Formulation of a fast-acting ibuprofen suspension by using nicotinamide as hydrotropic agent-application of DSC, spectroscopy and microscopy in assessment of the type of interaction

Lalit Mohan Oberoi
The University of Toledo

Follow this and additional works at: http://utdr.utoledo.edu/theses-dissertations

Recommended Citation
Oberoi, Lalit Mohan, "Formulation of a fast-acting ibuprofen suspension by using nicotinamide as hydrotropic agent-application of DSC, spectroscopy and microscopy in assessment of the type of interaction" (2004). Theses and Dissertations. 1530.
http://utdr.utoledo.edu/theses-dissertations/1530

This Thesis is brought to you for free and open access by The University of Toledo Digital Repository. It has been accepted for inclusion in Theses and Dissertations by an authorized administrator of The University of Toledo Digital Repository. For more information, please see the repository’s About page.
A Thesis

entitled

Formulation of a fast-acting ibuprofen suspension by using nicotinamide as hydrotropic agent - application of DSC, spectroscopy and microscopy in assessment of the type of interaction

By

Lalit M. Oberoi

Submitted as partial fulfillment of the requirements for the Master of Science Degree in Pharmaceutical Sciences
(Industrial Pharmacy Option)

Kenneth S. Alexander, Ph.D.
(Thesis and Research Advisor)

Steven M. Peseckis, Ph.D.
(Thesis Committee)

Steven Martin, Pharm.D.
(Thesis Committee)

Graduate School

The University of Toledo

May 2004
The purpose of this study was to examine the nature of the solid-state interaction between ibuprofen and nicotinamide using thermal, spectroscopic and microscopic techniques. A secondary objective was to determine the solubility enhancement achieved by using nicotinamide as a hydrotropic agent and to develop a suitable efficacious formulation. A suspension was found to be the suitable choice for product formulation since it provided the required concentration of the hydrotropic agent to be effective as a solubilizer. Ibuprofen-nicotinamide binary mixtures were prepared by a solvent evaporation method. Differential Scanning Calorimetry (DSC) was used to investigate the stoichiometry of the possible complex between the drug and the carrier. A sharp single endotherm was
observed between the melting endotherms of the individual components at a composition of 60% ibuprofen and 40% nicotinamide by weight which is approximately a 1:1 molar ratio of the two components. Lowering of the melting point of the drug in its complexed state predicted greater solubility for the complex. A number of spectroscopic techniques such as Ultraviolet-Visible spectroscopy (UV-Vis), Fourier transform infrared spectroscopy (FTIR), Proton Nuclear Magnetic Resonance imaging (NMR) and Powder X-ray diffraction (PXRD) were used to investigate the type of interaction between the two components in the solid state as well as in the liquid state. Light microscopy was used to observe changes with regard to particle size and crystal habit. It was concluded that the interaction which occurred was physical in nature and too weak to sustain the integrity of the complex in the liquid state. The HPLC studies were performed to calculate the solubility enhancement for the complex in solution as well as in suspensions. It was observed that the solubility enhancement increased with increasing concentration of nicotinamide. The solubility of ibuprofen was enhanced by 51 times in the solution when the concentration of nicotinamide was 8 mg/ml. In a similar manner the solubility was enhanced by 62 times in the suspension when the concentration of nicotinamide was 13.3 mg/ml.
ACKNOWLEDGEMENTS

First and foremost, I want to thank Dr. Alexander for giving me an opportunity to do independent research. The most important thing I learnt from him is to think independently and have creativity in my work. As a good mentor he provided constructive criticism as well as encouragement as and when required. I want to thank Dr. Alan Riga for his support and enthusiasm all along the ups and downs of research. I also want to thank Dr. Curtis Black for all I learnt during the TA labs. I wish to express my gratefulness for Dr. Jeffrey Dunn for all the knowledge that he provided regarding thermal analysis. Thank you Dr. Steven Martin and Dr. Steven Peseckis for agreeing to be on my committee and taking time out from your busy schedules to read my thesis.

A special word of thanks for Dr. Timothy Mueser and Dr. Pannee Burckel who helped me with the microscope and PXRD respectively. I am indebted to Rahul Khupse and Vinu Johnson for the invaluable help that they provided with the instruments. I couldn’t have finished my work on time without their help. I am also very grateful to Pallabi Mitra for training me on the HPLC which eventually turned out to be the most important study of my project. Thank you Paroma (PP) for helping me out with the PXRD studies and lending me a listening ear when I needed.

I want to thank all my seniors especially Sumit, Pradyot and Rajesh from whom I learned a lot of things which are helpful in life. I thank all my classmates and wonderful juniors for making my stay here at Toledo a memorable experience. Thank you Marcia for all the encouragement that you provided all along in good and bad days.

