A SUPRAMOLECULAR DERIVATISED STUDY OF BIS(ADAMANTAN-1-AMINIUM) CARBONATE

By

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A thesis submitted in fulfilment of the requirements for the degree of Magister Scientiae (Pharmaceutical Science) in the School of Pharmacy, University of the Western Cape

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Abstract

In this study, new solid supramolecular derivatised forms of bis(adamantine-1-aminium) carbonate (ADTCO$_3$) were prepared. ADTCO$_3$ is a derivative of amantadine used for Parkinson’s disease and has antiviral properties against influenza-A, dengue fever and pharmacological activity towards Parkinson’s disease. The new forms prepared were polymorphic and co-crystal forms of ADTCO$_3$. Polymorphism is a phenomenon where the ability of a substance to exist in two or more crystalline forms occurs when crystallised under different conditions and co-crystallization is the process of formation of multicomponent crystals of a drug substance.

New solid forms often display different mechanical, physicochemical and thermal properties that can remarkably influence the bioavailability, hygroscopicity and stability of active pharmaceutical ingredients (APIs). For the formation of polymorphs of ADTCO$_3$, techniques such as dry grinding, solvent-drop grinding, co-precipitation, sublimation and vapour diffusion were applied. For the development of co-crystals and/or complex formation, ADTCO$_3$ was treated in combination with ten selected co-formers viz; benzoic acid, 4-hydroxybenzoic acid, cinnamic acid, 4-hydroxycinnamic acid, succinic acid, tartaric acid, salicylic acid, L-glutamic acid, citric acid monohydrate and L-glutaric acid using similar techniques as applied in the polymorphism study. The first four co-formers were selected for their potential biological activity and the latter six were selected for their generally regarded as safe (GRAS) status. All products were isolated and characterized using different analytical techniques to assess the thermal behaviour of the products by hot stage microscopy (HSM), differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). FTIR spectroscopy and proton-nuclear magnetic resonance ($^1$HNMR) were used to identify and determine the purity of the parent compounds and the modified forms. X-ray powder diffraction was used to determine the formation of a new phase and single crystal X-ray diffraction was applied at the initial stages to identify ADTCO$_3$ by its unit cell parameters. Furthermore, the Cambridge Structural Database (CSD) and other resources were used to generate information on the molecular structures of all elucidated parent compounds, their polymorphs and reported co-crystals.

Four different polymorphic forms of ADTCO$_3$ were identified (viz. ADTCO$_3$ Forms I to IV) and sixteen co-crystals (viz. ADTCO$_3$BA1 to ADTCO$_3$BA5, ADTCO$_3$HBA, ADTCO$_3$CIN, ADTCO$_3$HCIN, ADTCO$_3$SUC, ADTCO$_3$LTTA, ADTCO$_3$SA,
Abstract

ADTCO$_3$CA, ADTCO$_3$GLA, ADTCO$_3$GA) were synthesised. Of the sixteen co-crystals 5 were identified as ADTCO$_3$BA “salt” co-crystal polymorphic forms and 2 as ADTCO$_3$SUC co-crystal polymorphic forms. Two solvated “salt” co-crystal forms were also identified, namely; ADTCO$_3$GLA and ADTCO$_3$LTTA. ADTCO$_3$GLA had a mass loss of 10.3% (n = 2.4) and ADTCO$_3$LTTA had a mass loss of 5.25% (n = 0.86). Finally, the rest of the co-crystals ADTCO$_3$HBA, ADTCO$_3$CIN, ADTCO$_3$HCIN, ADTCO$_3$SA, ADTCO$_3$CA and ADTCO$_3$GA all crystallised as “salt” co-crystals.
Keywords

Salts
Polymorphism
Crystallisation
Co-crystallisation
Amantadine
Bis(adamantan-1-aminium) carbonate
Co-formers
Physicochemical characterisation
X-ray crystallography
Declaration

I, Jean Baptiste NGILIRABANGA, hereby declare that the work presented in this thesis is a product of my own efforts.

Signature………………………………………………
Acknowledgement

I would like to express my sincere gratitude to my supervisor, Dr. Halima Samsodien for her excellent guidance, supervision and for creating a suitable environment for learning.

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Finally, and most importantly, to my family and friends for their invaluable support and contribution and for always keeping me in their prayers.

Without all of you, this work would not have been accomplished.
Dedication

To almighty God, my family and friends, I dedicate this work.
List of abbreviations

b.p.: boiling point
m.p.: melting point
ADT: 1-adamantanamine
ADTHCl: 1-adamantanamine hydrochloride
ADTCO₃: Bis(adamantan-1-aminium) carbonate
BA: Benzoic acid
HBA: 4-hydroxybenzoic acid
CIN: Trans-cinnamic acid
HCIN: 4-hydroxycinnamic acid
SUC: Succinic acid
GLA: L-glutamic acid
GA: L-glutaric acid
LTTA: L-tartaric acid
SA: Salicylic acid
CA: Citric acid monohydrate
ADTCO₃BA: Bis(adamantan-1-aminium) carbonate-benzoic acid co-crystal
ADTCO₃HBA: Bis(adamantan-1-aminium) carbonate-4-hydroxybenzoic acid co-crystal
ADTCO₃CIN: Bis(adamantan-1-aminium) carbonate- cinnamic acid co-crystal
ADTCO₃HCIN: Bis(adamantan-1-aminium) carbonate-4-hydroxycinnamic acid co-crystal
ADTCO₃SUC: Bis (adamantan-1-aminium) carbonate-succinic acid co-crystal
ADTCO₃LTTA: Bis(adamantan-1-aminium) carbonate-L-tartaric acid co-crystal
ADTCO₃SA: Bis(adamantan-1-aminium) carbonate-salicylic acid co-crystal
ADTCO₃GLA: Bis(adamantan-1-aminium) carbonate-L-glutamic acid solvated co-crystal
List of abbreviations

ADTCO$_3$CA: Bis(adamantan-1-aminium) carbonate-citric acid monohydrate co-crystal
ADTCO$_3$GA: Bis(adamantan-1-aminium) carbonate-$L$-glutaric acid co-crystal
API: Active Pharmaceutical Ingredients
DNA: Deoxyribonucleic acid
DSC: Differential Scanning Calorimetry
HSM: Hot Stage Microscopy
H$^1$NMR: Proton Nuclear Magnetic Resonance
FTIR: Fourier Transform Infrared Spectroscopy
PXRD: Powder X-ray Diffraction
SXRD: Single X-ray Diffraction
CSD: Cambridge Structural Database
Table of Contents

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

OVERVIEW OF SUPRAMOLECULAR CHEMISTRY

POLYMORPHISM

SOLVATES

STOICHIOMETRIC SOLVATES

NON-STOICHIOMETRIC SOLVATES

HYDRATES

CLASSIFICATION OF HYDRATES

AMORPHOUS

POLYMORPH PREPARATION

POLYMORPH IDENTIFICATION

TYPES OF POLYMORPHS

KINETICS OF POLYMORPHS

PHYSICOCHEMICAL CHARACTERISATION OF POLYMORPHS

IMPACT OF POLYMORPHISM ON DRUG PRODUCT

POLYMORPH SELECTION

SALT FORMATION

INTRODUCTION

PROPERTIES OF SALTS

SALT SELECTION

CO-CRYSTALLISATION

INTRODUCTION TO CO-CRYSTALLISATION
**Table of contents**

- **PHARMACEUTICAL CO-CRYSTALS** ................................................................. 25
- **PHARMACEUTICAL CO-CRYSTAL FORMATION AND DESIGN** .................. 27
- **CO-CRYSTALS CHARACTERISATION** .......................................................... 29
- **PROPERTIES OF PHARMACEUTICAL CO-CRYSTALS** ................................. 30
- **CO-CRYSTAL POLYMORPHISM** ................................................................. 36
- **SCALE-UP** ..................................................................................................... 37
- **INTELLECTUAL PROPERTY AND MANAGEMENT OF LIFECYCLE** .......... 38
- **1-ADAMANTANAMINE** .................................................................................. 39
- **MOTIVATION OF THE STUDY** ..................................................................... 40
- **OBJECTIVES OF THE STUDY** ......................................................................... 41
- **REFERENCES** .................................................................................................. 42

**CHAPTER 2: MATERIALS AND EXPERIMENTATION** ........................................ 54

- **THE USED COMPOUNDS** ............................................................................... 54
- **PREPARATION OF POLYMORPHS** ............................................................... 54
  - **CRYSTALLISATION FROM SOLVENT EVaporation** .................................... 54
  - **VAPOUR DIFFUSION** ..................................................................................... 55
  - **THERMAL TREATMENT** .................................................................................. 55
  - **GRINDING METHOD** ....................................................................................... 56
  - **SUBLIMATION METHOD** ................................................................................ 56
- **PREPARATION OF CO-CRYSTALS/ COMPLEXES** ......................................... 57
  - **SOLVENT EVAPORATION** .............................................................................. 57
  - **VAPOUR DIFFUSION** ..................................................................................... 58
  - **GRINDING METHOD** ....................................................................................... 58
- **ANALYTICAL METHODS USED** ..................................................................... 59
  - **HOT STAGE MICROSCOPY (HSM)** ............................................................. 59
  - **DIFFERENTIAL SCANNING CALORIMETRY (DSC)** ..................................... 59
  - **THERMOGRAVIMETRIC ANALYSIS (TGA)** ................................................. 60
  - **FTIR SPECTROPHOTOMETRIC ANALYSIS** .................................................. 60
  - **‘H NMR ANALYSIS** ....................................................................................... 60
  - **X-RAY POWDER DIFFRACTION (PXRD)** .................................................... 61
  - **SINGLE X-RAYS DIFFRACTION (SXRD)** .................................................... 61
  - **OTHER USED RESOURCES** ......................................................................... 62
- **REFERENCES** .................................................................................................. 62

**CHAPTER 3: 1-ADAMANTANAMINE SALT FORMATION** ....................................... 64

- **INTRODUCTION TO 1-ADAMANTANAMINE HCl (ADTHCl)** ......................... 64
# Table of contents

1-ADAMANTANAMINE HCl (ADTHCl) CONVERSION TO THE FREE BASE ..................65
CHARACTERISATION OF THE CONVERTED SAMPLE..............................................65
PROTON-NMR ANALYSIS (¹HNMR) ........................................................................65
THERMAL ANALYSIS ..............................................................................................67
FTIR SPECTROPHOTOMETRY ANALYSIS ..............................................................69
SINGLE X-RAY DIFFRATION ..................................................................................71
EXPERIMENTAL POWDER X-RAY DIFFRATION ...................................................72
REFERENCES ...........................................................................................................73

CHAPTER 4: POLYMORPHISM OF BIS(ADAMANTAN-1-AMINIUM) CARBONATE ..........................................................75
INTRODUCTION .......................................................................................................75
POLYMORPHISM OF ADTCO₃ ..............................................................................76
PREPARATION OF ADTCO₃ POLYMORPHS .......................................................76
ANALYSES OF ADTCO₃ POLYMORPHIC FORMS ..............................................78
REFERENCES .........................................................................................................86

CHAPTER 5: SALT CO-CRYSTALLISATION OF BIS(ADAMANTAN-1-AMINIUM) CARBONATE .................................................87
INTRODUCTION .......................................................................................................87
REFERENCES ..........................................................................................................145

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS ....................................147
CONVERSION OF ADTHCl TO ADTCO₃ .............................................................147
POLYMORPHISM ..................................................................................................147
SALT CO-CRYSTALLISATION .............................................................................148
RECOMMENDATIONS ..........................................................................................149
REFERENCES .......................................................................................................150

Appendix A ........................................................................................................152
Appendix B .........................................................................................................154
List of figures

Figure 1.1: Representation of two strands of DNA hydrogen bonded. ........................................ 2
Figure 1.2: Polymorphs (A) and (B) arranged differently in the crystal lattice. ......................... 5
Figure 1.3: Structure of olanzapine. .................................................................................................................. 9
Figure 1.4: The packing arrangement of Olanzapine dihydrate with water molecules in blue (a) and Olanzapine anhydrate (b) molecules down “a” axis. ................................................. 10
Figure 1.5: Solvates (A) and hydrates (B) arranged in the crystal lattice. ..................................... 11
Figure 1.6: Structure representation of an amorphous form. ................................................................. 13
Figure 1.7: The molecular structure of novobiocin. .................................................................................. 13
Figure 1.8: Energy-temperature diagram for the identification of the enantiotropy dimorph. ............................................................................................................................................... 16
Figure 1.9: Energy-temperature diagram for identification of the monotropy dimorph. ....................... 16
Figure 1.10: Salt molecules arranged in the crystal lattice. ................................................................. 22
Figure 1.11: The structure of diclofenac vs sodium diclofenac hydrate. ............................................ 23
Figure 1.12: The co-crystal molecules arranged in the crystal lattice. .............................................. 26
Figure 1.13: Representation of homosynthons and heterosynthons. ................................................. 28
Figure 1.14: Illustration of co-crystal and co-former m.p. correlation. .............................................. 31
Figure 1.15: Schematic representation of a solubility curve caused by a form change and precipitation of a less stable form (curve A); at equilibrium, the curve will level out to the solubility of the less soluble form. Curve B represents the less soluble form. ................................. 34
Figure 1.16: 1-adamantanamine (ADT) structure. ................................................................................. 39
Figure 1.17: The schematic presentation of 1-adamantanamine activity. ........................................ 40
Figure 3.1: $^1$H NMR pattern of the converted sample. ................................................................. 66
Figure 3.2: HSM of the converted sample. ................................................................................................. 67
Figure 3.3: The DSC of the converted sample. ......................................................................................... 68
Figure 3.4: The TGA of the converted sample. ......................................................................................... 68
Figure 3.5: Spectra of ADTHCl, ADT and ADTCO$_3$ compared. ......................................................... 70
Figure 3.6: Experimental and calculated PXRD (YAXNOC) patterns of ADTCO$_3$. ...................... 72
Figure 4.1: Photographs of Forms I-IV crystals recorded at room temperature. ................................ 76
Figure 4.2: The HSM photographs of Form I to IV of ADTCO$_3$. ......................................................... 79
Figure 4.3: The DSC (a) and TGA (b) trace of Form I. ............................................................................ 80
Figure 4.4: The DSC trace of form II. ........................................................................................................... 81
Figure 4.5: The DSC trace of Form III. ................................................................................................. 81
List of figures

Figure 4.6: The DSC (a) and TGA (b) traces of Form IV ........................................................................... 82
Figure 4.7: FTIR-spectra of Forms I, II, III and IV of ADTCO$_3$. .............................................................. 83
Figure 4.8: The PXRD patterns of forms I-IV of ADTCO$_3$. .................................................................... 85
Figure 4.9: The DSC of forms I-IV ........................................................................................................... 85
Figure 5.1: ADTCO$_3$ structure along the b axis highlighting the proton donor and acceptor groups in (blue) with hydrogen bonds coloured in red............................................................................... 89
Figure 5.2: HSM photographs of ADTCO$_3$BA salt co-crystal forms at (a) room temperature, (b) first change in crystal, (c) onset of melting and (d) onset of decomposition .................................................................................................................. 93
Figure 5.3: DSC traces of ADTCO$_3$BA forms 1 to 5 .................................................................................. 94
Figure 5.4: TGA traces of ADTCO$_3$BA forms 1 to 5 .................................................................................. 95
Figure 5.5: FTIR spectra of 1-5 form of ADTCO$_3$BA forms 1 to 5 ............................................................ 96
Figure 5.6: Structure presenting the possible sites for interactions between ADTCO$_3$ and BA .................................................................................................................................................................................. 98
Figure 5.7: PXRD of 1-5 forms of ADTCO$_3$BA salt co-crystal ..................................................................... 99
Figure 5.8: HSM photographs of ADTCO$_3$HBA recorded at (a) room temperature, (b) the crystallisation of sample (c) the melting point (d) the completion of melt with immediate decomposition occurring .......................................................................................................................... 100
Figure 5.9: DSC trace of ADTCO$_3$, HBA and ADTCO$_3$HBA ................................................................. 101
Figure 5.10: TGA trace for ADTCO$_3$HBA .................................................................................................. 101
Figure 5.11: FTIR spectra of ADTCO$_3$, ADTCO$_3$HBA and HBA .............................................................. 103
Figure 5.12: The possible site of action for the interactions between ADTCO$_3$ and HBA .................................................................................................................................................................................. 104
Figure 5.13: PXRD of ADTCO$_3$, HBA and ADTCO$_3$HBA ......................................................................... 105
Figure 5.14: PXRD of ADTCO$_3$HBA prepared by various solvent assisted grinding .................................. 105
Figure 5.15: HSM photographs of ADTCO$_3$CIN at (a) room temperature (b) melting temperature, (c) the onset of decomposition and (d) completion decomposition ........................................... 106
Figure 5.16: DSC of ADTCO$_3$, CIN and ADTCO$_3$CIN ............................................................................. 106
Figure 5.17: TGA trace of ADTCO$_3$CIN .................................................................................................... 107
Figure 5.18: FTIR of ADTCO$_3$, CIN and ADTCO$_3$CIN ........................................................................... 108
Figure 5.19: The possible interactions sites for ADTCO$_3$ and CIN .......................................................... 109
Figure 5.20: PXRD of ADTCO$_3$, CIN and ADTCO$_3$CIN .......................................................................... 110
List of figures

Figure 5.21: HSM photographs of ADTCO$_3$HCIN at (a) room temperature, (b) the melting temperature, (c) onset of decomposition and (d) completion of decomposition. 111
Figure 5.22: DSC trace of ADTCO$_3$HCIN. 111
Figure 5.23: TGA trace of ADTCO$_3$HCIN. 112
Figure 5.24: FTIR of ADTCO$_3$, ADTCO$_3$HCIN and HCIN. 113
Figure 5.25: The possible sites for interactions between ADTCO$_3$ and HCIN. 114
Figure 5.26: PXRD of ADTCO$_3$, HCIN and ADTCO$_3$HCIN. 115
Figure 5.27: PXRD pattern of ADTCO$_3$HCIN prepared by different solvent-assisted grinding systems. 115
Figure 5.28: HSM photographs of ADTCO$_3$SUC recorded at (a) room temperature, (b) transition temperature to a 2nd form (metastable) (c) the melting temperature of the most stable form. 116
Figure 5.29: DSC trace of ADTCO$_3$SUC. 117
Figure 5.30: TGA trace of ADTCO$_3$SUC. 117
Figure 5.31: FTIR of ADTCO$_3$, ADTCO$_3$SUC and SUC. 118
Figure 5.32: The possible sites of interactions for ADTCO$_3$ and SUC. 119
Figure 5.33: PXRD of ADTCO$_3$, SUC and ADTCO$_3$SUC. 120
Figure 5.34: PXRD patterns of ADTCO$_3$SUC synthesised in various solvents. 121
Figure 5.35: HSM photographs of ADTCO$_3$LTTA at (a) ambient temperature (b) first change (bubbling), (c) melting and (d) decomposition. 122
Figure 5.36: DSC traces of ADTCO$_3$, ADTCO$_3$LTTA and LTTA. 122
Figure 5.37: TGA trace of ADTCO$_3$LTTA. 123
Figure 5.38: FTIR of ADTCO$_3$, ADTCO$_3$LTTA and LTTA. 124
Figure 5.39: The possible sites of intermolecular interactions of ADTCO$_3$ and LTTA. 125
Figure 5.40: PXRD patterns of ADTCO$_3$, LTTA and ADTCO$_3$LTTA. 126
Figure 5.41: HSM photographs of ADTCO$_3$SA recorded at (a) room temperature, (b) crystallisation, (c) the melting temperature. 127
Figure 5.42: DSC trace of ADTCO$_3$SA. 127
Figure 5.43: FTIR of ADTCO$_3$, ADTCO$_3$SA and SA. 129
Figure 5.44: The possible sites for ADTCO$_3$-SA interactions. 130
Figure 5.45: PXRD of SA, ADTCO$_3$SA and ADTCO$_3$. 131
Figure 5.46: PXRD of ADTCO$_3$SA synthesised by neat grinding and solvent-drop grinding. 131
List of figures

Figure 5.47: HSM photographs of ADTCO$_3$CA at (a) room temperature (b) melting starting point (c) melting and (d) decomposition.................................................................132
Figure 5.48: DSC trace of ADTCO$_3$CA........................................................................133
Figure 5.49: FTIR of ADTCO$_3$CA, ADTCO$_3$ and CA respectively..............................134
Figure 5.50: Possible sites for intermolecular between ADTCO$_3$ and CA...........................135
Figure 5.51: PXRD of CA, ADTCO$_3$CA and ADTCO$_3$. .................................................136
Figure 5.52: The HSM photographs of ADTCO$_3$GLA taken at (a) room temperature, (b) bubbling of the sample, (c) melting and (d) decomposition................................................137
Figure 5.53: DSC trace of ADTCO$_3$GLA. .......................................................................137
Figure 5.54: TGA trace of ADTCO$_3$GLA. ......................................................................138
Figure 5.55: FTIR spectra of ADTCO$_3$, ADTCO$_3$GLA and GLA.....................................139
Figure 5.56: The possible sites for ADTCO$_3$–GLA interactions........................................140
Figure 5.57: PXRD of ADTCO$_3$, ADTCO$_3$GLA and GLA..............................................140
Figure 5.58: HSM photographs of ADTCO$_3$GA recorded at (a) room temperature (b) first change (c) melting and (d) decomposition .................................................................141
Figure 5.59: The DSC of ADTCO$_3$, GA and ADTCO$_3$GA................................................142
Figure 5.60: TGA trace of ADTCO$_3$GA co-crystal............................................................142
Figure 5.61: FTIR spectra from ADTCO$_3$, ADTCO$_3$GA and GA. .....................................143
Figure 5.62: The possible binding sites for intermolecular interactions between ADTCO$_3$GA..........................................................................................................................144
Figure 5.63: PXRD of ADTCO$_3$GA, GA and ADTCO$_3$. .................................................145
## List of tables

Table 1.1: Different categories of bonding ................................................................. 4
Table 3.1: Summary of Pharmacological activities of ADTHCl................................. 64
Table 3.2: Specific bands assigned to given functional groups and key intensity shifts .... 71
Table 3.3: The space groups and unit cell data for ADTCO3. Experimental compared to theoretical at room temperature .................................................................................. 71
Table 4.1: Selected ADTCO3 polymorphism experiments conducted .......................... 75
Table 4.2: Shifts observed for forms I, II, III and IV of ADTCO3 ............................... 84
Table 5.1: The empiric formula, melting ranges/points and uses of ADTCO3 and selected co-formers ................................................................................................................................. 88
Table 5.2: Representation of the experiments conducted to prepare ADTCO3 co-crystals using the salt co-precipitation method ................................................................. 89
Table 5.3: Different band shifting (in wavenumber cm\(^{-1}\)) observed in the ADTCO3BA spectra Forms 1 to 5 ................................................................. 97
Table 5.4: Important shifts in the ADTCO3-HBA spectrum ........................................ 104
Table 5.5: Important shifts detected in the ADTCO3CIN spectrum ............................ 109
Table 5.6: Important shifts in the ADTCO3CIN spectrum ........................................... 114
Table 5.7: Functional groups compared in ADTCO3, SUC and ADTCO3SUC spectra .... 119
Table 5.8: Some shifts of bands in ADTCO3LTTA spectrum ...................................... 125
Table 5.9: Some important changes and shifts observed in ADTCO3SA spectrum ....... 130
Table 5.10: Shifts in the ADTCO3CA spectrum .......................................................... 135
Table 5.11: Shifts in the ADTCO3GLA spectrum ....................................................... 140
Table 5.12: Shifts observed in the ADTCO3GA spectrum .......................................... 144
Academic output

Part of this thesis was presented as a poster presentation:

A supramolecular study of Bis(adaman-tan-1-aminium) carbonate, Jean Baptiste, Dr. Samsodien H., Dr. Joubert J. and Prof. Caira M. at the 35th Annual Conference of the Academy of Pharmaceutical Sciences, 12-14 September, 2014, Nelson Mandela Metropole University, Port Elizabeth.
CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

This chapter describes the concept of supramolecular chemistry and events leading up to salt formation, polymorphism, solvates, hydrates and co-crystallisation from recrystallization studies. Motivation, rationale as well as objectives for this study are also presented in this chapter.

OVERVIEW OF SUPRAMOLECULAR CHEMISTRY

Supramolecular chemistry is an area of chemistry that mainly focuses on the non-covalent interactions of molecules.\textsuperscript{1,2} It is the study of weak and reversible molecular interactions such as hydrogen bonding, metal coordination, pi-pi interaction and Van der Waals forces. This range of intermolecular interactions originated from only a few attractive and repulsive forces and also includes electrostatic interactions such as ion-ion, ion-dipole, dipole-dipole interactions and coordinative (metal-ligand) bonding. The use of these interactions for the directed self-assembly of a given structure requires knowledge of their strength and of their dependence on distances and directionality. Therefore, even if a single intermolecular interaction is generally much weaker than a covalent bond, the cooperative action of many of such interactions may lead to supramolecular species that are thermodynamically and kinetically stable under a variety of conditions.\textsuperscript{2} It was reported that the explicitly designed study of weak molecular interactions is of extreme importance to the understanding of many biological processes and structure-activity relationships of compounds.\textsuperscript{3} Typical examples are the protein structure of cells, where the double helical structure of deoxyribonucleic acid (DNA) is made of two distinct strands of nucleotides having the complementary base connected by hydrogen bonds (figure 1.1).
Chapter 1

Figure 1.1: Representation of two strands of DNA hydrogen bonded. 5,6,7,8

This genetic material is necessary for cell replication and the polymeric strands must be unwound in order to be decoded and translated into instructions for cellular components, thus a reversible supramolecular process is necessary to read the DNA. 4

The H-bonding and supramolecular recognition are necessary for many biological functions including receptor binding, immunological responses, protein folding, protein function and coordination bonds responsible for the ability of haemoglobin to transport oxygen. 9

In principle there are two types of approaches to modify physicochemical properties of active pharmaceutical ingredients (API) at the molecular level at an early stage; one is the covalent approach such as organic lead optimization and the other is non-covalent approaches or supramolecular chemistry approaches such as polymorph formation 3 and salt formation 10 which this study will highlight.
Chapter 1

In comparison with covalent approaches, the most distinct advantage to apply these supramolecular non-covalent approaches is that the molecular structure of the APIs is retained so that essential structural features which are responsible for biological activities are retained accordingly.³

During this application, hydrogen bonds are the basis of molecular recognition phenomena in pharmaceutical systems and are responsible for the generation of families of molecular networks with the same molecular components (single component crystals and their polymorphs) or with different molecular components (multiple component crystals) in the crystalline¹¹ and amorphous state.
Details about such networks will be provided later, whereas hydrogen bonds, the most involved in these non-covalent interactions, are discussed.

