigma_{\text{ip} \text{HNC}}$</td>
</tr>
<tr>
<td>1618vs</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>$\nu_{CC}, \sigma_{\text{ip} \text{HNC}}, \sigma_{\text{ip} \text{HCC}}$</td>
</tr>
<tr>
<td>1609vs</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>$\sigma_{\text{ip} \text{HNC}}, \nu_{CC}, \nu_{\text{as} \text{CNC}}, \sigma_{\text{ip} \text{HCC}}$</td>
</tr>
<tr>
<td>1555s</td>
<td>1562w</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>$\sigma_{\text{ip} \text{HNC}}, \nu_{CC}, \nu_{\text{as} \text{CNC}}, \sigma_{\text{ip} \text{HCC}}$</td>
</tr>
<tr>
<td>1514w</td>
<td>1518w</td>
<td>1518w</td>
<td>1518w</td>
<td>1518vw</td>
<td>A</td>
<td>$\sigma_{\text{ip} \text{HCC}}, \sigma_{\text{CCC}}, \nu_{\text{CC}}$</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Aryl C$\text{\textendash}H$, C$\text{\textendash}H$ sym metric bends</td>
</tr>
<tr>
<td>1445w</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>$\sigma_{\text{as} \text{CH}_3}$</td>
</tr>
<tr>
<td>1371s</td>
<td>1383m</td>
<td>1383m</td>
<td>1383m</td>
<td>1383m</td>
<td>1383m</td>
<td>$\nu_{\text{CCC}}, \sigma_{\text{ip} \text{HCC}}, \sigma_{\text{ip} \text{HNC}}, \nu_{\text{C-O}}$</td>
</tr>
<tr>
<td>1324vvs</td>
<td>1333m</td>
<td>1333m</td>
<td>1333m</td>
<td>1333m</td>
<td>1333m</td>
<td>Amide III band (C-N stretch/ C-N-ph stretch/ C-N-H bend)</td>
</tr>
<tr>
<td>1278m</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>$\nu(C-N); \delta(N-H)$ amide III</td>
</tr>
<tr>
<td>1256m</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>$\nu_{\text{C-O}}, \sigma_{\text{ip} \text{HCC}}, \nu_{\text{CG}}, \sigma_{\text{CCC}}$</td>
</tr>
<tr>
<td>1236s</td>
<td>1236m</td>
<td>1236m</td>
<td>1233m</td>
<td>1233m</td>
<td>1230m</td>
<td>$\nu_{\text{CG}}, \sigma_{\text{ip} \text{HCC}}, \sigma_{\text{ip} \text{HCC}}, \nu_{\text{CNC}}$</td>
</tr>
<tr>
<td>1169s</td>
<td>1178m</td>
<td>1178m</td>
<td>1178m</td>
<td>1178m</td>
<td>1171m</td>
<td>$\sigma_{\text{ip} \text{HCC}}, \nu_{\text{CC}}$</td>
</tr>
<tr>
<td>1121vww</td>
<td>1121vw shoulder</td>
<td>1121vw shoulder</td>
<td>1121vw shoulder</td>
<td>1121vw shoulder</td>
<td>1121vw shoulder</td>
<td>$\nu_{\text{CC}}, \sigma_{\text{HOCC}}$</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>$\sigma_{\text{ip} \text{HCC}}, \nu_{\text{CC}}, \sigma_{\text{ip} \text{HOC}}$</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>$\sigma_{\text{ip} \text{HCC}}, \nu_{\text{CC}}, \sigma_{\text{ip} \text{HOC}}$</td>
</tr>
<tr>
<td>971w</td>
<td>976w</td>
<td>976w</td>
<td>976w</td>
<td>976w</td>
<td>976w</td>
<td>H-C-C bend</td>
</tr>
<tr>
<td>860 vs</td>
<td>A, observed as shoulder on peak at 880</td>
<td>A, observed as shoulder on peak at 880</td>
<td>A, observed as shoulder on peak at 880</td>
<td>A, observed as shoulder on peak at 880</td>
<td>A, observed as shoulder on peak at 880</td>
<td>Aromatic ring bend</td>
</tr>
<tr>
<td>---------</td>
<td>-----------------------------------------</td>
<td>-----------------------------------------</td>
<td>-----------------------------------------</td>
<td>-----------------------------------------</td>
<td>-----------------------------------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>836 m</td>
<td>836 w</td>
<td>836 w</td>
<td>836 w</td>
<td>836 w</td>
<td>836 w</td>
<td>Out-of-plane C—H bend (Aryl-1,4-disubstituted)</td>
</tr>
<tr>
<td>799 s</td>
<td>806 m</td>
<td>806 w</td>
<td>801 mw</td>
<td>801 vw</td>
<td>811 vw</td>
<td>Aromatic ring stretches</td>
</tr>
<tr>
<td>713 vw</td>
<td>714 w</td>
<td>714 w</td>
<td>721 w</td>
<td>721 w</td>
<td>Aromatic ring bend</td>
<td></td>
</tr>
<tr>
<td>709 w</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>-</td>
</tr>
<tr>
<td>685 w sh</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>Out of plane wagging of NH (amide group)</td>
</tr>
<tr>
<td>654 m</td>
<td>654 w</td>
<td>654 w</td>
<td>654 w</td>
<td>654 w</td>
<td>652 w</td>
<td>Aromatic ring bend</td>
</tr>
<tr>
<td>626 w</td>
<td>630 vw</td>
<td>630 vw</td>
<td>633 vw</td>
<td>633 vw</td>
<td>Aromatic ring bend</td>
<td></td>
</tr>
<tr>
<td>606 w</td>
<td>606 vv w</td>
<td>606 vv w</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>σ C—CH₃</td>
</tr>
<tr>
<td>503 m</td>
<td>512 w</td>
<td>512 w</td>
<td>512 w</td>
<td>512 vw</td>
<td>512 vw</td>
<td>Skeleton vibration</td>
</tr>
<tr>
<td>463 m</td>
<td>437 m</td>
<td>437 m</td>
<td>437 m</td>
<td>437 m</td>
<td>437 m</td>
<td>Aromatic ring bend</td>
</tr>
<tr>
<td>412 w</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>σ in-plane</td>
</tr>
<tr>
<td>393 s</td>
<td>393 m</td>
<td>393 m</td>
<td>393 m</td>
<td>393 m</td>
<td>390 m</td>
<td>C-N bend</td>
</tr>
<tr>
<td>328 m</td>
<td>330 w</td>
<td>330 w</td>
<td>330 w</td>
<td>330 vw</td>
<td>disappear</td>
<td>Stretching (IMHB)</td>
</tr>
<tr>
<td>217 m</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>-</td>
</tr>
</tbody>
</table>

A, observed; B, obscured by ethanol bands; C, appears as shoulder; s, symmetrical; as, asymmetrical; ip, in-plane σ, scissoring; v, stretching.
5.4.3 Salicylaldoxime (SALO)

In this study, the effect of pH on salicylaldoxime (Fig.5.16) was investigated by means of Raman spectroscopy and potentiometry.

![Chemical Structures](image)

Fig. 5.16. Chemical Structures: (a) salicylaldoxime, H$_2$L and (b and c) deprotonated ionic forms.

5.4.3.1 Determination of limit of detection (LOD)

Spectra were collected from salicylaldoxime solutions in the range 1.0 mol dm$^{-3}$ to 0.01 mol dm$^{-3}$ and at constant pH value; the spectra from the concentration range 1.0 mol dm$^{-3}$ to 0.02 mol dm$^{-3}$, show no change in peak positions. Each solution was measured in triplicate. Using GRAMS software the spectra were peak fitted to calculate the area of the peak at 1623 cm$^{-1}$, the peak areas were then averaged to give the mean peak area of each measurement. Calibration curves were then plotted of the mean peak area against concentration and the standard deviation (s) was calculated using the replicate of the blank solution. The theoretical limit of detection (LOD) was calculated. The Limit of Detection as determined by the peak area and peak intensity of the band at 1623 cm$^{-1}$ are 0.055 mol dm$^{-3}$ and 0.063 mol dm$^{-3}$, respectively, see figs 5.18 and 5.19. The use of the
peak area to estimate the LOD is considered more preferable than the use of the band intensity (Figs 5.17 and 5.18).

Fig.5.17. Plot of peak area versus concentration for the peak at 1623 cm\(^{-1}\) in the Raman spectra of SALO.

Fig.5.18. Plot of peak intensity versus concentration for the peak at 1623 cm\(^{-1}\) in the Raman spectra of SALO.
5.4.3.2 Potentiometric data

The potentiometric titrations of the salicylaldoxime solution (1.0 mol dm$^{-3}$, 50% wt/wt ethanol/water) in the presence of 0.1 mol dm$^{-3}$ (NO$_3^-$) started with a base solution (KOH, 1.0 mol dm$^{-3}$) at pH ca 2.93 and continued to pH ca. 12.66. An example of such a potentiometric titration is shown in Fig. 5.19. The deprotonation equilibria of salicylaldoxime based on its chemical structure can be written as follows:

\[
\begin{align*}
C_7H_7NO_2 & \rightarrow C_7H_6NO_2^- + H^+ \quad pK_{a1} = 10.12 \quad (1) \\
C_7H_6NO_2^- & \rightarrow C_7H_5NO_2^{2-} + H^+ \quad pK_{a2} = 13.0 \quad (2)
\end{align*}
\]

The potentiometric data were fitted to a diprotic acid (H$_2$L) model as described in the software section. It can be seen that a good agreement is observed between the experimental values and calculated values of pH. The calculated pK$_a$ values are given in Table 5.6. The results from spectroscopic data are in good agreement with those in the literature within the reported errors.

**Table.** 5.6. Equilibrium Dissociation Constants of salicylaldoxime

<table>
<thead>
<tr>
<th>Equilibrium</th>
<th>Spectrophotometric</th>
<th>Potentiometric</th>
</tr>
</thead>
<tbody>
<tr>
<td>H$_2$A ----- HA$^- + H^+$</td>
<td>pK$_{a1}$ = 10.13 ± 0.35</td>
<td>pK$_{a1}$ = 10.090 ± 0.31</td>
</tr>
<tr>
<td>HA$^-$ ----- A$^{2-} + H^+$</td>
<td>pK$_{a2}$ = 11.84 ± 0.50</td>
<td>pK$_{a2}$ = 12.65 ± 0.26</td>
</tr>
<tr>
<td>Sig r</td>
<td></td>
<td>0.276</td>
</tr>
</tbody>
</table>
Fig. 5.19. Potentiometric titration curve of salicylaldoxime in a 0.1 mol dm$^{-3}$ KNO$_3$, HNO$_3$ ionic medium at 30 $^\circ$C (line: calculated curve, points: experimental data)

### 5.4.3.3 Spectral changes and assignment

In the range 1630 cm$^{-1}$ to 300 cm$^{-1}$, the Raman spectra of solutions at different pHs differ mainly in intensity and position (Table 5.7). The spectra were recorded over the pH range 2.93 – 8.24, don’t show any change in the peak position or intensity; these spectra in the wavenumber range 1623 cm$^{-1}$ – 562 cm$^{-1}$ show many bands centred at 1623, 1276, 1238 and 650 cm$^{-1}$, corresponding to the $\nu$(C-N) stretching of the oxime group, $\nu$(O–H) bending (in-plane), aromatic (C–O) stretching vibration and (O-H) out-of-plane bending, respectively. [120,121]
In the pH range 8.76 to 9.75 two new bands were observed at about 1552 cm\(^{-1}\) and 1388 cm\(^{-1}\) with the intensity of these peaks increasing with increasing pH as shown in Fig.5.21. The spectral changes indicate the formation of HL\(^-\) from the H\(_2\)L species.

![Raman spectra](image)

**Fig.5.20.** Raman spectra of salicylaldoxime at different pHs range identify on spectra.

From the spectra in Fig.5.20, several well-defined isosbestic points can be observed. The presence of these isosbestic points demonstrates that the salicylaldoxime exhibits a series of spectral changes by proton exchange between the species present in the system. This fact is further consolidated by the Raman spectral data versus pH shown in Figs.5.21 and 5.22. The peak at 1582 cm\(^{-1}\) is shifted to 1586 cm\(^{-1}\) at pH 9.83 and appears only as a weak shoulder as shown in Fig.5.23. Furthermore, the peak has shifted to higher wavenumber and centred at 1597 cm\(^{-1}\) at pH 10.07 and becomes more intense with increasing pH.

On the other hand, the peak at 1218 cm\(^{-1}\) (OH deformation vibration) disappears at pH 10.14; also, the singlet centred at 650 cm\(^{-1}\) becomes a doublet at 650 and 633 cm\(^{-1}\) at pH
10.07 – 10.44, and then becomes a singlet again, centred at 532 cm⁻¹, at pH 10.75 and higher as shown in Figs.5.23 and 5.24.

![Fig.5.21. Plots of Raman Intensity at 1550 cm⁻¹ vs pH](image-url)
Fig. 5.22. Relationship between the 1582 and 1550 cm\(^{-1}\) peak intensity with pH.

Fig. 5.23. Raman spectra of salicylaldoxime at pHs 2.93, 8.76 and 9.75
Fig. 5.24. Raman spectra of salicylaldoxime at pHs 9.75, 9.83 and 10.07

The peak at 1622 cm\(^{-1}\) is shifted to lower wavenumber and becomes a doublet at 1618 and 1601 cm\(^{-1}\) at pH 10.14; additionally, the peak at 1218 cm\(^{-1}\) (OH deformation vibration) disappears. A new peak appears at 1566 cm\(^{-1}\) at pH 11.88 becoming more intense with increasing pH; at the same time the peak at 1241 cm\(^{-1}\) (aromatic C–O stretching vibration) disappears. The doublet peaks at 650 and 633 cm\(^{-1}\) become a single peak centred at 1634 cm\(^{-1}\) at pH 11.88 and higher pHs as shown in Fig. 5.25. These changes in the spectra are ascribed to the formation of the \(L^2^-\) species from \(HL^-\).
5.4.3.4 Acid dissociation constants

The calculated dissociation constants of salicylaldoxime determined by the potentiometric and Raman spectroscopic techniques are tabulated in Table 5.6.

The good agreement within one standard deviation between the Raman spectroscopic and potentiometric titration measurements indicates that Raman spectroscopy in principle is an excellent technique to monitor a titration, provided of course that some identifiable spectroscopic features vary with the titration. We have compared the literature values of $pK_a$ found for salicylaldoxime and our calculated $pK_a$ values are significantly lower than the corresponding $pK_a$ values reported by Terrier et al [118]. This discrepancy is attributed especially to an ionic medium and solvent mixture properties. The decrease of ionization found occurs on increasing the alcohol content in solution may be attributed to changes in the $\pi$ electron density of the C=O groups by solvent molecules [119].
species distribution curves given in Fig. 5.26 show that the major species in the acidic pH range in the system are $C_7H_7NO_2$ (aq) and $C_7H_6NO_2^-$, but in the highly basic pH range $C_7H_6NO_2^-$ loses a proton and becomes a $C_7H_6NO_2^{2-}$ species. These curves were calculated using the in-house MATLAB programme.

5.26. Distribution of the species in a 1.0 mol dm$^{-3}$ aqueous solution of salicylaldoxime as function of pH at 30 °C in 0.1 mol dm$^{-3}$ ionic medium.
Table 5.7 Salicylaldoxime (SALO) Raman assignments (cm\(^{-1}\)) at different pHs

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>3060w</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>v(C-H)</td>
</tr>
<tr>
<td>2988w</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>v(C-H)</td>
</tr>
<tr>
<td>1631sh</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>OH deform. vibr</td>
</tr>
<tr>
<td>1619s</td>
<td>1623s</td>
<td>1623s</td>
<td>1622s</td>
<td>1618m, 1601m</td>
<td>v(C-N) str. of oxime group</td>
</tr>
<tr>
<td>1580m</td>
<td>1582m</td>
<td>1582m</td>
<td>1582 w sh</td>
<td>-</td>
<td>v(C-N) str. of oxime group</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>1552m</td>
<td>1552m</td>
<td>1551m</td>
<td></td>
</tr>
<tr>
<td>1495w</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Ortho disubstituted benzene ring vibr.</td>
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<tr>
<td>1475w</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>1412w</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>O-H bending (in-plane)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>1388m</td>
<td>1388m</td>
<td>1388w</td>
<td></td>
</tr>
<tr>
<td>1310m</td>
<td>1312m</td>
<td>1312m</td>
<td>1312m</td>
<td>1312w</td>
<td>CH vibr. In plane</td>
</tr>
<tr>
<td>1286w</td>
<td>1276w</td>
<td>1276m</td>
<td>1276m</td>
<td>1275m</td>
<td>v(O-H) bending (in plane)</td>
</tr>
<tr>
<td>1239m</td>
<td>1238m</td>
<td>1238m</td>
<td>1238m</td>
<td>1241w</td>
<td>Aromatic C-O str.vibration</td>
</tr>
<tr>
<td>1211w</td>
<td>1218w</td>
<td>1218w</td>
<td>1218w</td>
<td>-</td>
<td>OH deform. vibr</td>
</tr>
<tr>
<td>1197w</td>
<td>1200w</td>
<td>1200w</td>
<td>1200w</td>
<td>1200w</td>
<td>N-O stretch. Vibr.</td>
</tr>
<tr>
<td>1158m</td>
<td>1157m</td>
<td>1157m</td>
<td>1157m</td>
<td>1157m</td>
<td>C-C deform.vibr. in disub. Benzene ring.</td>
</tr>
<tr>
<td>1036s</td>
<td>1038s</td>
<td>1038s</td>
<td>1038s</td>
<td>1038m</td>
<td>Benzene ring breathing</td>
</tr>
<tr>
<td>987w</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>v(OH) deformation (out of plane)</td>
</tr>
<tr>
<td>959w</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>N-O stretch. vibr.</td>
</tr>
<tr>
<td>935w</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>899w</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>v(N–O) str.</td>
</tr>
<tr>
<td>784s</td>
<td>782s</td>
<td>782s</td>
<td>782s</td>
<td>786s</td>
<td></td>
</tr>
<tr>
<td>762vs</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>C-H out of plane</td>
</tr>
<tr>
<td>646m</td>
<td>650m</td>
<td>650m</td>
<td>650w</td>
<td>650wsh</td>
<td>(O-H) o.p.bending</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>633w</td>
<td>633m</td>
</tr>
<tr>
<td>560vs</td>
<td>562s</td>
<td>562s</td>
<td>562s</td>
<td>562s</td>
<td></td>
</tr>
<tr>
<td>488w</td>
<td>489w</td>
<td>489w</td>
<td>489w</td>
<td>489w</td>
<td>o.p. skeletal deformation</td>
</tr>
<tr>
<td>467w</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>o.p. skeletal deformation</td>
</tr>
<tr>
<td>419s</td>
<td>424m</td>
<td>424m</td>
<td>424m</td>
<td>424m</td>
<td>o.p. skeletal deformation</td>
</tr>
<tr>
<td>336s</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>o.p. skeletal deformation</td>
</tr>
<tr>
<td>233w</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>216m</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>202m</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>
5.4.4 Citric acid

5.4.4.1 Determination of limit of detection (LOD)

Spectra were collected from citric acid solutions in the range 1.0 mol dm$^{-3}$ to 0.01 mol dm$^{-3}$ and at constant pH value; the spectra from the concentration range 1.0 mol dm$^{-3}$ to 0.03 mol dm$^{-3}$, show no change in peak positions. Each solution was measured in triplicate. Using GRAMS software the spectra were peak fitted to calculate the area of the peak at 797 cm$^{-1}$; the peak areas were than averaged to give the mean peak area of each measurement. Calibration curves were then plotted of the mean peak area against concentration Fig. 5.27 and the standard deviation ($s$) was calculated using the replicate of the blank solution. The theoretical limit of detection LOD was calculated. Also, the peak intensity was used to determine LOD Fig. 5.28; the Limit of Detection as determined by the peak area and peak intensity of the band at 797 cm$^{-1}$ are 0.07 mol dm$^{-3}$ and 0.078 mol dm$^{-3}$, respectively. The use of the peak area to estimate the LOD is considered more preferable than the use of band intensity.
Fig. 5.27. Plot of peak area versus concentration for the peak at 797 cm\(^{-1}\) in the Raman spectra of citric acid.

\[ y = 14513x \]
\[ R^2 = 0.9967 \]

Fig. 5.28. Plot of peak intensity versus concentration for the peak at 797 cm\(^{-1}\) in the Raman spectra of citric acid.

\[ y = 3847.5x \]
\[ R^2 = 0.9974 \]
5.4.4.2 Potentiometric data

The potentiometric titrations of the citric acid solution (0.8 mol dm\(^{-3}\), 50% wt/wt ethanol/water) in the presence of 0.1 mol dm\(^{-3}\) (NO\(^3\)) started with a base solution (KOH, 1.0 mol dm\(^{-3}\)) at pH ca 2.38 and continued to pH ca. 6.16. An example of such a potentiometric titration is shown in (Fig. 5.29) Table 5.8. There is a good agreement between the calculated curve and experimental data.