In the end I would like to thank my parents and sister Ritika for being so caring and understanding. Whatever I am today is because of them.
Table of Contents

Abstract ii
Acknowledgements iii
Table of Contents v
List of Tables x
List of Figures xi

1. INTRODUCTION 1

2. HYDROTROPY 5

2.1 Introduction 5

2.2 Models 8

  2.2a Maximum aromatic overlap model 8

  2.2b Two-Class models 10

  2.2c Partition coefficient models 12

  2.2d The π-donor and π-acceptor orbital overlap model 13

2.3 Overview 13

3. SOLID-STATE INTERACTION STUDIES 16

3.1 Differential Scanning Calorimetry 16

  3.1a Introduction 16

  3.1b Instrumentation 18

  3.1c Pans and crucibles 20

  3.1d Sample size 21

  3.1e Calibration 21
3.1f Applications 22

3.2 X-ray Powder Diffraction Spectroscopy 23

3.2a Introduction 23

3.2b Instrumentation 25

3.2c Applications 26

3.3 Fourier transform infrared spectroscopy 29

3.3a Introduction 29

3.3b Instrumentation 33

3.3c Application 35

3.4 Polarized light microscopy 37

3.4a Introduction 37

3.4b Instrumentation 39

3.4c Applications 40

4. SOLUTION-STATE STUDIES

4.1 High performance liquid chromatography (HPLC) 43

4.1a Introduction 43

4.1b Basic concepts of HPLC 44

4.1c Instrumentation 46

4.1d Applications 49

4.2 Nuclear magnetic resonance spectroscopy 50

4.2a Introduction 50

4.2b Instrumentation 54

4.2c Application 55
4.3  Ultraviolet/Visible absorption spectroscopy 57
   4.3a  Introduction 57
   4.3b  Instrumentation 59
   4.3c  Applications 60

5.  FORMULATION INGREDIENTS – IBUPROFEN-NICOTINAMIDE COMPLEX AND EXCIPIENTS

5.1  Ibuprofen 62
   5.1a  Introduction 62
   5.1b  Structural formula 62
   5.1c  Physicochemical properties 62
   5.1d  Mechanism of action and use 63
   5.1e  Available dosage forms and dose 64

5.2  Nicotinamide 64
   5.2a  Introduction 64
   5.2b  Structural formula 64
   5.2c  Physicochemical properties 65
   5.2d  Mechanism of action and use 65
   5.2e  Available dosage forms 65

5.3  Carboxymethylcellulose sodium 65

5.4  Veegum 66

5.5  Sorbitol Solution 70% 67

5.6  Saccharin Sodium 68

5.7  Disodium edetate (Disodium EDTA) 69
6. MATERIALS AND METHODS

6.1 Reagents and Chemicals

6.2 Methods and Instruments

6.2a Solvent evaporation method

6.2b Differential scanning Calorimetry studies

6.2c Powder x-ray diffraction (PXRD) studies

6.2d Fourier transform infrared (FTIR) studies

6.2e Microscopic studies

6.2f Ultraviolet-Visible spectroscopy (UV-Vis) studies

6.2g Nuclear magnetic resonance spectroscopy (NMR) studies

6.2h High performance liquid chromatography (HPLC) studies

6.2i Preparation of ibuprofen suspension

6.2j Sample preparation for the analysis of ibuprofen suspensions

7. RESULTS AND DISCUSSIONS

7.1 DSC Studies

7.2 UV-Vis Spectroscopy

7.3 FTIR Spectroscopy

7.4 $^1$H NMR Spectroscopy

7.5 Powder X-Ray Diffraction Studies

7.6 Microscopic Studies

7.7 High Performance Liquid Chromatography Studies

7.7a Solubility comparison of saturated solution of ibuprofen and complexed ibuprofen
7.7b Comparison of the extent of solubilized ibuprofen in various suspensions 88

8. CONCLUSIONS

Recommendations for future work 93
List of Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Calibration materials for DTA and DSC</td>
</tr>
<tr>
<td>6.1</td>
<td>Formula for the preparation of Ibuprofen suspension</td>
</tr>
<tr>
<td>7.1</td>
<td>Results from UV scans</td>
</tr>
<tr>
<td>7.2</td>
<td>Summary of the FTIR group frequencies</td>
</tr>
<tr>
<td>7.3</td>
<td>Comparison of the solubility of pure Ibuprofen and complexed Ibuprofen</td>
</tr>
<tr>
<td>7.4</td>
<td>Comparison of the solubility of Ibuprofen in various suspensions studies</td>
</tr>
<tr>
<td>7.5</td>
<td>Comparison of Ibuprofen solubility in various suspensions as compared to saturated Ibuprofen solution</td>
</tr>
<tr>
<td>7.6</td>
<td>Characteristics of various suspensions studied</td>
</tr>
</tbody>
</table>
# List of Figures