HYDROGEN BONDING

Hydrogen bonding as one of the above mentioned intermolecular interactions dates back to the early 1900’s. Pauling first defined hydrogen bonding as a largely ionic interaction between two electronegative atoms with hydrogen being attached to both as a bond (equation 1.1). However, this definition would include only N-H···O, O-H···O and F-H···F and excluded C-H···O and O-H···O bonds. However, these bonds were later identified as well, universally approved and received extensive attention due to their abundance in biomolecules.¹²,¹³,¹⁴ Different literature reviews debated on how a more conclusive definition of hydrogen bonding should be.¹⁵,¹⁶,¹⁷

\[
A + BH \rightarrow A − H \cdots B 
\]

Equation 1.1

H-bonding occurs between a proton donor group (A-H) and a proton acceptor group (B), with (A) being an electronegative atom such as O, N, S, P, Se and X taking the place of F, Br, I, Cl or C and the acceptor group (B) being a lone electron pair of an electronegative atom, or a π-electron orbital of a multiple bond or unsaturated system.¹⁸ Generally, a hydrogen bond can be characterized as a proton shared by the two lone electron pairs. The strength of hydrogen bonding is directly proportional to the dipole moment of the donor and the electron pair on the acceptor.
The strength of hydrogen bonds is typically around 20 kJ mol\(^{-1}\) but can even be as strong as 163 kJ mol\(^{-1}\) as has been reported for the F···H-F interaction between fluorine, the most electronegative element of the halogen group and hydrogen. This hydrogen bond is the strongest one known.\(^{18}\)

A detailed understanding of the supramolecular chemistry of the functional groups present in a given molecule is compulsory for designing organic non-covalent complexes because of its capability to facilitate the selection procedure of suitable crystal formers.\(^{19}\) Table.1.1 shows examples of different categories of hydrogen bonding.\(^{16}\)

**Table 1.1: Different categories of bonding.**

<table>
<thead>
<tr>
<th>Type of hydrogen bond</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ionic</td>
<td>N(^{+}) – H···O(^{-}), N(^{+}) – H···Cl(^{-})</td>
</tr>
<tr>
<td>Very strong</td>
<td>P = OH···O = P ,F – H ··· F(^{-})</td>
</tr>
<tr>
<td>Strong</td>
<td>O – H···O = O, N – H···O = O, O – H···O – H</td>
</tr>
<tr>
<td>Weak</td>
<td>C – H···O, C – H···N, C – H···F – C</td>
</tr>
</tbody>
</table>

The strength of hydrogen bonds varies considerably. The weakest possesses (1-2 kJ mol\(^{-1}\)) and the strongest which is also as strong as the covalent bond; possess (40.3756 kJ mol\(^{-1}\)). Typical values include; F···H···F (163 kJ mol\(^{-1}\)), O-H···N (29.288 kJ mol\(^{-1}\)), O-H···O (20.92 kJ mol\(^{-1}\)) and N-H···N (12.552 kJ mol\(^{-1}\)). Strength is temperature, pressure, bond angle and environment dependent.\(^{20}\)

**POLYMORPHISM**

Some chemical elements are known to have allotropes and the phenomenon known as allotropism, the existence of a chemical element in different forms. The most common example is carbon which is found in its two allotropic forms; graphite and diamond having very different properties. This is a distinctive case of how physical structure determines properties. When applied to compounds, the phenomenon of allotropism is scientifically referred to as polymorphism.
Polymorphism comes from the Greek words, Polus meaning “many” and morph meaning “shape.” Thus, it is defined as the ability of a substance to exist as two or more crystalline phases presenting different arrangements or conformations of the molecules in the crystal lattice.21-26

Figure 1.2 shows the different arrangements of neutral molecules in the crystal lattice. Because of these differences in molecular packing, energies holding molecules together in the crystal lattice differ significantly, therefore leading to differences in structures that subsequently confer the distinct physical, chemical and mechanical properties of the drugs.

![Figure 1.2: Polymorphs (A) and (B) arranged differently in the crystal lattice.](image)

Polymorphism is a phenomenon that preformulation investigators use to deliver necessary physicochemical, physicomechanical and biopharmaceutical data of drug substances, excipients and packaging materials. This may influence the formulation design of an active pharmaceutical ingredient (API) as well as the drug products pharmacokinetic or biopharmaceutical resultant properties.27

It essentially means that in different polymorphs, the same molecule exists in different ways. If this difference is because of packing, the phenomenon is termed as packing polymorphism and if it is due to a difference in conformation, it is called conformational polymorphism. As a result of polymorphism, molecules have different arrangements in the unit cell of its crystal and thus display different physical properties. These include different packing properties, thermodynamic properties such as solubility, free energy, melting point, spectroscopic properties, kinetic properties such as dissolution rate, stability, mechanical properties such as hardness, compatibility with excipients, tableting and tensile strength.26
Polymorphism is thus very important in areas of chemical research (e.g. in pharmaceutical, pigment, agrochemical, explosive and fine chemical industries) where a full characterization of a material has a pivotal role in determining its ultimate use. Most drugs are formulated and marketed in crystalline form. Many of the drug molecules are highly functionalized and can self-organize in several ways in the solid state with nearly the same lattice energies, these are naturally called polymorphs.

Research on polymorphism and material properties of active drug compounds and excipients which are ingredients included in a pharmaceutical preparation for the purpose of improving its physical qualities, is an integral part of drug development. The knowledge of solid-state properties in an early stage of drug development helps to avoid manufacturing problems, to fine-tune the performance of drugs and particularly provides space for innovations. Drugs that were previously known to exist only in a single form are now known to have various polymorphic forms.

This has enabled pharmaceutical companies to investigate crystal polymorphism in order to optimize the physical properties of a pharmaceutical solid before the drug development starts. Scientists in formulation or drug development need to select the appropriate polymorph for the development. For example, Paracetamol also known as acetaminophen, which is the most widely used antipyretic and analgesic in the world is known to exist in two polymorphic forms. The first form and the marketed one is the monoclinic Form I (P21/n) whereas Form II is orthorhombic (Pbca). Accordingly, Famotidine, an excellent histamine H2 receptor antagonist is shown to exist in two different polymorphic forms; the less stable (metastable) polymorph is named B and polymorph A is the stable form. Also, Piroxicam, a non-steroidal anti-inflammatory drug, widely prescribed all over the world, exists in three forms. Forms I, II and III.

The antiretroviral, Ritonavir® or Norvir® is a novel protease inhibitor for the Human Immunodeficiency Virus (HIV), the causative agent of Acquired Immune Deficiency Syndrome (AIDS), was also found to be another important example. The drug marketed as Norvir® was launched in 1996 and distributed for about 18 months without trouble, then, its manufacturing company, Abbott observed an unexpected occurrence. The final product did not show dissolution and the drug was precipitating. Investigations revealed that this was because of a new thermodynamically more stable and less soluble polymorph, Form-II.
Surprisingly, the company attempted to formulate Form-I thereafter; it turned out very difficult (perhaps the exact conditions could not be reproduced) and put the company almost into a market crisis. The drug is now often quoted as an example in pharmaceutical industries to show the importance of polymorphism in this field. Norfloxacin is also a good example of polymorphism understanding in the pharmaceutical field. It is a synthetic broad spectrum antibacterial fluoroquinolone widely used in the treatment of prostate and urinary tract infections. This drug was found to exist in two anhydrous polymorphs (A and B), an amorphous form and several hydrated forms. Of the two anhydrous polymorphs, Form B is the most stable at room temperature. But the commercial sample of norfloxacin used is the Form A, which is metastable at room temperature.

All these examples clearly show that it is highly important to make the required polymorphic form, as the other form may not show the desired effects. Usually the form that is most stable is preferred in market formulation as the metastable form may transform to other stable forms. But it is a universally accepted rule that the metastable form has a higher solubility than the stable form and this form converts into the stable form as a result of spontaneous change but the reverse never happens. Thus, whenever possible, metastable forms having high solubility that can survive for years without changing to the stable form are selected for formulation. This means that forms that have considerable activation barrier in moving from the metastable state to the stable state would be selected. A careful evaluation of both thermodynamic parameters and kinetic parameters is of significant importance in the crystallization process of such compounds.

Polymorphs present the same properties in the liquid and gaseous states but they are different in the ways they are arranged or packed in the solid states. Hence, due to these packing arrangements in the crystal lattice, physical properties of polymorphs such as solubility, melting point, density, hardness, crystal shape, optical and electrical properties, vapour pressure, hygroscopicity, dissolution rate and bioavailability may differ significantly. These properties may have a direct effect on the ability to process the drug, its stability and the bioavailability of the drug substance as well as the final product.

Since most organic polymorphs do not possess a particular name, their nomenclature is systematic and arranged in their order of discovery which normally starts from the most stable at the ambient conditions of environment (i.e. room temperature and pressure).
Chapter 1

It was reported however, that the breakthrough of the metastable form is eventually the first choice to introduce into the pharmaceutical development since it has a higher solubility compared to the stable one and thus bioavailability. Furthermore, the transformation of an unstable form into the stable one is also possible due to its higher Gibbs free energy.

The investigation of compounds exhibiting the polymorphism phenomenon requires much time, and the number of polymorphs discovered by different researchers working on the same compound differ due to the time and efforts spent, environmental conditions as well as technics used when studying such a compound.

SOLVATES

Apart from polymorphs, solvates are another type of pharmaceutical solid form to look at due to its presence in pharmaceutical formulations on the market. Solvates are complexes in which the crystallizing solvent is incorporated in its crystal system (lattice). One can distinguish two classes of solvates depending on the way in which the solvent is fixed or fitted in the crystal system.

STOICHIOMETRIC SOLVATES

Also called polymorphic solvates; this is a class containing a stoichiometric amount of solvent in their crystal lattice, whereby with desolvation results in a disordered non-crystalline form or a different crystalline solid form. The packing arrangement of molecules in the crystal lattice may contribute to the ability of a crystal to incorporate molecules of solvents for the stability of the crystal and for structure purposes. In this class of solvate, the solvent being tightly bound to the molecules of the compound, becomes an integral part of the crystal structure and holds the structure of the crystal intact. Therefore, desolvation of this crystal would be more difficult. However, if by any means necessary the crystal loses the solvent, it could collapse and lead to the formation of a new crystal form. Compared to non-stoichiometric solvates, this class of solvates presents a higher stability.

NON-STOICHIOMETRIC SOLVATES

In this class of solvates, solvent molecules are included in the crystal lattice to fill the intermolecular voids. Solvent is not in the crystal bonding within the crystal lattice.
Thus, this type of solvate is known for its capacity of freely losing the molecules of solvent over desolvation. This creates confusion since after the desolvation process; the crystal analysis of the resulting desolvated form shows no changes of the chemical structure. Compared to the polymorphic solvates, characterisation of the pseudo-polymorphic solvates is complicated by the lack of order.\textsuperscript{36}

Pressure, temperature and humidity change may lead to solvent exchange, loss or uptake. Owing to the risk of desolvation, toxicity with regards to organic solvents that are not regarded as safe (i.e. solvents that are not in the class III, and the ICH guidelines for limits on residual solvents) solvates, are not usually selected for development.\textsuperscript{34-36}

However, when the desolvation characteristic of a solvate from both the kinetic and thermodynamic point of view takes place before, developing a resulting crystalline or amorphous form may be decided and processing, handling and storage of this may then be impacted.

Due to this amorphicity occurrence upon desolvation, changes in physical properties are expected and chemical stability particularly may be a concern owing to the improved reactivity of the amorphous solid forms relative to its crystalline counterparts.\textsuperscript{38} Examples (such as 1.4 fold for indomethacin, 2 fold for cephalaxin, 2.5 fold for tetracycline, and more or less 10 fold reported for a macrolide antibiotic, as well as novobiocin acid) were reported.\textsuperscript{95-96}

Solvates can also exhibit the polymorphism phenomenon known as solvatromorphism. The case of Olanzapine compound\textsuperscript{39} (Figure 1.3) with the structure anhydrate and hydrate structure of Olanzapine and nitrofurantoin\textsuperscript{40} were reported (Figure 1.4).

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure1}
\caption{Structure of olanzapine.\textsuperscript{41}}
\end{figure}
HYDRATES

It is well known that solvates are formed by the incorporation of solvent molecules in the crystal structure of the active compound generally during the crystallisation process. However, when the included solvent is water, then in this case the crystallised solid would be referred to as a crystal hydrate. Due to the ubiquitous nature of water in the environment and its inclusion in solvent mixtures during the crystallisation process, the incorporation of water molecules into crystal structures is sometimes inevitable.

Hydration of an anhydrous crystal exposed to water can be explained by the following reaction:

$$A_{(c)} + x \cdot H_2O \rightarrow A.x.H_2O_{(c)}$$  \hspace{1cm} \text{Equation 1.2}

Where $A_{(c)}$ is the anhydrous crystal and $x$; the amount of water molecules participating in the formation of a hydrate ($A.x.H_2O_{(c)}$).

And this is only possible if water activity is sufficient enough to allow the hydration process. Like solvates, hydrates consist of two-component systems (figure 1.5) and they are disclosed through factors such as pressure, temperature and water activity. Properties of water such as its small size and the ability to act as proton donor (in hydrogen bond formation) and acceptor favour its incorporation of water molecules into many areas within a crystal lattice, either as a filler of a vacancy or empty space in the lattice or as a
Chapter 1

crystal stabilising force whose removal would substantially cause the crystal structure to collapse.\textsuperscript{42}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{solvates-hydrates.png}
\caption{Solvates (A) and hydrates (B) arranged in the crystal lattice.\textsuperscript{43,44,45,46}}
\end{figure}

Water molecules can also act as a link between molecules in the crystal structure. Thus, the ability of water in hydrogen bonding is to support the crystal structure of the hydrates. In the crystal, water molecules can bind to nearby water molecules or to functional groups of the compound. The change in volume of the unit cell is proportional to the volume of water molecules added or removed from the hydrate system. It is well understandable that any change of water activity during the life-time of the active drug can be of great concern. And this is the reason why a third of all pharmaceutical solids are crystal hydrates. A good understanding of how an active compound and excipients behave when exposed to water is very important.\textsuperscript{43,44,46}

\textbf{CLASSIFICATION OF HYDRATES}

Two aspects, crystal structure and crystal energy must be considered when classifying hydrates. The former which is mainly used in the pharmaceutical industry, classifies hydrates into three different classes\textsuperscript{46}:

\textbf{CLASS I}

In this class also known to the name of isolated site (lattice) hydrates, water molecules do not come into contact with each other and dehydration of this class of hydrates occurs very slowly even when heated to the actual dehydration temperature.\textsuperscript{46}
Chapter 1

CLASS II

Channel hydrates, also referred to as lattice channels, are hydrates in which water molecules are part of the crystal structure and they can be in contact with each other in the crystal lattice. The adjacency of these water molecules leads to the formation of channels that spread throughout the lattice of the crystal. When exposed to heating, the dehydration process (which does not impact the crystal structure) starts early. At a definite temperature the hydrated system will have a sufficient amount of free energy for the molecules of water on the surface to be removed first. According to Morris, after this removal, the channel is exposed and a thermodynamic gradient exists in the direction of the water being removed. This may finally lead to the formation of an amorphous form.\(^{46,47}\)

This class can be further subdivided into expanded (also referred to as non-stoichiometric) hydrates, planar hydrates (lattice planes) and dehydrated hydrates.\(^{45,46}\)

CLASS III

This class contains ion-associated hydrates. Its characterisation is based upon the coordination of water and metal ions. The strong interaction between water molecule and metal ion affects the structure of the crystal. Therefore, due to these interactions, dehydration commences at a very high temperature.\(^{46,47}\)

AMORPHOUS

Amorphous is another type of solid form characterised by a short range molecular packing in the structure. Research on the amorphous form has been of a great concern in the pharmaceutical industry due to the necessity to overcome the poor physical and chemical properties of crystalline solid forms.\(^{48}\)

Amorphous is also referred to as liquid-like solid forms since they present a similar structure to that of liquids while exhibiting solid properties.\(^{48,49}\) Variable intermolecular distances between molecules in the structure of amorphous solids are longer than those found in the crystalline solid form (figure 1.6). Thus, molecules have more energy and are free of motion in disorganised conformations throughout the amorphous solid form.\(^{43,50,51}\)
Chapter 1

Figure 1.6: Structure representation of an amorphous form.

Therefore, an amorphous solid:

- Presents different physical and chemical properties from those of crystalline solid forms of the same drug.
- They are isotropic since properties of solids depend on direction of dimension of molecular packing.
- They are characterised by their low stability compared to that of the crystalline form.
- They are unstable thermodynamically compared to their crystalline counterpart, due to the low chemical and structural stability.\(^{50}\)

However, if chemically and physically stable, the molecular mobility of an amorphous form is profitable since it leads not only to the improvement of the dissolution rate of the API and subsequently enhanced bioavailability after oral administration,\(^{45,50,52,53,54}\) but also to enhanced solubility as shown by novobiocin (figure 1.7).

Figure 1.7: The molecular structure of novobiocin.
Chapter 1

POLYMORPH PREPARATION

The existence of polymorphs is a critical problem for pharmaceutical companies. Therefore preparation as well as investigation, the rudimentary step in preformulation should be conducted to avoid later problems. Pharmaceutical companies are required to prove that prospective polymorphism of marketed drugs has been investigated in order to avoid undesired polymorphs filtering through to the consumer.

The knowledge about methods that are likely to produce different polymorphs of the same compound is also of utmost importance. Various methods are suggested in producing polymorphs, solvates, hydrates and amorphous forms. These methods include sublimation, crystallisation from a single or binary solvent system, vapour diffusion, thermal treatment on differential scanning calorimetry (DSC), crystallisation from the melt, rapid pH changes, thermal desolvation of crystalline solvates, growth in the presence of additives and grinding (either dry or solvent assisted grinding). Among these methods crystallisation from solution using different solvents is usually the first step in the search for polymorphic forms of a compound and has been very successful in many cases.

POLYMORPH IDENTIFICATION

TYPES OF POLYMORPHS

There are two types of polymorphs (figures 1.8 & 1.9) depending upon the stability of polymorphs with respect to their range of temperatures and pressures called monotropes and enantiotropes.

In the case where one of the polymorphs is stable (has lower Gibbs free energy content and thus poorly soluble) over a certain temperature range and pressure, whereas the other polymorph is stable over a different range of temperature and pressure, then the two polymorphs are said to be enantiotropes (figure 1.8).

Polymorphs are said to be monotropes when only one polymorph is stable at all temperatures below the melting point, while all the other polymorphs are unstable. Both types of polymorphs can be easily understood by looking at the graphs of figures 1.8 & 1.9 describing the variation of Gibbs free energy (G) against Enthalpy (H).
Chapter 1

The graphs (figures 1.8 & 1.9) not only help in the assessment of thermodynamic relationships between different polymorphs of the same active ingredient but also allows for the calculation of transition temperatures. Considering two polymorphs A and B, in figures 1.8, polymorph A is stable below the transition temperature (Tt) and therefore has a lower free energy (G_A) than polymorph B. But as the temperature increases and becomes more or higher than the Tt, the free energy (G_B) of polymorph B becomes less than that of A (G_A); thus polymorph B becomes more stable than polymorph A. This gives rise to an enantiotropic system of solid phases.

For an enantiotropic system, a reversible transition can be observed at a definite transition temperature at which the free energy curves cross, before the melting point is reached, hence the reversibility. Whereas in figure 1.9, polymorph A has its free energy less than the other polymorphs throughout the range below the melting point.

For a monotropic system, the free energy curves do not cross each other, and therefore no reversible transition can be observed below the melting point; the polymorph with higher free energy curve and solubility, is the unstable polymorph.

It is possible to distinguish between monotruses and enantiotropes from their heats of fusion. An endothermic polymorphic transition indicates enantiotropes whereas an exothermic one indicates monotruses. Other than differential scanning calorimetric (DSC) analysis, there are a number of efficient ways to characterize polymorphs, such as vibrational spectroscopy, thermogravimetric analysis (TGA), X-ray powder diffraction (PXRD) methods and the most preferred, single X-ray diffraction (SXRD). However, the combination of all these methods gives the most outstanding results.
In these figures (figure 1.8 and 1.9), $T$ refers to the absolute temperature. While $A$ and $B$ respectively denote form-$A$ and form-$B$, subscripts $L$, $m$ and $t$ refer to as liquid state, melting or fusion and transition temperature. $\Delta H_t$ and $\Delta H_m$ denote the polymorphic transition and fusion. The graphs (figure 1.8 & 1.9) explain and were constructed based on the $G = H - TS$ relationship where $G$ as previously mentioned is the Gibbs free energy, $H$ the enthalpy and $S$ being the entropy.
Apart from this graph, different rules were set to thermodynamically distinguish monotropy and enantiotropy polymorphs. The Burger and Ramberger rules\(^{61}\) are based on statistical mechanical arguments of an ideal model of molecular crystals and are as follows:

**Heat of transition rule:** states that if an endothermic phase change is observed at a particular temperature, the stability relationship is enantiotropic, otherwise it is monotropic.

**Heat of fusion rule:** states that when the higher melting polymorph has the higher heat of fusion, the stability relationship is monotropic, and if not so, it is enantiotropic.

**Entropy of fusion rule:** according to this rule if the higher melting polymorph has the higher entropy of fusion, the stability relationship is monotropic, otherwise it is enantiotropic.

**Heat capacity rule:** If the higher melting polymorph has the higher heat capacity at a given temperature then the stability relationship is enantiotropic, otherwise it is monotropic.

**Density rule:** The more stable polymorph/form at the absolute zero of temperature should have the higher density.

**Infrared rule:** this rule is applied to polymorphs having strong hydrogen bonds. The one that possesses the larger frequency for the highest frequency infrared absorption band will eventually have the larger entropy.\(^{61}\)

Pure thermodynamic arguments may also be used to assume, the difference in Gibbs free energy of the polymorphs, and their temperature gradients at the melting point of the lower melting polymorph as well as the extrapolation of the temperature gradients to their intersection, yielding the transition temperature \(T_r\). These lead to the stability relationship and transition temperature at the same time; an advantage to the former/previous rules.

Form 2 \((T_{f,2})\) → Form 1 \((T_{f,1})\) are:

\[
\Delta H_0 = \Delta H_{f,2} - \Delta H_{f,1} + (C_{p,L} - C_{p,1}) (T_{f,1} - T_{f,2}) \\
\Delta S_0 = \Delta H_{f,2}/T_{f,2} - \Delta H_{f,1}/T_{f,1} + (C_{p,L} - C_{p,1}) \ln (T_{f,1}/T_{f,2}) \text{ and} \\
\Delta G_0 = \Delta H_{f,1}(T_{f,2}/T_{f,1} - 1) + (C_{p,L} - C_{p,1}) [T_{f,1} - T_{f,2} - T_{f,2} \ln (T_{f,1}/T_{f,2})]
\]

\(^{1}\)
Where the subscript 0 is referred to as the function at the melting temperature of the lower melting form and $(C_p,L - C_p,1)$ is the difference in heat capacities of supercooled liquid and Form 1 between the temperatures $T_{f,2}$ and $T_{f,1}$. $\Delta G (T)$ having the linear dependence; 

$$\Delta G(T) = \Delta G_0 - \Delta S_0 (T-T_{f,2})$$ with $\Delta G(T_{tr}) = 0$

gives yield to $T_{tr} = \Delta H_0 / \Delta S_0$ equation 1.6

In exception of $(C_p,L - C_p,1)$ that was obtained from the equation $k = (C_p,L - C_p,1) / \Delta H_{f,1}$, where $k$ based on an empirical study of miscellaneous polymorphic systems, is approximately equal to $0.003/K$, all parameters in the above equations are directly attained from differential scanning calorimetry data.62

**KINETICS OF POLYMORPHS**

Apart from thermodynamic data that mainly deals with direction and conditions of solid to solid transformation as assessed by thermodynamic rules according to Burger and Ramberger, kinetic consideration is also important in polymorphic distinction of solid state drugs. This is how crystallisation of the same drug in various polymorphs occurs. If, for example a metastable form is found, consequently the kinetic pathway, determines which form is to be formed by transformation of that metastable form and how long this will take.96

Thermodynamic transformation of a metastable into a stable form which is normally forbidden by Gibbs energy of activation, is only possible if forces holding together the metastable phase are overcome and allow molecules in the crystal to rearrange into a stable crystalline phase.96

**PHYSICOCHEMICAL CHARACTERISATION OF POLYMORPHS**

The characterization of polymorphs is of utmost importance when studying polymorphism and there are several analytical methods ranging from simple melting point determination to complete structural determination of the different form.

The morphology of crystals is observed by microscopic methods, phase transition by thermal methods, interpretation of molecular motion and chemical environment by the use of vibrational spectroscopy and solid-state NMR.27,62-66
A successful single crystal X-ray diffraction analysis can provide unambiguous atomic positions and complete structural information, but obtaining a single crystal suitable for such a study often becomes the bottleneck. In such cases, powder X-ray diffraction studies using microcrystalline samples become a major tool. In fact, it has become routine to take powder diffractograms to ascertain the solid state nature and purity of every batch of synthetic drugs.  

Another quick and efficient method is to study the crystal morphology by optical microscopy. As unit cell repetition leads to crystal formation, this feature is reflected in the outer appearance of crystals that can be observed by simple hand lens or a microscope. A detailed study can be performed using polarizing optical microscopy, electron microscopy and thermal microscopy.

The third important method, which is widely used in pharmaceutical industries for characterization of polymorphism, solvation, purity, degradation and drug compatibility, is thermal analysis, which includes thermogravimetry, differential thermal analysis (DTA) and differential scanning calorimetry (DSC). The study of molecular motions by use of vibrational spectroscopy is also sometimes employed in the characterization of polymorphs. This method includes infrared absorption spectroscopy and Raman spectroscopy. Today solid state NMR is also used for characterization when studying the chemical environment of the nuclei which is different in polymorphs because of magnetic non-equivalence. Resonance peaks for the magnetically non-equivalent nuclei will differ in different polymorphs and this can yield very useful information.

**IMPACT OF POLYMORPHISM ON DRUG PRODUCT**

Knowing that most drug substances can exhibit the phenomenon of polymorphism, a special analytical concern should be made. This is because the phenomenon can affect each and every stage of the drug development stages, starting from the preclinical to the post marketing phase of the drug product, the reason why special care must be taken from the beginning. Particularly, a very careful attention on polymorphs and their effect on the drug product bioavailability and chemical decay during stability should be paid in the late investigation stages that follow the entire development.
Chapter 1

The same thing must be done to the post marketing stage to assess whether polymorphism can have an impact upon the quality of the final and finished product during storage, transport and distribution.\(^{63}\)

**POLYMORPH SELECTION**

The selection process of a polymorph for formulation is a critical and very important step. Therefore it requires the collaboration of different key personnel, such as process chemists, engineers, formulators and material scientists. Each physicochemical property for all solid phases must be carefully evaluated and compared to be able to decide which one is best for selection. Important physical properties such as chemical and physical stability, bioavailability, dissolution rate, solubility and hygroscopicity are diligently evaluated. Manufacturability and processability must not be ignored since these may have an implication on processing. Particle size of crystals is crystallisation process dependant. However, milling may also lead to the conversion of crystalline to amorphous phase.