![Potentiometric titration curve](image)

Fig. 5.29. Potentiometric titration curve of citric acid in a 0.1 mol dm\(^{-3}\) KNO\(_3\), HNO\(_3\) ionic medium at 30 \(^0\)C (line: calculated curve, points: experimental data)
Table 5.8. Equilibrium Dissociation Constants of citric acid

<table>
<thead>
<tr>
<th>Equilibrium</th>
<th>Spectrophotometric</th>
<th>Potentiometric</th>
</tr>
</thead>
<tbody>
<tr>
<td>$H_2A \rightleftharpoons H_2A^- + H^+$</td>
<td>$pK_{a1} = 3.57 \pm 0.21$</td>
<td>$pK_{a1} = 3.2 \pm 0.06$</td>
</tr>
<tr>
<td>$H_2A^- \rightleftharpoons HA^2^- + H^+$</td>
<td>$pK_{a2} = 4.4 \pm 0.53$</td>
<td>$pK_{a2} = 4.78 \pm 0.06$</td>
</tr>
<tr>
<td>$HA^2^- \rightleftharpoons A^3^- + H^+$</td>
<td>$pK_{a3} = 6.8 \pm 0.42$</td>
<td>$pK_{a3} = 6.02 \pm 0.03$</td>
</tr>
</tbody>
</table>

The results from these experimental data are in good agreement with those in the literature within the reported errors [122].

5.4.4.3 Spectral changes and assignment

Table 5.9 shows the wavenumbers and assignments of the Raman spectra of aqueous solutions of citric acid at various pHs values.

Citric acid is clearly different from the ions generated by its dissociation; initially, there is the strong band due to the carboxylic acid group ν (COOH) at 1748 cm$^{-1}$. Also, there is a very strong band at 1163 cm$^{-1}$ attributed to C-O-H deformation (see Table 2). The region 770-1000 cm$^{-1}$ is very important, since citric acid has two characteristic bands at 780 cm$^{-1}$ and 816 cm$^{-1}$ assigned to symmetric COO deformations [123]. The band at 933 cm$^{-1}$ is also characteristic of citric acid, this being assigned to an OH out-of-plane bend and strong band at 941 cm$^{-1}$ attributed to C-C symmetric stretch [89-92].

The peak at 780 cm$^{-1}$ in the solid sample is shifted to 797 cm$^{-1}$ in solution moving from the pH 2.38 to 3.80. The intensity of this peak decreases at increasing pH and transforms into a doublet with peaks at 797 and 837 cm$^{-1}$ in the pH range 3.95 – 6.16, whereby the peak at 837
cm\(^{-1}\) increases in intensity as observed in Figs. 5.30. and 5.31. This change assigned to dihydrogen citrate species (H\(_2\)C\(^-\)) demonstrates an intensity ratio of 1.16: 1.0. for the bands at 797 and 837 cm\(^{-1}\) respectively. On other hand the intensity of the peak at 715 cm\(^{-1}\) decreases with decreasing pH and disappears at pH 4. Furthermore, intensity of the peak at 936 cm\(^{-1}\) assigned to OH out-of-plane bend was decreasing with increasing pH and disappeared at pH 5.20 in the same time a new band appears at 951 cm\(^{-1}\) and increases in intensity with increasing pH, the intensity ratio of the 797: 837 cm\(^{-1}\) peaks is about 0.6: 1.0 the change corresponding to monohydrogen citrate (HC\(^-\)) as shown in Figs 5.31 and 5.32.

Fig. 5.30. relationship between Raman peak areas at 1721, 837 and 797 cm\(^{-1}\)
Fig. 5.31. Raman spectra of citric acid at different pH from 1100 to 500 cm\(^{-1}\)

Fig. 5.32. Relationship between Raman peak areas at 951 and 936 cm\(^{-1}\)
The peak of the citric acid solution at 1721 cm$^{-1}$ at pH 2.38 attributed to v(COOH) has decreased with increasing pH and disappears at pH 5.95 as shown in Figs.5.30 and 5.33; the intensity ratio of the 797: 837 cm$^{-1}$ peaks is about 0.32: 1.0 at pH 5.95. (Comparison with the corresponding intensity ratios of 1.16: 1.0 and 0.6: 1.0 for (H$_2$C$^-$) species and for the (HC$^-$) species; these ratios characterised the species in solution ((H$_2$C$^-$), (HC$^-$) and (C$^-$)).

Fig.5.33. Raman spectra of citric acid at different pH from 1800 to 1000 cm$^{-1}$
5.4.4.4 Acid dissociation constants

The calculated dissociation constants of citric acid determined by the potentiometric and Raman spectroscopic techniques are tabulated in Table 5.8.

The species distribution curves given in Fig. 5.34 show that the major species in the acidic pH range in the system are [H$_3$C] (aq), [H$_2$C$^-$] and [H$_2$C$^{2-}$], but in the highly basic pH range [H$_2$C$^{2-}$] loses a proton and becomes a [C$^{3+}$] species. These curves were calculated using the in-house MATLAB programme.

![Species distribution curves](image)

Fig. 5.34 Distribution of the species in a 0.8 mol dm$^{-3}$ aqueous solution of citric acid as function of pH at 30 °C and 0.1 mol dm$^{-3}$ ionic medium.
Table 2. Raman wavenumbers and vibrational assignment of citric acid (solid and aqueous solution at a range of pH values)

<table>
<thead>
<tr>
<th>pH 2.38-3.95</th>
<th>pH 4.00-5.76</th>
<th>pH 5.95-6.16</th>
<th>Assignments [89, 124]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1748 vs</td>
<td>1721 m</td>
<td>disappear</td>
<td>ν(COOH)</td>
</tr>
<tr>
<td>1719 w</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1677 vs</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1440 m broad</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>1430 m broad</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>1414 s</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>1392 s</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>1346 m</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>1312 m</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>1292 m</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>1255 m</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>1225 m</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>1196 w</td>
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<td>*</td>
<td>*</td>
</tr>
<tr>
<td>1163 vs</td>
<td>disappear</td>
<td>disappear</td>
<td>disappear</td>
</tr>
<tr>
<td>1104 s</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>1073 vs</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>1054 s</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>941 vs</td>
<td>936 m</td>
<td>938 m</td>
<td>disappear</td>
</tr>
<tr>
<td>933 v</td>
<td>-</td>
<td>-</td>
<td>951</td>
</tr>
<tr>
<td>902 m</td>
<td>disappear</td>
<td>disappear</td>
<td>disappear</td>
</tr>
<tr>
<td>816 m</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>780 s</td>
<td>broad at 797</td>
<td>796</td>
<td>disappear</td>
</tr>
<tr>
<td></td>
<td></td>
<td>837</td>
<td>837 m</td>
</tr>
<tr>
<td>701 w</td>
<td>715 w</td>
<td>715 w</td>
<td>disappear</td>
</tr>
<tr>
<td>677 m</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>648 w broad</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>588 m</td>
<td>broad at 582</td>
<td>broad at 582</td>
<td>broad at 582</td>
</tr>
<tr>
<td>666 m</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>518 m</td>
<td>broad at 527</td>
<td>broad at 527</td>
<td>broad at 527</td>
</tr>
<tr>
<td>499 w</td>
<td>shoulder 480</td>
<td>shoulder 480</td>
<td>shoulder 480</td>
</tr>
<tr>
<td>437 s</td>
<td>435 s</td>
<td>435 s</td>
<td>435 s</td>
</tr>
<tr>
<td>397 m</td>
<td>388 m</td>
<td>388 m</td>
<td>388 m</td>
</tr>
</tbody>
</table>
where

* disappear under the ethanol peaks.

** disappear under the NO$_3^-$ peaks.

5.5 Conclusion

The current work has established protocols for the investigation of pharmaceutically-relevant materials in solutions with *in-situ* Raman measurements. These have been applied successfully to the estimation of the limit of detection for Raman spectroscopic studies in solution, the pK$_a$ values of salicylic acid, paracetamol, salicylaldoxime and, citric acid for provision of structural data for the molecular speciation of these compounds in solution.

In the present work, the acidity constants of salicylic acid, paracetamol, salicylaldoxime and citric acid were determined using Raman spectroscopy for the first time and these have been compared with analogous values obtained from potentiometry and UV spectrophotometric measurements.

With no prior information, We have identified, characterized, and determined the abundance of the different species in the Raman spectra of aqueous salicylic acid by numeric treatment of the data using a multiwavelength curve fitting program, from which the ionic species, salicylic acid, monoanion, and bianion were identified. Also, we have identified, characterized, and determined the abundance of two different species in the Raman spectra of aqueous paracetamol, namely, C$_8$H$_9$NO$_2$ and O-C$_6$H$_4$-NH-COCH$_3$. we have identified, characterized, and determined the abundance of the different species in the Raman spectra of aqueous salicylaldoxime (SALO) by numeric treatment of the data.
using a multiwavelength curve fitting program, from which the ionic species, \( \text{H}_2\text{L} \), \( \text{HL}^- \) and \( \text{L}^{2-} \), were identified.

pH titration and Raman spectroscopy were successfully applied in combination to identify, characterize, and determine the abundance of the different species of aqueous citric acid by numeric treatment of the data using a multiwavelength curve fitting program. As a result, the first, second and third step dissociation constants of citric acid were \( 3.2 \pm 0.06, 4.78 \pm 0.06 \) and \( 6.02 \pm 0.03 \), respectively. These values were sufficiently close to those in the literature values.

5.6 References


47. E. Dreassi., P. Corti., S. Lonardi., P. L. Perruccio., Analyst., 1995. 120. p. 1005 - 1008.
71. Y.H. Hui., Food Biotechnology: Microorganisms: Wiley-IEEE.


Section B

Raman spectroscopic and structural studies of selective cocrystals in the solid state
Chapter 6

Identification of Cocrystals of Salicylic acid and the Pharmaceutical Relevance
6.1. Introduction

Co-crystallisation is a crystallisation method that allows the binding of two or more building blocks within one periodic crystalline lattice by hydrogen bonding, π-stacking and Van der Waal’s forces[1-3]. The synthesis of crystalline supramolecular structures mediated by hydrogen bonds is of considerable importance [4-7]; the main difference between cocrystals and salts, for example, is that in salts a proton is transferred from the acidic to the basic functionality of the constituent free base molecule, or vice-versa if applicable, whereas in co-crystals no such transfer occurs [8]. On the other hand, the primary difference between co-crystals and solvates/ hydrates is the physical state of the isolated pure components: if one component is a liquid at room temperature, the crystals are designated as solvates/hydrates; if both components are solids at room temperature, the crystals are designated as co-crystals [9].

In the pharmaceutical context, the attraction of the investigation of cocrystalline forms of drugs relates to four principal factors [10]; design – many Active Pharmaceutical Ingredients (API) contain functional groups pre-disposed to hydrogen bonding that can be exploited to form pharmaceutical cocrystals; discovery – several processes, including conventional solvent crystallisation, solvent drop grinding, melt crystallisation, can be used to produce diverse ranges of cocrystal forms of API; diversity – pharmaceutical cocrystals exhibit different properties compared with pure crystal forms of API; development – cocrystals represent non-obvious forms of APIs and therefore can represent intellectual property for future exploitation. Recently, there has been interest as a means of optimizing the physicochemical properties of solid dosage forms. In addition to potential improvements in solubility, dissolution rate, bioavailability and physical stability, pharmaceutical co-crystals frequently enhance other essential properties of the APIs such as hygroscopicity, chemical stability, compressability and flowability [11].
Co-crystals can be prepared through evaporation [12] or cooling [13] of a heteromeric solution, co-grinding the components,[14] sublimation and growth from the melt [15, 16] or slurry [17, 18]. Solution crystallization is the preferred method for cocrystal formation, particularly to obtain single crystals for structural analysis [19, 20].

Hydrogen bonding is one of the most important fundamental interactions that cause association of organic molecules, forming the building block units known as supramolecular synthons. One example of a supramolecular homosynthon is the molecular dimer that exists in crystals of carboxylic acids (formation I). However, the formation of (I) is unlikely in competitive situations. Allen et al. [21] determined the probability of formation of 75 bimolecular hydrogen bonded ring synthons in organic crystal structures. The probability of formation of I was found to be only 33%. This relatively low probability was attributed to competition with other hydrogen-bonded acceptors (e.g., COO\(^-\), pyridine N, Amide C=O, S=O, P=O).

Another supramolecular synthon of interest would be the molecular dimer that exists in crystals of organic amides, which may be typified by benzamide (formation II).

A third supramolecular synthon of original interest is heteromeric hydrogen-bond interactions between a phenyl-carboxylic acid and a phenyl-amide, such as these provided by a dimeric species containing O··H--N and O-H··O hydrogen bonds (formation III).

\[\text{Ab initio calculations support the idea that the acid-amide supramolecular heterosynthon (III) is favoured over (I) and (II) [22-27]. Also, a survey of hydrogen bonded cocrystal in the CSD [28] reveals that most of them have been prepared using strategies that utilize suitable combinations of chemical entities (or functional groups) located on different}\]
molecules such that they would prefer to interact and bind heteromerically, giving formation III, rather than with themselves (homomerically, formation I and II) [29].

These observations are particularly relevant for the design of cocrystals since robust supra molecular heterosynthons represent perhaps the most reliable and rational route to co-crystals. Furthermore, complementary supramolecular heterosynthons that seem to clearly favour formation of co-crystals are not limited to carboxylic acids.

The solid product of each reaction is commonly analyzed using X-ray powder diffractometry (PXRD) as the primary technique. PXRD detects changes in the crystal lattice and is therefore a powerful tool for studying polymorphism, pharmaceutical salts, and cocrystalline phases. PXRD can also be used as a component of automated robotic systems in high-throughput screening technologies [30, 31]. Consequently, Raman spectroscopy is now being advocated as an analytical alternative to PXRD for cocrystal characterisation [32-34]. Raman spectroscopy probes the effect of crystal structure on bond vibrational energies and is potentially able to selectively distinguish between the polymorphs of a given API. Furthermore, measurements are noninvasive, nondestructive, and rapid (data acquisition is achievable within seconds rather than minutes), which makes Raman spectroscopy ideal for automated high-throughput systems. Since Raman spectroscopy and PXRD are complementary techniques at the molecular level, in combination they can provided an increased understanding of solid-state phenomena. Also, the crystallization process and other physicochemical characteristics can be determined by differential scanning calorimetry (DSC) and infrared (IR) [35]. Extension of these studies to Raman spectroscopy was also undertaken. The principal benefit of TRS lies in its ability to provide rapid volumetric information on the content of pharmaceutical formulations including intact tablets and capsules eliminating the subsampling issues of conventional Raman spectroscopy and suppressing surface Raman
and fluorescence components (e.g. from capsule shell or tablet coating). These features are important in quality control within production environment [36] and from this point of view the cocrystals were also analysed with this procedure.

A number of salicylic acid cocrystals have been reported to date, [37-48]. Also, many researcher have studied nicotinic acid cocrystals [49-55].

Using these studies as a basis, a strategy was identified to prepare a range of examples of salicylic acid co-crystals using a range of co-crystal formers including benzamide, nicotinic acid, DL- phenylalanine and 6-hydroxynicotinic acid. These than provide a selection of well characterised H-bonded materials for detailed Raman spectroscopic study.
6.2 Identification of a New Cocrystal of Salicylic acid and Benzamide of Pharmaceutical Relevance

In order to prepare a system with potential amide-acid heterosystems studies into formation of a cocrystal between salicylic acid (6.2a) and benzamide (6.2b) were undertaken. In addition to the amide-acid functionality, this system was anticipated to provide an intramolecular motif in the salicylic acid moiety. Cocrystallisation studies were initiated with stoichiometric mixtures in ethanol which, after slow evaporation, afforded a white crystalline solid with significant differences in PXRD and vibrational spectroscopy.

Scheme 6.1. Molecular structures of (a) salicylic acid (SA) (b) benzamide (BA)
6.2.1 Results and discussion

6.2.1.1 Raman spectroscopy characterisation

The Raman spectra of salicylic acid, benzamide and the mixture are shown in Figs.1-3 and the vibrational wavenumbers and assignments are listed in Table 1. Raman spectroscopic data were utilized first to evaluate whether the complex is a simple physical mixture or component of molecular ions. Salicylic acid contains a carboxylic group and a hydroxyl group whereas benzamide contains a carboxyl group and primary amide group. It was found that very little perturbation of the wavenumbers of the carbon-carbon and carbon-hydrogen modes was observed in the fingerprint region of the Raman spectra, indicating that these units were little changed in the cocrystal. The peak at 3086 cm\(^{-1}\) (7b (C—H) stretching), 3075 cm\(^{-1}\) (2 (C—H) stretching and 3065 cm\(^{-1}\) (C-H) stretching mode in the spectra of salicylic acid and benzamide respectively, appear as a broad peak centred at 3070 cm\(^{-1}\) in the spectrum of the mixture as shown in Fig. 6.1.