The most thermodynamically stable polymorph is of preference for product development since risks of conversion during manufacturing, packaging and storage is low. The exception is made when the lowest-energy form (amorphous or metastable polymorph) (solid dispersion) is desired and allowed for development. In this instance, justifiable and efficient reasons must be given and guarantee on safety of the patient must be provided.\(^{68}\) However, the developability of a metastable form is more challenging compared to the most stable polymorph since the understanding of the impact of many additional factors such as humidity, process-induced stresses, processing variables on the phase transition and storage temperature is required.\(^{69}\)

**SALT FORMATION**

**INTRODUCTION**

A half of all drug molecules used in pharmaceuticals are administered as salts. The high percentage indicates the widespread use and justifies the abundance of crystalline salts available on the market.\(^{70-72}\) Salts constitute an alternative for drug delivery when the physicochemical properties of the parent compound are not suitable for a perfect formulation.
Salt formation is one of the most ancient traditional methods used to improve not only the physicochemical but also formulation, biopharmaceutical and therapeutic properties of the active pharmaceutical drug and this is achieved through the understanding of selected method.\textsuperscript{10,73}

Salt formation is a very old technique and dates back to the 1950’s when Nelson discovered and demonstrated that dissolution rates of salt forms of many weak acidic compounds were much higher than those of their respective free acid forms under gastrointestinal conditions.\textsuperscript{74-82} Since then and with the progress in medicinal chemistry, introduction of combinatorial chemistry and high-throughput screening in identification of new chemical entities, interest in salt formation has grown and become the most common technique applied in drug product development to increasing solubility and dissolution rate.\textsuperscript{74-82}

This is possible by enhancing the ionization which in turn enhances the molecule polarity, thus, the increased solubility of the drug molecule in aqueous solution. Furthermore, salts of weak acids and bases are often the solid-state equivalent of pH adjustment. A salt is normally produced from an anionic interaction between weak acidic or basic drug molecules and complementary basic or acidic counterion. Dissolving a salt in an aqueous solution dissociates the acidic and basic counterion. In other words, salt formation is defined as an acid-base reaction between an API and an acid or basic substance\textsuperscript{83} (Equation 1.6).

\begin{equation}
A - H + B \rightarrow (A^-)(B^+ - H) \tag{1.7}
\end{equation}

A difference of about 2.7 pKa units between the conjugate acid and base is necessary for the formation of a salt.

\begin{equation}
[pK_a \text{ base} - pK_a \text{ acid} \geq 2.7] \tag{1.8}
\end{equation}

A typical example is of succinic acid (pKa 4.2) forming a salt with L-lysine base (pKa 9.5). This explains well the reason why a salt is chosen from its free acid or base.

pK\textsubscript{a} being the negative base -10 logarithm of the acid dissociation constant.
Chapter 1

Brønsted-Lowry defined an acid as a substance that can donate a proton and a base as a substance that can accept a proton. The following equilibrium (equation 1.8) explains the dissociation of a monotropic acid \( HA \) in the aqueous medium.

\[
[HA] \rightleftharpoons [H^+] [A^-]
\]

Equation 1.9

Where, \( K_a \) is referred to as the equilibrium constant, and it is described by the following equation;

\[
K_a = \frac{[H^+][A^-]}{[HA]}
\]

Equation 1.10

The dissociation of the basic compound can be expressed by the following reaction

\[
[HB^+] \rightleftharpoons [H^+] + [B]
\]

Equation 1.11

And

\[
K_a = \frac{[H^+][B]}{[HB^+]}
\]

Equation 1.12

Where, \( K_a \) is the dissociation constant of \( [HB^+] \).

Therefore, isolating a drug as a particular salt form alters the nature of its crystal lattice (figure 1.10), leading to widely different physicochemical properties. Compared to the untreated compound of the same drug, the change mentioned above, modifies the dissolution rate which in turn modifies solubility of the untreated drug (free acid or free base of the same parent compound).

Figure 1.10: Salt molecules arranged in the crystal lattice.\(^{43, 44, 45, 46}\)
Chapter 1

When salts are subsequently allowed to dissolve and dissociate in water, the acidic or basic counterion from which they were derived causes a shift in the pH of the solution to provide an analogous endpoint to that obtained by pH adjustment. Diclofenac is a good example of the advantage of salt formation. The non-ionised form of diclofenac \(2-[(2,6\text{-dichlorophenyl})\text{amino}]\text{benzeneacetic acid}\) is poorly soluble in water (<0.02 mg/mL) (figure 1.11) while; the sodium or potassium salt forms of diclofenac are >400 times more soluble in water.\(^8^4\)

![Figure 1.11: The structure of diclofenac vs sodium diclofenac hydrate.](image)

PROPERTIES OF SALTS

Different salts of the same active drug rarely differ pharmacologically, only differences appear in their physical properties. In addition, Wagner elaborated on this statement and said that although the nature of biological responses provided by different salts of the same parent compound may not differ significantly, the intensity of response may clearly vary.\(^8^5\)

As mentioned above, salt formation affects physicochemical properties of the parent compound such as dissolution rate, solubility, stability, and hygroscopicity. Pharmaceutical industry formulators are systematically engaged in studying each new drug entity extensively in order to determine the most effective form for formulation.

DISSOLUTION

The dissolution of an API is a very important property in pharmaceutical formulation since this reflects the bioavailability of the compound especially with the poorly soluble drugs.\(^8^6\)

Pharmaceutical salts exhibit a higher dissolution rate than that of the corresponding conjugate acid or base at equal pH and equilibrium solubility.
Description of this property is given by a diffusion model in the following equation (Equation 1.12) developed by Nernst and Brunner.\(^8^6\)

\[
\frac{dW}{dt} = \frac{DS}{R} (Cs - C)
\]

Equation 1.13

Where \(W\) is the mass of solute dissolved at time \(t\), \(dW/dt\) is the rate of mass transfer per unit time, \(D\) is the solute molecule dissolution coefficient, \(S\) is the surface area of dissolving solid, \(h\) is the diffusion layer thickness, \(C\) is the concentration of the drug in the bulk solution at time \(t\), and \(Cs\) is the saturation solubility of the solute in the diffusion layer.\(^8^6\)

The difference between saturation solubility and concentration of the drug in the bulk solution being the driving force for dissolution, if the drug is not absorbed rapidly after dissolution, then its concentration \(C\) in the bulk solution approaches \(Cs\) and the dissolution is retarded. In this case the absorption rate (or membrane transport) is limited accordingly. The dissolution rate is proportional to saturation solubility (\(Cs\)) of the drug in the bulk solution.\(^8^7\)

However, when the absorption occurs rapidly (if absorption mass transfer is higher than \(DS/h\) (Equation 1.12) \(C\) becomes negligible compared to \(Cs\), and dissolution is said to occur under sink conditions (dissolution of the expected amount of drug). In this instance, absorption is dissolution limited (case of most poorly soluble drugs).\(^8^7\)

Salts frequently enhance dissolution by efficiently acting as their own buffers to change the pH of diffusion layer, therefore providing the parent compound with the increased solubility \(Cs\) over its intrinsic solubility at the pH of the dissolution medium.

**SALT SOLUBILITY**

Solubility of a drug depends essentially upon physical and chemical properties of the solute. (e.g., a lower melting point for a compound within a series reveals a reduced energy of the lattice, thus a higher solubility). Salts exhibit a higher solubility compared to their corresponding acid and base forms of the same compound. However, a careful study needs to be performed.\(^8^8,8^9\)
Chapter 1

SALT SELECTION

The choice of optimized form is a difficult task due to the fact that each salt imparts its unique behaviours to the parent compound of the same drug. In other words various salts of the same compound behave very differently due to physical, chemical and thermodynamic properties they impart to the parent compound. Therefore, a very good understanding of the physicochemical characteristics is required. However, most of the time this choice is based on the cost of raw materials, ease of crystallisation and percentage yield. Consideration of other properties such as stability, hygroscopicity, flowability of the resulting drug as well as toxicity is of outmost importance.\textsuperscript{93,94}

CO-CRYSTALLISATION

INTRODUCTION TO CO-CRYSTALLISATION

While crystallization/recrystallization is regarded as a key purification and separation technique used in the pharmaceutical and fine chemical industries, co-crystallization is a method of combining two or more former molecules using intermolecular interactions between the molecules without creating or breaking covalent bonds.\textsuperscript{27} This is often referred to as supramolecular synthesis. Therefore, to explore the co-crystallization potential around an API increases the intellectual property protection over a particular drug product; thus, reducing the risk of costly litigation and market erosion\textsuperscript{11}

PHARMACEUTICAL CO-CRYSTALS

Pharmaceutical co-crystals can be defined as crystalline molecular complexes of two or more neutral molecular constituents bound together in the crystal lattice through non-covalent interactions, primarily hydrogen bonding.\textsuperscript{95} The formation of co-crystals represents a potential route (and an alternative strategy to salt formation, polymorph formation that sometimes lead to solvate and hydrate formation by incorporation of solvent in the crystal system) to achieve pharmaceutical materials with improved properties of interest, including dissolution rate and stability under conditions of high relative humidity,\textsuperscript{96} properties that once administrated may lead to the product is poor performance if not well investigated.
Chapter 1

Pharmaceutical co-crystal technology is used to identify and develop new proprietary forms of widely prescribed drugs and offer a chance to increase the number of forms of an API.

The formation of a pharmaceutical co-crystal involves incorporation of an API with a pharmaceutically acceptable molecule in the crystal lattice and the resulting multicomponent crystalline phase maintains the intrinsic activity of the parent API. Hence, pharmaceutical co-crystals are referred to as non-ionic supramolecular complexes made by incorporation of two neutral molecules in the crystal lattice, one being an API and the other a co-former and due to their structure can be used to address physical property issues such as solubility, stability and bioavailability in pharmaceutical development without changing the chemical composition of the API.

The co-crystal former employed in co-crystallization studies may be an excipient or another drug and a clear understanding of the supramolecular chemistry of the functional groups present in a given molecule is mandatory for designing a co-crystal because it facilitates selection of relevant co-crystal formers. A number of pharmaceutical co-crystals have been reported to date with co-crystal formers selected from the list of GRAS (generally recognized as safe) compounds which includes various food additives, preservatives and pharmaceutical excipients. Figure 1.12 below shows how active pharmaceutical ingredients and a stoichiometric amount of a pharmaceutically acceptable co-crystal former are arranged in the crystal.

![Figure 1.12: The co-crystal molecules arranged in the crystal lattice.](image)

Because crystalline materials either (single or multiple components) obtain their principle physical properties from their molecular arrangements within the solid, any change that alters the placement and interactions between these molecules, can have a direct impact on the properties of the particular solid. Therefore, any disturbance either physical or chemical may lead to the formation of a new different product.
PHARMACEUTICAL CO-CRYSTAL FORMATION AND DESIGN

Crystal engineering is defined as “the understanding of intermolecular interactions in the context of crystal packing and in the utilization of such understanding in the design of new solids with desired physical and chemical properties”\(^1\)\(^2\). It offers a rational approach to the design of new composition and crystal structures. Though, the great understanding of how achievable crystalline forms are made. Intermolecular interactions play a significant role because they intervene during the crystal packing process and self-assembly, with hydrogen bonded networks being the primary and the most commonly studied since a certain degree of reliability and predictability exists regarding the interaction of donors and acceptors.

Different researchers have addressed the hierarchy of the supramolecular synthons that can occur for a range of common functional groups in order to design new co-crystals and certain functional groups such as carboxylic acids, amides and alcohols are particularly compliant to the formation of supramolecular heterosynthons (i.e. non-covalent bonds between different but complementary functional groups).

It is becoming evident that these interactions are quite essential to implement a design strategy for co-crystals in which a target molecule forms co-crystals with a set or sequence of co-crystal formers that are carefully selected for their ability to form supramolecular heterosynthons with the target molecule. The statistics that are provided by Cambridge Structural Database suggest that as illustrated in figure 1.13, the carboxylic–pyridine supramolecular heterosynthon (figure 1.13b) is much more likely to occur than the acid–acid supramolecular homosynthon (figure 1.13a) and supramolecular heterosynthon (figure 1.13d) is favoured over homosynthons (figure 1.13a) and (figure 1.13c). Being energetically more favoured, heterosynthon formation is the most consistent and robust route for co-crystals designing. This is the type of database mining that makes co-crystals designable because if the acid and pyridine moieties are in different molecules then it follows that a co-crystal is the likely outcome.\(^2\)\(^7\)-\(^10\)\(^2\)-\(^10\)\(^7\)

As a result of competing molecular associations through hydrogen bonds between similar molecules or homosynthons and different molecules or heterosynthons as to figure 1.13, pharmaceutical co-crystallization is therefore a reliable method to modify physical and
technical properties of drugs such as solubility, dissolution rate, stability, hygroscopicity and compressibility without alternating their pharmacological behaviour \(^{107,108}\).

**Figure 1.13: Representation of homosynthons and heterosynthons.**

Co-crystals in their pure states are solids at room temperature generally and by convention, these normally exclude salts. Co-crystals can have different properties to the crystals of individual components. They also have different crystal structures to the pure components and contain different intermolecular packing patterns. Therefore, co-crystals often exhibit widely different physical properties to the pure components.

Furthermore co-crystals are an alternative to salts when these do not have the appropriate solid state properties or cannot be formed due to the absence of ionization sites in the API.\(^{110}\) Thus, co-crystals of the same active pharmaceutical ingredient will thus have strikingly different pharmaceutical properties (\textit{viz.} melting point, solubility, dissolution, bioavailability, moisture uptake, chemical stability, etc.) depending on the nature of the second component or co-former. Here are examples of synthesized co-crystals in which some had higher melting points and others had lower as compared to their pure components. For example, succinic acid (m. p. 135.3 °C), urea (m. p. 188.9 °C), co-crystal of succinic acid and urea (m. p. 149.9 °C).\(^{111}\)

The physical and chemical property improvements through pharmaceutical co-crystals draw closer the fields of crystal engineering and pharmaceutical sciences.\(^{108,112}\)
CO-CRYSTALS CHARACTERISATION

The characterisation of every new compound is compulsory and must be performed in the early stage of drug development.

This is necessary to avoid problems later and consequences that may rise during pre-clinic or post-clinic phases as well as lifetime effects of the drug product. A co-crystal as a new multicomponent compound must undergo such identification.

Different analytical techniques are used when attempting to characterise a co-crystal, especially when establishing a co-crystal from a salt, a very difficult process due to the fact that the two crystalline materials are alike physically.

Single crystal X-ray diffraction being the preferred characterization technique is used in determining whether a co-crystal has been produced. However, suitable X-ray quality crystals cannot always be generated. Therefore other techniques may be necessary.

A variety of solid state spectroscopic techniques are also employed as an attempt to characterize potentially new co-crystalline materials. These include Raman, H\textsuperscript{1}NMR and solid state NMR spectroscopy which play a significant role especially when determining the proton transfer from acid to base (location of hydrogen atom determination), the process which may be enigmatic. Infrared spectroscopy is powerful tool in detecting co-crystal formation, especially when a carboxylic acid is used as a co-crystal former and/or when a neutral O-H⋯N hydrogen bond was established between an acid and a base. This is indicated by shifts in peaks as well as formation of new peaks in the pattern of the resulting co-crystal.

Distinct differences within the FTIR spectra can be observed between a neutral carboxylic acid moiety and a carboxylate anion. A neutral carboxylate (−COOH) displays a strong C=O stretching band around 1700 cm\textsuperscript{−1} and a weaker C–O stretch around 1200 cm\textsuperscript{−1}, while a carboxylate anion (−COO\textsuperscript{−}), due to resonance, displays a single C–O stretch in the fingerprint region of 1000–1400 cm\textsuperscript{−1}. Additionally, if a neutral intermolecular O-H⋯N hydrogen bond has formed between the components, then two broad stretches around 2450 and 1950 cm\textsuperscript{−1} will be observed. Observations about the state of the carboxylic moiety (neutral or ionic) can also be verified through measuring the C–O and C=O bond distances from the single-crystal X-ray data.
Chapter 1

A typical C=O bond distance is around 1.2 Å, while the C–O bond distance is around 1.3 Å; however, if deprotonation has occurred then the resonance stabilized C–O bond distances will be very similar.\textsuperscript{104,113}

PROPERTIES OF PHARMACEUTICAL CO-CRYSTALS

Physicochemical properties of co-crystals must be carefully investigated like other solid forms for the determination of reproducibility and hence the developability into the marketable dosage forms.

MELTING POINT

The determination of the melting point of a compound as a means of characterization or purity identification is an orthodox practice. Melting point plays a significant role in pharmaceutical sciences. The existence of a relationship between the melting point and aqueous solubility and vapour pressure guarantee a significant role played by the former in pharmaceutical sciences.\textsuperscript{114} Accordingly, in fact, the melting point has been directly correlated to the Log (logarithm) of solubility, although assumptions pertaining to the entropy of fusion had to be drawn.\textsuperscript{115}

In addition, being able to determine the melting point of a particular API before it is synthesized would be very beneficial in order to adjust its aqueous solubility toward a particular function. However, it is unfortunate that correlations relating the chemical structure directly to melting point data remain fugitive.\textsuperscript{116}

Given the various factors contributing to the melting point of a crystalline solid including the molecular arrangement within the crystal lattice, molecular symmetry, intermolecular interactions and conformational degrees of freedom for a molecule, one clearly sees the difficulties when attempting to draw stringent comparisons from molecular structure to crystalline lattice energy to melting point.\textsuperscript{117} The situation only becomes more complex when observing multicomponent (e.g. the case of co-crystal) systems, for each component has its own characteristic properties and those can influence the environment (and intermolecular interactions) around its neighbours.

In this section we will examine the thermal behaviour of co-crystals in which one component is an API, although findings and trends should be translatable to all co-crystalline materials.
Comparison of the melting points of 10 co-crystals to the API AMG517 and their respective co-formers was conducted.\textsuperscript{92} Each of the co-crystals displayed a melting point that fell between the melting point of AMG517 and their co-former. A plot was generated using the melting points of the co-crystals and co-formers and displayed a direct proportionality between the two.

A correlation coefficient was determined ($r = 0.7849$) which means that 78\% of the variability of melting point of the co-crystal can be attributed to the variability of the co-formers melting point. These data show that within the set of AMG517 co-crystals the melting point can typically be modified in accordance with the selected or chosen co-former. If a higher melting co-crystal is desired for example, then a higher melting co-former should be selected and vice versa.\textsuperscript{117} However higher melting point may lead to the poor solubility. Therefore, it is also necessary to assess stability properties.

![Figure 1.14: Illustration of co-crystal and co-former m.p. correlation.](image)

**STABILITY**

Stability comes among essential studied parameters during the development of a new chemical entity. Different types of stability are considered depending on the structure and characteristics of the molecule. Chemical and physical stability data are commonly obtained at accelerated stability conditions to determine the drug developability and shelf life.\textsuperscript{91}

Because water uptake is included from a handling and packaging point of view, the amount of water present can also lead to form changes and degradation. Therefore, the effect of the water uptake should be carefully investigated early in the process.\textsuperscript{92}
Chapter 1

Thermal stress studies are also incorporated, and extra work may be warranted for hydrates or thermally labile materials.

In the case of co-crystals and salts, solution stability may be a factor due to dissociation of the material resulting in precipitation of the less soluble parent compound or a less soluble form (such as a hydrate in aqueous media).  

**RELATIVE HUMIDITY STRESS**

The changes in relative humidity (RH) range are a key consideration when developing a co-crystal as observed in other solid state forms of a compound.

When studying co-crystals, it is necessary to measure the moisture uptake in order to establish principles regarding the environmental contact with the compound being investigated and thus come up with orientations and direction to more comprehensive studies.

In this process, data from X-ray powder diffraction (PXRD) of the solid are collected at the end of the moisture balance experiment and provides information on the final form, but not necessarily on any form conversions that may have occurred during the experiment. Significant moisture uptake during the course of the experiment may guarantee a longer exposure at a specific relative humidity by the use of a relative humidity chamber and subsequent analysis of the sample after equilibration.

For co-crystals, the limited water sorption/ desorption data was found. Different examples such as a 1:1 indomethacin/ saccharin co-crystal showed water uptake that is about < 0.05 % water. The obtained data suggested that there was no solid-state transformation that occurred, although no PXRD data were reported after the moisture balance experiment. Therefore, there is not a major concern for co-crystals. Attention should be taken when co-crystals are exposed to the RHS for a long period since this can create serious effects.

**THERMAL STRESS**

High temperature stress is also a common condition used to determine chemical and physical stability of a compound, based on accelerated stability conditions. This is applicable on co-crystals as well. For example, Paracetamol co-crystals were analysed by DSC.
Paracetamol/4, 4-bipyridine co-crystal sample did not show any loss of guest upon heating and exhibited a melting endotherm corresponding to the monoclinic form of Paracetamol. No additional data were included to confirm the conversion, but this type of study can provide very important information on high temperature transitions and processes such as drying and accelerated stability.\textsuperscript{111}

**SOLUTION STABILITY**

Solution stability is defined as the ability of the co-crystal components to stay in solution and not readily crystallize, it can be an important parameter to investigate during development, not only limited to solutions or suspensions, but also applied to solid dosage forms that will dissolve in the gastro-intestinal (GI) tract. A variety of vehicles can be employed including water, simulated gastric fluid (SGF), simulated intestinal fluid (SIF), formulation vehicles, and buffered solutions. In many cases, solubility or dissolution experiments can be incorporated in order to get a more complete picture of the behaviour and the solid form remaining at the end of the experiment. Because dissociation of a co-crystal can occur, then solution stability can be a key consideration for development. The results however should always be weighed with other properties and needs.

Studies of co-crystals in water can give an indication of possible dissociation and precipitation of another form such as a hydrate. During the study of caffeine co-crystals,\textsuperscript{123} the 2:1 caffeine/oxalic acid co-crystal was found to be stable at all relative humidities up to 98% RH for a seven week period. For further tests of the stability, the material was slurried in water at ambient temperature for two days. No change in physical form was observed, demonstrating the stability of the material. In order to determine the form present in aqueous solutions of a 1:1 carbamazepine/saccharin co-crystal, the material was slurried with equal parts carbamazepine dihydrate and saccharin in solution.\textsuperscript{121} After one day, only the co-crystal was evident based on PXRD data and the carbamazepine dihydrate was not detected.

**SOLUBILITY**

Improvement of a poorly soluble compound is one of the main reasons to investigate co-crystals. For neutral molecules, co-crystals can certainly expand the solid forms possible for development. For a free acid or free base, both salts and co-crystals can be used to improve the solubility.
However, it is not always straightforward to determine whether a salt or a co-crystal has been formed \(^{86}\) and a variety of techniques may be needed from the simplest to the more sophisticated in order to understand the system.

When studying solubility data, various considerations must be taken into account. The first one is the equilibrium versus kinetic solubility measurements. Kinetic solubility values are approximate values usually based on one measurement at one time point. For equilibrium solubility, a number of time points and measurements are taken to ensure that the solution has reached the equilibrium, as evidenced by a plateau in the concentration data. In other words, this is called powder dissolution. \(^{53}\)

The time required to reach the equilibrium solubility can also be a factor for development based on the residence time in the stomach and intestines. It is desirable to have the soluble drug while it is in the (GI) tract since very long dissolution times may result in less absorption of the drug.

Powder dissolution rates can also be particle size dependent. Another consideration is form changes during the experiment. When form changes occur, the solubility data obtained may not be relevant to the starting compound in the experiment. Form changes can be suggested by solubility data collected at different time points by a precipitous drop in concentration indicating crystallisation of a less soluble form. \(^{89,90}\) Suggested form changes can be confirmed by analysis of the solid form remaining at the end of the experiment (figure 1.15).

**Figure 1.15:** Schematic representation of a solubility curve caused by a form change and precipitation of a less stable form (curve A); at equilibrium, the curve will level out to the solubility of the less soluble form. Curve B represents the less soluble form.
INTRINSIC DISSOLUTION

Dissolution rate is influenced measuring intrinsic dissolution and is a technique in functionality and characterisation of bulk substances as well as excipients.

Intrinsic dissolution measures the rate of dissolution (the dissolution of a pure substance under the conditions of constant surface area)\(^{122}\) without the effect of particle size, the process that is accomplished by pressing a disk or pellet, normally using a Woods apparatus in a dissolution vessel.\(^{118}\) Over time the concentration of solution is measured to determine the dissolution rate (in mg/cm\(^2\).min). The disk must be left intact during the experiment, so compression pressures may be critical for poorly compressible powders. It is also important that there is no form change upon pressing the pellet or during the dissolution study. XRPD data can be obtained on the initial disk and the remaining disk after completion of the experiment to determine any major form changes that may affect the dissolution data.\(^{91}\)

A good example is that of glutaric acid where data for the glutaric acid co-crystal of 2-[4-(4-chloro-2-fluorophenoxy) phenyl]pyrimidine-4-carboxamide\(^{124}\) were collected in water over 90 min and showed that the co-crystal dissolution was more or less 18 times faster than that of the parent compound. XRPD of the remaining solid showed mainly the glutaric acid co-crystal, with only minor peaks for the parent material, indicating that the results were not skewed by remarkable form changes over the course of the experiment.\(^{126}\)

BIOAVAILABILITY

Bioavailability refers to the degree at which an active drug reaches the circulatory system when a given dose is administrated into the body.\(^{92}\) Animal bioavailability is an important factor to consider when preparing new forms of a compound, and studies are conducted in different ways in order to obtain specific information for development. Animals such as rodents, rabbits, dogs, pigs, and primates are commonly used in bioavailability studies. Small studies (4-6 animals) are usually performed during early development to determine pharmacokinetic data quickly on a new form. Normally the same animals are usually used for all forms/formulations with a week washout period between the doses. This gives a direct comparison within the same animals for all the materials in the study.\(^{92}\)
A study with both the parent material and the co-crystal will provide a direct appraisal of bioavailability enhancements due to the co-crystal. During bioavailability appraisal, the amount of drug in the blood is measured after oral administration of the original form and after administration of the co-crystal. Blood samples are taken periodically after dosing to investigate blood levels over time. It is important to note that the materials used in the study need to be formulated in the same way if a direct comparison is desired. Most dosing is done orally and formulation possibilities include powder in a capsule, powder and excipient in a capsule, or liquid formulations (solutions or suspensions). It is important to differentiate between solutions and suspensions since dissolution of the solid in a solution could significantly improve bioavailability.\textsuperscript{124}

For suspensions it is helpful to know how much of the compound may actually be dissolved in order to determine how that may affect the results. Absolute bioavailability includes not only the co-crystal formulation, but an IV formulation as well to determine maximum exposure based on the IV data and a comparison with the oral formulation.\textsuperscript{124}

As an example of how the co-crystal can improve bioavailability, 1:1 AMG 517/sorbic acid co-crystal\textsuperscript{124} was compared to the parent free base in a rat bioavailability study.

A significant increase in bioavailability was observed for the co-crystal. It was found that a 30 mg/kg dose of the co-crystal resulted in comparable exposure to a 500 mg/kg dose of the free base.\textsuperscript{124}

**CO-CRYSTAL POLYMORPHISM**

Investigation on different polymorphic forms of a particular compound is common in solid-state pharmaceutics.

Because these polymorphic forms can have different physical and chemical properties, steps should be taken to identify and characterize all forms during development. Polymorphic co-crystals also exist and possess different physicochemical properties between them.

As previously stated (polymorphism section), it was observed that the APIs form new species that present distinct physicochemical properties (polymorphs) due to crystallisation process.
Chapter 1

Therefore, like other solid state compounds, the existence of polymorphic co-crystals is also possible\cite{125} and present different physicochemical properties between forms.

A typical example of co-crystal polymorphism was observed when a chloroform solution of caffeine and glutaric acid were allowed to evaporate slowly and two polymorphs having different morphologies rods (form I) and blocks (Form II) were produced.\cite{126}

**SCALE-UP**

Scalable processes offering high co-crystal purity and yields must be designed and established for a pharmaceutical co-crystalline material to move from small scale screening exercises to large scale for development of drug products. Co-crystal quantities of milligrams to a few grams are typically produced depending on the types of characterization and/or initial physicochemical studies such as the rate of dissolution, solubility study, bioavailability, etc. the materials are subjected to.\cite{127} Slow evaporation and grinding are two reported common techniques used for co-crystal growth; unfortunately the two approaches possess some limitations upon scale-up. Therefore, additional routes must be utilized.\cite{127}

In one account, the preparation of a carbamazepine/saccharin co-crystal was produced on a scale of 30 g using solution crystallization,\cite{121} where the components were dissolved in a ethanol-methanol mixture (3:1 ratio) and refluxed at 70°C for 60 minutes. The solution was cooled down and then filtered, the precipitate was dried and characterized as the 1:1 co-crystal of carbamazepine and saccharin. Interestingly, no seeding applied under the reaction conditions selected for the production of the desired co-crystalline form in high purity.\cite{127}

Three criteria were outlined during the scale-up study of carbamazepine/nicotinamide co-crystal using solution-crystallization methodology: (a) determine an appropriate solvent system; (b) identify the pathway, through multicomponent solid–liquid phase equilibrium diagrams, to produce the desired form; (c) determine a mechanism to induce nucleation and control the desaturation kinetics of the process. Through a seeding strategy in ethanol, the co-crystal was successfully produced on a 1 L scale with yields in excess of 90%.

The use of ternary phase diagrams must be taken into consideration when scaling up co-crystals from solution. This is because information regarding the relationship between
equilibria of the solid phases and solvent choices is obtainable. Critically, the individual components’ solubility must be determined and mapped since the phase region where the most thermodynamically stable co-crystal is located will be altered due to whether or not they possess similar solubilities in a given solvent.\textsuperscript{104,108,119,125,128,129}

Scaleable co-crystal processes must be evaluated and optimized for the pharmaceutically based co-crystalline materials to move further into development and become viable options as marketed products.

**INTELLECTUAL PROPERTY AND MANAGEMENT OF LIFECYCLE**

Patents are the basic element of drug development, especially in solid forms. Pharmaceutical patents can cover different areas such as composition of matter (molecular structure, solid form or formulation) method of use (medical indication) and manufacturing processes.\textsuperscript{107} Novelty, utility and non-obviousness are three criteria to be satisfied for an invention to qualify for patent coverage.

Co-crystals represent a broad patent space since there is a large number of co-formers available based on the possible compounds in the EAFUS (Everything Added to Food in the US) and GRAS (Generally Regarded as Safe) lists.\textsuperscript{130,131} Because of the lack of predictability in the field, it is expected that in many circumstances patent coverage will be narrow.

Co-crystals can also play a role in lifecycle management\textsuperscript{90,132} that can involve drug product improvements along with new solid forms. Early in the development process, chemical structures are patented and additional IP protection can be obtained by patenting different solid forms throughout development.

If an approved drug product contains a new patented solid form, especially where the solid form offers a commercial advantage over the original form, the solid form patent might provide meaningful IP protection after the expiration of the original patent. However, a solid form that was not found by the innovator, but was found and patented by a competitor, could significantly alter this strategy.

Co-crystal screens for potential blockbuster drugs could end up being very large in order to protect, not only the co-crystals found, but also any polymorphs, hydrates, solvates, or other solid forms of the individual co-crystals.\textsuperscript{27}
1-ADAMANTANAMINE

1-Adamantanamine (ADT) free base, also called 1-aminoadamantane is an intermediate compound and it is an effective base for salt formation. The rate of activity of the free base compared to the salt forms might differ. Its trade names as the HCl salt form are Amantadine®, Midantan® and Symmetrel®. It is used for its antiviral activity in the prevention and the early-stage treatment of influenza A. The drug also increases dopamine level in the CNS; it is for this reason why it is often applied in medical practice for the treatment of dementia, Parkinson’s disease, Alzheimer’s diseases, stroke, hypoxic brain afflictions and neuroinfections. 133-138

ADT possesses an interesting structure consisting of the presence of an adamantane moiety linked to an amino group which confers to the molecule the special chemical property of a diamond-like structure (figure 1.16). 148 The system is rigid but strain-free demonstrating that there is no angle or torsional strain present. Consequently, it is a highly stable structure. 149

![Figure 1.16: 1-adamantanamine (ADT) structure.](image)

ADT hydrochloride chemically referred to as tricycle (3.3.1.1.3,7)decan-1-amine hydrochloride, is a stable, white or nearly white crystalline powder, freely soluble in water and soluble in alcohol and chloroform. 139

1-adamantanamine hydrochloride (ADTHCl) is a M2 inhibitor since it specifically inhibits replication of the influenza group A virus, 140,141 containing the M2 protein (absent in B group viruses figure 1.17). The M2 protein functions as an ion channel and prevent exposure of the viral hemagglutinin to low intracellular pH to which it is sensitive. 144,145 The apparent antiviral activity of ADTHCl results from the ability of the drug to prevent viral penetration into the host cell by blocking ion channel proteins existing in the virion lipid envelope of the group A viruses or suppressing the uncoating process. 142,143
Figure 1.17: The schematic presentation of 1-adamantanamine activity.  

The antiparkinson activity is less known, although it is also related to the ability of 1-adamantanamine to block neuromuscular transmission, which may, however depend on its capacity to increase the synthesis and release of dopamine. ADTHCl is also believed to be effective against the dengue virus. The dengue virus belongs to the family of Togoviruses and the subgroup Flaviviridae. The Flaviviruses are a family of at least 66 viruses, 29 of which cause human diseases including dengue, yellow fever, Murray Valley encephalitis and Japanese encephalitis. Dengue occurs mainly in tropical areas of Asia, Oceania, Africa, Australia and the Americas. Some outbreaks of dengue have involved one million or more cases with attack rates of 50-90%. The World Health Organization estimates 50 million cases of dengue infection occur each year, resulting in approximately 24,000 deaths (WHO 1998).

MOTIVATION OF THE STUDY

Several studies have reported that the change in physicochemical properties that may occur during the manufacturing process and storage of pharmaceutical formulations should have a direct impact on the bioavailability of a drug and hence, consequently on the patient.

Modifying the physicochemical properties of a pharmaceutical compound through salt formation, polymorphism and complexation/co-crystallisation may improve the
Chapter 1

performance of ADT. Therefore, due to its anti-parkinsonian and varied antiviral activities, a study of supramolecular derivatised forms of ADT is well placed in a preformulation exercise to investigate these forms for future formulation research.

OBJECTIVES OF THE STUDY

The objectives of this study were:

1. To give an account of the systematic conversion of ADTHCl to bis(adamantan-1-aminium) carbonate, with the initial intention having been to convert ADTHCl to its free base.
2. To give an account of the systematic conversion of bis(adamantan-1-aminium) carbonate into various polymorphs and to isolate and characterise these modified forms.
3. To give an account of the systematic conversion of bis(adamantan-1-aminium) carbonate into various salt complexes and/or co-crystals.
4. To conclude theoretically whether the polymorphic salts and salt complexes/co-crystals might present any pharmaceutical or technological advantages (viz. solubility, melting point, moisture uptake, chemical stability etc.) over ADT and ADTHCl.

Chapter two will discuss the various techniques used for the preparation of salts, polymorphs and non-covalent complexes/co-crystals. Furthermore, chapter two will discuss all the relevant analytical techniques applied in this study.
REFERENCES


Chapter 1


Chapter 1


40. Pienaar E, Caira M, Lotter A. Polymorphs of nitrofurantoin. Preparation and X-ray


52. Brittain H. Methods for the characterization of polymorphs and solvates. In:
Chapter 1


63. Gandhi S., Chandrul K. Pharmaceutical Solid Polymorphism in Abbreviated New Drug Application (ANDA)- A Regulatory Perspective. *Journal of Chemical and


Chapter 1


77. Furesz S. Blood levels following oral administration of different preparations of novobiocin. Antibiotics and Chemotherapy. 1958;8:446-449.


Chapter 1


122. *Pharmacopeial Forum and Biopharmaceutics* 05 USP29-NF24 p2923. 30(6).


Chapter 1


Chapter 1


CHAPTER 2: MATERIALS AND EXPERIMENTATION

This chapter contains a full description of the experimentation to prepare salts, polymorphs and complexes/co-crystals of 1-adamantanamine. It also presents the materials used in this study and describes all the analytical techniques (laboratory and computational) applied to critically analyse the various forms produced.

THE USED COMPOUNDS

1-adamantanamine (ADT), was purchased in its HCl salt form from Sigma-Aldrich Chimie GmbH (Steinheim, Germany). Benzoic acid, 4-hydroxybenzoic acid, trans-cinnamic acid, 4-hydroxycinnamic acid, succinic acid, 1-tartaric acid, 1-glutamic acid, 1-glutaric acid, salicylic acid and citric acid monohydrate were all purchased from Sigma-Aldrich Chimie GmbH (Steinheim, Germany) as well.

PREPARATION OF POLYMORPHS

Different techniques such as crystallization from solution and recrystallization from neat API (via; the sublimation technique, thermal treatment, crystallization from the melt and grinding)\(^1,2\) were used for polymorph screening into several polymorphic forms.

CRYSTALLISATION FROM SOLVENT EVAPORATION

SINGLE SOLVENT EVAPORATION

A measured mass of the compound was dissolved in a known volume of solvent. The mixture was stirred and heat was applied to temperatures set at 10°C below the boiling point of the solvent and below the melting point of the parent compound. Using a syringe, solvents were added drop-wise into a vial dovetailed with a magnetic stirrer while on a hot plate until the powders were completely dissolved.

Solutions of solvent and the compound being crystallised were filtered using a micro-filter (0.45 \(\mu\)m syringe filter to remove most nuclei and other impurities) and transferred to a clean and labelled vial and closed with Parafilm\(^\circledast\). A few holes were made in a closing piece of parafilm\(^\circledast\) using needles to facilitate the evaporation of the solvent. The solution was left undisturbed for a reasonable period of time, allowing for the evaporation process to occur\(^4,5,6\)
Chapter 2

EVAPORATION FROM A BINARY MIXTURE OF SOLVENTS

Multi-solvent evaporation methods which eventually depend on the difference in solubility of the solute in different solvents were also used. The second solvent, in which the solute is slightly soluble or insoluble was added to a saturated solution of the compound in a good solvent. The preparation was stored in a stable place to allow for evaporation.

As the solution evaporated, the volume of the solution was reduced. As the solvents evaporated at different rates, the composition of the solvent mixture and concentration changed over time, which facilitated the crystallisation process.4,5,6

VAPOUR DIFFUSION

A solution of the solute was dissolved in a good solvent such as methanol which was placed in a small vial that was kept in another big container in which a small quantity of a miscible and volatile non-solvent was added.

The big container was then sealed. The sample preparation was left for a reasonable period of time to allow diffusion of the non-solvent through the vapour phase into the solution of the small vial as the equilibrium was reached or until saturation or super-saturation was achieved. The solubility of the compound in a precipitant used in such a technique should be very low (much less than 1 mg/ml) and the precipitant (the solvent in which the compound is poorly soluble) should be miscible with the solvent and the saturated solution of the small vial. Due to the behaviour of a used solvent in the presence of the preparation, there will be crystallisation.4,5,6

THERMAL TREATMENT

THERMAL TREATMENT BY DSC

With the application of differential scanning calorimetry (DSC), there are possibilities to observe a variety of endotherms. The first one corresponds to a phase transition, followed by a second endothermic peak corresponding to the melt. Sometimes the observation of a third peak is possible. This is an exothermic peak that comes between the two endothermic ones and represents a crystallisation step.
These peaks (endothermic and/or exothermic) are associated with a series of thermal events, including guest release, phase transformation, recrystallization, melting, as well as decomposition. Hence, the onset temperatures and enthalpy changes ($\Delta H$) of these thermal events can be established and finally new forms may be insulated accordingly.\textsuperscript{1,2}

**CRYSTALLISATION FROM THE MELT**

When the cooling process is applied to the melts of polymorphic substances, it often gives the unstable form which subsequently rearranges into the stable form. Super-cooling is necessary to crystallise the metastable form from the melt. This is because the metastable form always has the lower melting point when compared to other forms. In this case, after melting has completed, the system is super-cooled below the melting point of the metastable form, while at the same time, the crystallisation of the more stable form or forms is achieved.\textsuperscript{3}

Further quenching or cooling a melt leads to the formation of an amorphous solid which on heating, subsequently undergoes the glass transition followed by crystallisation.\textsuperscript{4,5,6}

**GRINDING METHOD**

**NEAT GRINDING**

Neat grinding is one of the traditional methods of polymorph screening and complex formation. The sample powder was manually ground for a reasonable period of about 30 minutes using a mortar and a pestle. The powder was then transferred from the mortar to an airtight container.\textsuperscript{9}

**SOLVENT DROP-GRINDING**

The same process as applied in the previous paragraph was assisted by adding a few drops of solvent\textsuperscript{9} while grinding.

**SUBLIMATION METHOD**

A small amount of the compound, (20 mg) was placed in a petri dish covered with a watch glass. The petri dish was then gently heated on a hot plate and the watch glass was observed to determine if crystals were growing on it.\textsuperscript{10}
Chapter 2

PREPARATION OF CO-CRYSTALS/ COMPLEXES

Different techniques were applied during the process of complexation. These include vapour diffusion, solvent evaporation, dry grinding and solvent-assisted grinding.

SOLVENT EVAPORATION

SINGLE SOLVENT EVAPORATION

A stochiometric amount of two parent compounds were dissolved in a necessary volume (e.g. 2 mL) of solvent. With temperatures kept below the melting point of the lowest melting constituent and about 10°C below the appraised boiling point of the solvent system, the two samples were separately dissolved in the solvent while being stirred with a magnetic stirrer on a hot plate until the powders were completely dissolved. The two solutions were then mixed together in the vial containing the smaller quantity and was extracted using a syringe. The solution was then filtered through a 0.45 μm micro-filter and transferred to a clean and labelled vial and closed with Parafilm®. A few holes were made in the parafilm® using needles, to facilitate solvent evaporation.

CO-CRYSTALLISATION OR COMPLEX FORMATION VIA BINARY MIXTURE OF SOLVENTS

In this method, co-crystal/complex preparation was done using slow evaporation of a mixture of solvents in several stoichiometric ratios. The stoichiometric amounts of the compound and co-crystal formers were dissolved in a known volume of a mixture of solvents.

All temperatures were kept below the melting point of the lowest melting component and approximately 10°C below the estimated boiling point of the solvent system.

Samples were dissolved separately in the respective solvent systems, stirred with a magnetic stirrer while on a hot plate until the powders were completely dissolved.

The two solutions were then mixed together in the vial with the lesser amount of solvent, stirred, extracted via a syringe and filtered from the syringe through a 0.45 μm micro-filter and transferred to a clean and labelled vial and closed with Parafilm®. The parafilm® was perforated using needles to facilitate solvent evaporation.
Chapter 2

The solution was left undisturbed for a reasonable period of time allowing the evaporation process to take place in a desirable manner unless unforeseen circumstances arose.

**VAPOUR DIFFUSION**

A solution of the solute and co-former in a stoichiometric amount was prepared using a so-called good solvent (solute and co-former soluble solvent) and was placed in a small vial that was stored in another big container, containing a small amount of a miscible, volatile non-solvent.

The bigger container was tightly closed. The sample preparation was left for a reasonable period of time to allow diffusion of the non-solvent vapour phase into the solution of the small vial creating an equilibrium in vial contents. As the equilibrium was reached, saturation or super-saturation was achieved. The solubility of the compound in a precipitant used in such a technique should be as low as possible (much less than 1 mg/ml), and the precipitant (the solvent in which the compound is poorly soluble) should be miscible with the solvent and the saturated solution in the small vial.

**GRINDING METHOD**

**NEAT GRINDING**

Neat grinding, also called dry grinding, consists of mixing stoichiometric amounts of crystal components together and grinding them either manually using a mortar and pestle or mechanically. Several pharmaceutical complexes/co-crystals have been successfully synthesised by neat grinding.

**SOLVENT-DROP GRINDING**

By the addition of a small amount of suitable solvent to the ground mixture (API and co-former) complexation is accelerated.

This is a particularly promising preparation method of which the efficacy of the method has been reported. The solvent-drop grinding method avoids excessive use of crystallisation solvent compared to the solvent evaporation method, hence it can be regarded as a green process. The solvent-drop grinding method could also prove useful for polymorph control and selective polymorph transformation.
Chapter 2

ANALYTICAL METHODS USED

HOT STAGE MICROSCOPY (HSM)

The use of this technique allows the observation of different thermal events while heating or cooling the sample crystal or powder on a hot stage.

A Linkam TP92 temperature control unit connected to a Linkam TH MS600 hot stage was used to heat samples at a controlled rate. Observations and visual characterisation were taken by a real-time Sony Digital Hyper HAD colour video camera fitted to a Nikon SMZ-10 stereoscopic microscope. Images were recorded and were analysed by the Soft Imaging system (Essential Stream) Analysis. This interconnected system proves to be very useful and allows distinguishing new solid phases such as polymorphs, co-crystals and/or complexes to be identified.

HSM results and the DSC analysis were correlated to TGA analyses that was also performed to assess % solvent content in samples. During the heating process on the HSM, the appearance of bubbles coming from the sample in an inert medium (e.g. silicone oil), usually indicated solvent loss. Other thermal changes such as polymorphic transition and decomposition were indicated by colour changes in the sample being analysed.

DIFFERENTIAL SCANNING CALORIMETRY (DSC)

A Perkin-Elmer PC-7 series thermal analysis system at a scanning rate of 10°C per min was used under N₂ gas purged with a flow rate of 20 mL/min. DSC traces were recorded over a temperature range between 30 and 460°C. Sample sizes were in the range of 1-4 mg for all compounds. Crystalline samples were taken from the vial, dried on filter paper and crushed to an appropriate particle size and sealed in a aluminium pan.

A sealed and empty pan was used as a reference. The DSC analyser was calibrated by measuring the onset temperatures of the melting of indium (m.p. 156.6°C) and zinc (m.p. 419.5°C) while the heat flow was calibrated from the enthalpy of melting of indium (28.62 J/g). Endothermic and exothermic peaks appearing in the DSC graphs were analysed in terms of their onset temperatures, temperature range of the peak (determined from the first derivative of the trace) and the enthalpy of the peak (measured in J/g).
Various endothermic and exothermic peaks observed in the DSC trace are associated to various thermal behaviours and were interpreted in correspondence with HSM to be associated with solvent loss, phase changes in the sample and decomposition.

**THERMOGRAVIMETRIC ANALYSIS (TGA)**

The TGA analysis on a Perkin-Elmer PC thermal system was used at a scanning rate of 10°C, under N\textsubscript{2} purged at a flow rate of 20 mL per minute. The calibration of the instrument was performed using indium (m.p. = 156.6°C) and aluminium (m.p. = 660.3°C).

This technique was used to measure the mass loss of samples as a function of temperature or time and the decomposition of desolvated materials. Crystalline samples were first dried on filter paper to remove the superficial solvent. An empty porcelain pan was weighed, and then the instrument balance was tarred. Sample (crystalline or powder) were then placed in a porcelain pan and the weight was recorded. The programmed TG technique was carried out over a temperature range between 30°C and 400°C at a predetermined heating rate of 10°C per minute. Samples were continuously purged by a stream of dry nitrogen gas, the solvent stoichiometry of the compounds was determined from the percentage mass loss.

**FTIR SPECTROPHOTOMETRIC ANALYSIS**

Infra-Red spectra of the drug substance as well as products were obtained using a Perkin-Elmer 100 FTIR instrument fitted with UATR and controlled with Spectrum\textsuperscript{®} software version 6.3.5.0176 for the analysis of samples.

Samples were analysed mostly in a powder form or crystalline form over the range 600-4000 cm\textsuperscript{-1}. The percentage transmittance was recorded against frequency. This analytical technique was used to investigate different functional groups in samples and prove the existence of intermolecular interactions during the co-crystal/complexation processes.

**\textsuperscript{1}HNMR ANALYSIS**

NMR is one of the most powerful spectrometric techniques used for structure analysis of compounds due to its ability to access information of the chemical structure at molecular level. \textsuperscript{1}HNMR was performed in this study to identify the product of the conversion (parent compound). The tubes were imported from Sigma-Aldrich WILMAD\textsuperscript{®}, USA.
Chapter 2

The spectrum was recorded on a Varian XR200 MHZ spectrometer, using CD$_3$OD as NMR solvent and internal standard.

**X-RAY POWDER DIFFRACTION (PXRD)**

Powder X-ray diffraction was used to determine the characteristic fingerprint traces of crystalline materials. This technique can uniquely identify materials therefore allowing for the identification of new species of polymorphs, complexes and co-crystals. This technique is particularly used in the absence of good quality crystals for single crystal structure determination.

Polymorphism can be detected due to different packing arrangements that polymorphs exhibit. Powder patterns of ground samples were recorded on a Philips PW1050/25 vertical goniometer with Ni-filtered CuKα radiation (λ = 1.5418 Å) produced at 40 kV and 25 mA. Samples were packed in aluminium or glass sample holders and step scans of 0.1° 20, with 2 seconds counting times, in the range 4°-45° 20 being employed.

In cases where sample material was limited, PXRD patterns were recorded using a Huber Imaging Plate Guinier Camera 670. Some of the samples were in powder form, manually ground and were analysed using a flat method. Other preparations were packed into Lindermann capillaries with an internal diameter of 1 mm and a glass thickness of 0.01 mm. Nickel-filtered CuKα radiation (λ = 1.5418 Å), produced at 48 kV and 30 mA by a Philips PW1120/00 generator fitted with a Huber long fine-focus tube PW2273/20 and a Huber Guinier Monochromator Series 611/15, was employed. A 2θ range of 4 to 100.0° was used with a step (Generation of polymorphs, hydrates, solvates and amorphous solids, 1999) size of 0.005° 20. Temperature Controller HTC 9643 unit was not used during this process.

**SINGLE X-RAYS DIFFRACTION (SXRD)**

Bruker KAPPA APEX II DUO diffractometer was employed for crystal structure determination of the Single Crystal. Good crystals (essentially found between 0.2 and 0.5 mm in all dimensions, were selected for their ability to consistently extinguish plane-polarised light. The Bruker KAPPA APEX II DUO diffractometer used graphite-monochromated Mo-Kα radiation (λ= 0.71069 Å).
Chapter 2

After their removal from polytope vial, the unit cells of the crystals were determined at low temperature (100 ±2 K). Further, the data reduction was performed using the program SAINT.\textsuperscript{11}

The space group determination was carried out by analysing the systematic absence and matching conditions observed to a well-known space group.

OTHER USED RESOURCES

The Cambridge Strctural Database (CSD) Conquest 1. 16. Ink as well as Mercury 3.3. Ink was used in this research to search for relevant structures reported in this work. ACD/ChemSketch\textsuperscript{®} freeware was also employed to draw different structures relevant to the dissertation.

REFERENCES


11. SAINT, Version 7.60a, WI, USA: Bruker AXS Inc. 2006.
CHAPTER 3: 1-ADAMANTANAMINE SALT FORMATION

This chapter presents the conversion of ADTHCl into its free base form (ADT), which however, lead to formation of the ADT salt known as bis(adamantan-1-aminium) carbonate (ADTCO$_3$). Identification of the newly formed salt is discussed in this chapter where different methods were used and contributed to the conclusive ADTCO$_3$ result.

INTRODUCTION TO 1-ADAMANTANAMINE HCl (ADTHCl)

The 1-adamantanamine hydrochloride (ADTHCl) is commercially available as Amantadine® or Symmetrel® and its derivative rimantadine is known as α-methyl-1-adamantanemethylamine and flumadine®. Both are symmetric tricyclic amine compounds that inhibit strains of influenza A by blocking the M2 protein that functions as an ion channel.1-3 The transmembrane domain of the M2 protein is a transmembrane domain which is highly conserved in all avian viruses.5,6

ADTHCl and rimantadine are both effective at low doses against H1N1, H2N2 and H3N2 strains. Both were reported to be effective against viruses which use an M2 ion channel in the initial uncoating steps of replication.1-3

It was also found that ADTHCl due to its ability of blocking neuromuscular transmission, depending on its capacity to increase the synthesis and release of dopamine, has antiparkinson activity.4 A summary of the pharmacological activities of ADTHCl is presented in table 3.1.

Table 3.1: Summary of Pharmacological activities of ADTHCl.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antiviral</td>
<td>7, 8, 9, 10, 11, 12</td>
</tr>
<tr>
<td>Anti-parkinsonian</td>
<td>13</td>
</tr>
<tr>
<td>Anti-dengue</td>
<td>14</td>
</tr>
</tbody>
</table>

For the purposes of this study, ADTHCl was purchased from Sigma-Aldrich. ADTHCl was converted to its free base form, ADT (figure 3.1). The free base was considered as an appropriate starting material for subsequent experimentation for reasons such as; lower physical properties (melting temperature) in comparison to ADTHCl, the pharmacological activity of the adamantane ring, and the availability of amine functional group for intermolecular interactions.
1-ADAMANTANAMINE HCl (ADTHCl) CONVERSION TO THE FREE BASE

The conversion of ADTHCl to its free base (ADT) was synthesised by the following chemical precipitation reaction. The reaction had to proceed as follows:

\[ C_{10}H_{18}N \cdot \text{Cl} + \text{NaHCO}_3 \text{(Aq)} \rightarrow C_{10}H_{17}N \uparrow + [\text{Na}^+\text{Cl}^- + \text{CO}_3^{2-} + \text{H}_2\text{O}]\text{(Aq)} \]  

Equation 3.1

Distilled water was used to prepare a saturated or supersaturated solution with sodium hydrogen carbonate (NaHCO\(_3\)) powder. An amount of ADTHCl was added to the solution. The mixture was stirred for 15 to 30 minutes, using a magnetic stirrer at room temperature. A precipitate formed was collected from the solution using a 0.45 \(\mu\)m sized filter paper. Since the free base does not dissolve in water, the collected precipitate was the “assumed” free base desired.

The precipitate was dried in an oven that was maintained at 40°C for 1-2 days until consistant white mass was produced. This fine, dry powder was collected and removed from the oven and kept in an airtight container at room temperature. Identification tests were completed on the precipitate collected to verify the physicochemical identity of the compound. Several analytical techniques were applied to the powder.

CHARACTERISATION OF THE CONVERTED SAMPLE

To characterise the converted sample, different analytical techniques such as DSC, HSM, TGA, FTIR, \(^1\)H-NMR, PXRD, single X-ray diffraction. A literature search was conducted and after careful examination of all data, the following reaction was in fact the resultant reaction:

\[ C_{10}H_{17}N \cdot \text{HCl} + \text{NaHCO}_3 \text{(Aq)} \rightarrow [2(C_{10}H_{18}N^+)\text{CO}_3^{2-} + \text{Na}^+\text{Cl}^-]\text{(aq)} \]  

Equation 3.2

The following analytical techniques provided evidence for the resultant reaction which suggests a carbonate salt instead of the free base precipitating.

PROTON-NMR ANALYSIS (\(^1\)HNMR)

NMR is one of the most powerful spectrometric techniques used for structure analysis of compounds due to its ability to access information of the chemical structure at molecular level. \(^1\)HNMR was initially performed in this study to identify the product of the conversion (starting material).
The tubes were bought from Sigma-Aldrich WILMAD®, USA. The $^1$HNMR spectrum was recorded on a Varian XR200 MHZ spectrometer using CD$_3$OD as the solvent: $\rho$: 1.4-1.6, (12H, triplet)

$\rho$: 1.8-2, (3H, multiplet)

Physical data: molecular formula $2C_{10}H_{18}N^+\cdot CO_3^{2-}$; m.p: 160-180°C; $^1$HNMR (200MHz, CD$_3$OD). $\rho_H$: 2.0-1.8 (multiplet, 3H), 1.6-1.4 (triplet, 12H). The additional patterns observed were due to the interaction of solvent with other hydrogen atoms of that molecule as well as the existence of intramolecular interactions in it. The molecule has 3 types of non-equivalent hydrogen atoms (figure 3.1). This findings did not show whether the result of the conversion is indeed $2(C_{10}H_{18}N^+\cdot CO_3^{2-})$ or $C_{10}H_{17}N$ since these two have the same number of non-equivalent hydrogen atoms.