However, the vibrational bands associated with the amide group of benzamide and the bands associated with the carboxyl group of salicylic acid were found to undergo significant changes as shown in Table 6.1. As shown in Figures (6.1-6.4) and Table 1, during the formation of a SA-BA crystal the O-H and C=O bands of salicylic acid and benzamide are shifted to higher or lower wavenumbers by 10 to 30 cm\(^{-1}\), which suggests that the molecular complex of salicylic acid and benzamide is a cocrystal and not simply a mixture of these components [51]. While intramolecular and intermolecular hydrogen bonding modes are shown by both benzamide and salicylic acid [56, 57], the changes in the positions of these bands point to the formation of intermolecular amide-acid interactions as evidenced in the crystal structure of the cocrystal.
Fig. 6.1 Raman spectra in the 3120 – 3000 cm\(^{-1}\) region: (a) BA, (b) SA and (c) SA-BA.
Fig. 6.2 Raman spectra in the 1750 – 1400 cm\(^{-1}\) region: (a) BA, (b) SA and (c) SA-BA

On the other hand, if the amide carbonyl group of benzamide is involved in an intermolecular hydrogen bond that would also be expected to shift to lower wavenumber. As shown in figure 6.2 Table 1, the Raman spectra for pure benzamide in the starting material has bands at 1685 cm\(^{-1}\) and 1569 cm\(^{-1}\), corresponding to the amide-I band and amide-II band, respectively. During the co-crystallization of benzamide with salicylic acid, these bands in the cocrystal were shifted to 1655 cm\(^{-1}\) and 1548 cm\(^{-1}\), respectively. The decrease in amide-I frequency of benzamide from 1685 cm\(^{-1}\) to 1655 cm\(^{-1}\) and 1569 cm\(^{-1}\) to 1548 cm\(^{-1}\) in the SA component indicates that the amide carbonyl group is participating in strong hydrogen bonding.

The Raman spectra for pure salicylic acid in the starting material has strong bands at 1632 cm\(^{-1}\), 1322 cm\(^{-1}\) and medium peak at 1383 cm\(^{-1}\) corresponding to C=O stretching,
(C—O)$_e$ stretching and (O—H)$_h$ in-plane bend, respectively. During the co-crystallization, the band at 1632 cm$^{-1}$ was shifted to 1612 cm$^{-1}$, the band at 1383 cm$^{-1}$ disappears and the peak at 1322 cm$^{-1}$ appears as triplet peaks at 1331, 1322 and 1315 cm$^{-1}$. These observations indicate that the carboxylic group is participating in strong hydrogen bonding as shown in Figs 6.2 and 6.3. The peaks in the spectrum of benzamide occurring at 1179, 1171 and 1122 cm$^{-1}$ can be attributed to an in-plane C-H deformation mode (9a), in-plane C-H deformation mode (7a) and in-plane C-H deformation mode, respectively. In the co-crystal, these bands were shifted to 1174 cm$^{-1}$, 1164 cm$^{-1}$ and 1115 cm$^{-1}$, respectively.

![Raman spectra](image)

Fig. 6.3 Raman spectra in the 1400-1100 cm$^{-1}$ region: (a) BA, (b) SA and (c) SA-BA.
The (18b(C—C)) in-plane bending band at 1029 cm$^{-1}$ in the spectrum of the salicylic acid is shifted to higher wavenumbers and appears at 1035 cm$^{-1}$ in the spectrum of the mixture. Moreover, new peaks at 746 cm$^{-1}$ and 540 cm$^{-1}$, which do not occur for either salicylic acid or benzamide, appear for the SA-BA cocrystal. The peaks at 449 cm$^{-1}$ (9b mode), 432 cm$^{-1}$ (16a mode) and 283 cm$^{-1}$ (C—O) in-plane bend are shifted to 444 cm$^{-1}$, 420 cm$^{-1}$ and 276 cm$^{-1}$, respectively. In the cocrystal the peaks at 256 cm$^{-1}$ (10a mode) in the spectrum of the salicylic acid and 242 cm$^{-1}$ in the spectrum of the benzamide appear as a single peak at 249 cm$^{-1}$ in the spectrum of the co-crystal. Two new peaks appear in the spectrum of the cocrystal at 189 cm$^{-1}$ and 160 cm$^{-1}$. The Raman spectrum of the SA-BA 1:1 physical mixture is shown in Fig. 6.8; it can be observed that the physical mixture has very similar band signatures with the raw material (SA and BA). Hence, the Raman data show a clear distinction between the physical mixture and the SA-BA cocrystal.

**Fig. 6.4** Raman spectra in the 1070-110 cm$^{-1}$ region: (a) BA, (b) SA and (c) SA-BA.
Fig. 6.5 Raman spectra in the 550-200 cm\(^{-1}\) region: (a) BA, (b) SA and (c) SA-BA.
Fig. 6.6 Raman spectra in the 1750-1300 cm\(^{-1}\) region: (a) BA, (b) SA, (c) SA-BA and (d) SABA 1:1 physical mixture

Fig. 6.7 Raman spectra in the 1200-400 cm\(^{-1}\) region: (a) BA, (b) SA, (c) SA-BA and (d) SABA 1:1 physical mixture
6.2.1.2 X-Ray powder diffraction (PXRD)

The powder XRD diffractograms of the ethanol-recrystallised product of the specimen from (Fig.6.8 and 6.9) are clearly different from those of the starting materials and confirm the presence of a new crystalline phase.

The XRD pattern of the cocrystal was compared with the physical mixture, Fig.6.10; it can be observed that the cocrystal has a different XRD pattern from the physical mixture.

On the other hand, the diffraction pattern for the physical mixture is a superposition of those of the raw materials Fig.6.10. These observations coupled with the Raman data indicate the new phase in a cocrystal.

Fig. 6.8 The powder X-ray diffraction pattern in region (6-20) 2θ: (a) SA-BA, (b) SA and (c) BA
Fig. 6.9 The powder X-ray diffraction pattern in region (20-40) 2θ: (a) SA-BA, (b) SA nd (c) BA

Fig. 6.10 The powder X-ray diffraction in region (5-30) 2θ: (a) SA-BA 1:1 physical mixture, (b) SA-BA co-crystal, (c) BA and (d) SA
6.2.1.3 Differential Scanning Calorimetry (DSC)

The DSC thermogram of the product exhibits a melting endotherm maximum at 118 °C providing further support for a new crystal phase (Fig. 6.11). The thermal behaviour of the new phase is significantly different from salicylic acid (melting endotherm maximum at 160 °C) and benzamide (melting endotherm maximum at 127.8 °C) and provides further support for assignment of the cocrystal. Interestingly, the melting point of the cocrystal phase is lower than either starting material.

Fig. 6.11 DSC melting curves of the cocrystal, BA and SA.
6.2.1.4 Single crystal X-ray diffraction

A single crystal was selected from the product and X-ray structure determination confirmed the existence of a 1:1 cocrystal formed from salicylic acid and benzamide. Crystal data is summarised in Table 6.2. The structure of the asymmetric unit in the structure (Fig. 6.12) establishes the stoichiometry of the cocrystal and shows the primary structural motif as an amide-acid dimer established through hydrogen bonding. In this case; the dimer motif is augmented by an intramolecular hydrogen bond in the salicylic acid moiety, formed by the phenolic hydrogen and the carbonyl of the acid group. The crystal packing (Fig.6.13) is dominated by chains of dimer units propagating parallel to the $b$-axis of the cell along the $2_1$-screw axis and formed through hydrogen bonds between the second hydrogen of the amide and the carbonyl of the amide group of the adjacent dimer unit. The characteristics of the hydrogen-bonded network are summarised in Table.6.3.
Fig. 6.12 Hydrogen bonded acid-amide dimers motifs in the 1:1 cocrystal of benzamide and salicylic acid showing the numbering of the asymmetric unit in the crystal.
Fig. 6.13 The crystal packing in the 1:1 cocrystal of benzamide and salicylic acid viewed in orthographic projection down the $a$-axis of the unit cell showing chains of dimer units linked through C=O…HN hydrogen bonds.
### Table 6.1 Assignments of major bands of Raman spectra (cm\(^{-1}\)) of salicylic acid and benzamide and their 1:1 cocrystal products

<table>
<thead>
<tr>
<th>Salicylic acid</th>
<th>SA: BA</th>
<th>Benzamide</th>
<th>Assignment [58-61]</th>
</tr>
</thead>
<tbody>
<tr>
<td>3086m</td>
<td>----</td>
<td>3070m</td>
<td>7b (C—H) str</td>
</tr>
<tr>
<td>3075m</td>
<td>----</td>
<td></td>
<td>2 (C—H) str</td>
</tr>
<tr>
<td>----</td>
<td>3065m</td>
<td>1655m</td>
<td>(C-H) str</td>
</tr>
<tr>
<td>----</td>
<td></td>
<td>1685m</td>
<td>amide-I</td>
</tr>
<tr>
<td>----</td>
<td>1548m</td>
<td>1569m</td>
<td>amide—II</td>
</tr>
<tr>
<td>1632s</td>
<td>1612m</td>
<td></td>
<td>C=O str</td>
</tr>
<tr>
<td>----</td>
<td>1657m</td>
<td></td>
<td></td>
</tr>
<tr>
<td>----</td>
<td>1613 sh</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1430w</td>
<td>----</td>
<td></td>
<td>( O—H (_h) ) i.p.bend</td>
</tr>
<tr>
<td>----</td>
<td>1498w</td>
<td>1499w</td>
<td>(CH) i.p.bend , (CC)str , (phCONH2) str</td>
</tr>
<tr>
<td>----</td>
<td>1174m</td>
<td>1179m</td>
<td>((CH) i.p.bend) mode 9a</td>
</tr>
<tr>
<td>1383m</td>
<td>----</td>
<td></td>
<td>( O—H (_h) ) i.p.bend</td>
</tr>
<tr>
<td>1320 vs</td>
<td>Triplet 1331,1322, 1315</td>
<td>1171m</td>
<td>(C—O (_c) ) c str</td>
</tr>
<tr>
<td>----</td>
<td>1164m</td>
<td></td>
<td>(in-plane C-H def) mode7a</td>
</tr>
<tr>
<td>1241s</td>
<td>1238s</td>
<td>1171m</td>
<td>( C—O (_n) ) str</td>
</tr>
<tr>
<td>----</td>
<td>1115w</td>
<td>1122w</td>
<td>((CH) i.p.bend) mode 7a</td>
</tr>
<tr>
<td>1150m</td>
<td>1154m</td>
<td>----</td>
<td>15(C—C) i.p.bend</td>
</tr>
<tr>
<td>1029</td>
<td>1035</td>
<td>----</td>
<td>18b(C—C)</td>
</tr>
<tr>
<td>873m</td>
<td>----</td>
<td>----</td>
<td>(C—O)h torsion</td>
</tr>
<tr>
<td>805m</td>
<td>----</td>
<td>----</td>
<td></td>
</tr>
<tr>
<td>----</td>
<td>769s , 778 sh</td>
<td>774s</td>
<td>((CH) o.p.bend) mode 6a</td>
</tr>
<tr>
<td>769s</td>
<td>----</td>
<td></td>
<td>(C—C) i.p.bend</td>
</tr>
<tr>
<td>----</td>
<td>746m</td>
<td></td>
<td></td>
</tr>
<tr>
<td>----</td>
<td>540m</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Str, stretching; i.p bend, in plane bending; def, deformation; o.p.bend, out-of-plane bending; ph, phenyl ring;

c= vibrational of the carboxylic group.
h= vibrational of the hydroxylic group.
**Table 6.2.** Crystal data and structure refinement for (C7H6O3)(C7H7NO).

<table>
<thead>
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<th>Property</th>
<th>Value</th>
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<tr>
<td>Identification code</td>
<td>me_salbenza_0m</td>
</tr>
<tr>
<td>Empirical formula</td>
<td>C14H13N04</td>
</tr>
<tr>
<td>Formula weight</td>
<td>259.25</td>
</tr>
<tr>
<td>Temperature</td>
<td>296(2) K</td>
</tr>
<tr>
<td>Wavelength</td>
<td>0.71073 Å</td>
</tr>
<tr>
<td>Crystal system</td>
<td>Monoclinic</td>
</tr>
<tr>
<td>Space group</td>
<td>P2(1)/c</td>
</tr>
<tr>
<td>Unit cell dimensions</td>
<td>a = 5.4523(3) Å, α = 90°, b = 9.6459(7) Å, β = 92.716(2)°, c = 23.9008(15) Å, γ = 90°.</td>
</tr>
<tr>
<td>Volume</td>
<td>1255.59(14) Å³</td>
</tr>
<tr>
<td>Z</td>
<td>4</td>
</tr>
<tr>
<td>Density (calculated)</td>
<td>1.371 Mg/m³</td>
</tr>
<tr>
<td>Absorption coefficient</td>
<td>0.102 mm⁻¹</td>
</tr>
<tr>
<td>F(000)</td>
<td>544</td>
</tr>
<tr>
<td>Crystal size</td>
<td>0.799 x 0.254 x 0.234 mm³</td>
</tr>
<tr>
<td>Theta range for data collection</td>
<td>2.71 to 29.79°.</td>
</tr>
<tr>
<td>Index ranges</td>
<td>-7 ≤ h ≤ 7, -13 ≤ k ≤ 13, -33 ≤ l ≤ 33</td>
</tr>
<tr>
<td>Reflections collected</td>
<td>14890</td>
</tr>
<tr>
<td>Independent reflections</td>
<td>3583 [R(int) = 0.0211]</td>
</tr>
<tr>
<td>Completeness to theta = 29.79°</td>
<td>99.4 %</td>
</tr>
<tr>
<td>Absorption correction</td>
<td>None</td>
</tr>
<tr>
<td>Refinement method</td>
<td>Full-matrix least-squares on F²</td>
</tr>
<tr>
<td>Data / restraints / parameters</td>
<td>3583 / 0 / 224</td>
</tr>
<tr>
<td>Goodness-of-fit on F²</td>
<td>1.033</td>
</tr>
<tr>
<td>Final R indices [I &gt; 2σ(I)]</td>
<td>R1 = 0.0422, wR2 = 0.1156</td>
</tr>
<tr>
<td>R indices (all data)</td>
<td>R1 = 0.0559, wR2 = 0.1263</td>
</tr>
<tr>
<td>Largest diff. peak and hole</td>
<td>0.278 and -0.181 e Å⁻³</td>
</tr>
</tbody>
</table>
Table 6.3 Hydrogen bond dimensions (d /Å; $\angle /^\circ$) in the 1:1 cocrystal of salicylic acid and benzamide.

<table>
<thead>
<tr>
<th>D-H</th>
<th>d(D-H)</th>
<th>d(H…A)</th>
<th>$\angle$(D-H…A)</th>
<th>d(D.A)</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>O(1A)-H(1AO)</td>
<td>0.941</td>
<td>1.684</td>
<td>168.85</td>
<td>2.613</td>
<td>O(1B)</td>
</tr>
<tr>
<td>O(3A)-H(3AO)</td>
<td>0.903</td>
<td>1.807</td>
<td>146.73</td>
<td>2.609</td>
<td>O(2A)</td>
</tr>
<tr>
<td>N(1B)-H(1N)</td>
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<td>2.243</td>
<td>167.80</td>
<td>3.118</td>
<td>O(1B)'(^a)</td>
</tr>
<tr>
<td>N(1B)-H(2N)</td>
<td>0.918</td>
<td>2.055</td>
<td>166.41</td>
<td>2.955</td>
<td>O(2A)</td>
</tr>
</tbody>
</table>

\(^a\) Symmetry operation: -x-1, y+\(\frac{1}{2}\), -z+\(\frac{1}{2}\)
6.3 Characterization of New Cocrystals of salicylic acid with nicotinic acid, DL-phenylalanine and 6-hydroxynicotinic acid by Raman spectroscopy, PXRD, DSC and Transmission Raman Spectroscopy

To further extend the samples set preparation of a series of heterotopic acid….acid complexes from salicylic acid was attempted. A series of cocrystallisation studies were prepared with stoichiometric mixtures of salicylic acid and nicotinic acid (6.2a), 6-hydroxynicotinic acid (6.2b) and DL-phenylalanine (6.2c). In each case, white crystalline solids, with significant differences in PXRD and Raman from their starting materials were obtained.

Scheme 6.2. The starting components
6.3.1 Characterization of the cocrystals

6.3.1.2 Salicylic acid and Nicotinic Acid

6.3.1.2.1 Raman spectroscopy characterisation

The Raman spectra of salicylic acid, nicotinic acid and cocrystal are presented in Figures (6.14-6.16). The assignments for the most characteristic vibrational bands are listed in Table 6.6. The Raman spectra obtained in the fingerprint region for salicylic acid, nicotinic acid, and their cocrystals contained additional information regarding the intermolecular interactions associated with the cocrystal formation. Assignments for the observed Raman bands are collected in Table 6.8.

Fig. 6.14- Raman spectra obtained for (a) salicylic acid, (b) nicotinic acid and (c) the cocrystal.
A comparison of the spectra reveals that there are several band shifts occurring between the individual components and the cocrystal; as shown in Figure 14, Table 8, the Raman spectrum for pure nicotinic acid in the starting material has bands at 1694 cm\(^{-1}\), 1599 cm\(^{-1}\) and 1588 cm\(^{-1}\), corresponding to \(\nu(C=O)\), \(\nu(C=\text{C})\text{N}\) and \(\nu_2(C=\text{C})\text{N}\), respectively. During the cocrystallization of nicotinic acid with salicylic acid, these bands in the cocrystal were shifted to 1675 cm\(^{-1}\), 1603 cm\(^{-1}\) and 1586 cm\(^{-1}\), respectively. The decrease in the \(\nu(C=O)\) frequency of nicotinic acid from 1694 cm\(^{-1}\) to 1675 cm\(^{-1}\) and 1536 cm\(^{-1}\) to 1533 cm\(^{-1}\) in the SA component indicates that the carbonyl group is participating in hydrogen bonding.

The peaks at 1321 cm\(^{-1}\) ((C\text{-O} ) str) of the carboxylic group and 1318 cm\(^{-1}\) in pure salicylic acid and nicotinic acid spectra, respectively, appeared as a broad peak at 1324 cm\(^{-1}\) as shown in Figure 6.14. Also, the peak at 1306 cm\(^{-1}\) (\(\beta\text{OH} + \beta\text{CH} (9a) + \nu\text{C-O}\)) in the pure nicotinic acid spectrum appears as a shoulder in the cocrystal spectrum. The Raman spectra for pure salicylic acid in the starting material has strong bands at 1245 cm\(^{-1}\) ((C––O str) of the hydroxylic group.
Fig. 6.15 - Raman spectra obtained for (a) salicylic acid, (b) nicotinic acid and (c) the cocrystal.

Upon cocrystallization, this band was shifted to 1247 cm\(^{-1}\) with a decrease in the intensity; this observation indicates that the carboxylic group is participating in hydrogen bonding. The peak at 1163 cm\(^{-1}\) in the spectrum of salicylic acid is shifted to 1159 cm\(^{-1}\) in the spectrum of the cocrystal and becomes stronger. Moreover, new bands at 1169 cm\(^{-1}\) and 850 cm\(^{-1}\), which do not occur in either salicylic acid or nicotinic acid, appear in the salicylic acid – nicotinic acid cocrystal.

The Raman spectra for pure nicotinic acid in the starting material has strong bands at 1042 cm\(^{-1}\), 810 cm\(^{-1}\) and medium peaks at 1031 cm\(^{-1}\) and 385 cm\(^{-1}\) corresponding to \(\sigma\) ring (12) + \(\beta\)CH (18a), \(\sigma\) ring (6a) + \(v\)(C-X) +\(\sigma\) (COO)) and \(\sigma\) ring (12) +\(v\) ring (1)), (\(v\)(C-X) +\(\sigma\) ring (6a) \(\sigma\) (COO)) modes, respectively. During the cocrystallization, the bands at 1031 cm\(^{-1}\) and 810 cm\(^{-1}\) were shifted to 1036 cm\(^{-1}\) and 818 cm\(^{-1}\), respectively, and the bands at 1042 cm\(^{-1}\) and 385 cm\(^{-1}\) disappear as shown in Figs 6.15 and 6.16.
Fig. 6.16 - Raman spectra obtained for (a) salicylic acid, (b) nicotinic acid and (c) the cocrystal.
6.3.1.2.2 X-Ray powder diffraction (PXRP)

The powder XRD diffractograms of the products of the specimen recrystallised from ethanol (Figs. 6.17 and 6.18) show significant differences between the diffraction patterns of the raw material and the cocrystal product.