![Figure 3.1: $^1$HNMR pattern of the converted sample.](image)

66
Chapter 3

THERMAL ANALYSIS

HOT STAGE MICROSCOPY ANALYSIS

An assessment on the physical and chemical properties was conducted. In this process, thermal analysis by HSM was performed under silicon oil at a heating rate of 10°C per minute. Changes associated with heating of the sample were observed under a microscope over the temperature range from 20°C until 230°C. The melting temperature of the converted sample by HSM was found to be in the range between 157°C and 170°C. However, bubbling occurred, which in fact was created by fumes released between the two glass slides of the sample holder and cover slide, commencing early around 136°C until the completion of melting at 170°C (figure 3.2). The gaseous fumes given off were accounted for as a sublimation process occurring in the temperature range of 136°C to 170°C. Decomposition of the sample followed immediately at about 185°C.

![Figure 3.2: HSM of the converted sample.](image)

DSC AND TGA ANALYSES

The DSC analysis was performed at a constant rate of 10°C per minute under nitrogen gas purged at 20 mL per minute. A trace produced showed a single broad endotherm in the temperature range between 140°C and 180°C, peaking at 170.67°C with an onset at 146.86°C. In addition, DSC analysis confirmed the sublimation results that were observed when using HSM analysis of this compound since the DSC pan and cover were covered with powder sublimates after each experiment run. Figure 3.3 also confirms the decomposition immediately after the melt at 183°C.
Figure 3.3: The DSC of the converted sample.

TGA analysis was also performed at a constant rate of 10°C per minute, under nitrogen purged at 20 mL per minute and proved that there was no solvent (water) included in the structure since there was negligible mass loss in the 30-170°C temperature range. The TGA calculated a mass loss of 0% (n = 0) (figure 3.4).

This correlates well with the DSC result since the more pronounced mass loss was observed in the melting range followed immediately by the decomposition of the sample.

Figure 3.4: The TGA of the converted sample.
Chapter 3

FTIR SPECTROPHOTOMETRY ANALYSIS

FTIR analysis of the converted sample and that of the purchased free base were conducted to elucidate the chemical functional groups of the converted sample as well as the purchased free base.

The following spectra (transmittance versus wavelength) were produced and comparatively presented, where the amine and other functional groups were identified in specific regions of the spectra (figure 3.5).

The analysis of the converted sample spectrum was identified as bis(adamantan-1-aminium) carbonate (ADTCO$_3$) using FTIR based on the key intensity shifts in comparison to the expected (ADT) free base and ADTHCl FTIR spectra. New peaks and peak shifts were identified in the fingerprint and functional groups regions of the converted sample spectrum (table 3.2).

The absence of the expected two peaks in the 3300 cm$^{-1}$ region of the ADTCO$_3$ spectrum, attributed to N-H stretching vibration of the primary amine group that originates from the participation of hydrogens of the amine functional group into intramolecular hydrogen bonding found in this compound. Furthermore, the amine group presents an extra hydrogen atom and formed an aminium group. The medium intensity band at 2910 cm$^{-1}$ and the weak one at 2852 cm$^{-1}$ are assigned to the stretching vibration of the C-H group. The strong bands at 1639.64 and 853.41 cm$^{-1}$ were assigned to the N-H deformation bands and 1550.51 cm$^{-1}$ band was attributed to the -C=O of the carbonate attached to the adamantane ring. The -C-O vibrations were observed in the fingerprint of this spectrum at around 1000 cm$^{-1}$. The absorption bands 2267.81 and 1456 cm$^{-1}$ were attributed to the –C=N or –C=O attached to the ring by the hydrogen interactions. The intramolecular hydrogen bonding in this compound was mainly observed at 2564.96 cm$^{-1}$ created shifts and overlapping in functional groups.
Figure 3.5: Spectra of ADTHCl, ADT and ADTCO$_3$ compared.
Table 3.2: Specific bands assigned to given functional groups and key intensity shifts.

<table>
<thead>
<tr>
<th>Functional groups</th>
<th>ADT</th>
<th>ADTCO₃</th>
<th>ADTHCl</th>
<th>Comments/shifts</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-H</td>
<td>3339 cm⁻¹</td>
<td>-</td>
<td>-</td>
<td>Primary amine</td>
</tr>
<tr>
<td>C-H</td>
<td>2898 cm⁻¹</td>
<td>2910 cm⁻¹</td>
<td>2915 cm⁻¹</td>
<td>adamantane ring</td>
</tr>
<tr>
<td></td>
<td>1450 cm⁻¹</td>
<td>1456 cm⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C=O</td>
<td>-</td>
<td>1639 cm⁻¹</td>
<td>-</td>
<td>Carbonate ion</td>
</tr>
<tr>
<td>NHCl</td>
<td>-</td>
<td>-</td>
<td>2063 2097 cm⁻¹</td>
<td>Influence of HCl</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1604 cm⁻¹</td>
<td></td>
</tr>
<tr>
<td>NH₃⁺</td>
<td>-</td>
<td>2564 cm⁻¹</td>
<td>-</td>
<td>H-bonding area</td>
</tr>
<tr>
<td>C-O/ C-N</td>
<td>-</td>
<td>2267 cm⁻¹</td>
<td>-</td>
<td>H-bonding effect</td>
</tr>
</tbody>
</table>

**SINGLE X-RAY DIFFRACTION**

A full data set was collected for the sample by single X-ray diffraction. The unit cell data for ADTCO₃ which ultimately confirmed the formation of ADTCO₃ are recorded in table 3.3. However, all data are not presented since literature review shows that ADTCO₃ had already been isolated and elucidated by single X-ray diffraction.¹⁵

Table 3.3: The space groups and unit cell data for ADTCO₃. Experimental compared to theoretical at room temperature.

<table>
<thead>
<tr>
<th></th>
<th>Experimental</th>
<th>REFCODE (YAXNOC)¹⁵</th>
</tr>
</thead>
<tbody>
<tr>
<td>Space group</td>
<td>P -3 c1</td>
<td>P -3 c1</td>
</tr>
<tr>
<td>Number of molecule per Unit cell</td>
<td>Z = 2</td>
<td>Z = 2</td>
</tr>
<tr>
<td>Unit cell axis lengths</td>
<td>a = 6.578 Å</td>
<td>a = 6.434 (&lt;1) Å</td>
</tr>
<tr>
<td></td>
<td>b= 6.492 Å</td>
<td>b= 6.434 (&lt;1) Å</td>
</tr>
<tr>
<td></td>
<td>c= 25.789 Å</td>
<td>c= 25.474 (2) Å</td>
</tr>
<tr>
<td>Unit cell angles</td>
<td>γ = 89.952°</td>
<td>γ = 90°</td>
</tr>
<tr>
<td></td>
<td>β = 90°</td>
<td>β = 90°</td>
</tr>
<tr>
<td></td>
<td>γ = 119.8°</td>
<td>γ = 120°</td>
</tr>
</tbody>
</table>
EXPERIMENTAL POWDER X-RAY DIFFRACTION

PXRD was performed to assess not only the crystallinity but also to confirm the identity of ADTCO$_3$ (figure 3.6). The obtained pattern shows a very sharp signal at 20 value 17.245° with smaller peaks at 20 values 6.685°, 15.72°, 20.78°, 28.425° and 35°. The distinctiveness of the compound in question was also investigated by comparing the experimental pattern to literature\textsuperscript{6} which confirmed the identity of the structure.

![Experimental and calculated PXRD (YAXNOC) patterns of ADTCO$_3$.](image)

**Figure 3.6:** Experimental and calculated PXRD (YAXNOC) patterns of ADTCO$_3$. The experimental PXRD showed a very good correlation to the calculated PXRD pattern\textsuperscript{15} (figure 3.6), confirming the integrity of the ADTCO$_3$ sample.

In conclusion, we see that the expected conversion of ADTHCl to ADT free base led to a conversion to ADTCO$_3$. ADTCO$_3$ was conclusively confirmed using HSM, DSC, TGA, $^1$HNMR, FTIR, single XRD and PXRD. Even though a conversion of ADTHCl to free base is a well-known technique, ADTCO$_3$ formed because of the ability of the primary amine to absorb CO$_2$ in the atmosphere which was also confirmed by Nowakoska et al\textsuperscript{15}, 2012. Thus, it is recommended to prepared a free base under nitrogen and perform all experimentation in neutral atmosphere to prevent a further conversion of the free base into ADTCO$_3$. The alternative conversion using NaOH is also advised.
REFERENCES


CHAPTER 4: POLYMORPHISM OF BIS(ADAMANTAN-1-AMINIUM) CARBONATE

This chapter describes the occurrence of the polymorphism phenomenon\textsuperscript{1-6} of bis(adamantan-1-aminium) carbonate (ADTCO\textsubscript{3}). The experimentation revealed the appearance of 4 different polymorphic forms of ADTCO\textsubscript{3}, where Form I was identified as the converted ADTCO\textsubscript{3} presented in chapter 3. Form I was recrystallized from a single solvent (methanol) while Form IV was also recrystallized from a single solvent (ethanol). Form II and Form III were obtained from binary mixtures of solvents, methanol-chloroform and methanol-ethyl acetate, respectively. Conclusions drawn were based on the application of several different analytical techniques which provided the results and confirmed the different polymorphic forms.

INTRODUCTION

ADTCO\textsubscript{3}, the converted salt form (discussed in chapter 3) showed the ability to crystallise into new polymorphic forms. Table 4.1 is representative of experiments done for this study with regards to polymorphism of ADTCO\textsubscript{3}.

Table 4.1: Selected ADTCO\textsubscript{3} polymorphism experiments conducted.

<table>
<thead>
<tr>
<th>METHOD 1: SLOW EVAPORATION USING DIFFERENT SINGLE SOLVENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOLVENT</td>
</tr>
<tr>
<td>Ethanol</td>
</tr>
<tr>
<td>Methanol</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>METHOD 2: SLOW EVAPORATION USING A BINARY MIXTURE OF SOLVENTS IN 1:1 RATIO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent 1</td>
</tr>
<tr>
<td>Methanol</td>
</tr>
<tr>
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</tr>
<tr>
<td>Methanol</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>METHOD 3: GRINDING TECHNIQUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent</td>
</tr>
<tr>
<td>Neat grinding</td>
</tr>
<tr>
<td>Methanol</td>
</tr>
</tbody>
</table>

INTRODUCTION

ADTCO\textsubscript{3}, the converted salt form (discussed in chapter 3) showed the ability to crystallise into new polymorphic forms. Table 4.1 is representative of experiments done for this study with regards to polymorphism of ADTCO\textsubscript{3}.

Table 4.1: Selected ADTCO\textsubscript{3} polymorphism experiments conducted.

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<td>SOLVENT</td>
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<tr>
<td>Ethanol</td>
</tr>
<tr>
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<tr>
<td>Methanol</td>
</tr>
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</tr>
<tr>
<td>Neat grinding</td>
</tr>
<tr>
<td>Methanol</td>
</tr>
</tbody>
</table>
A full representation of all experiments conducted is presented in Appendix A.

Using various techniques such as DSC, HSM, FTIR and X-ray diffraction, four polymorphic forms of ADTCO$_3$ were identified and are discussed below.

**POLYMORPHISM OF ADTCO$_3$**

The starting material, ADTCO$_3$ will be referred to as **Form I**. Several techniques were employed to investigate the polymorphism effects of ADTCO$_3$. The preparation and isolation of the new forms will follow with a full characterisation of each form being fully illustrated and discussed.

**PREPARATION OF ADTCO$_3$ POLYMORPHS**

The following methods were used to prepare the four polymorphs of ADTCO$_3$: single solvent evaporation and crystallisation using binary mixtures of solvents.

**Form I-Single solvent evaporation**

Single crystals of **Form I** were prepared by solubilizing the ADTCO$_3$ powder mass in the solvent, methanol. The mixture was placed on a hot plate to approximately 10°C below the boiling point of methanol and stirred continuously with a magnetic stirrer until the ADTCO$_3$ powder solubilised. The saturated solution was filtered through a 0.45 μm filter and kept in a vial under a secured and undisturbed area. Vials were covered by perforated parafilm® to allow for evaporation of the solvent to occur so that the crystallisation process at ambient environmental conditions would be ensured. Clear, hexagonal crystals were formed and fully characterised (figure 4.1).

![Figure 4.1: Photographs of Forms I-IV crystals recorded at room temperature.](image)

**Form II- Recrystallization using binary mixtures of solvents**

Form II was prepared by recrystallizing 20 mg of the starting material (ADTCO$_3$) in a binary mixture of methanol and chloroform in a 1:1 ratio.
The mixture was placed on a hot plate to approximately a 10°C below the boiling point of chloroform and stirred continuously with a magnetic stirrer until the ADTCO$_3$ powder solubilised. The saturated solution was filtered through a 0.45 $\mu$m filter and kept in a vial under a secured area. Vials were covered by perforated parafilm$^\text{®}$ to allow for evaporation of the solvent to occur at 20°C. Quartz-like crystals of irregular shapes were harvested and further analysed (figure 4.2).

**Form III**

Form III was prepared by recrystallizing ADTCO$_3$ in a binary mixture of methanol:acetone in a 1:1 ratio. 20 mg of ADTCO$_3$ powder was carefully weighed and transferred into a clean polytop vial. Firstly, 1 mL of methanol (solvent) was added to solubilise the powder. Then, 1 mL of acetone (precipitant) was added drop-wise while the solution was heated on a hot plate and continuously stirred using a magnetic stirrer to 10°C below the boiling point of acetone (the least stable solvent). The solution was filtered using a 0.45 $\mu$m filter with a syringe, then the solution was kept in a vial covered by perforated parafilm$^\text{®}$ to facilitate evaporation of the solvent at ambient temperature. Flat glass plate-like crystals were produced (figure 4.1).

**Form IV - Single solvent evaporation**

Form IV was synthesised by solubilizing 20 mg ADTCO$_3$ in ethanol. The mixture was placed on the hot plate at approximately 10°C below the boiling point of ethanol and stirred continuously until all powder was dissolved. The solution was then 0.45 $\mu$m filtered in the vial covered by perforated parafilm$^\text{®}$ to allow for evaporation at 20°C. Small, hexagonal crystals formed and were kept in a vial to be further analysed (figure 4.1).

The synthesis of Form I could also be achieved by recrystallization of ADTCO$_3$ in binary mixtures of solvents. Equal amounts of methanol:acetone, methanol:1,4-dioxane, methanol: ethanol, ethanol:acetone and ethanol:propane-2-ol (1:1 ratio), were individually added to 20 mg of ADTCO$_3$ and gently heated to 10°C below the boiling point of the lower b.p. solvent. The saturated solution was filtered and solvent evaporation proceeded as in the production of form I with methanol solvent. Hexagonal crystals were obtained though much smaller in size. The smaller crystal size was possibly due to factors such as concentration of the recrystallization solution and the rate of solvent evaporation.
Furthermore, **Form I** could also be prepared by both neat and methanol assisted grinding of 20 mg of ADTCO$_3$ in a mortar with a pestle for 30 minutes.

By recrystallizing ADTCO$_3$ in a binary mixture of methanol and diethyl ether, **Form II** was reproduced.

**Form III** was also prepared by solubilising ADTCO$_3$ powder in binary mixtures of methanol:ethyl acetate and ethanol:ethyl acetate in a 1:1 ratio.

Finally, **Form IV** synthesis was achieved by recrystallizing ADTCO$_3$ in a binary mixture of methanol and ethanol (1:1 ratio).

**ANALYSES OF ADTCO$_3$ POLYMORPHIC FORMS**

Physicochemical characterisation of the obtained crystal forms was determined by HSM, DSC, TGA, FTIR spectroscopy and PXRD.

**THERMAL ANALYSES**

**HOT STAGE MICROSCOPIC ANALYSIS (HSM)**

To analyse the thermal behaviour of different crystal forms, HSM was performed and each product was exposed to the heating process at a constant rate of 10°C/min under silicone oil in order to assess the presence of “so-called” impurity (water/solvent) that could be entrapped in the molecular structure of the formed crystals. Changes were observed under the microscope and analysed using the Essential Stream software® (Figure 4.2).

For **Form I** hexagonal crystals showed with bubbling at 130°C. At this stage bubbling was accounted for due to the fragmentation of large hexagonal crystals. Furthermore, phase change occurred as the temperature increased with melt taking place between 168°C and 173°C, until 182°C where no more crystal was left on the slide fixed to the hot stage. No discoloration or any other sign of decomposition was observed.

All photographs were taken at (a) ambient temperature (b), the first sign of change (c) clear melting (d) and the completion of melting or decomposition (figure 4.2).

For **Form II**, bubbling commenced from 140°C, crystal size reduction continued and melting occurred between 183-185°C.
Form III showed bubbling from 157°C with a melt between 185-190°C.

Finally, Form IV showed an onset of melting at 180°C. The melting process was identified as crystal fragmentation and bubble production occurred until complete phase change between 205°C and 209°C.

<table>
<thead>
<tr>
<th></th>
<th>Form I</th>
<th>Form II</th>
<th>Form III</th>
<th>Form IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Room temper-ature</td>
<td>25 °C</td>
<td>25 °C</td>
<td>25 °C</td>
<td>25 °C</td>
</tr>
<tr>
<td>130 °C</td>
<td>100-129 °C</td>
<td>138 °C</td>
<td>170 °C</td>
<td></td>
</tr>
<tr>
<td>(b) 1st change</td>
<td>168 °C</td>
<td>180 °C</td>
<td>185 °C</td>
<td>203 °C</td>
</tr>
<tr>
<td>(c) Melting</td>
<td>180 °C</td>
<td>192 °C</td>
<td>195 °C</td>
<td>205 °C</td>
</tr>
<tr>
<td>(d) Degradation/ completion melting</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 4.2: The HSM photographs of Form I to IV of ADTCO₃.

DSC and TGA analyses of ADTCO₃ Forms I to IV

Thermal analysis was performed on a Perkin Elmer Differential Scanning Calorimeter DSC7 using TA Pyris software version 9.0.00148. The instrument was calibrated using Indium. Aluminium pans were used as sample holders. An empty pan was sealed and used as the reference. The flow of inert nitrogen at a rate of 20 mL/min was used while heating samples in the DSC from 30°C to the required temperatures depending on the stability of the analytical sample. For all the ADTCO₃ forms, DSC was performed at a constant rate of 10°C/min in the temperature range 30-260°C.
Chapter 4

To support HSM and DSC findings and ensuring the purity of samples, TGA was performed. Crystals were weighed (1–3 mg) into a ceramic crucible using a micro balance. The TGA scans were obtained and recorded on a Perkin Elmer TGA 4000, using Pyris software version 11.0.3.0470. TGA traces were recorded at a heating rate of 10°C per minute.

Form I crystals showed a broad endotherm in the temperature range of 147-180°C peaking at 170°C. This compared well with the starting material and is therefore called Form I (figure 4.3a).

The TGA analysis for this form indicated a negligible mass loss in the range 30-178°C, thus confirming that there is no solvent or water present in the molecular structure of Form I crystals. The TGA trace (figure 4.3b) presented a 0.0% mass loss (n = 0.0) before melting. This correlates with the HSM & DSC results. Decomposition of the sample commenced immediately after the melt from 180°C with a sharp decline in mass loss seen in by TGA analysis.

Figure 4.3: The DSC (a) and TGA (b) trace of Form I.

For Form II, the DSC analysis showed a broad endotherm in the temperature range 170-200°C peaking at 185°C (figure 4.4). No solvent release was detected by DSC analysis. Therefore, bubble production experienced during the HSM analyses was created by crystal fragmentation prior to melt.

TGA analysis indicated negligible mass loss in the range of 30-170°C, thus confirming that these crystals are not solvates. The TGA trace showed that there was only 1.1% mass loss (n = 0.23).
Chapter 4

Figure 4.4: The DSC trace of form II.

DSC analysis for Form III showed a double headed endotherm in the temperature range of 140-200°C, peaking at 170°C suggestive of Form I and 192°C suggestive of Form III. The two peaks do not suggest a conversion from Form I to Form III since this would have been seen in all other DSC analysis. It does however indicate that not all samples (i.e. form I) had changed to Form III during preparation (figure 4.5).

The TGA analysis for this form also showed a negligible mass loss. Thus, confirming the absence of solvent in the molecular structure of these crystals form. The TGA trace was recorded and presented a 0% (n = 0) mass loss in the range 30-140°C. There was a good correlation between the thermal analytical results.

Figure 4.5: The DSC trace of Form III.

The DSC analyses for Form IV showed a trace with a very broad endotherm between 187.54 and 220°C peaking at 203°C (figure 4.6a). The broad endotherm correlates with the HSM result characterised by complete phase change between 205-209°C (i.e. melt).
TGA for this form also confirmed the absence of solvent in the molecular structure of the crystal since the analysis of the trace recorded in the temperature range 30-205°C showed negligible loss of mass 2% (n = 0.7) (figure 4.6b).

![Figure 4.6: The DSC (a) and TGA (b) traces of Form IV.](image)

**FTIR SPECTROSCOPY**

FTIR analysis was performed to identify the amine and other important functional groups in the different forms of ADTCO₃. Patterns obtained were compared to the originally converted spectrum. Spectra of these forms appeared to be quite similar since they possess identical functional groups, with only some slight differences observed (figure 4.7).

The lack of ADTCO₃ FTIR spectrum in the literature led to the initial identification of the spectrum for Form I. Subsequently, when spectra of Forms II, III and IV were compared to Form I only small differences were observed and that was sufficient to identify the polymorphs of ADTCO₃.

The characteristic absorption bands at 2910 cm⁻¹ and 2852 cm⁻¹ are assigned to the C-H (sp³ and sp²) vibration of adamantane ring. The weak bands at 2722 cm⁻¹, 2563 cm⁻¹ may be attributed to the NH₃⁺.

A very weak absorption band at 1730 cm⁻¹ is ascribed to C=O vibrations for the carbonate. The absorption band at 2250 cm⁻¹ is assigned to the C–N vibration, the absorption bands at 1639 cm⁻¹, 1548 cm⁻¹, 1456 cm⁻¹ may be assigned to the C=O of the carbonyl while the rest of bands found in the fingerprint of the spectrum (1381-1290 cm⁻¹, 1142-1087 cm⁻¹, 1039 cm⁻¹, 979 cm⁻¹, 932 cm⁻¹, 852 cm⁻¹, 815 cm⁻¹, 780 cm⁻¹, 752 cm⁻¹, 722 cm⁻¹ and 667 cm⁻¹) may be attributed to the C-O of the carbonyl and C-H deformation (table 4.2).
Figure 4.7: FTIR-spectra of Forms I, II, III and IV of ADTCO₃.
From the FTIR results, it is evident that IR analysis is not a good technique to distinguish polymorphs from one another; however, slight shifts in the bands and/or intensity differences are due to the intermolecular forces between the differences in packing arrangements of the molecules in the unit cell.

Furthermore, it is possible to ascertain whether the different forms had indeed crystallised as salts by identifying the NH$_3^+$ group within each spectrum.

**PXRD ANALYSIS**

Experimental PXRD patterns of the four forms presented a very close match since only a few variations in patterns were detected (figure 4.8). Along the Form I trace the intense peaks in the regions 17.56°, 20.8° and 34.51° and the short peaks at 13.79°, 15.77° and 42.96° are presented. Along the Form II pattern different peaks at 2θ values 5.815°, 16.7°, 21.03°, 31.015° and 34.99° are identified. Along the Form III new peaks at 17.19°, 20.98°, 35.05°, 43.0°, 44.19° and 49.5° are presented. Form IV trace presented distinct peaks at 2θ values of 13.7°, 15.75°, 17.23°, 32° and 35, 21°.
Figure 4.8: The PXRD patterns of forms I-IV of ADTCO$_3$.

Figure 4.9: The DSC of forms I-IV.

Even though small changes such as new peaks, additional shoulders or shifts in the position often imply the presence of different polymorphs, the PXRD patterns stacked (figure 4.8) together with the DSC curves stacked (figure 4.9) is a clear and undeniable indication of the four polymorphic forms of ADTCO$_3$. 

85
REFERENCES

CHAPTER 5: SALT CO-CRYSTALLISATION OF BIS(ADAMANTAN-1-AMINUM) CARBONATE

This chapter describes the preparation and results of the co-crystallisation experiments of bis(adamantan-1-aminium) carbonate (ADTCO₃) with 10 selected co-formers presenting complementary accepter or donor functional groups. A full physicochemical characterisation of the obtained ADTCO₃ products is discussed with reasonable conclusions drawn.

INTRODUCTION

Co-crystallisation is an effective crystal engineering approach used to modify structures and properties of crystalline drugs. The approach is normally applied to neutral drugs such as free acids and bases. However, with the discovery of polymorphs of piroxicam-4-hydroxybenzoic acid co-crystal (1:1, v/v) in both neutral and zwitterion forms, an overlap rose between definitions of a co-crystal and a salt. Thus, the salt co-crystallisation approach¹ was incorporated.

ADTCO₃, a zwitterion² was unexpectedly derived from ADTHCl by conversion using the precipitation reaction of ADTHCl with sodium bicarbonate solution during an attempt to synthesise the ADT free base (Chapter 3). Subsequently, salt co-crystallisation experimentation was a newly defined approach added to the study.

ADTCO₃ is insoluble in water. Co-crystallising ADTCO₃ with different co-crystal formers may significantly impart not only solubility enhancement but also other physicochemical properties such as pharmaceutical manufacturing and biological activity enhancement.

Co-crystallisation of ADTCO₃ was performed using: benzoic acid (BA), 4-hydroxybenzoic acid (HBA), trans-cinnamic acid (CIN) and 4-hydroxycinnamic acid (HCIN) selected for their antiviral and antioxidant activities. Succinic acid (SUC), L-glutamic acid (GLA), L-glutaric acid (GA), L-tartaric acid (LTTA), salicylic acid (SA) and citric acid monohydrate (CA) were selected from the Generally Regarded as Safe (GRAS) list³, for their good co-forming properties as carboxylic acids.
Table 5.1: The empiric formula, melting ranges/points and uses of ADTCO$_3$ and selected co-formers.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Abbrev.</th>
<th>Structure</th>
<th>Melting range</th>
<th>Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bis(adamantan-1-aminium) carbonate</td>
<td>ADTCO$_3$</td>
<td><img src="image" alt="Structure" /></td>
<td>170-180°C</td>
<td>Antiviral, antiparkinson</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>BA</td>
<td><img src="image" alt="Structure" /></td>
<td>122.4°C</td>
<td>Antioxidant</td>
</tr>
<tr>
<td>4-Hydroxybenzoic acid</td>
<td>HBA</td>
<td><img src="image" alt="Structure" /></td>
<td>210°C</td>
<td>Antioxidant</td>
</tr>
<tr>
<td>Trans-cinnamic acid</td>
<td>CIN</td>
<td><img src="image" alt="Structure" /></td>
<td>133°C</td>
<td>Antimicrobial</td>
</tr>
<tr>
<td>4-hydroxycinnamic acid</td>
<td>HCIN</td>
<td><img src="image" alt="Structure" /></td>
<td>214°C</td>
<td>Antioxidant</td>
</tr>
<tr>
<td>Succinic acid</td>
<td>SUC</td>
<td><img src="image" alt="Structure" /></td>
<td>185-187°C</td>
<td>Flavourant</td>
</tr>
<tr>
<td>L-Glutamic acid</td>
<td>GLA</td>
<td><img src="image" alt="Structure" /></td>
<td>205°C</td>
<td>Flavourant</td>
</tr>
<tr>
<td>L-Glutaric acid</td>
<td>GA</td>
<td><img src="image" alt="Structure" /></td>
<td>98°C</td>
<td>Virucidal activity</td>
</tr>
<tr>
<td>L-Tartaric acid</td>
<td>LTTA</td>
<td><img src="image" alt="Structure" /></td>
<td>171-174°C</td>
<td>Antioxidant</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>SA</td>
<td><img src="image" alt="Structure" /></td>
<td>159°C</td>
<td>Keratolytic</td>
</tr>
<tr>
<td>Citric acid monohydrate</td>
<td>CA</td>
<td><img src="image" alt="Structure" /></td>
<td>135-152°C</td>
<td>Preservative</td>
</tr>
</tbody>
</table>

ADTCO$_3$ is poorly soluble (or insoluble) in most organic solvents; for this reason, slow solvent evaporation was not frequently used for salt co-crystallisation. However, a few experiments were conducted using this technique. The ADTCO$_3$ structure (figure 5.1) lends itself to co-crystallisation due to the possible intermolecular interactions of one proton of the aminium group together with hydrogen atoms of the amantadine ring and one atom of the carbonate that is free to participate in further interactions.
Figure 5.1: ADTCO$_3$ structure along the b axis highlighting the proton donor and acceptor groups in (blue) with hydrogen bonds coloured in red.

Table 5.2 is a representation of the experiments conducted to prepare ADTCO$_3$ salt co-crystals. A complete set of the experimental data is presented in Appendix B. All experiments commenced with Form I of ADTCO$_3$, the starting material identified in ‘chapter 3’. For co-crystal experimentation, it was of utmost importance to ensure that all analytical results exclude the different polymorphic forms (I-IV) of ADTCO$_3$ identified.

Table 5.2: Representation of the experiments conducted to prepare ADTCO$_3$ co-crystals using the salt co-precipitation method.

<table>
<thead>
<tr>
<th>Host</th>
<th>Co-former</th>
<th>Solvent 1</th>
<th>Solvent 2</th>
<th>Ratio</th>
<th>Salt co-crystal</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADTCO$_3$</td>
<td>BA</td>
<td>Methanol</td>
<td>-</td>
<td>1:1</td>
<td>ADTCO$_3$BA</td>
<td>Positive</td>
</tr>
<tr>
<td>ADTCO$_3$</td>
<td>BA</td>
<td>Ethanol</td>
<td>-</td>
<td>1:1</td>
<td>ADTCO$_3$BA</td>
<td>Positive</td>
</tr>
<tr>
<td>ADTCO$_3$</td>
<td>BA</td>
<td>Ethanol</td>
<td>Ethyl ether</td>
<td>1:1</td>
<td>ADTCO$_3$BA</td>
<td>Positive</td>
</tr>
<tr>
<td>ADTCO$_3$</td>
<td>BA</td>
<td>Methanol</td>
<td>Ethyl ether</td>
<td>1:1</td>
<td>ADTCO$_3$BA</td>
<td>Positive</td>
</tr>
<tr>
<td>ADTCO$_3$</td>
<td>BA</td>
<td>Amyl alcohol</td>
<td>-</td>
<td>1:1</td>
<td>ADTCO$_3$BA</td>
<td>Positive</td>
</tr>
</tbody>
</table>

PREPARATION AND ANALYSIS OF ADTCO$_3$ CO-CRYSTALS

ADTCO$_3$BA (FORMS 1 to 5) – Polymorphism of ADTCO$_3$BA salt co-crystals

The co-precipitation and grinding techniques are traditional methods that are reported to be effective in crystal screening since they very often lead to valuable results.\(^4\)
In this study, ADTCO$_3$ salt co-crystals were generally prepared by these two techniques inclusive of co-precipitation by single and binary mixtures and grinding by the neat and solvent-drop method.

**PREPARATION**

**ADTCO$_3$BA1**

A stoichiometric amount of ADTCO$_3$ (20 mg) and BA (18 mg) in a 1:1 ratio was dissolved in 2 mL of methanol in two separate polytop vials. A hot plate was used to heat the two solutions to approximately 10°C below the boiling point of methanol while continuously stirring using magnetic stirrers. The two samples were then transferred into one vial and stirred again for a period of 10 to 15 minutes. The final solution was filtered using a 0.45 μm micro-filter and kept in a clean polytop vial sealed with perforated parafilm® to facilitate the evaporation of the solvent.

Long, thin fibre-like crystals were produced.

**ADTCO$_3$BA2**

To prepare ADTCO$_3$BA2 co-crystal, a stoichiometric amount of the two samples (ADTCO$_3$ and BA powders in 1:1 ratio) was solubilised in ethanol in two different polytop vials. Using a hot plate, the same procedure as applied to ADTCO$_3$BA1 was pursued. Thin needle-like, clear crystals were produced after a few days and were fully analysed.

**ADTCO$_3$BA3**

The ADTCO$_3$BA3 was synthesised by the co-precipitation method using ethanol and ethyl acetate. During this process, a stoichiometric amount of ADTCO$_3$ (20 mg) and 16.5 mg of BA in 1:1 ratio were weighed, transferred each in separate polytop vials and dissolved each in a binary mixture of ethanol and ethyl acetate (50:50). With constant stirring on the hot plate using a magnetic bar, the two solutions were heated to 10°C below the boiling point of the least stable solvent; ethanol for this instance. The two solutions were transferred to the same polytop vial, stirred for a further 20 minutes and filtered using a syringe and a 0.45 μm microfilter. The final solution was kept in a secure and stable area to allow for evaporation at 20°C. Thin fibre-like crystals of ADTCO$_3$BA3 were harvested after 4 days for further analyses.
ADTCO$_3$BA4

The ADTCO$_3$BA4 co-crystal was synthesized through recrystallization of both ADTCO$_3$ and BA from a mixture of methanol and ethyl acetate in a 1:1 ratio. ADTCO$_3$ (20 mg) was transferred to a polytop vial and 16.5 mg of BA was added to another. With consistent stirring using magnetic stirrers, the two solutions were heated to 10$^\circ$C below the boiling point of the lowest boiling solvent (methanol in this case). Then, the two solutions were mixed into one polytop vial using a syringe and continuously stirred for approximately 15 minutes. Filtration was performed using a syringe and a 0.45 $\mu$m micro-filter. The resulting solution was kept in a secured space for evaporation to occur at ambient temperature and pressure. Holes were made in parafilm® covering the solution to allow for evaporation. As evaporation occurred, saturation increased and these resulted to the formation of crystals. Long, thin and hair-like crystals were produced and fully characterized.

ADTCO$_3$BA5

ADTCO$_3$BA5 was synthesised by neat grinding. During this process, a stoichiometric amount of ADTCO$_3$ (20 mg) and BA (16.5 mg) in a 1:1 ratio; was weighed, transferred in a mortar and manually grounded together for 30 minutes using a pestle. This salt co-crystal form was reproduced by solvent drop grinding as well. Solvents used were propan-2-ol, methanol, ethanol, acetone, ether acetate, diethyl ether and chloroform.

THERMAL ANALYSIS

HSM and DSC ANALYSIS

An investigation on the thermal behaviour of the produced crystals as one of the most important steps in drug pre-formulation was performed. The stability (both, physical and chemical) of a pharmaceutical substance directly affects its solubility and plays an important role during the drug development process which could lead to serious consequences during the clinical bioavailability and storage periods if not monitored.

From literature, the melting point range of BA is 121-123$^\circ$C whereas melting for ADTCO$_3$ and its decomposition occur between 150$^\circ$C and 180$^\circ$C peaking at 170$^\circ$C with an emission of gas.$^2$
Chapter 5

The experimental results showed a melting range of 150-180°C also peaking at 170°C with bubbling and sublimation occurring between 120°C and 130°C. Hence, the theoretical melt for ADTCO$_3$ corresponded well with the experimental melt obtained.

HSM analysis was performed on ADTCO$_3$BA1 with the sample immersed in silicone oil to assess the presence of solvent in the crystal. This would be indicated by bubble formation prior to melting. Melting occurred in the temperature range 180-250°C. The crystal decomposed with an emission of gas bubbles immediately thereafter (figure 5.2).

To prepare ADTCO$_3$BA2, the sample was submerged into silicone oil and was gently heated at a constant rate of 10°C/min. Melting occurred slowly in the temperature range from 209°C to 240°C. Emission of bubble gas from the sample and particle size reduction implied that decomposition occurred soon after melting (figure 5.2).

Thermal behaviour by HSM analysis at a constant rate of 10°C per minute with ADTCO$_3$BA3 crystals submerged in silicon oil was performed. The product melts in the temperature range of 181-240°C. Decomposition characterised by sample discoloration was observed around 240°C.

ADTCO$_3$BA4

HSM analysis of the results showed a melt in the temperature range between 200-238°C (figure 5.2). Bubbles observed at 137°C, is a sign of solvent evaporation. However, this was a surficial solvent since TGA analysed showed a negligible mass loss at that temperature. This HSM also confirmed that no solvent is present in the molecular structure of the crystal since bubbling only occurred during the melting and decomposition of crystals on the hot stage.

HSM analysis was also conducted to analyse the thermal behaviour of the produced ADTCO$_3$BA5. The sample was submerged in silicone oil on the stage of the microscope. Heating was applied at a constant rate of 10°C per minute and melting characterised by sample size reduction was observed in the temperature range 190-250°C. The powder degraded in the temperature range of 260-264°C as indicated by spontaneous bubbling. HSM photographs of ADTCO$_3$BA1 to ADTCO$_3$BA5 are recorded at different temperatures in figure 5.2.
ADTCO$_3$BA1 DSC analysis showed a melt of 239°C in the temperature range of 180-245°C with a very broad endothermic peak as shown in figure 5.3. Decomposition followed the melt immediately.

ADTCO$_3$BA2 DSC analysis showed superficial solvent (ethanol) coming off at 77°C with an onset at 72.37°C. The melting point of ADTCO$_3$BA2 occurred in the temperature range of 200-225°C illustrated by a sharp endothermic peak at 212°C with an onset at 205°C. Degradation was clearly seen commencing within the melt peak from 225°C identified as an irregular baseline.

ADTCO$_3$BA3 DSC analysis presents a melt in the temperature range between 197.73°C and 237°C as indicated by a broad endothermic peaking at 229°C.

ADTCO$_3$BA4 DSC trace showed a melt in the temperature range of 214-245°C with a broad endotherm peaking at 241°C followed immediately by degradation of the sample.

ADTCO$_3$BA5 DSC analysis produced a trace with a broad endotherm presenting a melt in the temperature range of 225-260°C, peaking at 250°C.
TGA ANALYSIS

Thermogravimetric analysis was performed to assess possible included solvent in the molecular structure of the five forms of ADTCO$_3$BA (figure 5.4).

ADTCO$_3$BA1 showed a negligible mass loss of 0.3% ($n$~0.04). Thus, bubbling observed over heating on the hot stage was due to superficial solvent loss on the crystal not adequately removed prior to analysis.

ADTCO$_3$BA2 showed a 0% mass loss in the temperature range 30-205°C prior to the melt of the sample. This finding proved the material to be a non-solvate/hydrate. The first endotherm at 77°C on the DSC graph was indeed just superficial solvent given off due to sample that was not fully dried prior to its analysis.

ADTCO$_3$BA3 TGA trace was analysed and presented a mass loss of 4.6% ($n = 0.161$) in the temperature range between 30°C and the onset of the melt of ADTCO$_3$BA3. The initial mass loss was accounted for as unidentifiable impurities present in the sample which contaminated the product during preparation.

ADTCO$_3$BA4 TGA analysis was 1.87% ($n = 0.077$). The mass loss was negligible and does not make any contribution to the structure of this compound.

ADTCO$_3$BA5 also showed a negligible mass loss in the range 30-179°C, confirming the absence of solvent in the molecular structure of this product.
FTIR ANALYSIS

FTIR spectroscopy analysis\textsuperscript{6} was performed on ADTCO\textsubscript{3}BA (forms 1-5) to investigate firstly, the interaction between the functional groups of the two parent compounds and secondly, the identification of salt forming co-crystals (or salt forming solvated co-crystals). Finally, the polymorphism phenomenon of forms 1 to 5 was characterised.

Based on different band shifts presented in the table 5.3, it has been confirmed that there are interactions occurring between the pure drug and the excipient. Salt formation was justified by the presence of NH\textsubscript{3}\textsuperscript{+} bands which shifted differently from one form to another due to its participation in interactions with OH or C=O of carbonyl group (-COOH). According to figure 5.6, the probable interaction occurring are: NH\textsubscript{3}\textsuperscript{+}···OH, NH\textsubscript{3}\textsuperscript{+}···C=O and possibly C=O···H–O.
Figure 5.5: FTIR spectra of 1-5 form of ADTCO₃BA forms 1 to 5.
Table 5.3: Different band shifting (in wavenumber cm\(^{-1}\)) observed in the ADTCO\(_3\)BA spectra Forms 1 to 5.

<table>
<thead>
<tr>
<th>Group</th>
<th>ADTCO(_3)</th>
<th>BA</th>
<th>ADTCO(_3)BA1</th>
<th>ADTCO(_3)BA2</th>
<th>ADTCO(_3)BA3</th>
<th>ADTCO(_3)BA4</th>
<th>ADTCO(_3)BA5</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH(_3^+)</td>
<td>2564 cm(^{-1})</td>
<td>-</td>
<td>2568 cm(^{-1})</td>
<td>-</td>
<td>2562 cm(^{-1})</td>
<td>2567 cm(^{-1})</td>
<td>2570 cm(^{-1})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1601 cm(^{-1})</td>
<td>2623 cm(^{-1})</td>
<td>2612 cm(^{-1})</td>
<td>2623 cm(^{-1})</td>
<td>2623 cm(^{-1})</td>
<td>2624 cm(^{-1})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1582 cm(^{-1})</td>
<td>1593 cm(^{-1})</td>
<td>-</td>
<td>1586 cm(^{-1})</td>
<td>1592 cm(^{-1})</td>
<td>1561 cm(^{-1})</td>
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<tr>
<td>C=O</td>
<td>1679 cm(^{-1})</td>
<td>-</td>
<td>-</td>
<td>1667 cm(^{-1})</td>
<td>1668 cm(^{-1})</td>
<td>1701 cm(^{-1})</td>
<td></td>
</tr>
<tr>
<td>C=O</td>
<td>1639 cm(^{-1})</td>
<td>-</td>
<td>1627 cm(^{-1})</td>
<td>1611 cm(^{-1})</td>
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<tr>
<td></td>
<td>1550 cm(^{-1})</td>
<td>-</td>
<td>1544 cm(^{-1})</td>
<td>1533 cm(^{-1})</td>
<td>1513 cm(^{-1})</td>
<td>1523 cm(^{-1})</td>
<td>1521 cm(^{-1})</td>
</tr>
<tr>
<td>C-H</td>
<td>2910 cm(^{-1})</td>
<td>-</td>
<td>2907 cm(^{-1})</td>
<td>2913 cm(^{-1})</td>
<td>2907 cm(^{-1})</td>
<td>2907 cm(^{-1})</td>
<td>2908 cm(^{-1})</td>
</tr>
<tr>
<td>O-H</td>
<td>-</td>
<td>3071 cm(^{-1})</td>
<td>3387 cm(^{-1})</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Figure 5.6: Structure presenting the possible sites for interactions between ADTCO$_3$ and BA.

The absorption band at 1639 cm$^{-1}$ ascribed to the C=O vibration of ADTCO$_3$ carbonate shifted to 1627 cm$^{-1}$, 1611 cm$^{-1}$, 1629 cm$^{-1}$, 1629 cm$^{-1}$ and 1627 cm$^{-1}$ the spectra of ADTCO$_3$BA forms 1-5 respectively, suggesting the interaction with OH of –COOH functional group. The 1550 cm$^{-1}$ band that was assigned to the C=O vibration of the carbonate has shifted to 1544 cm$^{-1}$, 1533 cm$^{-1}$, 1513 cm$^{-1}$, 1523 cm$^{-1}$ and 1521 cm$^{-1}$ in the spectra of ADTCO$_3$BA forms 1-5 respectively, suggesting the effect of the same intermolecular interactions. NH$_3^+$ band found at 2564 cm$^{-1}$ in the ADTCO$_3$ spectrum has shifted differently in the spectrum of each ADTCO$_3$BA form due to its interaction involvement with BA functional groups as shown (figure 5.6). Furthermore, the 3071 cm$^{-1}$ band assigned to the OH of the carboxylic acid (COOH) was not present in the entire range of ADTCO$_3$BA forms spectra except for the ADTCO$_3$BA1 spectrum where this peak shifted to 3387 cm$^{-1}$, suggesting the interactions with NH$_3^+$. All these changes confirmed the co-crystallisation process between ADTCO$_3$ and BA and formation of ADTCO$_3$BA as a salt co-crystal in different forms.

PXRD ANALYSIS

To further compare and sustain thermal and spectroscopic findings 5 patterns of 5 ADTCO$_3$BA forms were compared. Firstly, they all showed good crystallinity. Secondly, patterns differ from those of both ADTCO$_3$ and BA.

Furthermore, each of ADTCO$_3$BA forms produced a unique PXRD pattern compared to others with peaks appearing at different 2θ values (figure 5.7). This provided evidence of the co-crystallisation of ADTCO$_3$ and BA into different forms.
While the BA trace presented peaks appearing at $2\theta$ 8.5°, 16.14°, 16.99°, 19°, 21.06°, 23.52°, 25.61°, 27.53° and 29.98°, along the ADTCO₃ curve, peaks are found at 6.84°, 15.79°, 17.25°, 20.73°, 28.48° and 35°.

ADTCO₃BA₁ co-crystal presents a trace with peaks at 8.4°, 15.88°, 18.35°, 19.28° and 21.43° (figure 5.7).

New intense peaks along ADTCO₃BA₂ pattern are formed at $2\theta$ 14°, 17° and 29°.

For ADTCO₃BA₃, the pattern showed different peaks in the region of $2\theta$ 15.74°, 16.2°, 16.65°, 18.4°, 19.25°, 21.76°, 27.8° and 34.4°.

Along ADTCO₃BA₄ curve, different intense peaks appeared in the region of $2\theta$ values 16.5°, 19° and 22.5°.

For ADTCO₃BA₅, intense peaks appear at $2\theta$ values 15.79° and 21.4°. Additional peaks are formed at $2\theta$ 5.6°, 8.3°, 18.3°, 19.2° and 29.6°.

Compared to ADTCO₃ and BA PXRD patterns (figure 5.7), it is clearly evident that all five forms of ADTCO₃BA differ in their packing arrangements from one another in the unit cell. Hence, the five polymorphic forms of ADTCO₃BA are undeniably proven.

![Figure 5.7: PXRD of 1-5 forms of ADTCO₃BA salt co-crystal.](image)
ADTCO$_3$HBA

PREPARATION

A stoichiometric amount of ADTCO$_3$ (20 mg) and HBA (29 mg) in a 1:1 ratio was transferred to a mortar and ground using a pestle for 30 minutes. The process was assisted by a few drops of methanol. The product was reproduced by grinding with a few drops of 1, 4-dioxan, ethanol, diethyl ether, acetone, chloroform, ethyl acetate and amyl alcohol. A fine white powder resulted as ADTCO$_3$HBA.

THERMAL ANALYSIS

HSM AND DSC ANALYSIS

A small amount of ADTCO$_3$HBA was submerged in silicone oil on a glass slide and analysed by HSM. Heating was applied at a constant rate of 10°C per minute and thermal changes of ADTCO$_3$HBA were observed under the microscope. HSM photographs (figure 5.8) were recorded and analysed using Essentials stream software$^\text{®}$.

HSM clearly showed the recrystallization process of the sample (a phase change of powder to crystal) around 206 °C. This continued until the sample melted in the temperature range 235-239 °C. Decomposition was characterised by discoloration and was observed from 250 °C.

(a) 25°C     (b) 208°C     (c) 239°C     (d) 250 °C

Figure 5.8: HSM photographs of ADTCO$_3$HBA recorded at (a) room temperature, (b) the crystallisation of sample (c) the melting point (d) the completion of melt with immediate decomposition occurring.

Analysis on DSC was performed and showed a melt in the temperature range between 236°C-245°C with a very sharp endotherm peaking at 238°C (figure 5.9).
Chapter 5

A slight exothermic curve preceding the melt peak suggested that the powder firstly re-crystallised around 200°C, then proceeded to melt at 238°C. These results are consistent with the HSM findings.

ADTCO₃HBA was confirmed as a new form since a single peak was seen on the DSC trace which differed from both the parent compounds melting points (ADTCO₃ m.p.: 170°C and HBA m.p.: 214.5°C).

Figure 5.9: DSC trace of ADTCO₃, HBA and ADTCO₃HBA.

TGA ANALYSIS

The TGA trace for ADTCO₃HBA (figure 5.10) presented a 0% mass loss in the range 30-200°C. This proved that there was no solvent present in the molecular structure of the product. A significant mass loss did however occur immediately after the melt of the sample suggesting decomposition of the sample.

Figure 5.10: TGA trace for ADTCO₃HBA.
Chapter 5

FTIR ANALYSIS

Infrared spectrum for ADTCO$_3$HBA presented various shifts of bands, intensity changes and the appearance of new bands (figure 5.11) providing evidence of intermolecular interactions between ADTCO$_3$ and HBA.

Formation of ADTCO$_3$HBA was evidenced by various band shifts in figure 5.11. The most probable intermolecular interactions between ADTCO$_3$ and HBA are as follows:

\[ \text{NH}_3^+ \cdots \text{O-H}, \text{HN}_3^+ \cdots \text{C}=\text{O} \ (\text{COOH}), \text{CO}_3^{2-} \cdots \text{OH}, \text{CO}_3^{2-} \cdots \text{O-H} \ (\text{COOH}), \text{C-H} \cdots \text{O-H}, \text{C-H} \cdots \text{C}=\text{O} \ (\text{COOH}). \]

Based on table 5.9, ADTCO$_3$HBA firstly presents the key intermolecular interactions between ADTCO$_3$ and HBA. Secondly, it is observed that ADTCO$_3$HBA retains its salt behaviour (NH$_3^+$ ion) due to the presence of 2608 cm$^{-1}$ having shifted from 2564 cm$^{-1}$. The appearance of 2162 cm$^{-1}$ is an indication that neutral N–H···O–H hydrogen bonding has occurred. Thus, ADTCO$_3$HBA will henceforth be referred to as a salt co-crystal.

Figure 5.11 and Table 5.9 suggests that NH$_3^+$ ion interacts with the –OH group since the NH$_3^+$ ion band shifts significantly and the –OH band for HBA is lost completely in the spectrum. The interactions also had an effect on 2910 cm$^{-1}$ absorption band ascribed to the stretching vibrations of C-H shifted to 2921 cm$^{-1}$. Displacements of C=O of ADTCO$_3$ from 2267 cm$^{-1}$ to 2162 cm$^{-1}$, C=O of HBA from 1669 cm$^{-1}$ to 1682 cm$^{-1}$ and 1607 cm$^{-1}$ to 1605 cm$^{-1}$ in ADTCO$_3$HBA originated from.

Further, one could see from figure 5.11 that ADTCO$_3$HBA salt co-crystal formation is also identified by 1519 cm$^{-1}$, 1496 cm$^{-1}$ and 1425 cm$^{-1}$ absorption bands attributed to the C-H deformation or C-C stretching vibration. The characteristic absorption bands at 1374 cm$^{-1}$ and 1309 cm$^{-1}$ assigned to the C-O stretching vibration of the carbonyl group, 1278 cm$^{-1}$, 1222 cm$^{-1}$ and 1206 cm$^{-1}$ of the aromatic C-O, 1164 cm$^{-1}$, 1137 cm$^{-1}$ and 1095 cm$^{-1}$ attributed to C-H bending vibration. Furthermore, 975 cm$^{-1}$, 855 cm$^{-1}$, 806 cm$^{-1}$ and 790-716 cm$^{-1}$ absorption bands are ascribed to aromatic C-H deformation of methyl groups that were all affected by the above interactions.
Figure 5.11: FTIR spectra of ADTCO$_3$, ADTCO$_3$HBA and HBA.
Shifts in the FTIR spectrum of ADTCO$_3$HBA compared to both ADTCO$_3$ and HBA are tabulated in the following table (table 5.4). The table was further used to predict the possible interaction sites for ADTCO$_3$ and HBA (figure 5.12).

**Table 5.4: Important shifts in the ADTCO$_3$HBA spectrum.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>ADTCO$_3$</th>
<th>HBA</th>
<th>ADTCO$_3$HBA</th>
<th>Shifts</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH$_3^+$</td>
<td>2564 cm$^{-1}$</td>
<td>-</td>
<td>2608 cm$^{-1}$</td>
<td>43 cm$^{-1}$</td>
</tr>
<tr>
<td>C=O</td>
<td>1639 cm$^{-1}$</td>
<td>1669 cm$^{-1}$</td>
<td>1682 cm$^{-1}$</td>
<td>27 cm$^{-1}$</td>
</tr>
<tr>
<td>C–H</td>
<td>2910 cm$^{-1}$</td>
<td>-</td>
<td>2921 cm$^{-1}$</td>
<td>11 cm$^{-1}$</td>
</tr>
<tr>
<td>O–H</td>
<td>2267 cm$^{-1}$</td>
<td>-</td>
<td>2162 cm$^{-1}$</td>
<td>105 cm$^{-1}$</td>
</tr>
</tbody>
</table>

**Figure 5.12: The possible site of action for the interactions between ADTCO$_3$ and HBA.**

**PXRD ANALYSIS**

To further investigate the formation of ADTCO$_3$HBA salt-co-crystal and support the previous analytical conclusions, powder X-ray diffraction analysis was performed.

A distinctive pattern was compared to both starting materials ADTCO$_3$ and HBA (figure 5.13). ADTCO$_3$HBA differed to its parent compounds at 2θ 16°, 17.9°, 19.1°, 20.3° and 20 27.1° (figure 5.14). Figure 5.14 also showed that ADTCO$_3$HBA could be reproduced using the solvent-drop grinding techniques with several solvent systems.
ADTCO$_3$CIN

PREPARATION

To synthesise ADTCO$_3$CIN, ADTCO$_3$ and trans-cinnamic acid (CIN) powders were ground together in a mortar using a pestle. The stoichiometric amount of ADTCO$_3$ (20 mg) together with CIN (19.5 mg) in a 1:1 ratio were manually ground for 30 minutes, with continuous introduction of methanol drops while grinding. ADTCO$_3$CIN was produced and fully characterised using different techniques.

The same product was reproduced by grinding with a few drops of a variety of organic solvents including acetone, ethanol, water, propane-2-ol, ethyl acetate, diethyl ether and amyl alcohol.
Chapter 5

THERMAL ANALYSIS

HSM and DSC

HSM analysis was performed to assess the thermal behaviour of ADTCO$_3$CIN. Photographs were recorded using Essential stream software$^\circledR$. Melting accompanied by bubbling commenced at 255°C and was characterised by a phase change from solid to liquid on the hot stage (figure 5.15). Decomposition was characterised by spontaneous bubbling and discoloration of the sample observed at 263°C.

![Figure 5.15: HSM photographs of ADTCO$_3$CIN at (a) room temperature (b) melting temperature, (c) the onset of decomposition and (d) completion decomposition.](image)

DSC analysis was performed at a constant rate of 10°C per minute. The DSC trace showed a melt in the temperature range 245-256°C illustrated by an endothermic peak at 254°C with an onset at 230°C (figure 5.16). Both parent compounds have significantly lower melting points compared to the new product (ADTCO$_3$ m.p. is 150-180°C and CIN m.p is 133°C).

![Figure 5.16: DSC of ADTCO$_3$, CIN and ADTCO$_3$CIN.](image)
Chapter 5

TGA ANALYSIS

To further support the previous analysis and assess the purity of ADTCO$_3$CIN, TGA was conducted. The TGA trace (figure 5.17) presented a mass loss 0% in the range 30-183°C, prior to the melt. A significant mass loss commenced with the onset of melting and continued with the sample degradation process.