Fig. 6.17. Powder X-ray diffraction patterns for (a) salicylic acid, (b) nicotinic acid and (c) the cocrystal for full range
Fig. 6.18. Powder X-ray diffraction patterns for (a) salicylic acid, (b) nicotinic acid and (c) the cocrystal for full range

6.3.1.2.3 Differential scanning Calorimetry (DSC)

The DSC thermograms of nicotinic acid, salicylic acid and their 1:1 cocrystal product are presented in Fig 6.19. The thermogram of salicylic acid shows a melting endotherm maximum at 159.83 °C and the thermogram of nicotinic acid melting endotherm maximum at 236.86 °C. The DSC thermogram of the 1:1 stoichiometric product proved that the nicotinic acid-salicylic acid cocrystal had been formed; this substance exhibits a melting endotherm maximum at 128.69 °C. Thereafter, the trace shows broad endothermic events consistent with decomposition of the mixture.
Fig. 6.19. DSC melting curves of cocrystal (---), nicotinic acid (——) and salicylic acid (-----).
6.3.1.3 Salicylic acid and DL-Phenylalanine

6.3.1.3.1 Raman spectroscopy 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 (6.20-6.22). The assignments for the most characteristic vibrational bands are listed in Table 6.7. 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 21 cm\(^{-1}\), 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-O)\(_h\) stretching (vibrational of hydroxyl 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-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\(^{-1}\).

The spectra of pure DL-phenylalanine has strong bands at 1609 corresponding to ([NH3]\(^+\) asym deformation phenyl ring quadrant. ring str (v8a)) 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 (\(\nu_9\a\)); 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.

Fig 6.20. Raman spectra obtained for (a) salicylic acid, (b) the cocrystal and (c) DL-phenylalanine
Fig 6.21. Raman spectra obtained for (a) salicylic acid, (b) the cocrystal and (c) DL-phenylalanine

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 (\(\nu_{5a}\)) 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 (Figs 6.20-6.22).
Fig 6.22. Raman spectra obtained for (a) salicylic acid, (b) the cocrystal and (c) DL-phenylalanine

6.3.1.3.2 Transmission Raman Spectroscopy (TRS)

The Transmission Raman Spectroscopy (TRS) spectra of salicylic acid, the DL-phenylalanine and the cocrystal are shown in Fig 6.23 and the vibrational wavenumbers and assignments are listed in Table 6.8.

The Raman spectrum of DL-phenylalanine in the starting material has bands at 1611, 1604,1561,1510,1214 and 1193 cm\(^{-1}\). In the cocrystal the bands at 1611 and 1604 cm\(^{-1}\) appear as a broad band at 1606 cm\(^{-1}\), whereas at the same time the other bands at 1561 and 1510 cm\(^{-1}\) have disappeared in the cocrystal and the bands at 1214 and 1193 cm\(^{-1}\) have shifted to lower wavenumbers at 1208 and 1185 cm\(^{-1}\), respectively. The bands at 827, 217 and 149 cm\(^{-1}\) have disappeared in the cocrystal, while, the peaks at 114 and 66 cm\(^{-1}\)
have shifted to 127 and 71 cm\(^{-1}\), respectively. On the other hand, the Raman spectrum for salicylic acid has bands at 1632, 1473, 1385, 1152, 1028, and 188 cm\(^{-1}\). For the cocrystal the peak at 1632 cm\(^{-1}\) has disappeared and the other peaks in the cocrystal were shifted to 1365, 1142, 1031 and 177 cm\(^{-1}\), respectively. Moreover, new peaks at 1670, 1659 and 950 cm\(^{-1}\), are observed which do not occur in either salicylic acid or DL-phenylalanine (Figs 6.23 and 6.24)

![Transmission Raman spectra](image)

**Fig 6.23.** Transmission Raman spectra obtained for (a) salicylic acid, (b) the cocrystal and (c) DL-phenylalanine
Fig 6.24. Transmission Raman spectra obtained for (a) salicylic acid, (b) the cocrystal and (c) DL-phenylalanine

6.3.1.3.3 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 6.25 and 6.26. 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.
Fig. 6.25. Powder X-ray diffraction patterns for (a) salicylic acid, (b) DL-phenylalanine and (c) the cocrystal from 52° to 122°.

Fig. 6.26. Powder X-ray diffraction patterns for (a) salicylic acid, (b) DL-phenylalanine and (c) the 1:1 cocrystal in the range 20 15 - 40°.
6.3.1.3.4 Differential Scanning Calorimetry (DSC)

The DSC thermograms of benzamide, salicylic acid and their 1:1 cocrystal product are presented in (Fig. 6.27.) 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.

Fig. 6.27. DSC melting curves of DL- phenylalanine (—), cocrystal (—) and salicylic acid (—).
6.3.1.3.5 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 (fig.6.28a and 6.28b) 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 (Table 6.4). The network may best be described as comprising two distinct phenylalanine-phenylalanine ladder motifs (labelled l1 and l2) which are based on H-bonded ring structures between amide and carboxylate functions of the zwitterions (Figure 6.29 and 6.30). The ladder form l2 essentially lies perpendicular to the central l1 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 6.28a and 6.28a b. 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 6.29. 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 6.30. 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 \( l1 \) axis based on a crystallographic centrosymmetric dimer and \( l2 \) is formed from opposite enantiomers in the asymmetric unit.
(a) r1 - down b-axis

Figure (6.31) 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
Figure 6.32. 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 6.4. Crystal data and structure refinement for the co-crystal of salicylic acid and DL-phenylalanine.

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<th>Value</th>
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<td>Empirical formula</td>
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<tr>
<td>Formula weight</td>
<td>303.31</td>
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<tr>
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<tr>
<td>Wavelength</td>
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<tr>
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<td>Space group</td>
<td>P-1</td>
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<td>Unit cell dimensions</td>
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<tr>
<td></td>
<td>α = 83.822(2)°, β = 80.281(2)°, γ = 86.986(2)°</td>
</tr>
<tr>
<td>Volume</td>
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<tr>
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<tr>
<td>Density (calculated)</td>
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<tr>
<td>Theta range for data collection</td>
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<tr>
<td>Index ranges</td>
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</tr>
<tr>
<td>Reflections collected</td>
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<tr>
<td>Independent reflections</td>
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<td>Refinement method</td>
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<td>Goodness-of-fit on F²</td>
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<tr>
<td>Largest diff. peak and hole</td>
<td>0.159 and -0.218 e.Å⁻³</td>
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</table>
Table 6.5 Hydrogen bond dimensions ($d$ /Å; $\angle$ /°) in the co-crystal of salicylic acid and DL-phenylalanine.

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<th>$d$(D-H)</th>
<th>$d$(H…A)</th>
<th>$\angle$(D-H…A)</th>
<th>$d$(D…A)</th>
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<td>O1A-H1OA</td>
<td>0.955</td>
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<td>2.907</td>
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<td>0.947</td>
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<td>2.875</td>
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<td>1.733</td>
<td>168.00</td>
<td>2.761</td>
<td>O1D$^a$</td>
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$^a$ [ x-1, y, z ]  
$^b$ [ -x+1, -y+1, -z+1 ]  
$^c$ [ -x+2, -y+1, -z+1 ]
6.3.1.4 Salicylic acid and 6-hydroxynicotinic acid

6.3.1.4.1 Raman spectroscopic characterisation

The Raman spectra obtained in the fingerprint region for salicylic acid, 6-hydroxynicotinic acid (6HNA) and their cocrystal contained additional information regarding the intermolecular interactions associated with the cocrystal formation. Assignments for the observed Raman bands are collected in Table 6.9.

Raman spectra of the (6HNA), the in-plane $\beta$(C-OH) band is now missing at $ca.1280$ cm$^{-1}$.

Also, the strong band around 1023 cm$^{-1}$, attributed to the pyridine ring, [16] is not seen. These features suggest that the (6HNA) could possibly have adopted the ketonic rather than the hydroxylated form. Furthermore, the presence of the v(C=O) stretching vibration at 1699 cm$^{-1}$ and the in-plane $\beta$(NH) bend at 1622 cm$^{-1}$, in the Raman spectra suggest that the ketonic form predominates in the solid state [17].

![Fig.6.33 Ketonic form and hydroxyl tautomeric form of (6HNA).](image-url)
The Raman spectrum for pure (6HNA) in the starting material has bands at 1699 cm\(^{-1}\) and 1467 cm\(^{-1}\) corresponding to C=O stretching and \(\nu_{\text{a,s}}\) (CC/CN), respectively, in the cocrystal formed between salicylic acid and 6-hydroxynicotinic acid, these bands were shifted to 1686 cm\(^{-1}\) and 1455 cm\(^{-1}\) and become shoulders. The decrease in the C=O stretching wavenumber of 6-hydroxynicotinic acid from 1699 cm\(^{-1}\) to 1686 cm\(^{-1}\) indicates that the carbonyl group is participating in hydrogen bonding. The Raman spectrum for pure salicylic acid in the starting material has strong bands at 1632 cm\(^{-1}\), 1322 cm\(^{-1}\) and a medium intensity peak at 1383 cm\(^{-1}\) corresponding to C=O stretching, (C—O)c stretching and (O—H )h in plane bend, respectively. In the co-crystal, the band at 1632 cm\(^{-1}\) was shifted to 1642 cm\(^{-1}\), the band at 1383 cm\(^{-1}\) disappears and the peak at 1322 cm\(^{-1}\) appears as a very weak broad band; these observations indicate that the carboxylic group is participating in hydrogen bonding as shown in Fig 6.36. The peaks in the spectrum of (6HNA) occurring at 1275 cm\(^{-1}\) \(\nu (\text{C-O})\) str, 1237 cm\(^{-1}\) and 642 cm\(^{-1}\) \(\sigma\) ring (6b) + \(\sigma\) (COO), in the cocrystal were shifted to 1265 cm\(^{-1}\), 1231 cm\(^{-1}\) as shoulder and a doublet at 638 cm\(^{-1}\) and 635 cm\(^{-1}\), respectively. Also, the peak at 859 cm\(^{-1}\) now appears as a doublet at 858 cm\(^{-1}\) and 845 cm\(^{-1}\). Furthermore, the Raman spectra for salicylic acid in the starting material has bands at 1163 cm\(^{-1}\), 564 cm\(^{-1}\) and 530 cm\(^{-1}\), corresponding to (3)mode, (O-H)c o.p.bend and (C-O)\(_h\) torsion, respectively. In forming the co-crystal, the band at 1163 cm\(^{-1}\) disappears and the other two bands were shifted to 559 cm\(^{-1}\) with (decreased intensity) and 527 cm\(^{-1}\) are broading, respectively. Moreover, a strong band at 846 cm\(^{-1}\), medium intensity bands at 1355 cm\(^{-1}\), 1140 cm\(^{-1}\), and 332 cm\(^{-1}\) and a weak band at 1343 cm\(^{-1}\), which do not occur in either salicylic acid or (6HNA) (Figs 6.34-6.36), now appear in the salicylic acid -(6HNA) cocrystal.
Fig. 6.34. Raman spectra obtained for (a) 6-dihydroxynicotinic acid, (b) salicylic acid and (c) the cocrystal.

Fig. 6.36. Raman spectra obtained for (a) 6-dihydroxynicotinic acid, (b) salicylic acid and (c) the cocrystal.
Fig. 6.36. Raman spectra obtained for (a) 6-dihydroxynicotinic acid, (b) salicylic acid and (c) the cocrystal.
6.3.1.4.2 Transmission Raman Spectroscopy (TRS)

The Transmission Raman (TRS) spectra of salicylic acid, 6-hydroxynicotinic acid and the cocrystal are shown in Figs 6.37-6.39 and the vibrational wavenumbers and assignments are listed in Table 6.10.

Fig. 6.37. Transmission Raman spectra in the 1700 – 1100 cm\(^{-1}\) region obtained for (a) salicylic acid, (b) 6-hydroxynicotinic acid and (c) the cocrystal

The Raman spectrum for 6-hydroxynicotinic acid in the starting material has bands at 1702 cm\(^{-1}\), 1651 cm\(^{-1}\) and 1510 cm\(^{-1}\). In the cocrystal the bands at 1651 cm\(^{-1}\) and 1510 cm\(^{-1}\) have disappeared whilst that at 1702 cm\(^{-1}\) was shifted to lower wavenumber at 1686 cm\(^{-1}\). The Raman spectrum for pure salicylic acid has bands at 1634 cm\(^{-1}\), 1473 cm\(^{-1}\), 1386 cm\(^{-1}\) and 1322 cm\(^{-1}\), corresponding to C=O str., 19a, O-H)\(^{\text{i.p.bend}}\) and (C—O )\(^{\text{c str.}}\) modes, respectively.
In the cocrystal formed between salicylic acid and (6HNA), these bands were appear as broad bands at 1635 cm\(^{-1}\), 1469 cm\(^{-1}\), 1386 cm\(^{-1}\) and 1323 cm\(^{-1}\), respectively. On the other hand, the Raman spectrum for (6HNA) has bands at 1235 cm\(^{-1}\), 155 cm\(^{-1}\), 120 cm\(^{-1}\), 96 cm\(^{-1}\) and 46 cm\(^{-1}\), which do not occur in the cocrystal. Also, the peak of medium intensity at 1308 cm\(^{-1}\) in pure salicylic acid has disappeared during the cocrystal formation. Moreover, new bands at 938 cm\(^{-1}\) and 591 cm\(^{-1}\) which do not occur in either salicylic acid or (6HNA) (Figs. 6.37-6.39) appear in the salicylic acid-(6HNA) cocrystal.

Fig. 6.38. Transmission Raman spectra in the 1100 – 500 cm\(^{-1}\) region obtained for (a) salicylic acid, (b) 6-hydroxynicotinic acid and (c) the cocrystal
Fig 6.39. Transmission Raman spectra in the 500 – 100 cm\(^{-1}\) region obtained for (a) salicylic acid, (b) 6-hydroxynicotinic acid and (c) the cocrystal.
6.3.1.4.3 X-ray powder diffraction (PXRD)

The powder XRD diffractograms of 6HNA, salicylic acid and of the product from their cocrystallization via slow evaporation from ethanol, are compared in (Fig. 6.40 and 6.41). The intensity of the characteristic peaks at 27.9° and 11° of (6HNA) and salicylic acid, respectively; were decreased during the cocrystal. Furthermore, the cocrystal has a strong peak at 4.42°, which indicates that a new phase was formed during the cocrystallization of (6HNA) and salicylic acid.

![Fig. 6.40. Powder X-ray diffraction patterns for (a) 6HNA, (b) Salicylic acid and (c) the cocrystal](image)

Fig. 6.40. Powder X-ray diffraction patterns for (a) 6HNA, (b) Salicylic acid and (c) the cocrystal
Fig. 6.41. Powder X-ray diffraction patterns for (a) (6HNA), (b) Salicylic acid and (c) the cocrystal

6.3.1.4.4 Differential Scanning Calorimetry (DSC)
The DSC of (6HNA), salicylic acid and cocrystallization product from slow evaporation are presented in Fig. 6.42. The new molecular complex has a melting points at 153.6 and 202.2 °C; while, salicylic acid has a melting endotherm maximum at 160 °C and the (6HNA) has melting endotherm maximum at 300 °C.
Fig 6.42. DSC melting curves of 6-hydroxynicotinic acid (---), cocrystal (---) and salicylic acid (—).
Table. 6.6 Assignments of major bands of Raman spectra of salicylic acid and nicotinic acid and their 1:1 cocrystal products

<table>
<thead>
<tr>
<th>Salicylic acid</th>
<th>Nicotinic acid</th>
<th>Cocrystal</th>
<th>Assignment [60,62,63]</th>
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<td>C=O str</td>
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</tr>
<tr>
<td>1321s</td>
<td>1318m</td>
<td>1324b broad</td>
<td>(C—O) c str</td>
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<td>1306m</td>
<td>shoulder</td>
<td>βOH+ βCH(9a)+νC-O</td>
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Table. 6.7 Assignments of major bands of Raman spectra of salicylic acid, DL-phenylalanine and their 1:1 cocrystal products

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<td>( \varphi ) i.p. ring. def (( \nu_6b ))</td>
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<td>- 468m</td>
<td>- 448w�</td>
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<td>- 365m</td>
<td>- ( \sigma(C-C), \sigma(C-C-C) )</td>
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<td>- 166,160m broad</td>
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Table 6.8. Major bands of transmission Raman Spectroscopy (TRS) of salicylic acid, DL-phenylalanine and their 1:1 cocrystal products

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Lattice modes
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<tr>
<td>-</td>
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<td>1642s</td>
<td>ant(COOH) str</td>
</tr>
<tr>
<td>1632s</td>
<td>-</td>
<td>1622s</td>
<td>i.p. β(NH)</td>
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<tr>
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<td>1583w</td>
<td>8a (C-C) str</td>
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<td>1555m</td>
<td>vas (CC/CN)</td>
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<td>1455sh</td>
<td>vas (CC/CN)</td>
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<td>1335m</td>
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<td>(C-O)hstr</td>
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<td>-</td>
<td>1033s</td>
<td>18b(CC) i.p.bend</td>
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<tr>
<td>-</td>
<td>859vs</td>
<td>Doublet 859vs, 845s</td>
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<td>846s</td>
<td>-</td>
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<td>806vw</td>
<td>-</td>
<td>795sh</td>
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<td>722w</td>
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<td>642w</td>
<td>Doublet 638s,635s</td>
<td>σ ring (6b)+σ (COO)</td>
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<td>-</td>
<td>559m</td>
<td>(O-H)c o.p.bend</td>
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<td>o.p.bend π (NH)</td>
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<td>(C-O)h torsion</td>
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<td>Salicylic acid</td>
<td>6-hydroxynicotinic acid</td>
<td>Cocrystal</td>
<td>Assignment [60, 68, 69-71]</td>
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<td>1686w</td>
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<td>-</td>
<td>anti(COOH)str</td>
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<td>8a</td>
</tr>
<tr>
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<td>1557w</td>
<td>(\nu_\text{as} ) (CC/CN)</td>
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<td>19a</td>
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<td>1468m broad</td>
<td>(\nu_\text{as} ) (CC/CN)</td>
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<td>(O-H)(_\text{h} ) i.p.bend</td>
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<td>-</td>
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<td>-</td>
<td>1323 w broad</td>
<td>(\nu_\text{(C—O)} ), str</td>
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<td>14</td>
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<td>1277s</td>
<td>1268 m</td>
<td>(\nu ) (C-O) str</td>
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<tr>
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<td>1246m</td>
<td>(\nu_\text{(C—O)} )_h str</td>
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<td>1152s</td>
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<td>15 (C-C)i.p.bend</td>
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<td>-</td>
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<td>18b(C-C)i.p.bend</td>
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<td>Doublet 859m, 848m</td>
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<td>-</td>
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<td>637m</td>
<td>(\sigma ) ring (6b)+(\sigma ) (COO)</td>
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<td>-</td>
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<td>-</td>
<td>96s</td>
<td>-</td>
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<td>72s</td>
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<td>53m</td>
<td>-</td>
<td>53w shoulder</td>
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</tr>
<tr>
<td>-</td>
<td>46m</td>
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</tr>
</tbody>
</table>

Where:

vs, very strong; s, strong; m, medium; w, weak; vw, very weak; sh, shoulder; v, stretching; \(\sigma \), scissoring; \(\beta \) in-plane deformation; a, antisymmetric; s, symmetric; as, asymmetric; i.p, in plane; o.p, out of plane; \(\varphi \), phenyl ring; def, deformation; str, stretch;
6.4 Conclusions

Cocrystals of salicylic acid with nicotinic acid, DL- phenylalanine and 6-hydroxynicotinic acid (6HNA), have been prepared for the first time and characterised by PXRD and substantiated by Raman spectroscopy, DSC and where available 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.

Transmission Raman spectroscopy (TRS), which is well suited for rapid volumetric probing of intact samples, has also been applied to study these cocrystal systems. Again spectral changes can be correlated with the presence of a different crystal structure in the cocrystal compared with the two precursor materials. Spectral differences are particularly pronounced in regains in these spectra, clearly consistent with phonon (lattice) modes.
6.5 References


Chapter 7

Preparation and solid-state characterization of stoichiometric cocrystals of a citric acid and pharmaceutical relevant
7.1 Introduction

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) [1-11]. Cocrystals, as well referred to as molecular complexes, contain two or more different components and regularly rely on hydrogen bonded assemblies between neutral molecules. Cocrystals with the same active pharmaceutical ingredient (API) can have remarkably different pharmaceutical properties; depending on the nature of the second component[12, 13]. The cocrystals are a homogeneous phase of stoichiometric composition and not a mixture of pure component crystalline phases.

The cocrystals are stabilized through a variety of different intermolecular interactions including hydrogen bonds, aromatic π-stacking, and van der Waal’s forces, and unlike salt formation, no proton transfer occurs between the API and the guest molecule[14].

Scheme 7.1. shows that slow evaporation and grinding are the most commonly used techniques for producing cocrystals,[15] expressing some 63% from the overall total.

To extend the set of cocrystal systems for study in this thesis, a series of studies utilising citric acid as cocrystal formers were initiated.

![Citric acid](image)
The molecule has two distinct hydrogen-bonding functions the hydroxyl and acid groups. In addition, absence of any aromatic functions offers the opportunity to probe directly the aromatic functions of aromatic amide in the cocrystal: paracetamol and benzamide. To date no cocrystals with these systems have been reported.

Karki et al. have reported an anhydrous cocrystal of citric acid – caffeine and hydrated cocrystal of citric acid - theophylline. Also, Myz et al. have studied a 1:1 citric acid – meloxicam cocrystal [16]. A number of paracetamol cocrystals have been reported to date,[17-19]; Schantz et al. have been studied citric acid anhydrous and paracetamol, prepared as crystalline physical mixtures using Solid-State NMR,[20] but a systematic synthesis and vibrational spectroscopy characterization of the citric acid – paracetamol (CIT- Pa) cocrystal has not, to our knowledge, been studied hitherto.

**Scheme 7.1.** Breakdown of techniques used for cocrystallization.
Acetaminophen (4-Acetamidophenol), also called paracetamol, is used worldwide as an analgesic and antipyretic. It is a white, odourless and crystalline powder. It possesses a bitter taste. Two explicit polymorphs, form (I) and form (II) are reported in the literature. The molecular structure of paracetamol is shown in (Scheme.7.1(a). [21, 22] Acetaminophen is also an important intermediate in manufacturing of azo-dyes and photographic chemicals. The importance of acetaminophen in industry and literature [23, 24] is the incentive for us to choose acetaminophen as our API here, and citric acid is most widely used in biotechnology and the pharmaceutical industry.

The largest use of citric acid in the pharmaceutical industry is for the effervescent effect it produces when combined with bicarbonates or carbonates in antacids and dentrifices. Effervescence, besides improving palatability, can greatly improve the solubility of co-constituents.

We have focused our attention here on the novel components assembled from citric acid and paracetamol in a 1:1 stoichiometry ratio. The goals of this work were to: (1) describe novel structural studies of new cocrystals, (2) determine the vibrational modes that were most affected by formation and assembly of the supramolecular synthons, and (3) determine the magnitude of perturbation of the vibrational frequencies of the involved modes. These goals necessitated the assignment of most of the observed spectral features in the vibrational bands of the citric acid and reactant, and tracking the energies of these bands in a stoichiometric mixture. The spectroscopic results were supported by single-crystal X-Ray diffraction, X-ray powder diffraction and differential scanning calorimetry studies of the same materials.
7.2 Identification of Cocrystal of citric acid and paracetamol

White crystalline solids were isolated on slow evaporation of ethanolic solutions containing stoichiometric mixtures of citric acid (7.2 b) and paracetamol (7.2a). The solids showed significant differences in PXRD patterns and hence were studied as potential cocrystal of the two molecular forms.

![Molecular Structure](image)

Scheme 7.2. Molecular structure of (a) paracetamol and (b) citric acid.

7.3 Characterization of the cocrystals

7.3.1 Raman spectroscopy characterisation

Since cocrystal formation is a result of interactions between different molecular components that also exist in the single-component crystalline states, vibrational spectroscopy is an excellent technique to characterize and study cocrystallization. Differences in hydrogen bond interactions of the CIT-Pa cocrystals, lead to significant changes in Raman spectra as shown in Figures 7.1, 7.2 and 7.3, and the vibrational wavenumbers and assignments are listed in Table 7.1. Raman spectroscopic data were utilized first to evaluate whether the complex is a simple physical mixture or component of molecular ions. Anhydrous citric acid with the chemical formula $\text{H}_3\text{C}_6\text{H}_5\text{O}_7$ is a tribasic acid with an OH group attached to the middle carbon atom, whereas paracetamol
contains a benzene ring core, substituted by one hydroxyl group and the nitrogen atom of an amide group in the para-position; the amide group is (an acetamide stretching). There are two activating groups that make the benzene ring highly reactive toward electrophilic aromatic substitution. Paracetamol has three crystalline polymorphs[25, 26]; monoclinic type 1 is the thermodynamically stable polymorph which has characteristic peaks at 1325 and 1234 cm$^{-1}$ [27] attributed to the Amide III band (C-N stretch/ C-N- aromatic stretch/ C-N-H bend) and $\nu$C-O, $\sigma$ipHCC,$\nu$CC, $\sigma$ CCC, respectively.

![Raman spectra](image)

**Fig.7.1.** Raman spectra of in the 3150-2850 cm$^{-1}$ region (A) citric acid, (B) paracetamol and (C) CIT-Pa cocrystal prepared from solution.

The Raman spectrum of pure CIT (Fig.7.1.) in the starting material shows peaks at 3001, 2964, 2956 and 2949 cm$^{-1}$. Through cocrystal formation of citric acid with paracetamol
the bands at 3001 and 2964 were shifted to 2998 and 2978, respectively, while the peaks at 2956 and 2949 cm\(^{-1}\) appear at 2953 cm\(^{-1}\) as a broad band.

The paracetamol has peaks at 3110, 3058 and 2935 cm\(^{-1}\) and in those in the complex at 3110 and 3058 cm\(^{-1}\) appeared as very weak and broad at the same position. At the same time as the peak at 2935 cm\(^{-1}\) was shifted to 2953 cm\(^{-1}\) to appear as a broad peak in the same region as the individual peaks at 2956 and 2949 cm\(^{-1}\) of the citric acid alone.

Pure citric acid has bands at 1734 and 1691 cm\(^{-1}\), corresponding to the \(\nu\)(COOH) and (C=O stretch), respectively. During cocrystallization these bands in the cocrystal were shifted to 1718 cm\(^{-1}\) as a weak broad band and 1668 cm\(^{-1}\) as a weak shoulder, respectively. The decreases in the \(\nu\)(COOH) and C=O stretching wavenumbers of citric acid from 1734 to 1718 cm\(^{-1}\) and from 1691 to 1668 cm\(^{-1}\) indicate that the carboxyl group is participating in a strong hydrogen bonding. Furthermore the broad peak at 1630 cm\(^{-1}\) disappears in the cocrystal as shown in Fig.7.2

![Fig.7.2. Raman spectra of in the 1750-1350 cm\(^{-1}\) region (A) citric acid, (B) paracetamol and (C) CIT-Pa cocrystal prepared from solution.](image_url)
The peaks in the spectrum of paracetamol at 1644, 1618, 1609 and 1555 cm\(^{-1}\), attributed to the Amide I band (C=O stretch), \(\nu\text{CC}, \sigma\text{CCC}, \sigma\text{ipHNC}, \nu\text{CC}, \sigma\text{ipHNC}, \sigma\text{ip HCC}\) and \(\sigma\text{ipHNC}, \nu\text{CC}, \nu\text{asCNC}, \sigma\text{ipHCC}, \sigma\text{ip HNC}\), respectively; during the cocrystal formation these peaks were shifted to 1654, 1611, 1611, and weak broad band at 1547, respectively. As shown in Fig. 7.2. and Table 7.1, during the formation of a CIT-Pa cocrystal the (C=O), (COOH) and (NH) bands of citric acid and paracetamol are shifted to higher or lower wavenumbers by 8 to 23 cm\(^{-1}\) accompanied by decreases in the band intensities; which suggests that the molecular complex of citric acid and paracetamol is a cocrystal and not simply a physical mixture of these components. In addition, the doublet at 1514 and 1505 cm\(^{-1}\) in the spectrum of pure paracetamol now appears as a single band at 1508 cm\(^{-1}\) in the cocrystal spectrum.

The \(\text{CH}_2\) scissors band at 1466 cm\(^{-1}\) in the spectrum of citric acid and (\(\sigma\text{asCH}_3\)) band at 1445 cm\(^{-1}\) in the spectrum of paracetamol are shifted to appear as a single broad band at 1453 cm\(^{-1}\). In the citric acid spectrum the peaks at 1430 (C-OH def.) and 1387 cm\(^{-1}\) disappeared during cocrystal formation, while the peak at 1367 cm\(^{-1}\) in the pure paracetamol was shifted to a higher wavenumber and centred at 1375 cm\(^{-1}\).
Fig. 7.3. Raman spectra of in the 1400-950 cm$^{-1}$ region (A) citric acid, (B) paracetamol and (C) CIT-Pa cocrystal prepared from solution.

The spectrum of citric acid showed a peak corresponding to the O-CO bending of the carboxylic group at 1346 cm$^{-1}$ and this band also appears in the cocrystal spectrum. In the spectrum of paracetamol, the bands observed at 1256, 1234 and 1269 cm$^{-1}$ were assigned to the ($\nu$C-O, $\sigma$ipHCC, $\nu$CC, $\sigma$ CCC), ($\nu$CC, $\sigma$ ipHOC, $\sigma$ipHCC, $\nu$CNC) and $\sigma$ipHCC, $\nu$CC, respectively. Throughout these bands in the cocrystal were shifted to 1246 cm$^{-1}$ as weak broad band, 1233 cm$^{-1}$ as broad band with decreasing intensity and 1268 cm$^{-1}$ as a weak shoulder, respectively. Moreover, two new medium bands are observed at 1175 and 776 cm$^{-1}$, which do not occur in the either citric acid or paracetamol (Figs. 7.3 and 7.4)
The Raman spectra of citric acid in the starting material has bands at 1050, 939 and 900 cm\(^{-1}\) assigned to C-O stretching, C-C symmetric stretching and C-C bending and OH out-of-plane bending, respectively. During the cocrystal formation, the band at 1050 cm\(^{-1}\) was shifted to 1061 cm\(^{-1}\) and now appears as a broad weak band, while the peaks at 939 and 900 cm\(^{-1}\) disappear all together.

Fig. 7.4. Raman spectra of in the 1000-550 cm\(^{-1}\) region (A) citric acid, (B) paracetamol and (C) CIT-Pa cocrystal prepared from solution

On the other hand, the Raman spectrum of paracetamol has a single peak at 966 cm\(^{-1}\) attributed to H-C-C bending; through cocrystal formation this peak becomes a doublet.
with intensity increasing as shown in Fig. 7.4. The peak at 682 cm\(^{-1}\) corresponding to C=O stretching was observed at 692 cm\(^{-1}\) as a weak broad band in the cocrystal spectrum.

![Raman spectra](image)

Fig. 7.5. Raman spectra of in the 550-100 cm\(^{-1}\) region (A) citric acid, (B) paracetamol and (C) CIT-Pa cocrystal prepared from solution

Furthermore, the peaks at 636 and 550 cm\(^{-1}\) disappear in the cocrystal (Fig. 7.5.). The peaks at 626 cm\(^{-1}\) (H-N-C deformation) and 463 cm\(^{-1}\) (aromatic ring bend); cocrystal formation are now centred at 648, 623 and 389 cm\(^{-1}\) as broad, weak peaks. Hydrogen bonding is a significant intermolecular interaction, which is responsible for the different crystal Packing [28]. The Raman spectroscopy results suggest that the citric acid and paracetamol are now in the cocrystal form and they are not single physical mixture.
Table 7.1. assignments of major bands of Raman spectra of citric acid paracetamol, and their cocrystal products

<table>
<thead>
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<th>CIT: Para</th>
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<td>2953w</td>
<td>-</td>
<td>σ\text{ipHNC}, vCC, vasCCN, σ\text{ipHCC}</td>
</tr>
<tr>
<td>2949w</td>
<td>-</td>
<td>* 2931w</td>
<td>Asymmetric CH\text{3} stretch</td>
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<tr>
<td>-</td>
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<td>1718vw</td>
<td>v(COOH)</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>( C=O stretch)</td>
</tr>
<tr>
<td>-</td>
<td>1691vs</td>
<td>1668 sh</td>
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</tr>
<tr>
<td>1630 m broad</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>1614m</td>
<td>1618vs</td>
<td>CH\text{2 sciss.}</td>
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<td>-</td>
<td>1609vs</td>
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<td>1514w</td>
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<td>O-CO bending of COOH</td>
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</tr>
<tr>
<td>1346w</td>
<td>*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>1321s</td>
<td>1325vvs</td>
<td>Amide III band (C-N stretch/ C-N-ph stretch / C-N-H bend)</td>
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<td>1276 broad</td>
<td>1278m</td>
<td>v(C-N); δ(N-H) amide III</td>
</tr>
<tr>
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<td>1246w</td>
<td>1256m</td>
<td>vC-O, σipHCC,vCC, σ CCC</td>
</tr>
<tr>
<td>-</td>
<td>1235 broad</td>
<td>1234s</td>
<td>vCC, σ ipHOC, σipHCC, vCNC.</td>
</tr>
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<td>1222m</td>
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</tr>
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<td>1203w</td>
<td>-</td>
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</tr>
<tr>
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<td>-</td>
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<tr>
<td>-</td>
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<td>σipHCC, vCC</td>
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<tr>
<td>1165w</td>
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<td>1129w</td>
<td>-</td>
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<td>*</td>
<td>1121vww</td>
<td>vCC, σHOC</td>
</tr>
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<td>1080m</td>
<td>-</td>
<td>1103vww</td>
<td>σ ipHCC, vCC, σ ip HOC</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>1106w</td>
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221
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<tr>
<th>Wave Number (cm⁻¹)</th>
<th>Interpretation</th>
<th>Description</th>
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<tr>
<td>-</td>
<td>* 1014 w</td>
<td>σCH3, σCCC</td>
</tr>
<tr>
<td>- 968 m</td>
<td>966 m</td>
<td>H-C-C bend</td>
</tr>
<tr>
<td>900 m</td>
<td>* C-C symmetric stretch</td>
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<td>860 vs</td>
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</tr>
<tr>
<td>- 834 w</td>
<td>836 m</td>
<td>Out-of-plane C—H bend (Aryl-1,4-disubstituted)</td>
</tr>
<tr>
<td>877 w</td>
<td>*</td>
<td>Aromatic ring stretches</td>
</tr>
<tr>
<td>- 788 s</td>
<td>796 s</td>
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</tr>
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<td>780 s</td>
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</tr>
<tr>
<td>- 776</td>
<td></td>
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</tr>
<tr>
<td>- 699 w broad</td>
<td>710 m</td>
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</tr>
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<td>682 m</td>
<td>697vw broad</td>
<td>C=O stretching</td>
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<td>626w</td>
<td>Amide IV band (H-N-C deformation)</td>
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<tr>
<td>539 sh</td>
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</tr>
<tr>
<td>- 497 w broad</td>
<td>503 m</td>
<td>Skeleton vibration</td>
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<td>417 m</td>
<td>412 w</td>
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</tr>
<tr>
<td>- * 412 w</td>
<td>412 w</td>
<td>σ in-plane</td>
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<td>- 389s broad</td>
<td>388s</td>
<td>C-N bend</td>
</tr>
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<td>- 324</td>
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<td>258mbroad</td>
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</tr>
<tr>
<td>212</td>
<td>211w broad</td>
<td>215 m</td>
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</table>

where * disappeared during the cocrystal.
7.3.2 X-ray powder diffraction (PXRD)

PXRD was used to identify crystalline phases and to qualitatively examine changes in crystallinity. The PXRD diffractograms of the citric acid, paracetamol and of the products from cocrystallization via slow evaporation from ethanol are compared in Figures 7.6 and 7.7.

The transformation to the cocrystalline phase is indicated by the diffraction peaks at positions 2θ = 7.5°, 17.5° and 22.7°, furthermore, some characteristic diffraction peaks of the raw material have disappeared in the PXRD diffractograms of the product via slow evaporation. Low intensity broad peaks around 13.7°, 14°, 18.1° and 26.5° corresponding to CIT and Pa suggest the presence of unreacted crystalline material. In addition, from the XRD patterns can be confirmed as the formation of a new complex phase.
Fig. 7.6. Powder x-ray diffraction pattern of the CIT-Pa system (a) citric acid, (b) paracetamol and (c) the cocrystal.

Fig. 7.7. Powder x-ray diffraction pattern of the CIT-Pa system (a) citric acid, (b) paracetamol and (c) the cocrystal

7.3.3 Differential Scanning Calorimetry (DSC)

The DSC of the cocrystallization product from slow evaporation is presented in Figure 7.8. The presence of unreacted component would cause a decrease in the melting point. The DSC traces were observed and the results are presented in Figure 7.8. The results show a single endothermic event at 154.5°C for pure citric acid and the pure paracetamol has an endothermic event at 170.5°C.

Interestingly, the product cocrystal shows two endotherms at 72.05 and 94.10°C separated by a broad exotherm. This can be interpreted as a transition between enantiotropic polymorphic forms of the cocrystal. While such behaviour is relatively uncommon, further study of this system was beyond the scope of this project.
7.3.4 Single-crystal X-ray diffraction

The single crystal X-ray structure confirmed the formation of a cocrystal and showed the structural centre piece of the crystal system to be 2:1 with respect to paracetamol and citric acid (Fig.7.9). The asymmetric unit of the crystal contains two paracetamol molecules hydrogen-bonded to the citric acid. One acts as a phenolic-OH hydrogen bond donor to the carbonyl of a carboxylic acid arm of citric acid. In contrast, the other phenolic-OH acts as a hydrogen bond acceptor from the quaternary C-OH of citric acid.

The structure centre piece in the crystal packing is the citrate chain formed by centrosymmetric carboxylic acid dimers. The chain propagates parallel to the c-axis (Fig.7.10).