![TGA Trace of ADTCO$_3$CIN](image)

Figure 5.17: TGA trace of ADTCO$_3$CIN.

FTIR ANALYSIS

The FTIR analysis performed on the ground sample produced a spectrum with shifts in bands, changes in intensity and formation of new bands all observed in comparison to parent compounds (figure 5.18). This justified the effect of intermolecular interactions between ADTCO$_3$ and CIN.

Generally, the absorption bands decreased or shifted to the left in the ADTCO$_3$CIN spectrum. The important shifts observed in the ADTCO$_3$CIN spectrum were attributed to the effect of intermolecular interactions predicted in figure 5.18 and conclusively confirmed the new entity.

From the FTIR results based on table 5.5 and figure 5.19, the most plausible interactions between the parent compounds could be O-H⋯NH (NH$_3^+$), C=O⋯NH$_3^+$ and C-H⋯O=C had occurred.
Figure 5.18: FTIR of ADTCO₃, CIN and ADTCO₃CIN.
Table 5.5: Important shifts detected in the ADTCO$_3$CIN spectrum.

<table>
<thead>
<tr>
<th>Groups</th>
<th>ADTCO$_3$</th>
<th>CIN</th>
<th>ADTCO$_3$CIN</th>
<th>Shifts</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH$_3^+$</td>
<td>2564 cm$^{-1}$</td>
<td>-</td>
<td>2628 cm$^{-1}$</td>
<td>63 cm$^{-1}$</td>
</tr>
<tr>
<td>C=O</td>
<td>1639 cm$^{-1}$</td>
<td>-</td>
<td>1638 cm$^{-1}$</td>
<td>1 cm$^{-1}$</td>
</tr>
<tr>
<td>C-H</td>
<td>2910 cm$^{-1}$</td>
<td>-</td>
<td>2912 cm$^{-1}$</td>
<td>2 cm$^{-1}$</td>
</tr>
<tr>
<td>C-C</td>
<td>2267 cm$^{-1}$</td>
<td>-</td>
<td>2162 cm$^{-1}$</td>
<td>105 cm$^{-1}$</td>
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<tr>
<td>Aromatic</td>
<td>-</td>
<td>1601 cm$^{-1}$</td>
<td>1593 cm$^{-1}$</td>
<td>7 cm$^{-1}$</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>1495 cm$^{-1}$</td>
<td>1503 cm$^{-1}$</td>
<td>8 cm$^{-1}$</td>
</tr>
</tbody>
</table>

Figure 5.19: The possible interactions sites for ADTCO$_3$ and CIN

Firstly the presence of 2162 cm$^{-1}$ band in ADTCO$_3$CIN spectrum is an indication that O-H⋯NH has occurred. The loss of bands at 3067 cm$^{-1}$, 3027 cm$^{-1}$ and 2543 cm$^{-1}$ ascribed to the O-H of the carbonyl (-COOH) in the ADTCO$_3$CIN spectrum is a result of the possible hydrogen bonding (O-H⋯NH$_3^+$) interactions and evidence of co-crystal formation. The presence of 2564 cm$^{-1}$ band (NH$_3^+$ of ADTCO$_3$) which shifted to 2528 cm$^{-1}$ in the ADTCO$_3$CIN spectrum was due to the hydrogen bonding with OH of the carbonyl (O-H⋯NH$_3^+$) and confirmed that the produced ADTCO$_3$CIN is a salt co-crystal.

Further, 1639 cm$^{-1}$ and 1551 cm$^{-1}$ bands assigned to the C=O stretching vibration slightly shifted as a result of its participation into H-bonding with the NH (NH$_3^+$) group. Furthermore, the appearance of characteristic absorption bands at 1391 cm$^{-1}$, 1363 cm$^{-1}$, 1241 cm$^{-1}$, 1196 cm$^{-1}$, 1170 cm$^{-1}$, 1127 cm$^{-1}$, 1117 cm$^{-1}$ and 1093 cm$^{-1}$ band attributed to the C-O stretching vibrations confirmed the deprotonating of the carbonyl group (-COOH).
POWDER X-RAY DIFFRACTION ANALYSIS

Experimental PXRD analysis was performed to confirm the formation of the ADTCO$_3$CIN salt co-crystal. The ADTCO$_3$CIN pattern presents new peaks at 2θ 17.35° and 19.6° while new short peaks were formed at 2θ of 7.1°, 10.5°, 14.4°, 15.5°, 15.7°, 22.1° and 24.9° (figure 5.20). The pattern differs significantly from the parent compounds patterns, confirming the newly derivatised salt co-crystal.

ADTCO$_3$HCIN PREPARATION

For the synthesis of ADTCO$_3$HCIN, a stoichiometric amount of ADTCO$_3$ (20 mg) and HCIN (21.65 mg) in a 1:1 ratio was weighed and added together in a mortar and ground for a 30 minute period using a pestle. This is referred to as neat grinding.

Neat grinding was a preferred technique from a pharmaceutical perspective because it ensured the use of few additives. The same product was reproduced by grinding with a few drops of chloroform, methanol, ethanol, chloroform, acetone and amyl alcohol. The produced white powder was kept in an airtight polytope vial for analysis.

THERMAL ANALYSIS

HSM and DSC

HSM analysis was performed with the sample submerged in silicon oil to assess the thermal behaviour and composition of ADTCO$_3$HCIN. The thermal behaviour was observed upon heating at a constant rate of 10°C per minute.
Slight bubbling occurred prior to the melt between 150°C and 180°C. Melt was characterised by bubbling and a phase change to liquid in the temperature range of 227-250°C (figure 5.21). Discoloration and spontaneous bubbling occurred at 255°C indicating the commencement of decomposition.

Figure 5.21: HSM photographs of ADTCO\textsubscript{3}HCIN at (a) room temperature, (b) the melting temperature, (c) onset of decomposition and (d) completion of decomposition.

DSC analysis of ADTCO\textsubscript{3}HCIN was performed and produced a trace with a unique broad melting endotherm between 240 °C and 270°C peaking at 250.80°C, with an onset at 240°C (figure 5.22). Melting was preceded by a solvent loss indicated by a small bump at 185°C which was evident during the HSM analysis by the occurrence of bubbling.

Figure 5.22: DSC trace of ADTCO\textsubscript{3}HCIN.

THERMOGRAVIMETRIC ANALYSIS

The HSM and DSC analyses were supported by thermogravimetric analysis performed to further investigate the purity and composition of ADTCO\textsubscript{3}HCIN. The TGA trace (figure 5.23) showed a mass loss of 3.45% in the temperature range 30-200°C and thus, confirmed the presence of possible water molecules in the molecular structure since no solvent was used to prepare the sample.
FTIR ANALYSIS

Infrared spectroscopic analysis for ADTCO$_3$HCIN produced a distinct spectrum compared to that of ADTCO$_3$ and HCIN (figure 5.24). Different functional groups from both parent compounds are observed with various shifts, changes in intensity of bands and the appearance of new bands in the ADTCO$_3$HCIN spectrum. This therefore confirmed the existence of interactions (e.g. hydrogen bonds) between the two parent compounds (salt co-crystallisation).

The ADTCO$_3$HCIN salt co-crystal was identified by FTIR analysis since the majority of bands in the spectrum decreased in wave length. From figure 5.25 and table 5.6, it can be confirmed that the possible intermolecular interactions occurring are C=O⋯NH$_3^+$, H-O⋯NH or C=O⋯H-O. Firstly, 2162 cm$^{-1}$ band in the ADTCO$_3$HCIN spectrum is an indication that H-O⋯NH (NH$_3^+$) has occurred. Secondly, the loss of 3353 cm$^{-1}$ and 2576 cm$^{-1}$ of HCIN assigned to the O-H stretching vibration some important bands in the ADTCO$_3$HCIN spectrum is due to the interaction with NH (O-H⋯NH$_3^+$). And the presence of weak absorption bands at 2628 cm$^{-1}$ assigned to the NH$_3^+$ in the ADTCO$_3$HCIN spectrum which shifted from 2564 cm$^{-1}$ confirmed the interaction with OH (H-O⋯NH$_3^+$) and retention of salt characteristic by this new entity.
Figure 5.24: FTIR of ADTCO₃, ADTCO₃HCIN and HCIN.
Table 5.6: Important shifts in the ADTCO₃CIN spectrum.

<table>
<thead>
<tr>
<th>Groups</th>
<th>ADTCO₃</th>
<th>HCIN</th>
<th>ADTCO₃HCIN</th>
<th>Shifts</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₃⁺</td>
<td>2564 cm⁻¹</td>
<td>-</td>
<td>2628 cm⁻¹</td>
<td>63 cm⁻¹</td>
</tr>
<tr>
<td>C=O</td>
<td>1639 cm⁻¹, 1550 cm⁻¹</td>
<td>-</td>
<td>1638 cm⁻¹, 1547 cm⁻¹</td>
<td>1 cm⁻¹, 3 cm⁻¹</td>
</tr>
<tr>
<td>C–H</td>
<td>2910 cm⁻¹</td>
<td>-</td>
<td>2911 cm⁻¹</td>
<td>1 cm⁻¹</td>
</tr>
<tr>
<td>C–C</td>
<td>2267 cm⁻¹</td>
<td>-</td>
<td>2162 cm⁻¹</td>
<td>105 cm⁻¹</td>
</tr>
<tr>
<td>Aromatic</td>
<td>-</td>
<td>1601 cm⁻¹</td>
<td>1593 cm⁻¹</td>
<td>7 cm⁻¹</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1495 cm⁻¹</td>
<td>1503 cm⁻¹</td>
<td>8 cm⁻¹</td>
</tr>
</tbody>
</table>

Figure 5.25: The possible sites for interactions between ADTCO₃ and HCIN.

The effects of the above interactions is seen on C–H stretching vibration of the methyl (sp³ or sp²) at 2910 cm⁻¹ and 2852 cm⁻¹ which shifted slightly to 2912 cm⁻¹ and 2853 cm⁻¹ respectively. The 1639 cm⁻¹ bands of C=O in ADTCO₃ has shifted to 1638 cm⁻¹ in the ADTCO₃HCIN spectrum due to C=O⋯NH₃⁺ interactions. The 1601 cm⁻¹ and 1495 cm⁻¹ bands of the aryl in HCIN spectrum also shifted to 1593 cm⁻¹ and 1503 cm⁻¹ respectively. Furthermore, the changes in location of various bands observed in the fingerprint region of the ADTCO₃HCIN spectrum also confirmed salt co-crystal formation.

EXPERIMENTAL PXRD

The salt co-crystal ADTCO₃HCIN formation is confirmed in figure 5.26 at 2θ 6.7°, 8.1°, 10.2°, 15.3°, 16.5° and 19.43°. This was compared to both ADTCO₃ and HCIN PXRD patterns. Figure 5.27 illustrates the reproducibility of ADTCO₃HCIN salt co-crystal using other several solvents.
Figure 5.26: PXRD of ADTCO$_3$, HCIN and ADTCO$_3$HCIN.

Figure 5.27: PXRD pattern of ADTCO$_3$HCIN prepared by different solvent-assisted grinding systems.

**ADTCO$_3$SUC PREPARATION**

ADTCO$_3$SUC was prepared by neat grinding of ADTCO$_3$ (20 mg) with SUC (18 mg) in a 1:1 ratio for 30 minutes in a mortar using a pestle. Solvent drop-grinding with methanol, ethanol, chloroform, acetone, amyl alcohol, butan-2-ol, diethyl ether, 1,4-dioxan, ethyl acetate and propane-2-ol also reproduced the same product. A full characterisation was conducted using a combination of different analytical techniques.
Furthermore, the product was prepared by the co-precipitation technique as well. The equimolar amount of ADTCO$_3$ and SUC was separately dissolved in minimum volumes of methanol at approximately 10°C below the boiling point of the solvent. The two solutions were then mixed and the resultant solution was stirred for up to 15 minute using a magnetic bar, filtered (0.45 µm microfilter) and allowed to crystallise at room temperature. Large cubic crystals were harvested and fully analysed.

THERMAL ANALYSIS

HSM analysis was conducted at a constant rate of 10°C per minute, with the sample submerged in silicon oil to analyse the thermal behaviour of ADTCO$_3$SUC. HSM photographs are presented in figure 5.28. Phase transition was observed at around 140°C and the melting process occurred at 197°C which was immediately followed by spontaneous bubbling (decomposition). This analysis identified the existence of two different forms of this product.

![Figure 5.28: HSM photographs of ADTCO$_3$SUC recorded at (a) room temperature, (b) transition temperature to a 2nd form (metastable) (c) the melting temperature of the most stable form.](image)

The DSC analysis of ADTCO$_3$SUC showed a curve with two endotherms. The DSC curve is presented in figure 5.29. The first endotherm appears in the temperature range 135-144°C peaking at 140.67°C with an onset at 139.64°C (figure 5.29) establishing the phase transition [metastable (form II) to stable (form I)] and the second endotherm corresponding to the melting process appears between 180°C and 200°C, peaking at 195°C. A very good correlation between HSM and the DSC analysis resulted.

Succinic acid was reported to exhibit two different polymorphs, α-succinic acid which is triclinic and only stable above 137°C and β-succinic acid which is monoclinic 188°C.$^6,7$
Figure 5.29: DSC trace of ADTCO$_3$SUC.

**TGA ANALYSIS**

The HSM and DSC findings are additionally supported by thermogravimetric analysis performed to further investigate the purity and composition of ADTCO$_3$SUC co-crystal. The TGA trace showed a 0% mass loss in the temperature range 30-176°C (figure 5.30) and thus, confirmed the absence of water/solvent in the molecular structure of this compound. The mass loss step is observed in the melting and decomposition range.

Figure 5.30: TGA trace of ADTCO$_3$SUC.

**FTIR ANALYSIS**

Thermal analysis findings are additionally supported by FTIR analysis. The comparison of ADTCO$_3$SUC spectrum to that of ADTCO$_3$ and SUC is presented (figure 5.31).
Figure 5.31: FTIR of ADTCO$_3$, ADTCO$_3$SUC and SUC.
Chapter 5

The following table 5.7 presents the important shifts observed in the spectrum of ADTCO₃SUC.

Table 5.7: Functional groups compared in ADTCO₃, SUC and ADTCO₃SUC spectra.

<table>
<thead>
<tr>
<th>Groups</th>
<th>ADTCO₃</th>
<th>SUC</th>
<th>ADTCO₃SUC</th>
<th>Shifts</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₃⁺</td>
<td>2564 cm⁻¹</td>
<td>-</td>
<td>2629 cm⁻¹</td>
<td>64 cm⁻¹</td>
</tr>
<tr>
<td>C=O</td>
<td>1682 cm⁻¹</td>
<td>1690 cm⁻¹</td>
<td>7 cm⁻¹</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1550 cm⁻¹</td>
<td>-</td>
<td>1556 cm⁻¹</td>
<td>6 cm⁻¹</td>
</tr>
<tr>
<td></td>
<td>1639 cm⁻¹</td>
<td>-</td>
<td>1611 cm⁻¹</td>
<td>28 cm⁻¹</td>
</tr>
<tr>
<td>C-H</td>
<td>2910 cm⁻¹</td>
<td>-</td>
<td>2905 cm⁻¹</td>
<td>5 cm⁻¹</td>
</tr>
<tr>
<td></td>
<td>2629 cm⁻¹</td>
<td>2633 cm⁻¹</td>
<td>4 cm⁻¹</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1456 cm⁻¹</td>
<td>-</td>
<td>1450 cm⁻¹</td>
<td>6 cm⁻¹</td>
</tr>
<tr>
<td>C-O-H</td>
<td>-</td>
<td>1409 cm⁻¹</td>
<td>1401 cm⁻¹</td>
<td>7 cm⁻¹</td>
</tr>
<tr>
<td>O-H</td>
<td>-</td>
<td>2930 cm⁻¹</td>
<td>3078 cm⁻¹</td>
<td>148 cm⁻¹</td>
</tr>
</tbody>
</table>

Figure 5.32: The possible sites of interactions for ADTCO₃ and SUC.

Based on figure 5.32, the interactions O-H⋯NH₃⁺, O-H⋯C=O and C=O⋯NH₃⁺ were expected. Shifts of bands presented in table 5.7 clearly confirmed the existence of the interactions between ADTCO₃ and SUC functional groups. Figure 5.31 showed a loss of 2564 cm⁻¹ band assigned to the NH₃⁺ in ADTCO₃SUC spectrum, thus confirming the product ADTCO₃SUC as a co-crystal not as a salt. The ADTCO₃SUC spectrum displays a weak band at 3078 cm⁻¹ band assigned to the O-H of the carbonyl group (COOH), which as a result of O-H⋯NH₃⁺ interaction, has shifted from 2930 cm⁻¹.

The C-H vibrations at 2910 cm⁻¹ and 1456 cm⁻¹ in ADTCO₃ spectrum have shifted to 2905 cm⁻¹ and 1450 cm⁻¹ respectively.
Chapter 5

The 1682 cm$^{-1}$ band attributed to the C=O of SUC has shifted to 1690 cm$^{-1}$ in the ADTCO$_3$SUC spectrum as a result of the possible C=O⋯NH$_3^+$ interactions. All these changes confirmed the formation of the ADTCO$_3$SUC co-crystal.

PXRD ANALYSIS

The PXRD pattern in figure 5.33 is quite different from the PXRD patterns of the starting materials ADTCO$_3$ and SUC. In addition to thermal as well as spectroscopic analysis, this confirmed the co-crystallisation between the two parent compounds. The pattern also matches other patterns of the same product obtained from grinding with various solvent systems (figure 5.34) and the crystal resulting from co-precipitation. The most stable phase presented new intense peaks at 2θ = 15.33°, 18.1° and 19°. Weaker peaks identified at 2θ = 21.7°, 24°, 28.5°, 32.6° and 34.2° confirming the crystallinity of the produced entity.

![Figure 5.33: PXRD of ADTCO$_3$, SUC and ADTCO$_3$SUC.](image)

The same pattern was produced by PXRD analysis of ADTCO$_3$SUC samples prepared through grinding, assisted by a few drops of methanol, ethanol, chloroform, acetone, amyl alcohol, diethyl ether, ether acetate, butan-2-ene and propane-2-ol, butan-2-ol and 1,4-dioxan.
In conclusion, due to the HSM and DSC findings reported, a second form for ADTCO$_3$SUC was definitely identified. However, variable temperature PXRD together with the HSM and DSC analysis would have resulted in a more conclusive result.

**ADTCO$_3$LTTA**

**PREPARATION**

A stoichiometric amount of ADTCO$_3$ (20 mg) and LTTA (19.8 mg) in a 1:1 ratio was transferred to a motor and ground manually for 30 minutes using a pestle. A white powder was produced and kept in the airtight polytope vial for analysis. The same product was obtained from solvent evaporation with a 1:1 of both compounds solubilised (in separate vials) in methanol at 10°C below the b.p. of the solvent. The two solutions were then added together and magnetically stirred on a hot plate, filtered (0.45µm) and the resulting solution was allowed to crystallise at room temperature.

**THERMAL ANALYSIS**

**HSM and DSC ANALYSIS**

HSM analysis was performed with the sample submerged in silicon oil. The analysis showed bubbling at ~135°C and continued as the temperature increased until the melt occurred in the range of 197°C-210°C.
Chapter 5

Decomposition occurred immediately after melting which was observed by spontaneous bubbling and discoloration of the sample. HSM photographs recorded at different temperatures due to thermal events observed are presented in the figure 5.35.

Figure 5.35: HSM photographs of ADTCO$_3$LTTA at (a) ambient temperature (b) first change (bubbling), (c) melting and (d) decomposition.

DSC analysis of the parent compounds (figure 5.36) showed a melt in the temperature range of 171-174°C for L-tartaric acid and the melt of ADTCO$_3$ was observed in the range of 155-180°C. ADTCO$_3$LTTA showed two endothermic peaks with firstly a small endotherm appearing at 140.9°C, suggesting that some water was coming off since no solvent was used to prepare this product. Secondly, a broad endothermic peak at 206.1°C corresponding to the melt of the product was observed. Both parent compounds melting points were nowhere close to the melt of the product produced.

Figure 5.36: DSC traces of ADTCO$_3$, ADTCO$_3$LTTA and LTTA.

DSC and HSM results consistently presented a different melt compared to both parent compounds, thus confirming the new entity.
Chapter 5

TGA ANALYSIS

The TGA trace (figure 5.37) presented a mass loss of 5.25 % (n = 0.86) in the range of 30-203°C which confirmed the presence of solvent in the molecular structure of ADTCO₃LTTA. The second mass loss step is a result of the melt and immediate decomposition of the sample.

![TGA Trace of ADTCO₃LTTA](image)

**Figure 5.37: TGA trace of ADTCO₃LTTA.**

FTIR ANALYSIS

In order to qualitatively analyse various functional groups in ADTCO₃LTTA, FTIR spectrum was recorded and compared to the parent compounds, ADTCO₃ and LTTA as shown in figure 5.38. The absorption bands in the spectrum of ADTCO₃LTTA differ significantly to the other two spectra. Shifts and changes of band intensities were carefully studied since these would appear as a result of intermolecular interactions. Particular differences in the spectra are presented in table 5.8.

From the FTIR results; table 5.8 and figure 5.38, the possible N-H⋯O-H, N-H⋯C=O, O-H⋯C=O interactions occurred. The ADTCO₃LTTA spectrum (figure 5.38) showed a loss of 3328 cm⁻¹ and 3096 cm⁻¹ bands of LTTA ascribed to O-H of hydroxyl and COOH groups and 2564 assigned to NH₃⁺ vibration from ADTCO₃, suggesting that this was due to the interaction with H−O⋯NH₃⁺. Furthermore, the loss of NH₃⁺ also confirmed that ADTCO₃LTTA was synthesised as a co-crystal.
Figure 5.38: FTIR of ADTCO$_3$, ADTCO$_3$LT and LT.
Table 5.8: Some shifts of bands in ADTCO\textsubscript{3}LTTA spectrum.

<table>
<thead>
<tr>
<th>Groups</th>
<th>ADTCO\textsubscript{3}</th>
<th>LTTA</th>
<th>ADTCO\textsubscript{3}LTTA</th>
<th>Shifts</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH\textsubscript{3}\textsuperscript{+}</td>
<td>2852 cm\textsuperscript{-1}</td>
<td>-</td>
<td>weak</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1639 cm\textsuperscript{-1}</td>
<td>-</td>
<td>1661 cm\textsuperscript{-1}</td>
<td>21 cm\textsuperscript{-1}</td>
</tr>
<tr>
<td>C-H</td>
<td>2910 cm\textsuperscript{-1}</td>
<td>-</td>
<td>2903 cm\textsuperscript{-1}</td>
<td>6 cm\textsuperscript{-1}</td>
</tr>
<tr>
<td></td>
<td>1456 cm\textsuperscript{-1}</td>
<td>-</td>
<td>1450 cm\textsuperscript{-1}</td>
<td>6 cm\textsuperscript{-1}</td>
</tr>
<tr>
<td>O-H</td>
<td>-</td>
<td>3399 cm\textsuperscript{-1}</td>
<td>3387 cm\textsuperscript{-1}</td>
<td>11 cm\textsuperscript{-1}</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>3328 cm\textsuperscript{-1}</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>3096 cm\textsuperscript{-1}</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Figure 5.39: The possible sites of intermolecular interactions of ADTCO\textsubscript{3} and LTTA.

The overlapping bands around 3387 cm\textsuperscript{-1} was due to the moisture (water molecules) also detected by thermal analysis. The 2903 cm\textsuperscript{-1} and 2853 cm\textsuperscript{-1} bands attributed to the C-H sp\textsuperscript{3} and sp\textsuperscript{2} stretching vibration of cycloalkanes slightly shifted from 2910 cm\textsuperscript{-1} and 2852 cm\textsuperscript{-1}. The characteristic bands at 1661 cm\textsuperscript{-1} assigned to the C=N vibration and come from the proton transfer from nitrogen of NH\textsubscript{3}\textsuperscript{+} to the oxygen atom of the carbonyl group through hydrogen bonding. The C=O bond also shifted to the carbon-nitrogen.

Differences of ADTCO\textsubscript{3}LTTA to pure compounds were also observed at 1629 cm\textsuperscript{-1}, 1585 cm\textsuperscript{-1}, 1547 cm\textsuperscript{-1} and 1512 cm\textsuperscript{-1}. These were attributed to the C=O vibration of the carboxylate salt. Furthermore, shifting of weak bands in the region between 1450 cm\textsuperscript{-1}, 1426 cm\textsuperscript{-1} and 1366 cm\textsuperscript{-1} which were attributed to the C-H bending of methyl groups also confirmed the effect of interactions, hence the formation of a new product ADTCO\textsubscript{3}LTTA.

PXRD ANALYSIS

Remarkable differences of PXRD patterns of ADTCO\textsubscript{3}, LTTA and ADTCO\textsubscript{3}LTTA (figure 5.40) confirmed a definite solvated salt co-crystal as identified by both thermal and spectroscopic analyses. However, ADTCO\textsubscript{3}LTTA pattern does not show a strong
crystallinity characteristic. This could have been compromised during the grinding process while preparing the sample. New intense peaks appear at 2θ 14.69° and 18.1°. Other weak peaks were identified at 2θ 7.38°, 13.1°, 16.6° and 15.7°.

![Figure 5.40: PXRD patterns of ADTCO₃, LTTA and ADTCO₃LTTA.](image)

**ADTCO₃SA**

**PREPARATION**

Salicylic acid with its keratolytic character was used to co-crystallise ADTCO₃. Neat grinding was used to synthesise ADTCO₃SA. A stoichiometric amount of ADTCO₃ (20 mg) and SA (18 mg) in a 1:1 ratio was manually ground into a mortar using a pestle for 30 minutes. A white powder, ADTCO₃SA complex was reproduced by the same procedure assisted by a few drops of methanol, ethanol and 1,4-dioxan.

**THERMAL ANALYSIS**

**HSM and DSC analysis**

Ab initio HSM analysis was performed upon heating the sample at a constant rate of 10°C/min with the sample submerged in silicon oil to assess the possible inclusion water and/or solvent in the molecular structure of ADTCO₃SA. The first change was observed at 160°C, where crystallisation of the powdered sample commenced and the melt followed in the temperature range of 207-215°C (figure 5.41).
Figure 5.41: HSM photographs of ADTCO$_3$SA recorded at: (a) room temperature, (b) crystallisation, (c) the melting temperature.

DSC analysis produced a trace with the unique sharp endotherm peaking at 211°C corresponding to the melt of ADTCO$_3$SA and an onset at 208.56°C. Decomposition occurred immediately after melting (figure 5.42).

Figure 5.42: DSC trace of ADTCO$_3$SA.

A different melt was consistently reported by both HSM and DSC compared to both parent compounds, thus confirming the formation of ADTCO$_3$SA.

TGA ANALYSIS

The TGA trace showed a 0% mass loss in the temperature range 30–205°C, thus confirming the absence of solvent in the molecular structure of this compound. TGA further confirmed that the melt proceeded around 208–212°C followed immediately by decomposition.
FTIR ANALYSIS

FTIR analysis was performed for the parent compounds and ADTCO$_3$SA, the prepared salt co-crystal. FTIR spectra are presented in figure 5.43. ADTCO$_3$SA was identified by FTIR spectrum showing differences at various regions of which some are shown in table 5.9. These changes are an indication of new bonds and formation of the new chemical entity, the ADTCO$_3$SA salt co-crystal.