Fig.7.8. DSC curve of the citric acid, paracetamol and cocrystal product.
Citric acid molecules cross-link through COOH…OH hydrogen bonds to the COH of an adjacent chain to form a sheet structure (Fig. 7.11). Pairs of paracetamol molecules, hydrogen bonded through intermolecular amide….amide bonds (Fig. 7.12) span every other citric acid molecule in the chain forming phenolic OH to carbonyl H-bond at one end of the pair and citric OH…O of the phenolic OH at the other end of the pair (Fig. 7.13). Curiously one NH amide does not appear to be involved in hydrogen bonding.

Figure 7.9 Asymm_unit of Viewed down b-axis of the unit cell showing the numbering scheme adopted.

Figure 7.10 The chain of citric acid molecules formed from centrosymmetric acid-acid dimer motifs propagating parallel to the c-axis of the unit cell.
Figure 7.11 Cross-linking of citric acid chains viewed down the \( a \)-axis of the unit cell.

Figure 7.12 Amide NH…O hydrogen bonding linking pairs of paracetamol molecules in the crystal.
Figure 7.13 The attachment of pairs of paracetamol molecules to the citric acid molecular chain viewed down the $b$-axis of the unit cell. The paracetamol phenolic OH also cross links the chain structures. The phenol with H-bonding through the oxygen to the citrate chain also acts as an H-bond donor to the amide oxygen (O2B) of the adjacent chain. This relationship corresponds with the $c$- glide plane of the crystal symmetry (Fig.7.14).
Figure 7.14 The crystal packing of the 2:1 co-crystal of paracetamol and citric acid showing the ‘crosslinking’ between chains of paracetamol-citric acid units viewed down the $a$-axis of the unit cell.
| **Table 7.2.** Crystal data and structure refinement for [para]2[cit]. |
| Identification code               | me_para_citric_0m |
| Empirical formula                | C22 H26 N2 O11   |
| Formula weight                   | 494.45           |
| Temperature                      | 296(2) K         |
| Wavelength                       | 0.71073 Å        |
| Crystal system                   | Monoclinic       |
| Space group                      | C2/c             |
| Unit cell dimensions             |                 |
| a                               | 24.2864(10) Å   |
| b                               | 11.3217(5) Å    |
| c                               | 16.9668(7) Å    |
| Volume                           | 4437.2(3) Å³    |
| Z                                | 8                |
| Density (calculated)             | 1.480 Mg/m³      |
| Absorption coefficient           | 0.120 mm⁻¹       |
| F(000)                           | 2080             |
| Crystal size                     | 0.35 x 0.27 x 0.23 mm³ |
| Theta range for data collection  | 2.00 to 27.49°   |
| Index ranges                     | -27<=h<=24, -14<=k<=12, -12<=l<=22 |
| Reflections collected            | 10850            |
| Independent reflections          | 4056 [R(int) = 0.0350] |
| Completeness to theta = 27.49°   | 79.6 %           |
| Absorption correction            | None             |
| Max. and min. transmission       | 0.9731 and 0.9587 |
| Refinement method                | Full-matrix least-squares on F² |
| Data / restraints / parameters   | 4056 / 0 / 349   |
| Goodness-of-fit on F²            | 1.011            |
| Final R indices [I>2sigma(I)]    | R1 = 0.0474, wR2 = 0.0811 |
| R indices (all data)             | R1 = 0.0839, wR2 = 0.0920 |
| Largest diff. peak and hole      | 0.221 and -0.249 e.Å⁻³ |
Table 7.3 Hydrogen bond dimensions ($d$ /Å; $\angle$ /°) in the 2:1 co-crystal of citric acid and paracetamol.

<table>
<thead>
<tr>
<th></th>
<th>$d$(D-H)</th>
<th>$d$(H…A)</th>
<th>$\angle$(D-H…A)</th>
<th>$d$(D…A)</th>
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<td>171.57</td>
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<tr>
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<td>1.809</td>
<td>169.65</td>
<td>2.681</td>
<td>O3&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
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<td>1.622</td>
<td>176.83</td>
<td>2.617</td>
<td>O7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>O8-H8O</td>
<td>0.942</td>
<td>1.677</td>
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<td>2.618</td>
<td>O9&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
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<td>3.141</td>
<td>O1B&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
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<td>1.839</td>
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<td>2.656</td>
<td>O2B&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
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<td>145.79</td>
<td>2.896</td>
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<tr>
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<td>176.29</td>
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</tr>
</tbody>
</table>

<sup>a</sup> $[-x+\frac{1}{2}, y+\frac{1}{2}, -z+\frac{1}{2}]$

<sup>b</sup> $[-x+\frac{1}{2}, -y+\frac{1}{2}, -z+1]$

<sup>c</sup> $[x, -y, z-\frac{1}{2}]$

<sup>d</sup> $[x, -y+1, z-\frac{1}{2}]$

<sup>e</sup> $[x, y, z+1]$
7.4 Vibrational Spectroscopic Studies of Cocrystal Products formed by citric acid and benzamide (CIT-BA)

White crystalline solids were isolated on slow evaporation of ethanolic solutions containing stoichiometric mixtures of citric acid (7.3 b) and benzamide (7.3a), the solids showed significant differences in PXRD patterns and hence were studied as potential cocrystal of the two molecular forms

![Molecular structures of benzamide and citric acid](image)

Scheme 7.3. Molecular structures of (a) benzamide and (b) citric acid

7.4.1 Characterization of the cocrystals

7.4.1.1 Raman spectroscopy characterisation

The Raman spectra of citric acid, benzamide, and their 1:1 cocrystal product were found to exhibit a number of differences (Figs 7.15-7.17.). In order to evaluate the trends in the spectra more effectively, the origins of the major bands were first assigned through the use of published compilations, studies specifically conducted on citric acid acids, and on benzamide in particular. The results of this analysis are provided in Table 7.4.

As shown in Table 7.2, the Raman spectrum for pure citric acid in the starting material has strong bands at 1734 cm\(^{-1}\) and 1691 cm\(^{-1}\), corresponding to COOH stretching and C=O stretching, respectively. During the cocrystallization of citric acid with benzamide, the band at 1734 cm\(^{-1}\) in the cocrystal was shifted to 1715 cm\(^{-1}\) as broad band, while the peak...
at 1691 cm\(^{-1}\) has disappeared. The decrease in the COOH stretching of citric acid from the 1734 cm\(^{-1}\) to 1715 cm\(^{-1}\) indicates that the carboxylic group is participating in strong hydrogen bonding. The medium band at 1630 cm\(^{-1}\) in the spectrum of pure citric acid was shifted to higher wavenumber by 15 cm\(^{-1}\) and centred at 1645 cm\(^{-1}\), which suggests that the molecule is in a complex. The Raman spectrum of benzamide has bands at 1569 cm\(^{-1}\), 1180 cm\(^{-1}\) and 805 cm\(^{-1}\) attributed to Amide-II, in-plan C-H mode and g(CH), g(CC), g(C=O), t(NH\(_2\)), respectively. During the cocrystal these bands were shifted to 1553 cm\(^{-1}\) as broad band, 1183 cm\(^{-1}\) and 779 cm\(^{-1}\), respectively. The decrease in the amide wavenumber of benzamide from 1569 cm\(^{-1}\) to 1553 cm\(^{-1}\) indicates that amide group is participating in strong hydrogen bonding.

![Raman spectra of in the 1800-110 cm\(^{-1}\) region](image)

Fig.7.15. Raman spectra of in the 1800-110 cm\(^{-1}\) region (A) benzamide, (B) citric acid and (C) CIT-BA cocrystal prepared from solution
In the Raman spectrum of citric acid there are bands at 1430, 1050 and 682 cm\(^{-1}\) assigned to C-OH deformation, C-O stretching and C=O stretching, respectively. Wherever cocrystallization between citric acid and benzamide occurs, these bands appear as broad bands at 1425 cm\(^{-1}\), 1051 cm\(^{-1}\) and 684 cm\(^{-1}\), respectively.

![Raman spectra](image)

Fig. 7.16. Raman spectra of in the 700-500 cm\(^{-1}\) region (A) benzamide, (B) citric acid and (C) CIT-BA cocrystal prepared from solution.

The peaks of the pure citric acid at 1466 cm\(^{-1}\) attributed to (CH\(_2\) scissors) and the pure benzamide at 1450 cm\(^{-1}\) assigned to \(\sigma(CH)\), \(\nu(CC)\), \(\sigma(NH)\), during cocrystal these bands appear as single broad band at 1445 cm\(^{-1}\). Also, the peaks 1410 cm\(^{-1}\) (C-N stretching) and 1387 cm\(^{-1}\) (CH\(_2\) scissors) for pure citric acid and benzamide, respectively; during the cocrystal these bands appear as single broad peak at 1402 cm\(^{-1}\). The Raman spectrum of
citric acid has peaks at 1346 (O-CO bending of COOH), 1205 (C-C stretching), 1080 (C-O stretching), 877, 636, 550 and 212 cm\(^{-1}\). During the cocrystallization of citric acid with benzamide, these bands in the cocrystal disappear. On other hand, the peak at 155 cm\(^{-1}\) of the citric acid was shifted to 167 cm\(^{-1}\) in the cocrystal spectrum as shown in (Fig.7.17). These observations suggest that citric acid and benzamide are in cocrystal formation. Moreover, new bands at 813, 711, 703, 697, 655, 580, 577 cm\(^{-1}\), which don’t occur in either citric acid or benzamide (Fig.7.16), appear in the CIT-BA cocrystal.

![Raman spectra](image)

*Fig.7.17. Raman spectra of in the 400-150 cm\(^{-1}\) region (A) benzamide, (B) citric acid and (C) CIT-BA cocrystal prepared from solution.*
Table 7.4. Assignments of major bands of Raman spectra of citric acid, benzamide, and their 1:1 cocrystal products

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<th>citric acid</th>
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<th>Benzamide</th>
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</tr>
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<td>*</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>2959m</td>
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</tr>
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<td>1715w</td>
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<td>ν(COOH)</td>
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</tr>
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<td>1645w</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>----</td>
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<td>1602s</td>
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<td>1569m</td>
<td>Amide-II</td>
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<td>1499w</td>
<td>σ(CH), ν(CC), ν(ph–CONH2)</td>
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<td>1425m</td>
<td>---</td>
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<td>998 vs</td>
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<td>805m</td>
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<tr>
<td>257m</td>
<td>257w broad</td>
<td></td>
<td></td>
</tr>
<tr>
<td>212m</td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>177w</td>
<td></td>
<td>177sh</td>
<td>177m</td>
</tr>
<tr>
<td>155m</td>
<td>167m broad</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

where:
* disappeared during the cocrystal.
7.4.1.2  X-ray powder diffraction (PXRD)

The PXRD diffractograms of the pure citric acid, benzamide and of the cocrystal via slow evaporation from ethanol/methanol are shown in Fig.7.18. It was observed that most of the characteristic scattering peaks of the citric acid and benzamide reactant become broadened and reduced in intensity, new diffraction peaks appeared at scattering angles of 5.4, 10.7, 21.4 and 20.2 degrees 20.

Fig.7.18. Powder x-ray diffraction pattern of the citric acid, benzamide and CIT-BA cocrystal
### 7.4.1.3 Differential Scanning Calorimetry (DSC)

The DSC of the cocrystallization product formed from slow evaporation of the solution is presented in Figure 7.19. The thermogram of this cocrystal contained a sharp, well defined, endothermic transition at approximately 134.7 °C that was attributed to the melting point of the CIT-BA cocrystal component. The results show a single endothermic event at 154.5°C for pure citric acid and 127.5°C for pure paracetamol.

![DSC curve of the citric acid, benzamide and the cocrystal product.](image)

Fig. 7.19 DSC curve of the citric acid, benzamide and the cocrystal product.
Pharmaceutical cocrystals of citric acid with paracetamol and benzamide were designed employing crystal engineering strategies. Citric acid-paracetamol and citric acid-benzamide cocrystals were prepared via a slow evaporation method and formed 1:2 and 1:1 complexes, respectively. The single crystal structure of citric acid-paracetamol cocrystal was determined. Also, DSC, PXRD, and Raman data confirmed the formation and stability of the citric acid-paracetamol and citric acid-benzamide cocrystals. Raman spectroscopy was found to be a useful spectroscopic technique for characterization of these products.

Formation of the cocrystal results in changes in the carbonyl band region that is diagnostic for the existence of the citric acid-paracetamol and citric acid-benzamide cocrystals. This mode of interaction appears to perturb the force constants of the carbonyl groups involved in the interaction nearly as much as the perturbation experienced by the -NH group of the amide group.
7.6 References


Chapter 8

Synthesis of a cocrystal of 3,4-dihydroxybenzoic and oxalic acid
8.1 Introduction

The synthesis of co-crystals representing supramolecular complexes of two or more components is very attractive especially in pharmaceutical chemistry due to the possibility of influencing the stability and dissolution of drugs without changing the molecular structure of the active pharmaceutical ingredient. Co-crystals are generally prepared by solution crystallization, less commonly by growth from the melt[1-3].

Modern methods of analysis, most of which are spectroscopic, allow full characterization of the synthesized target at a molecular level in a short time. However, full information on the molecular assembly in the solid state is less readily available.

Shan et al. [4] have studied the cocrystal structure of 4,7-phenanthroline and oxalic acid obtained by slow evaporation at room temperature from a 1:1 oxalic acid/ 4,7-phenanthroline solution in dimethylsulfoxide.

Olenik et al. [5] have studied 1:1 cocrystals of oxalic acid with quinoxaline, phthalazine and naphthyridine and X-ray structure analyses are reported. Child et al. [6] have reported cocrystals of chlorzoxazone with 2,4-dihydroxybenzoicacid. Bucar et al. [7] have described the cocrystals of 3,4- dihydroxybenzoicacid with caffeine by using a slurry/suspension technique that is based on thermodynamically driven solution-mediated phase transformation. Cocrystals of 2,5-dihydroxybenzoic acid and (2,3 / 2,4 / 2,6 / 3,4 and 3,5) dihydroxybenzoic acids with piracetam have been investigated [8, 9]; it was reported that the position of the functional groups has a significant impact on the formation of co-crystals and their physicochemical properties. 2:1 caffeine/oxalic acid cocrystals have been studied by Trask et al. [10].
The structures of the crystals obtained by mixing oxalic acid, phenol, p-cresol, hydroquinone, β-naphthol, and cholesterol have been studied by Lopez et al. [11]; it was shown that oxalic acid forms co-crystals in 1:2 stoichiometry with phenol, p-cresol and cholesterol, but in 2:2 and 1:4 molar ratios for hydroquinone and β-naphthol, respectively. Wenger et al. [12] have designed a cocrystal of γ-amino butyric acid (GABA) with oxalic acid and benzoic acid; it was reported that, from a calculation of the molar fraction for GABA and oxalic acid, the crystallization for a 1:1 GABA/oxalic acid cocrystal should be carried out in the pH range 0 – 4.19 and crystallization for a 2:1 cocrystal ratio should be carried out in the pH range 4.19 – 10.43. Experimentally, oxalic acid and GABA yielded a 1:2 co-crystal, GABA is protonated and the oxalic acid is a dianion. Charge neutrality therefore requires a 1:2 ratio of GABA and oxalic acid. Wenger et al. [13, 14] have studied the cocrystal of oxalic acid with L-glutamine, L-asparagine monohydrate and gabapentin by slow evaporation at room temperature from aqueous solutions containing a 1:1 stoichiometric ratio of oxalic acid/ L-glutamine, oxalic acid/ L-asparagine monohydrate and 2:1 cocrystal of gabapentin/oxalic acid. Tojiboev et al. [15] studied cocrystals of oxalic acid with tricyclic quinazolone derivatives (tri-, tetra- and pentamethylene-3, 4-dihydroquinazolin-4-one); the results show that the tri- and tetra- species cocrystals with oxalic acid are formed without protonation of the quinazolin-4-one molecule in the ratio 2:1, in contrast to a first crystallization of the pentamethylene-3,4-dihydroquinazolin- 4-one with oxalic acid which gives a salt type crystal in the 1:1 ratio. Varughese et al. [16] studied molecular complexes (co-crystals and salts) of alprazolam with oxalic acid, 2,6-dihydroxybenzoic acid and 3,5-dihydroxybenzoic acid.
In this work, the formations of a cocrystal of 3,4-dihydroxybenzoic acid with oxalic acid via evaporation from ethanol solution is studied (Scheme 8.1).

Scheme 8.1. Compounds used in cocrystallization
8.2 Result and discussion

The molecular interaction between 3, 4-dihydroxybenzoic acid and oxalic acid was examined by Raman spectroscopy, and the vibrational wavenumbers and assignments are listed in Table 8.1. The spectrum of oxalic acid showed a peak corresponding to the carbonyl $\nu_{\text{a}}(\text{C}=\text{O})$ of carboxylic acid at 1724 cm$^{-1}$. The decrease in the C=O stretching wavenumber from 1724 cm$^{-1}$ to 1704 cm$^{-1}$ indicates that the carbonyl carboxylic group is participating in strong hydrogen bonding. This band was shifted in the Raman spectrum of the co-crystal to a lower wavenumber at 1704 cm$^{-1}$ and appears as a broad peak as shown in Fig.8.1. The Raman spectrum for pure oxalic acid in the starting material has bands at 1481 cm$^{-1}$, corresponding to the $\delta$(C-OH) + $\nu$(C-O) modes, and 1173 cm$^{-1}$. During the cocrystallization of the oxalic acid with 3, 4 -dihydroxybenzoic acid, the band at 1481 cm$^{-1}$ appears at 1480 cm$^{-1}$ as a broad peak and the peak at 1173 cm$^{-1}$ has disappeared in the cocrystal.
Fig 8.1. Raman spectra obtained for (a) 3, 4- dihydroxybenzoic acid, (b) oxalic acid, and (c) the cocrystal.

The Raman spectrum of pure 3, 4- dihydroxybenzoic acid in the starting material has bands at 1604 cm\(^{-1}\) \(\nu(\text{CC})\text{ar}\), 1599 cm\(^{-1}\) and 1514 cm\(^{-1}\) \(\nu(\text{CC})\text{ar}\) upon co-crystallization, these bands in the cocrystal were shifted to 1616 cm\(^{-1}\), 1604 cm\(^{-1}\) and disappear, respectively. Also, the bands at 1291 cm\(^{-1}\), 1242 cm\(^{-1}\), 1097 cm\(^{-1}\) and 754 cm\(^{-1}\), corresponding to the \(\beta(\text{OH})\text{carboxylic } , (\text{CC})\text{ar}, \text{CH bending}, \text{CO stretch (COOH)}\) and CH wagging, respectively, upon co-crystal formation, these bands were shifted to higher wavenumbers with the exception of the band at 1097 cm\(^{-1}\) which has disappeared, as shown in fig.8.2.
Fig. 8.2. Raman spectra obtained for (a) 3, 4- dihydroxybenzoic acid, (b) Oxalic acid, and (c) the cocrystal.

Furthermore, the O-H stretching mode of the 3, 4- dihydroxybenzoic acid shifted from 1291 cm\(^{-1}\) to 1313 cm\(^{-1}\) in the cocrystal indicating that the intermolecular hydrogen bonds between 3, 4- dihydroxybenzoic acid molecules were broken. The bands at 463 cm\(^{-1}\), 455 cm\(^{-1}\) and 449 cm\(^{-1}\) in the spectrum of oxalic acid disappeared in the spectrum of the co-crystal. Furthermore, the peaks in the spectrum of 3, 4- dihydroxybenzoic acid the peaks occurring at 438 cm\(^{-1}\), 343 cm\(^{-1}\) (OH wagging) and 230 cm\(^{-1}\) for the cocrystal, the bands at 438 cm\(^{-1}\) and 230 cm\(^{-1}\) were broadened, decreased in intensity and shifted to 429 cm\(^{-1}\) and 225 cm\(^{-1}\), while the peak at 343 cm\(^{-1}\) appears as a broad peak at the same position. Moreover, a new peak at 478 cm\(^{-1}\), which does not occur in oxalic acid or 3, 4- dihydroxybenzoic acid, is observed. (Fig. 8.3.)
Fig. 8.3. Raman spectra obtained for (a) 3, 4'-dihydroxybenzoic acid, (b) oxalic acid, and (c) the cocrystal.

The Raman spectral data also confirmed that only oxalic acid is present in the complex, as no bands can be assigned to the oxalate [17] or hydrogen oxalate [18, 19] ions. Furthermore, the $\nu$(C–C) stretching wavenumber, 837 cm$^{-1}$, is in agreement with that reported for H$_2$C$_2$O$_4$ molecule [20], but this value is too low for HC$_2$O$_4^-$ or C$_2$O$_4^{2-}$ ions where $\nu$(C–C) appears at about 880 cm$^{-1}$ [17-19].
Using a synthetic standard containing a 1:1:1 mixture of KNO$_3$, oxalic acid and 3,4-dihydroxybenzoic acid, for which each sample was analysed at seven random positions with each point sampled twice, the ratio of the 834:754 cm$^{-1}$ bands is 0.327; in the cocrystal the ratio of these bands is 0.329, confirming that it is a 1:1 cocrystal of oxalic acid and 3,4-dihydroxybenzoic acid.

The Transmission Raman (TRS) spectra of oxalic acid, 3,4-dihydroxybenzoic acid and the co-crystal are shown in Figs 8.4-8.5 and the vibrational wavenumbers and assignments are listed in Table 6.2. The Raman spectrum for 3, 4-dihydroxybenzoic acid in the starting material has bands at 1611 cm$^{-1}$ and 1299 cm$^{-1}$. In the co-crystal, these bands in the cocrystal were shifted to 1605 cm$^{-1}$ and 1315 cm$^{-1}$, respectively, while the peaks at 1173 cm$^{-1}$, 1130 cm$^{-1}$ and 1105 cm$^{-1}$ disappear from the cocrystal spectrum. The Raman spectrum for pure oxalic acid has bands at 1796 cm$^{-1}$, 1725 cm$^{-1}$, 1156 cm$^{-1}$ and 543 cm$^{-1}$. whereas in the co-crystal the bands at 1796 cm$^{-1}$, 1725 cm$^{-1}$ and 1156 cm$^{-1}$ have disappeared; and the band at 543 cm$^{-1}$ is broadened but centred on 542 cm$^{-1}$. On the other hand, the peak at 156 cm$^{-1}$ in the spectrum of pure salicylic acid has shifted to a lower wavenumber at 142 cm$^{-1}$. 
Fig. 8.4 Transmission Raman spectra from 180 -40 cm\(^{-1}\) region obtained for (a) 3,4-dihydroxybenzoic acid, (b) oxalic acid and (c) the cocrystal.
Fig. 8.5 Raman spectra from 500 -150 cm⁻¹ region for

(a) 3,4-dihydroxybenzoic acid, (b) oxalic acid and (c) the cocrystal.

The cocrystal formed between oxalic acid and 3, 4-dihydroxybenzoic acid at a molar ratio of 1:1 were investigated by other solid state analysis methods. The PXRD data for the oxalic acid 3, 4-dihydroxybenzoic acid system are depicted in Figs.8.6 and 8.7; the PXRD pattern of the (oxalic acid-3,4dihydroxybenzoic acid) differed from those of the constituents, confirming the formation of a new complex phase.
Fig. 8.6. Powder X-ray diffraction patterns for (a) 3, 4-dihydroxybenzoic acid, (b) oxalic acid, (c) and cocrystal.

Fig. 8.7. Powder X-ray diffraction patterns for (a) 3, 4-dihydroxybenzoic acid, (b) oxalic acid, (c) and cocrystal.
Furthermore, the DSC thermogram of the product exhibits a melting endotherm maximum at (96.57 ± 0.37 °C and 159.97 ± 0.46 °C) providing further support for a new crystal phase (Fig 8.8.). The thermal behaviour of the new phase is significantly different from oxalic acid (melting endotherm maximum at 100.1 ± 1.2 °C) and 3, 4-dihydroxybenzoic acid (melting endotherm maximum at 203.45 °C).

Fig 8.8. DSC melting curves of cocrystal, oxalic acid and 3,4-dihydroxybenzoic acid.
**Table 8.1.** Assignments of major bands of Raman spectra of 3,4 dihydroxybenzoic acid, oxalic acid and and their 1:1 cocrystal products

<table>
<thead>
<tr>
<th>Oxalic acid</th>
<th>3,4-dihydroxybenzoic acid</th>
<th>Cocrystal</th>
<th>Assignment [21-25]</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>3077w</td>
<td>3094 vw broad</td>
<td>v(CH)</td>
</tr>
<tr>
<td>-</td>
<td>2584vw</td>
<td>2584 vw 1792m</td>
<td>vOH</td>
</tr>
<tr>
<td>2584vw</td>
<td>-</td>
<td>-</td>
<td>vOH</td>
</tr>
<tr>
<td>1792m</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1778m</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1724s</td>
<td>-</td>
<td>1704 broad</td>
<td>va (C=O)</td>
</tr>
<tr>
<td>-</td>
<td>1604s</td>
<td>1616w</td>
<td>v(CC)ar</td>
</tr>
<tr>
<td>-</td>
<td>1599s</td>
<td>1604s</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>1514w</td>
<td>-</td>
<td>v(CC)ar</td>
</tr>
<tr>
<td>1481m</td>
<td>-</td>
<td>1480 w broad</td>
<td>δ(COH) + v(C-O)</td>
</tr>
<tr>
<td>1454vw</td>
<td>-</td>
<td>1454 vw</td>
<td>vs (C–O) + v (C–C)</td>
</tr>
<tr>
<td>-</td>
<td>1444w</td>
<td>-</td>
<td>CH3 bend C ring st</td>
</tr>
<tr>
<td>-</td>
<td>1379w</td>
<td>1373m</td>
<td>CH3 bend C ring st.</td>
</tr>
<tr>
<td>-</td>
<td>1364w</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>1338w</td>
<td>-</td>
<td>v (OH) phenolic</td>
</tr>
<tr>
<td>-</td>
<td>1291m</td>
<td>1313m</td>
<td>β(OH)carboxylic ,(CC)ar</td>
</tr>
<tr>
<td>-</td>
<td>1242m</td>
<td>1249m</td>
<td>CH bending</td>
</tr>
<tr>
<td>1173m</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>1097m</td>
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<td>β(CH)</td>
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<tr>
<td>852w</td>
<td>-</td>
<td>-</td>
<td>vC–C</td>
</tr>
<tr>
<td>837m</td>
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<td>837s</td>
<td>vC–C</td>
</tr>
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<td>824m</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>794m</td>
<td>794w broad</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>771w</td>
<td>-</td>
<td>Ring breathing</td>
</tr>
<tr>
<td>-</td>
<td>754vs</td>
<td>761w</td>
<td>CH wagging</td>
</tr>
<tr>
<td>-</td>
<td>636m</td>
<td>-</td>
<td>Ring deformation</td>
</tr>
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<td>-</td>
<td>593m</td>
<td>-</td>
<td>scissors (COOH)</td>
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<td>541w</td>
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<td>535w</td>
</tr>
<tr>
<td>-</td>
<td>531w</td>
<td>-</td>
<td>Ring deformation</td>
</tr>
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<td>-</td>
<td>-</td>
<td>478m</td>
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</tr>
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<td>463m</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>455m</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>449m</td>
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<td>-</td>
</tr>
<tr>
<td>-</td>
<td>449m</td>
<td>429vw</td>
<td>Ring wagging</td>
</tr>
<tr>
<td>-</td>
<td>438m</td>
<td>-</td>
<td>Ring deformation</td>
</tr>
<tr>
<td>-</td>
<td>382s</td>
<td>382vw</td>
<td>Ring deformation</td>
</tr>
<tr>
<td>-</td>
<td>343s</td>
<td>343w broad</td>
<td>OH wagging</td>
</tr>
<tr>
<td>230m</td>
<td>225w broad</td>
<td>Lattice mode</td>
<td></td>
</tr>
</tbody>
</table>

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### Table 8.2. Major bands of Transmission Raman Spectroscopy (TRS) of oxalic acid, 3,4-dihydroxybenzoic acid and their 1:1 cocrystal products.

<table>
<thead>
<tr>
<th>Oxalic acid</th>
<th>3,4-dihydroxybenzoic acid</th>
<th>Cocrystal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1796m</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1781m</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1725s</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1631w</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>1621sh</td>
<td>1620m</td>
</tr>
<tr>
<td>-</td>
<td>1611s</td>
<td>1605s</td>
</tr>
<tr>
<td>-</td>
<td>1299s</td>
<td>1315s</td>
</tr>
<tr>
<td>-</td>
<td>1173m</td>
<td>-</td>
</tr>
<tr>
<td>1156m</td>
<td>-</td>
<td>1155m</td>
</tr>
<tr>
<td>-</td>
<td>1130w</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>1105s</td>
<td>-</td>
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<tr>
<td>807m</td>
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<td>791s</td>
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<td>435m</td>
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<td>-</td>
<td>466s, 456s doublet</td>
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<td>362w</td>
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<td>238s</td>
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<td>-</td>
<td>243s</td>
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</tr>
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<td>456m</td>
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<td>156m</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>117s</td>
<td>-</td>
<td>-</td>
</tr>
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</table>

vs, very strong; s, strong; m, medium; w, weak; vw, very weak; sh, shoulder; v, stretching; σ, deformation or in-plane bending; β in-plane deformation; a, antisymmetric; s, symmetric.
8.3 Conclusion

The increasing interest in cocrystal formation and structures in recent years is due to the recognition of the important potential commercial value of generating new crystal forms, with improved properties and intellectual property value. We have obtained cocrystals of 3,4-dihydroxybenzoic acid with oxalic acid by slow evaporation at room temperature from ethanol solution in a 1:1 stoichiometric ratio. There has been considerable discussion of the definition of a cocrystal with regard to the nature of both the starting components and the resultant molecules through the use of Raman spectroscopy (dispersive and transmission), X-ray powder diffraction and thermal analysis. Raman spectroscopy can be used to demonstrate a number of important aspects regarding the nature of the interactions in the cocrystal. Formation of the cocrystal causes broadening in the carbonyl band region that is diagnostic for the existence of cocrystals. Alterations in the energies of bands associated with the carbonyl vibrations of the carboxyl group were shown to be sensitive to fine details of the solids. We have used a synthetic standard to confirm that the ratios of cocrystal components were in a 1:1 molar ratio. Transmission Raman spectroscopy (TRS), which is well suited for rapid volumetric probing of intact samples, is applied to study cocrystals for the first time, from which we can identify the presence of a different crystal structure in the cocrystal compared with the two precursor materials can be identified especially from low wavenumber Raman bands.
8.4 References

Chapter 9
Conclusions and further work
9.1 Conclusions

In this body of work, the development of several methods for analysis of pharmaceutically relevant organic molecular species has been realised. In particular, pH titration using Raman spectroscopy for pharmaceutically relevant materials, gave rise to several objectives. A combination of potentiometric and Raman spectroscopic measurement of aqueous solutions of pharmaceutically relevant materials over different pH values provides a set of vibrational data that can be used to determine and monitor pH-induced molecular structural changes.

The data collection allows a thorough integrated interpretation of the complementary spectral data to be made, which leads to a higher reliability of the peak assignments. We have demonstrated that the set of Raman vibrational data obtained can be used to determine the possible states of protonation, i.e. $\text{H}_3\text{A}$, $\text{H}_2\text{A}^-$, $\text{HA}^2-$, $\text{A}^3-$. Furthermore, a multivariate computational method has been adopted to deduce the $\text{pK}_a$ values from the Raman spectra recorded at different pH values. The technique is exemplified by using several drugs and molecule analogues, namely, salicylic acid, paracetamol, salicylaldoxime, and citric acid. The $\text{pK}_a$ values obtained agree well with those derived from the pH-metric titrations. It has been demonstrated that the $\text{pK}_a$ values and the molar fraction coefficients of the individual species at different wavenumbers can be derived with high accuracy in mutiwellavelength spectroscopic titration experiments.

Also, this study highlights structural and analytical aspects of the novel structures of seven new cocrystals. The systems have been prepared and characterised using several techniques, providing the opportunity to understand the mechanism of the molecular interactions in these systems.

The results have shown that molecules of similar shapes and sizes are not necessarily required to form cocrystals. Instead, the arrangement and number of the hydrogen
bonding groups are much more significant in the cocrystal formation. The results have also shown that the heteromeric associations are not always favoured over homomeric associations in all the cocrystals prepared here; and have pointed to new ways of thinking in solid form preparation in particular, the conceptual of docking hetero-molecules on homomeric assemblies through unsaturated hydrogen bond donor/acceptor functions has been introduced. The homomeric ladder/ribbon assemblies of the citric acid and paracetamol cocrystals reported here, point to this design approach. The knowledge gained from these studies provides an insight into the understanding and predicting the behaviour of the hydrogen bonding in these supramolecular compounds. This thesis has shown that Raman spectroscopy could be used to demonstrate a number of important aspects regarding the nature of the interactions in the cocrystal; also, TRS, which is well suited for the rapid volumetric probing of intact samples, has been applied here to study cocrystals for the first time, from which we can identify the presence of a different crystal structure in the cocrystal compared with the two precursor materials, especially from the analysis of the low wavenumber Raman bands.
9.2 Further work

The use of vibrational spectroscopy in conjunction with a multivariate computational method to determine pK\textsubscript{a} values from the Raman spectra recorded at different pH values has not been reported hitherto, therefore, further work should be carried out to include a larger volume of pharmaceutical compounds from different chemical structure to allow a better understanding for relevant pharmaceutical systems. More development needs to be carried out on the UV/Raman coupled technique with an Automatic Titrator Dosimat (where the pH-meter and \textit{automatic titrator} are connected and managed with a computer by specialised software).

The combined measurement by Raman spectroscopic and DSC methods give us the possibility to obtain a complete picture of the chemical and physical changes occurring in the thermal process of molecular conversion. So, a further use for the method in cocrystal research is the understanding of the thermal behaviour of pharmaceutical cocrystals during the heating of the samples.
Appendices (I)
Appendix (I)
This Appendix included a copy of the computational program which has been used for the
determination of the acidity constant for citric acid as an example.

% Main_EQAHn
clc clear
% the following lines define a model for citric acid as a H3A
s.spec_names = {'A3-' 'H' 'AH' 'AH2' 'AH3' 'OH'};
s.Model = [ 1 0 1 1 1 0 ; ... % component A
    0 1 1 2 3 -1 ]; % component H
s.log_beta = [ 0 0 6 10 -13.7 ]; % component H
s.c_0 = [.8 2.404]; % conc initial solution, Atot, Htot
s.c_added = [0. -1.0]; % conc titration solution, Atot,OH
s.v_0 = 25.0; % Initial volume
s.v_added = [.05 0.1 0.16 0.2 0.25 0.3 0.35 0.5 0.6
    0.71 0.8 0.92 1 1.3 1.5 1.7 2 2.55
    3 3.5 4 4.5 5 5.55 6 6.54 7.1
    8 9 10 11 12 13 14 14.5 16
    17 18.53 20 22 24 26.1 28 30 32.5
    35 38 42 46 50 54 60 63 65.1
67]'; % added volumes
s.v_tot = s.v_0 + s.v_added; % total volumes
s.nvol = length(s.v_added); % number of additions
s.ncomp = size(s.Model,1); % number of components, 2
s.c_comp_guess0 = [1.e-10 1.e-10]; % init. guess for Newton-Raphson
s.c_comp_guess = s.c_comp_guess0; % species concentrations
beta = 10.^s.log_beta;
s.fname = 'Rcalc_EQAHn'; % file to calc residuals
s.par_str = {'s.log_beta(3)' 's.log_beta(4)' 's.log_beta(5)' 's.log_beta(6)' 's.c_0(1)' 's.c_0(2)'} % variables to be fitted
s.log_beta(3:6) = [7 12 14 -15]; % [logB1, logB2, ... ,-pKw] initial estimates
s.c_0(1:2) = [.800 2.404 ]; % Atot, Htot, initial estimates
s.par = get_par(s); % collects variable param. into s.par
s.nlm3(s); % call ngl/m
s.sig_r = sqrt(s.ssq/(prod(size(s.pH))-length(s.par))); % sigma_r
s.sig_par = s.sig_r * sqrt(diag(inv(s.Curv))); % sigma_par
for i=1:length(s.par)
fprintf(1,'s: %g\n',s.par(i));
s.sig_r=
plot(s.v_added,s.pH,'.',s.v_added,s.pH_calc);
xlabel('ml');ylabel('pH');

function [r,s]=Rcalc_EqAHn(s)

s.v_tot=s.v_0+s.v_added;
s.C_tot=(s.v_0*repmat(s.c_0,s.nvol,1)+s.v_added*s.c_added) ... 
./repmat(s.v_tot,1,s.ncomp);

beta=10.^s.log_beta;
c_comp_guess=s.c_comp_guess0; % species concentrations
for i=1:s.nvol
  s.C(i,:)=NewtonRaphson(s.Model,beta,s.C_tot(i,:), ... 
  c_comp_guess,i);
  c_comp_guess=s.C(i,1:s.ncomp);
end

s.pH_calc=-log10(s.C(:,s.ncomp));
r=s.pH-s.pH_calc;
s.ssq=sum(r.*r);
if nargout==2
  figure(2);plot(s.v_added,s.pH,'.',s.v_added,s.pH_calc);
  xlabel('ml');ylabel('pH');drawnow
end

function s=put_par(s)

% updates all parameter variables in s (e.g. s.log_beta, etc)
for i=1:length(s.par_str)
  eval([s.par_str(i) ' = s.par(i);']);
end

function par=get_par(s)