Noticeable shifts in the ADTCO$_3$SA spectrum confirmed the existence of intermolecular interactions between ADTCO$_3$ and SA, thus confirming the new entity. The possible interactions according to figure 5.44 would be NH$_3^+$⋯C=O, NH$_3^+$⋯O-H and O-H⋯O-H.

Initially, the presence of NH$_3^+$ bands in the ADTCO$_3$SA spectrum confirmed that the product ADTCO$_3$SA retained the salt characteristic. The loss of characteristic absorption bands at 3231 cm$^{-1}$ and 1654 cm$^{-1}$ assigned to O-H and C=O of the carbonyl group (COOH) in the ADTCO$_3$SA spectrum is an indication that a new salt co-crystal has formed. This loss was probably due to either NH$_3^+$⋯O-H of the hydroxyl group and/or NH$_3^+$⋯C=O of the carbonyl (–COOH) group.

These interactions also affected the C-H vibrations of the adamantane ring methyl slightly shifted from 2910 and 2854 in the ADTCO$_3$ spectrum to 2909 cm$^{-1}$ and 2854 cm$^{-1}$ in the ADTCO$_3$SA spectrum. The 1659 cm$^{-1}$ band assigned to C=O vibration of SA carbonyl group (-COOH) disappeared as a result of its possible interaction with NH$_3^+$. The C=O of the ADTCO$_3$ carbonate group CO$_3^{2-}$ shifted slightly to 1638 cm$^{-1}$. Furthermore, various displacements observed in the fingerprint region of ADTCO$_3$SA spectrum justified the formation of the salt co-crystal.
Figure 5.43: FTIR of ADTCO$_3$, ADTCO$_3$SA and SA.
Table 5.9: Some important changes and shifts observed in ADTCO$_3$SA spectrum.

<table>
<thead>
<tr>
<th>Groups</th>
<th>ADTCO$_3$</th>
<th>SA</th>
<th>ADTCO$_3$SA</th>
<th>Shifts</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH$_3^+$</td>
<td>2564 cm$^{-1}$</td>
<td>-</td>
<td>Disappeared</td>
<td>-</td>
</tr>
<tr>
<td>C=O (-COOH)</td>
<td>-</td>
<td>1654 cm$^{-1}$</td>
<td>Disappeared</td>
<td>-</td>
</tr>
<tr>
<td>C=O</td>
<td>1639 cm$^{-1}$</td>
<td>-</td>
<td>1638 cm$^{-1}$</td>
<td>1 cm$^{-1}$</td>
</tr>
<tr>
<td>C-H</td>
<td>2910 cm$^{-1}$</td>
<td>-</td>
<td>2909 cm$^{-1}$</td>
<td>1 cm$^{-1}$</td>
</tr>
<tr>
<td>2852 cm$^{-1}$</td>
<td>-</td>
<td>2854 cm$^{-1}$</td>
<td>2 cm$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>aryl</td>
<td>-</td>
<td>1579 cm$^{-1}$</td>
<td>1582 cm$^{-1}$</td>
<td>3 cm$^{-1}$</td>
</tr>
<tr>
<td>O-H</td>
<td>-</td>
<td>3231 cm$^{-1}$</td>
<td>Disappeared</td>
<td>-</td>
</tr>
</tbody>
</table>

The possible sites for intermolecular interactions are presented in the following figure (see next page):

Figure 5.44: The possible sites for ADTCO$_3$-SA interactions.

POWDER X-RAY DIFFRACTION (PXRD)

The PXRD pattern of ADTCO$_3$SA is presented in figure 5.45 and show differences at 7.5°, 14.4°, 15.4°, 15.58°, 16° and 21.1° 2θ values. These results support thermal and FTIR spectroscopic findings, confirming the formation of ADTCO$_3$SA as a new chemical entity, viz. a salt co-crystal.

The same pattern was observed by grinding the two parent compounds ADTCO$_3$ and SA with several solvents (figure 5.46).
ADTCO$_3$CA

**PREPARATION**

To synthesise ADTCO$_3$CA, a stoichiometric amount of ADTCO$_3$ (20 mg) and CA (27.7 mg) in a 1:1 ratio was neatly ground in a mortar using a pestle for 30 minutes. ADTCO$_3$CA was reproduced by grinding assisted by a few drops of methanol or ethyl acetate. A white crystalline powder was produced and kept in a polytop vial and further analysed using a combination of various analytical techniques.
Furthermore, the same material in single crystal form was produced by dissolving the same amount of ADTCO₃ and CA powder in minimum volumes of methanol separately at approximately 10°C below the b.p. of the solvent.

The two solutions were added together and the resultant mixture was stirred magnetically for 15 min, filtered (using a 0.45 µm filter) and allowed to crystallise at 20°C.

THERMAL ANALYSIS
HSM & DSC analysis

Thermal analysis by HSM was performed upon heating the sample at a constant rate of 10°C/min and the sample was submerged in silicon oil to investigate the purity of ADTCO₃CA. The melt occurred at 138°C and decomposition was characterised by discoloration and spontaneous bubbling immediately after the melt. Photographs of ADTCO₃CA are presented in figure 5.47. The melting temperature of ADTCO₃CA differed from that of ADTCO₃ and CA, thus confirming the formation of a new form, viz. ADTCO₃CA.

![HSM photographs of ADTCO₃CA at different temperatures](image)

**Figure 5.47:** HSM photographs of ADTCO₃CA at (a) room temperature (b) melting starting point (c) melting and (d) decomposition.

The HSM findings were additionally supported by DSC analysis where a generated curve shows the unique melt at 142.50°C and an onset at 137.24°C while ADTCO₃ melts at 170°C and CA at 155°C. The first endotherm in CA curve is due to water coming off since the former is a monohydrate drug. Decomposition comes immediately after melting (Figure 5.48). DSC results are consistent to HSM findings and, thus confirmed the formation of the complex.
**Figure 5.48: DSC trace of ADTCO$_3$CA.**

**TGA ANALYSIS**

The TGA showed a negligible mass loss of 0% in the temperature range 30-135°C.

**FTIR ANALYSIS**

FTIR is a very good technique to give an insight into the kind of interactions occurring between an API and a co-former. FTIR spectra of ADTCO$_3$, CA and ADTCO$_3$CA are presented in figure 5.49. Noticeable shifts, changes in intensity of bands and new bands are observed in ADTCO$_3$CA spectrum in comparison to ADTCO$_3$ and CA. This confirmed the existence of intermolecular interactions between the complementary functional groups of ADTCO$_3$ and CA as highlighted in figure 5.50, hence the formation of ADTCO$_3$CA salt co-crystal.

Based on table 5.10 and figure 5.50, the plausible intermolecular interactions are: C=O⋯NH$_3^+$, NH$_3^+$⋯O-H, NH$_3^+$⋯OH (-COOH), C=O⋯HO (H$_2$O) or NH$_3^+$⋯OH (H$_2$O).

Shifts in FTIR spectrum of ADTCO$_3$CA were observed as a result of the above interactions. Firstly, one can see that the salt characteristic of ADTCO$_3$ was lost since NH$_3^+$ bands completely disappeared in ADTCO$_3$CA spectrum and the appearance of 1981 cm$^{-1}$ band is an indication of NH⋯O-H neutral hydrogen bonding, thus formation of ADTCO$_3$CA co-crystal. Secondly, a small bump around 3500 cm$^{-1}$ attributed to OH was observed as a result of the possible interactions that have been mentioned.
Figure 5.49: FTIR of ADTCO$_3$CA, ADTCO$_3$ and CA respectively.
Table 5.10: Shifts in the ADTCO$_3$CA spectrum.

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<thead>
<tr>
<th>Groups</th>
<th>ADTCO$_3$</th>
<th>CA</th>
<th>ADTCO$_3$-CA</th>
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<tr>
<td>C-H</td>
<td>2852 cm$^{-1}$</td>
<td>-</td>
<td>2887 cm$^{-1}$</td>
<td>35 cm$^{-1}$</td>
</tr>
<tr>
<td></td>
<td>2910 cm$^{-1}$</td>
<td>-</td>
<td>2913 cm$^{-1}$</td>
<td>3 cm$^{-1}$</td>
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<tr>
<td>NH$_3^+$</td>
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<td>-</td>
<td>lost cm$^{-1}$</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>2544 cm$^{-1}$</td>
<td>2518 cm$^{-1}$</td>
<td>26 cm$^{-1}$</td>
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<td>1550 cm$^{-1}$</td>
<td>-</td>
<td>1559 cm$^{-1}$</td>
<td>8 cm$^{-1}$</td>
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<td>1721 cm$^{-1}$</td>
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<tr>
<td></td>
<td>-</td>
<td>1752 cm$^{-1}$</td>
<td>lost</td>
<td>-</td>
</tr>
<tr>
<td>O-H</td>
<td>-</td>
<td>3295 cm$^{-1}$</td>
<td>3150 cm$^{-1}$</td>
<td>144 cm$^{-1}$</td>
</tr>
</tbody>
</table>

Given a structure of CA with different O-H, C=O and COOH functional groups as well as a H$_2$O molecule, there is a very high probability of intermolecular interactions around the molecule.

![Figure 5.50: Possible sites for intermolecular between ADTCO$_3$ and CA.](image)

The characteristic absorption band at 3150 cm$^{-1}$ assigned to the O-H stretching vibration of the carbonyl group (-COOH) shifted from 3295 cm$^{-1}$ due to the interactions with NH$_3^+$ of ADTCO$_3$.

The 2913 cm$^{-1}$ and 2887 cm$^{-1}$ bands assigned to the C-H stretching vibration of the cycloalkanes shifted from 2910 cm$^{-1}$ and 2952 cm$^{-1}$ respectively. Furthermore, 2518 cm$^{-1}$ band in ADTCO$_3$CA spectrum assigned to OH of (-COOH) has shifted from 2544 cm$^{-1}$ band (present in CA spectrum) as a result of interaction suggested in figure 5.50.

Furthermore, 1706 cm$^{-1}$ and 1559 cm$^{-1}$ absorption bands that can be assigned to the C=O of the carbonyl functional group have shifted from 1721 cm$^{-1}$ (CA) and 1550 cm$^{-1}$ (ADTCO$_3$), suggesting the interaction with NH$_3^+$ of ADTCO$_3$ (C=O···H-N). All these changes and other different displacements in the fingerprint region of the spectrum confirmed the formation of ADTCO$_3$CA salt co-crystal.
Chapter 5

EXPERIMENTAL PXRD

To further support the previous analyses, experimental X-ray powder diffraction analysis provide us with a definite conclusion with regards to the formation of ADTICO\textsubscript{3}CA. Compared to both ADTICO\textsubscript{3} and CA, new intense peaks in ADTICO\textsubscript{3}CA spectrum appear at $6.9^\circ$, $17.35^\circ$, $23.3^\circ$, $24.8^\circ$, $26^\circ$ and $31^\circ 20$ values (figure 5.51).

![Figure 5.51: PXRD of CA, ADTICO\textsubscript{3}CA and ADTICO\textsubscript{3}.]

ADTICO\textsubscript{3}GLA

PREPARATION

To synthesize ADTICO\textsubscript{3}GLA, a grinding technique was performed. A stoichiometric amount of ADTICO\textsubscript{3} (20 mg) and GLA (19.4 mg) in a 1:1 ratio were manually ground in a mortar with pestle for a period of 30 minutes. The process was assisted by a few drops of chloroform. A white powder was obtained and fully characterised.

ADTICO\textsubscript{3}GLA in a crystal form was also produced by solvent evaporation method using propan-2-ol or methanol.

THERMAL ANALYSIS

HSM & DSC analysis

HSM analysis for ADTICO\textsubscript{3}GLA was conducted with the sample submerged in silicon oil and heat applied at a constant rate of 10°C per minute. Bubbling was observed as the solvent evaporated at 135°C and melting occurred at around 160°C. Decomposition was indicated by explosive bubbling immediately after the melt (figure 5.52). HSM
photographs recorded at different temperatures according to changes observed are presented below.

Figure 5.52: The HSM photographs of ADTCO\textsubscript{3}GLA taken at (a) room temperature, (b) bubbling of the sample, (c) melting and (d) decomposition.

Analysis by DSC of the sample produced a graph with two broad endotherms (figure 5.53). The first endotherm peaking at 139.17°C with an onset at 131.56°C was due to solvent loss. The second endotherm formed at 168°C with an onset at 161.92°C was accounted for as the melting point of the sample which was immediately followed by decomposition.

Figure 5.53: DSC trace of ADTCO\textsubscript{3}GLA.

HSM and DSC findings were further supported by TGA analysis performed to investigate the amount of solvent present in ADTCO\textsubscript{3}GLA. The TGA trace (figure 5.54) showed a 10.3% (n = 2.4) mass loss in the temperature range of 30-144°C, thus confirming that the product is a solvate.
FTIR ANALYSIS

Infrared spectroscopic analysis also produced a different spectrum compared to both host and guest compounds. Shifts and changes in band intensities of the parent compounds are a result of intermolecular interactions between ADTCO$_3$ and GLA as well as the solvent that is incorporated in the molecular structure of ADTCO$_3$GLA (figure 5.55).

Based on FTIR shifts presented in table 5.11 and figure 5.56, the most probable interactions would be; NH$_3^+$⋯NH$_2$, NH$_2$⋯O=C, NH$_3^+$⋯OH (-COOH), NH$_3^+$⋯C=O (-COOH) and C=O⋯HO. The decrease in intensity of NH$_3^+$ in ADTCO$_3$GLA spectrum suggests its implication into the above interactions. The loss of 3012 cm$^{-1}$ and 2739 cm$^{-1}$ assigned to the O-H functional group in ADTCO$_3$GLA spectrum is a result of their possible interactions with NH$_3^+$. Furthermore, shifting of -NH$_3^+$ function group suggests that there is an interaction with the carbonyl (-COO) of GA and retention of salt characteristic. In the ADTCO3 spectrum, bands at 1639, 1550 cm$^{-1}$ that can be assigned to C=O of the carbonate shifted to 1608 cm$^{-1}$ and 1529 cm$^{-1}$ in the ADTCO$_3$GLA spectrum, suggesting the interaction with OH of (-COOH). All these interactions confirmed the formation of ADTCO$_3$GLA as a solvated salt co-crystal.
Figure 5.55: FTIR spectra of ADTCO$_3$, ADTCO$_3$GLA and GLA.
Chapter 5

Table 5.11: Shifts in the ADTCO$_3$GLA spectrum.

<table>
<thead>
<tr>
<th>Groups</th>
<th>ADTCO$_3$</th>
<th>GLA</th>
<th>ADTCO$_3$GLA</th>
<th>Shifts</th>
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<tbody>
<tr>
<td>NH$_3^+$</td>
<td>2564 cm$^{-1}$</td>
<td>-</td>
<td>weak</td>
<td>-</td>
</tr>
<tr>
<td>C=O</td>
<td>1639 cm$^{-1}$</td>
<td>-</td>
<td>1608 cm$^{-1}$</td>
<td>29 cm$^{-1}$</td>
</tr>
<tr>
<td>-</td>
<td>1637 cm$^{-1}$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1550 cm$^{-1}$</td>
<td>-</td>
<td>1529 cm$^{-1}$</td>
<td>21 cm$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>NH$_2$</td>
<td>-</td>
<td>3012 cm$^{-1}$</td>
<td>lost</td>
<td>-</td>
</tr>
<tr>
<td>OH</td>
<td>-</td>
<td>2739 cm$^{-1}$</td>
<td>lost</td>
<td>-</td>
</tr>
</tbody>
</table>

The possible sites for intermolecular interaction between ADTCO$_3$ and GLA are shown in the figure 5.56.

![Figure 5.56: The possible sites for ADTCO$_3$–GLA interactions.](image)

EXPERIMENTAL PXRD

In figure 5.57 the ADTCO$_3$GLA pattern is different form the PXRD patterns of the starting compounds labelled ADTCO$_3$ and GLA. This is consistent with the results from thermal and spectroscopic analyses, which confirm that this is a new salt complex (solvated form).

New intense peaks along the ADTCO$_3$GLA curve appear at 2θ 15.4°, 15.86°, 16.2°, 16.56°, 18.5° values.

![Figure 5.57: PXRD of ADTCO$_3$, ADTCO$_3$GLA and GLA.](image)
ADTCO$_3$GA

PREPARATION

A minimum volume of solvent (methanol) at approximately 10°C below the b.p. of the solvent was used for the synthesis of ADTCO$_3$GA. Stoichiometric amount of ADTCO$_3$ and GA in a 2:1 ratio was separately dissolved, mixed, filtered (using a 0.45 μm microfilter) and allowed to crystallise at 20°C. Irregularly shaped crystals were produced and characterized.

THERMAL ANALYSIS

HSM and DSC ANALYSIS

The thermal analysis of ADTCO$_3$GA by HSM was conducted by heating the sample at a constant rate of 10°C/min, with the sample submerged in a drop of silicon oil. Changes of the sample on hot stage were carefully observed and photographs were recorded at different temperatures due to physical changes as shown in figure 5.58.

![Figure 5.58: HSM photographs of ADTCO$_3$GA recorded at (a) room temperature (b) first change (c) melting and (d) decomposition.](image)

The DSC trace for ADTCO$_3$GA (figure 5.59) indicates a melting point at 185°C with a single endotherm (180-187°C). This finding correlates well to the HSM observations.

Furthermore, the melt differs from that of both starting materials ADT CO$_3$ (170°C) and GA (98°C). The analysis suggests that this is due to the higher stability of the newly formed salt co-crystal or strong intermolecular interactions created by the preparation process.
Figure 5.59: The DSC of ADTCO$_3$, GA and ADTCO$_3$GA.

The TGA trace (figure 5.60) shows a negligible mass loss of 0.68% (n~1) in the temperature range of 30-165°C and thus confirms the absence of a solvent in the molecular structure of this complex. The significant mass loss step is observed from the onset of melting to the decomposition.

Figure 5.60: TGA trace of ADTCO$_3$GA co-crystal.

FTIR SPECTROSCOPIC ANALYSIS

FTIR analysis produced a unique spectrum in comparison to that of the parent compounds ADTCO$_3$ and GA. Shifts and changes in intensity (figure 5.61) are seen in the spectrum of ADTCO$_3$GA, providing evidence of the intermolecular as well as the formation of the new chemical ADTCO$_3$GA. Table 5.17 presents the important shifts observed in ADTCO$_3$GA spectrum.
Figure 5.61: FTIR spectra from ADTCO$_3$, ADTCO$_3$GA and GA.
Table 5.12: Shifts observed in the ADTCO$_3$GA spectrum.

<table>
<thead>
<tr>
<th>Groups</th>
<th>ADTCO$_3$</th>
<th>GA</th>
<th>ADTCO$_3$GA</th>
<th>Shifts</th>
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<tbody>
<tr>
<td>NH$_3^+$</td>
<td>2564 cm$^{-1}$</td>
<td>-</td>
<td>2566 cm$^{-1}$</td>
<td>2 cm$^{-1}$</td>
</tr>
<tr>
<td>C=O$^-$</td>
<td>1639 cm$^{-1}$</td>
<td>-</td>
<td>1614 cm$^{-1}$</td>
<td>24 cm$^{-1}$</td>
</tr>
<tr>
<td>C=O</td>
<td>1550 cm$^{-1}$</td>
<td>-</td>
<td>1546 cm$^{-1}$</td>
<td>4.7 cm$^{-1}$</td>
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<tr>
<td>O-H...N</td>
<td>-</td>
<td>1690 cm$^{-1}$</td>
<td>1714 cm$^{-1}$</td>
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</tr>
<tr>
<td>O-H</td>
<td>-</td>
<td>2953 cm$^{-1}$</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>3010 cm$^{-1}$</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Figure 5.62: The possible binding sites for intermolecular interactions between ADTCO$_3$GA.

Based on FTIR shifts presented in table 5.12 and figure 5.62, the most probable interactions would be; NH$_3^+$···OH (-COOH), NH$_3^+$···C=O (-COOH) and C=O···HO.

The presence on NH$_3^+$ at 2566 cm$^{-1}$ showed that the salt characteristic of ADTCO$_3$ is retained in the product and shifting of this function group suggested that there is an interaction with the carbonyl (-COOH) of GA. Furthermore, the loss of 3010 cm$^{-1}$ and 2953 cm$^{-1}$ bands assigned to OH of the carbonyl group (COOH) of GA is a result of possible interactions with NH$_3^+$.

The appearance of 1930 cm$^{-1}$ band in ADTCO$_3$GA spectrum is an indication of the existence of NH···OH neutral hydrogen bonding.

Bands at 1639 cm$^{-1}$ and 1550 cm$^{-1}$ assigned to C=O of the carbonate in the ADTCO$_3$ spectrum shifted to 1614 cm$^{-1}$ and 1546 cm$^{-1}$ in the ADTCO$_3$GA spectrum; suggesting the interaction with OH of (-COOH). The C=O (-COOH) band at 1690 cm$^{-1}$ also shifted to 1714 cm$^{-1}$. All these changes and shifts confirmed the formation of ADTCO$_3$GA as a salt co-crystal.
EXPERIMENTAL PXRD

PXRD analysis of ADTCO$_3$GA pattern supports the results from the previous analyses with regards to the co-crystal formation. The pattern shows co-crystallisation occurring between the two parent compounds, with most of peaks disappearing from the parent compounds (figure 5.63). The new intense peaks appear at 13.1°, 14.6°, 16.64° and 18° 2θ values.

Figure 5.63: PXRD of ADTCO$_3$GA, GA and ADTCO$_3$. 

REFERENCES


CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

The present study was undertaken to investigate the possible impact of polymorphism and co-crystallisation on the physicochemical properties of bis(adamantan-1-aminium) carbonate (ADTCO$_3$).

Initially, ADTCO$_3$ was unexpectedly produced in an attempt to convert 1-adamantanamine hydrochloride (ADTHCl) into the free base (ADT) and was fully characterised. Then, nineteen supramolecular derivatised forms were synthesised of which four were identified as polymorphic forms of ADTCO$_3$ and fifteen salt co-crystals forms were synthesised and characterised.

CONVERSION OF ADTHCl TO ADTCO$_3$

1-adamantanamine (ADT free base), the antiviral and anti-parkinsonian agent was the desired product of the conversion process. However, even though the conversion procedure was correct, ADTCO$_3$ was produced instead due to the ability of the primary amine to absorb carbon dioxide (CO$_2$) from the atmosphere. This changed the focus of the research to investigate different aspects of supramolecular chemistry with regard to polymorphism and salt co-crystallisation of the converted ADTCO$_3$.

The converted ADTCO$_3$ was analysed by DSC and PXRD and the results were similar to what has been reported in the literature. Structure elucidation was not included in this study since all the information about the ADTCO$_3$ structure had already been reported by Nowakowska et al.

Thermal analysis presented a ADTCO$_3$ melt in the temperature range (150-175°C) which correspond to the melting range reported. All other analytical data such as PXRD and basic single unity cell dimension analysis by X-ray diffraction were similar to the reported data. ADTCO$_3$ was then selected for further studies.

POLYMORPHISM

Polymorphism is a common phenomenon studied during pharmaceutical preformulation studies.

It should be noted that from previous literature searches, no study on solid state characterisation of neither ADT nor ADTCO$_3$ had been reported at the time of writing this
thesis. Four different polymorphic forms of ADTCO$_3$ were identified during the polymorphism study.

By recrystallization from methanol, ADTCO$_3$ presented a single crystal (Form I) having the same melt as the starting material, Form II was obtained from a binary mixture of methanol-chloroform (1:1), Form III from a binary mixture of methanol and ethyl acetate (1:1) and Form IV was obtained from ethanol solubilisation.

Forms I, II, III and IV were isolated as crystals and characterised by combination of several analytical techniques including DSC, HSM, TGA, FTIR and PXRD. Thermal, spectroscopic and PXRD analysis showed that form I matches the originally converted material. In addition, Form II, III and IV showed different melting points. Form I melts at 170°C and was considered as least stable while form IV with a melt of 203°C was considered the most stable thermodynamically. Form II and III showed a melt at 185°C and 193°C, respectively.

These melting temperatures differ significantly and together with PXRD patterns were sufficient to confirm polymorphism of ADTCO$_3$.

**SALT CO-CRYSTALLISATION**

Making co-crystals of ADT with selected co-crystal formers from the GRAS list was one of the objectives of this study. Introduction of the unexpected ADTCO$_3$ led to salt co-crystallisation studies. Ten co-formers and salt co-crystals were synthesised by co-precipitation, neat grinding and solvent assisted grinding. Fifteen salt co-crystals were synthesised and analysed by HSM, DSC, TGA, FTIR and PXRD analysis.

FTIR results presented the intermolecular interactions highlighting the significance of the hydrogen bond interactions during non-covalent bonding. Not only were the products seen to interact intermolecularly but together with the PXRD results, it was clearly evident that molecules were packing in different ordered systems and co-crystals were indeed different to their parent compounds.

The stability of each polymorph and co-crystal was investigated by DSC and TGA analysis. Degradation of a compound generally occurs after its melt barring no form change occurs during the melting process. Degradation would then commence immediately after the melt of the most stable form.
Chapter 6

RECOMMENDATIONS

First of all, it would be of interest to fill in the gaps in the current study. Physicochemical characterisation of all supramolecular forms synthesised during the present study is an important step to be further supported. Therefore, we recommend that future work should include solubility studies, dissolution testing and the study of the antiviral activity on all synthesised products. Findings can then be compared to the untreated compounds: amantadine hydrochloride and 1-adamantanamine.

Hydrogen bonding is an important factor for molecular co-crystallization, and played a significant role in the co-crystallisation of the different synthesised products. Structure elucidation by single X-ray diffraction and refinements would be of interest to further study the molecular interactions and packing arrangement within the co-crystals.

Primary amines are sustainable to capture CO₂ in the atmosphere. Therefore, performing any conversion to the free base and recrystallization procedure must be done under nitrogen flow to obtain the free base of amantadine. However, alternative methods may also include protecting the amine functional group from air. A typical example of this is sublimation. Sublimating ADTCO₃ may generate the glassy form of amantadine free base.

In terms of preparative techniques, solvent screening is a useful tool when studying the impact of supramolecular chemistry on physicochemical properties of a drug substance since the formation of single crystals by recrystallization offers an opportunity to further study the structure of the new products. However, the grinding method would be an easy and a preferable technique to synthesise salt co-crystals especially for those with a limited solubility. Therefore, a combination of these techniques will lead to more results and will provide vital information on these structures.

Investigation of polymorphism phenomenon in a compound is of utmost importance since different polymorphs of the same drug impart different physicochemical, kinetic and pharmaceutical properties. Therefore, apart from solubility study, dissolution test and antiviral activity, selection on the best polymorph and co-former is recommended.

An outstanding knowledge of the active pharmaceutical ingredient and excipients is very important for the prediction of the expected results. A typical example is that of functional groups and their complementarity in the prediction of intermolecular interactions in the
product. The understanding of a compounds’ molecular reactivity is a key factor to protect it from any degradation caused by temperature, light, air etc and identifying the best processing method. Therefore, stability studies under different conditions also need to be carried out.

REFERENCES


Appendix

Appendix A

Appendix A presents a list of all experiments for the production of ADTCO₃ polymorphic forms.

Recrystallization outcomes

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</tr>
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<table>
<thead>
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### Appendix

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**Method 4: Vapour diffusion**

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Appendix

Appendix B

Appendix B presents a range of experiments conducted for the preparation of salt complexes including (co-crystals and solvates).

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**Method 2: Crystallisation from the binary solvent mixture**

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### Method 3: (a): Neat grinding

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### Method 4: Solvent assisted grinding

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