% collects variable parameters into s.par
for i=1:length(s.par_str)
  par(i,1)=eval(s.par_str(i));
end
function c_spec=NewtonRaphson(Model, beta, c_tot, c,i,s)

ncomp=length(c_tot);                       % number of components
nspec=length(beta);                        % number of species
c_tot(c_tot==0)=1e-15;                    % numerical difficulties if c_tot=0

it=0;
while it<=200
    it=it+1;
    c_spec    =beta.*prod(repmat(c',1,nspec).^Model,1); %species conc
    c_tot_calc=sum(Model.*repmat(c_spec,ncomp,1),2); %comp ctot calc
    d         =c_tot-c_tot_calc; % diff actual and calc total conc
    if all(abs(d) <1e-15)       % return if all diff small
        return
    end
    for j=1:ncomp                 % Jacobian (J_s=J*)
        for k=j:ncomp
            J_s(j,k)=sum(Model(j,:).*Model(k,:).*c_spec);
            J_s(k,j)=J_s(j,k); % J_s is symmetric
        end
    end
    delta_c=(d/J_s)*diag(c); % equation (2.43)
    c=c+delta_c;
    while any(c <= 0)            % take shift back if conc neg.
        delta_c=0.5*delta_c;
        c=c-delta_c;
        if all(abs(delta_c)<1e-15)
            break
        end
    end
if it>200; fprintf(1,'no conv. at C_spec(%i,:)
',i); end
end
function s=nglm3(s)

ssq_old=1e50;
mp=0;                 % Marquardt parameter
mu=1e-4 ;             % convergence limit
delta=1e-6;           % step size for numerical diff
it=0;

while it<50
    [r0,s]=feval(s.fname,s);   % calculation of residuals
    conv_crit=(ssq_old-s.ssq)/ssq_old;  % convergence criterium
    fprintf(1,'it=%i, ssq=%g, mp=%g, conv_crit=%g\n', ... 
        it,s.ssq,mp,conv_crit);
    if abs(conv_crit) <= mu        % ssq_old=ssq, minimum reached
        if mp==0
            break
        else
            mp=0;
            r0_old=r0;
        end
    elseif conv_crit > mu         % convergence
        mp=mp/3;
        ssq_old=s.ssq;
        r0_old=r0;
        for i=1:length(s.par)  % num. differentiation
            eval(['s.par_str{i} ='' s.par_str{i} ''*(1+delta);']);
            r=feval(s.fname,s);
            eval(['s.par_str{i} ='' s.par_str{i} ''/(1+delta);']);
            J(:,i)=(r-r0)/(delta*s.par(i));
        end
    elseif conv_crit < -mu       % divergence
        if mp==0
            mp=1;
        else
            mp=mp*5;
        end
        s.par=s.par-delta_par;  % and take shifts back
    end
    J_mp=[J;mp*eye(length(s.par))]; % augment Jacobian matrix
    r0_mp=[r0_old;zeros(size(s.par))]; % augment residual vector
    delta_par=-J_mp\r0_mp;  % calculate parameter shifts
    s.par=s.par+delta_par; % add parameter shifts
    s=put_par(s);  % updates parameter variables in s (e.g. s.log_beta)
    it=it+1;
end
s.Curv=J'*J;               % curvature matrix
The following m-files used for Spectroscopic data processing

1- Example of structure of a data file.

```
fuction [D,v_added,pH]=salidoxamine
v_added=[0 0.044 0.064 0.1 0.13 0.15 0.2 0.3 0.4
0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 6
];
pH=[2.92 3.86 5.44 6.46 6.95 7.16 7.48 7.81 8.05
12.66 ];

%Strcture of D=[wavelength spectra intensity for solutions
#1...#lastspectra
D=[90.757011 1437 1613.9 1693.9 1494.7 1615.5 1583.7 1600.7 1527
1531.8 1581.1 1884 1880.9 1780.2 1694.4 1702.9 1737.8 1735.2
1678.5 1636.1 1580.5 1528.1 1486.8 1425.9 1595.9 1474.6 1600.1
1233.6 1127.6 1086.3 1051.4 1045 1030.7 778.1 801.94 817.3
681.17 636.68 94.244905 2566.7 2998.8 3002.1 2665.9 2844.6 2866.4 2897.9 2752.6
2755.4 2821.9 3405.9 3366.7 3172.2 3042.9 3149.2 3125.9 3095.8
3043.4 3010.9 2861.7 2794.8 2708.4 2585.3 2867.7 2612.5 2857.8
2228.6 2002.9 1942 1880.4 1854.1 1762.7 1345.6 1409.8 1444.7
1182.6 1110.7 97.732799 4473.2 5204.8 5266.4 4654.7 4984.6 5028.7 5044.9 4800.2
4809.7 4923.4 5948.2 5859 5595.1 5364.9 5496.8 5461.2 5407.2
5310.4 5257.7 4982.5 4867.8 4716.1 4507.7 5003.9 4557.3 5050.5
3886.8 3516.9 3397.3 3278.9 3219.6 3071.5 2309.2 2448 2533.5
2052.6 1918.8 101.220692 7104.5 8235 8402.2 7413 7983.2 7999.5 8007.4 7652.7
7650.2 7843.2 9499.9 9340.2 8962.9 8592.9 8746.7 8730 8659.8
8478.9 8379 7958.1 7751.2 7535.2 7228.6 8026.5 7308.4 8154.7
6231.7 5644.7 5445 5265.6 5137.3 4944.3 3693.2 3924.9 4084.6
3287.6 3066.1 104.708586 10453 12163 12372 10943 11845 11755 11809 11356
11285 11595 14132 13871 13243 12715 12977 12993 12921
12625 12452 11880 11518 11268 10862 10309 10933 12217
9352.6 8406 8126 7915.4 7648.7 7412.8 5565.5 5889.9 6138.7
4913.6 4588.2 ];
```
% Main_spect
clear
clc
%### define the model and get data in a s structre, e.g H2A ,a diprotic acid###
s.spec_names =  {'A2-','AH','AH2','H','OH'};
s.Model = [
    1 1 1 0 0;  % component A
    0 1 2 1 -1];  % component H
s.ncomp = size(s.Model,1);
s.log_beta = [0 12 19 0 -15.1];
s.c_0 = [0 12 19 0 -15.1];  % conc titration solution, Atot,OH
s.v_0 = 25.0;

[D,s.v_added,s.pH]=salidoxamine;  %files containing titration data
s.v_tot = s.v_0+s.v_added;  % total volumes
s.ns = length(s.v_added);  % number of spectra
s.c_comp_guess0 = [1e-10 1e-10];  % default guess for Newton-Raphson
s.c_comp_guess=s.c_comp_guess0;  % init. comp. conc.
s.lam = D(:,1)';

D=D(:,2:s.ns+1);
s.nl = length(s.lam);
s.A_sim(4,:)=0.*D(:,1)';  %define a zero intensity for species #4 based on above model i.e. H+ s.A_sim(5,:)=0.*D(:,1)';  %define a zero intensity for species #5 based on above model i.e. OH-
s.A_sim(3,:) = D(:,1')./s.c_0(1);
s.A_sim(1,:) = D(:,s.ns')./65*25;
D=D';

uu,ss,vv=svd(D);  %perform Singular value decomposition
nc=4;  %number of pc
D=uu(:,1:nc)*ss(1:nc,1:nc)*(vv(:,1:nc)');  %reconstruct data file with reduced some PCs
s.Y=D;

s.fname = 'Rcalc_spect';  % file to calc. residuals
s.par_str = {'s.log_beta(2)' 's.log_beta(3)'};
s.log_beta(2:3)=[11.8 23];  % [logB1, logB2 -pKw] initial estimates
s.par=get_par(s);  % collects variable param. into s.par
s.known = [0 0 1 1 1];  % 0 for unknown spectra, 1 for known spectra

s.A_k(1:sum(s.known),:) = s.A_sim(s.known==1,:);
s=nglm3(s);

s.sig_r=sqrt(s.ssq/(s.ns*s.nl-

sum(s.known==0)*s.nl)));
s.sig_par=s.sig_r*sqrt(diag(inv(s.Curv))));  % sigma_par

for i=1:length(s.par)
    fprintf(1,'%s: %g +- %g\n',s.par_str(i)(3:end),s.par(i), ...
    s.sig_par(i));
end

fprintf(1,'sig_r: %g\n',s.sig_r);
plot(s.lam,s.A_k,'.',s.lam,s.A);
xlabel('wavelength'); ylabel('mol. abs');

C1=s.Y*pinv(s.A);
figure(2)
plot(s.pH,s.C(:,1:3),'-')
legend('A-','AH','AH2')

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
g=s.C(:,1:5)*s.A;
[sr,sc]=size(s.A);
for i=1:sr
    for j=1:sc
        if s.A(i,j)<0
            s.A(i,j)=0;
        end
    end
end
figure(3)
plot(RSS1')
p=RSS1';
figure (4)
plot(s')
figure (5)
plot(g', 'r')
hold on
plot(D', 'b')
hold off

3-Rcalc_spect.m (calculate the residuals and..)

function [r,s]=Rcalc_spect(s)

s.v_tot=s.v_0+s.v_added;
s.C_tot=(s.v_0*repmat(s.c_0,s.ns,1)+s.v_added*s.c_added) ... 
    ./repmat(s.v_tot,1,s.ncomp);

beta=10.^s.log_beta;
c_comp_guess=s.c_comp_guess0;     % reinit. comp. conc.
for i=1:s.ns
    s.C(i,:)=NewtonRaphson(s.Model,beta,s.C_tot(i,:), ... 
        c_comp_guess,i);
    c_comp_guess=s.C(i,1:s.ncomp);
end

if sum(s.known)>0

    C_k =s.C(:,s.known==1);     % conc with known spectra
    C_uk=s.C(:,s.known==0);     % conc with unknown spectra

    Y_k=C_k*s.A_k;     % known part of Y
    Y_uk=s.Y-Y_k;     % unknown part of Y

    A_uk=C_uk*Y_uk;     % unknown spectra
    A_uk=nonneg(Y_uk',C_uk');     % non-negative spectra
R = Y_{uk} - C_{uk}A_{uk};

s.A(s.known==1,:) = s.A_k;
s.A(s.known==0,:) = A_{uk};

else
    s.A = s.C \backslash s.Y;
    R = s.Y - s.C*s.A;
end

r = R(:);
s.ssq = sum(r.*r);

if nargout == 2
    figure(3);plot(s.pH, s.C, '-');drawnow
    figure(4);plot(s.pH, s.Y, '.', s.pH, s.Y-R, '-');drawnow
    figure(4);plot(s.lam, s.A, s.lam, s.A_org, '.');drawnow
end
function [F,SS]=nonneg1(X,M);%,Options);
%NONNEG1 alternative to NNLS
%
%function [F,SS]=nonneg1(X,M,F,Options);
%
% 'nonneg.m'
% $ Version 0.01 $ Date 6. Aug. 1997 $ Not compiled $
%
% This algorithm requires access to:
%
'','
% --------------------------------------------------------
%             Fast! Non-negativity Regression
% --------------------------------------------------------
%
% [F,SS]=nonneg(X,M,F,Options);
% [F,SS]=nonneg(X,M);
%% X        : Matrix of regressors.
%% M        : Matrix of regressands.
%% F        : The non-negativity constrained solution matrix.
%% Options  : Type 'help Options' at the prompt.
%
% Given X and M this algorithm solves for the optimal
% F in a least squares sense, using that
% X = F*M
% in the problem
% min ||X-F*M||, s.t. F>=0, for given X and M.
% This version does not accept missing values.
%
% Copyright
% Claus A. Andersson 1995-1997
% Chemometrics Group, Food Technology
% Department of Food and Dairy Science
% Royal Veterinary and Agricultural University
% Rolighedsvej 30, T254
% DK-1958 Frederiksberg
% Denmark
% E-mail claus@andersson.dk
format long
format compact
Show=0;
SS=[];
[aX bX]=size(X);
[aM bM]=size(M);
aF=aX;
bF=aM;
w=bF;
Xc=X;
W=eye(w);
itimax=100;
itomax=100;
SSimax=1e-10;
SSomax=1e-12;
SSiOld=realmax;
SSoOld=realmax;
%Initialize F
if ~exist('F')
    F=X*M'/(M*M');
    FOld=F;
    I=find(F<0);
    FOld(I)=0;
    SSiOld=sum(sum( (F-FOld).^2 ));
    F=FOld;
end;
FOld=F;
SSX=sum(sum(X.^2));
MMT=M*M';
XMT=X*M';
InvMMT=1./diag(MMT);

ito=0;
convo=0;
while ~convo,
    ito=ito+1;
    convi=0;
    iti=0;
    while ~convi,
        iti=iti+1;
        %Iterate on variables for non-negativity
        for i=1:w,
            W(i,i)=0;
            f=XMT(:,i)-F*W*MMT(:,i);
            f=InvMMT(i)*f;
            I=find(f<0);
            if ~isempty(I),
                f(I)=zeros(1,length(I));
            end;
            F(:,i)=f;
            W(i,i)=1;
        end;
        %Estimate error now
        SSi=sum(sum( (F-FOld).^2 ));
        if SSi < sum(sum( FOld.^2 ))*SSimax | iti>itimax,
            convi=1;
        end;
        FOld=F;
    end;
    %Estimate error on the transformed LS problem
    SSo=sum(sum( (XMT-F*MMT).^2 ));
    if (SSoOld-SSo)/SSX<SSomax,
        convo=1;
    end;
    if ito>itomax,
        convo=1;
    end;
    SSoOld=SSo;
end;
forma
Appendix (II)
This Appendix included tables of Single -crystal X-Ray diffraction.

1- Salicylic acid – benzamide cocrystal.

Table 1. Atomic coordinates ( x 104) and equivalent isotropic displacement parameters (Å2 x 103) for (C7H6O3)(C7H7NO). U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

<table>
<thead>
<tr>
<th></th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>U(eq)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C(1A)</td>
<td>3339(2)</td>
<td>6636(1)</td>
<td>3956(1)</td>
<td>37(1)</td>
</tr>
<tr>
<td>C(2A)</td>
<td>3663(2)</td>
<td>7599(1)</td>
<td>4392(1)</td>
<td>41(1)</td>
</tr>
<tr>
<td>C(3A)</td>
<td>5597(3)</td>
<td>7432(2)</td>
<td>4792(1)</td>
<td>52(1)</td>
</tr>
<tr>
<td>C(4A)</td>
<td>7178(2)</td>
<td>6329(2)</td>
<td>4753(1)</td>
<td>55(1)</td>
</tr>
<tr>
<td>C(5A)</td>
<td>6882(2)</td>
<td>5367(2)</td>
<td>4323(1)</td>
<td>53(1)</td>
</tr>
<tr>
<td>C(6A)</td>
<td>4974(2)</td>
<td>5524(1)</td>
<td>3927(1)</td>
<td>45(1)</td>
</tr>
<tr>
<td>C(7A)</td>
<td>1287(2)</td>
<td>6814(1)</td>
<td>3534(1)</td>
<td>40(1)</td>
</tr>
<tr>
<td>O(1A)</td>
<td>1003(2)</td>
<td>5794(1)</td>
<td>3171(1)</td>
<td>50(1)</td>
</tr>
<tr>
<td>O(2A)</td>
<td>-56(2)</td>
<td>7840(1)</td>
<td>3527(1)</td>
<td>56(1)</td>
</tr>
<tr>
<td>O(3A)</td>
<td>2175(2)</td>
<td>8712(1)</td>
<td>4451(1)</td>
<td>59(1)</td>
</tr>
<tr>
<td>C(1B)</td>
<td>-6107(2)</td>
<td>6760(1)</td>
<td>1908(1)</td>
<td>36(1)</td>
</tr>
<tr>
<td>C(2B)</td>
<td>-8217(2)</td>
<td>7575(1)</td>
<td>1862(1)</td>
<td>44(1)</td>
</tr>
<tr>
<td>C(3B)</td>
<td>-9782(2)</td>
<td>7465(2)</td>
<td>1387(1)</td>
<td>53(1)</td>
</tr>
<tr>
<td>C(4B)</td>
<td>-9247(3)</td>
<td>6558(2)</td>
<td>963(1)</td>
<td>54(1)</td>
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<tr>
<td>C(5B)</td>
<td>-7158(3)</td>
<td>5747(1)</td>
<td>1007(1)</td>
<td>50(1)</td>
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<tr>
<td>C(6B)</td>
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<td>5838(1)</td>
<td>1481(1)</td>
<td>42(1)</td>
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<tr>
<td></td>
<td>C(7B)</td>
<td></td>
<td>N(1B)</td>
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<td>-------</td>
<td>---</td>
<td>-------</td>
<td>---</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>-4331(2)</td>
<td>6848(1)</td>
<td>2402(1)</td>
<td>38(1)</td>
</tr>
</tbody>
</table>
**Table 2.** Bond lengths [Å] and angles [°] for (C7H6O3)(C7H7NO).

<table>
<thead>
<tr>
<th>Bond</th>
<th>Length [Å]</th>
</tr>
</thead>
<tbody>
<tr>
<td>C(1A)-C(6A)</td>
<td>1.3982(17)</td>
</tr>
<tr>
<td>C(1A)-C(2A)</td>
<td>1.4020(16)</td>
</tr>
<tr>
<td>C(1A)-C(7A)</td>
<td>1.4803(14)</td>
</tr>
<tr>
<td>C(2A)-O(3A)</td>
<td>1.3570(15)</td>
</tr>
<tr>
<td>C(2A)-C(3A)</td>
<td>1.3977(17)</td>
</tr>
<tr>
<td>C(3A)-C(4A)</td>
<td>1.375(2)</td>
</tr>
<tr>
<td>C(3A)-H(3A)</td>
<td>0.983(18)</td>
</tr>
<tr>
<td>C(4A)-C(5A)</td>
<td>1.390(2)</td>
</tr>
<tr>
<td>C(4A)-H(4A)</td>
<td>1.007(18)</td>
</tr>
<tr>
<td>C(5A)-C(6A)</td>
<td>1.3813(17)</td>
</tr>
<tr>
<td>C(5A)-H(5A)</td>
<td>0.960(18)</td>
</tr>
<tr>
<td>C(6A)-H(6A)</td>
<td>0.972(16)</td>
</tr>
<tr>
<td>C(7A)-O(2A)</td>
<td>1.2310(15)</td>
</tr>
<tr>
<td>C(7A)-O(1A)</td>
<td>1.3154(14)</td>
</tr>
<tr>
<td>O(1A)-H(1AO)</td>
<td>0.94(2)</td>
</tr>
<tr>
<td>O(3A)-H(3AO)</td>
<td>0.90(2)</td>
</tr>
<tr>
<td>C(1B)-C(6B)</td>
<td>1.3919(16)</td>
</tr>
<tr>
<td>C(1B)-C(2B)</td>
<td>1.3934(16)</td>
</tr>
<tr>
<td>C(1B)-C(7B)</td>
<td>1.4950(14)</td>
</tr>
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Table 3. Anisotropic displacement parameters (Å² x 10³) for (C7H6O3)(C7H7NO). The anisotropic displacement factor exponent takes the form: \(-2\pi² \sum h² a^* U^{11} + ... + 2 h k a^* b^* U^{12}\)

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Table 4. Hydrogen coordinates \((x \times 10^4)\) and isotropic displacement parameters \((\AA^2 \times 10^{-3})\) for \((\text{C7H6O3})(\text{C7H7NO})\). 

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2- Salicylic acid – DL-phenylalanine cocrystal

Table 1. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (Å^2 x 10^3) for [salacid][phenala]. U(eq) is defined as one third of the trace of the orthogonalized U^ij tensor.

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Table 2. Bond lengths [Å] and angles [°] for [salacid][phenala].

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Symmetry transformations used to generate equivalent atoms:
Table 3. Anisotropic displacement parameters (Å² x 10³) for [salacid][phenala]. The anisotropic displacement factor exponent takes the form: \(-2\pi^2 [h^2 a^* U^{11} + \ldots + 2hk a^* b^* U^{12}]\)

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C(6B)-C(5B)-C(4B)  120.0(2)
C(5B)-C(6B)-C(1B)  120.98(19)
O(2B)-C(11B)-N(1B) 122.2(2)
O(2B)-C(11B)-C(12B) 121.8(2)
N(1B)-C(11B)-C(12B) 115.96(19)

Symmetry transformations used to generate equivalent atoms:
Table 3. Anisotropic displacement parameters (Å² x 10³) for [para]2[cit]. The anisotropic displacement factor exponent takes the form: $-2\pi^2 [h^2 a^* U_{11} + ... + 2hk a^* b^* U_{12} ]$

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