<table>
<thead>
<tr>
<th>Figures</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Molecular structure for nicotinamide</td>
<td>3</td>
</tr>
<tr>
<td>1.2</td>
<td>Molecular structure for ibuprofen</td>
<td>4</td>
</tr>
<tr>
<td>2.1</td>
<td>The graphical depiction of the solubility curves to differentiate between</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>hydrotropy and complexation</td>
<td></td>
</tr>
<tr>
<td>2.2</td>
<td>Hypothetical benzoic acid complexes: (a) in water; (b) in benzene or in a</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>cyclodextrin cavity</td>
<td></td>
</tr>
<tr>
<td>2.3</td>
<td>General category of Class A compounds</td>
<td>10</td>
</tr>
<tr>
<td>2.4</td>
<td>Examples of Class A compounds</td>
<td>11</td>
</tr>
<tr>
<td>2.5</td>
<td>General category of Class B compounds</td>
<td>11</td>
</tr>
<tr>
<td>3.1</td>
<td>Representation of the results of DSC scan of a typical polymeric material</td>
<td>17</td>
</tr>
<tr>
<td>3.2</td>
<td>Schematic of DSC sample holder and furnaces for power compensation DSC</td>
<td>18</td>
</tr>
<tr>
<td>3.3</td>
<td>Schematic of a heat flux DSC</td>
<td>20</td>
</tr>
<tr>
<td>3.4</td>
<td>Resultant reflection of x-rays by single lattice array (above) and two</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>different, intermeshed lattice arrays (below)</td>
<td></td>
</tr>
<tr>
<td>3.5</td>
<td>X-ray monochromator and detector</td>
<td>26</td>
</tr>
<tr>
<td>3.6</td>
<td>A segment of the electromagnetic spectrum comparing infrared energy to other</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>forms of radiation</td>
<td></td>
</tr>
<tr>
<td>3.7</td>
<td>Types of molecular vibrations; + indicates motion from the plane towards the</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>reader; - indicates motion from the reader</td>
<td></td>
</tr>
<tr>
<td>3.8</td>
<td>Schematic of a double-beam spectrophotometer</td>
<td>35</td>
</tr>
<tr>
<td>3.9</td>
<td>Configuration of polarized light microscope</td>
<td>40</td>
</tr>
</tbody>
</table>
3.10 Polarized light Photomicrographs of Ranitidine Hydrochloride Form I (top) and Form II (bottom) 41

4.1 Resolution of two peaks at Rs values of 0.75, 1.0, and 1.5, respectively 45

4.2 Schematic of a modern HPLC system 47

4.3 The effect of external magnetic field on spin quantum state of nuclei 52

4.4 The effect of gyromagnetic ratio and external magnetic field on larmor frequency 53

4.5 Block diagram of various components of FT-NMR spectrometer 55

4.6 A hypothetical energy diagram depicting the energy transitions 58

4.7 Schematic of a typical manual double-beam spectrophotometer for the ultraviolet/visible region 60

5.1 Structure of ibuprofen 62

5.2 Structure of nicotinamide 64

5.3 Structure of sodium saccharin 68

7.1 Overlay of the DSC scans for the ibuprofen and nicotinamide binary mixtures 77

7.2 Overlay of the wavelength scans for nicotinamide, ibuprofen and ibuprofen-nicotinamide complex 78

7.3 FTIR spectrum for ibuprofen 79

7.4 FTIR spectrum for nicotinamide 79

7.5 FTIR spectrum for complex 80

7.6 $^1$H NMR imaging for ibuprofen 81

7.7 $^1$H NMR imaging for nicotinamide 81
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.8</td>
<td>$^1$H NMR imaging for the complex</td>
<td>82</td>
</tr>
<tr>
<td>7.9</td>
<td>The overlay of the PXRD-patterns of ibuprofen, nicotinamide and the complex</td>
<td>83</td>
</tr>
<tr>
<td>7.10</td>
<td>The microscopic picture for ibuprofen</td>
<td>84</td>
</tr>
<tr>
<td>7.11</td>
<td>The microscopic picture for nicotinamide</td>
<td>84</td>
</tr>
<tr>
<td>7.12</td>
<td>The microscopic picture for the complex</td>
<td>84</td>
</tr>
<tr>
<td>7.13</td>
<td>The microscopic picture of ibuprofen in polarized light</td>
<td>85</td>
</tr>
<tr>
<td>7.14</td>
<td>The microscopic picture of nicotinamide in polarized light</td>
<td>85</td>
</tr>
<tr>
<td>7.15</td>
<td>The microscopic picture of complex in polarized light</td>
<td>85</td>
</tr>
<tr>
<td>7.16</td>
<td>HPLC chromatogram showing the peak separation and solubility enhancement for ibuprofen (A) in the presence of nicotinamide (B)</td>
<td>86</td>
</tr>
<tr>
<td>7.17</td>
<td>HPLC chromatogram showing the peak for ibuprofen (A) obtained by injecting saturated ibuprofen solution</td>
<td>87</td>
</tr>
</tbody>
</table>
CHAPTER ONE
INTRODUCTION

Poor solubility of the drugs is a problem that still confounds the pharmaceutical scientist and is still the biggest reason for the failure of new chemical entities (NCE’s). There have been a number of methodologies that have been used to improve the bioavailability by enhancing the solubility of these drugs. Some of these methods include size reduction, chemical modification, pH modification, use of surfactants, cosolvents and hydrotropes and use of amorphous and polymorphic forms\(^{1-3}\). The majority of the new highly potent drugs that are being discovered using the modern techniques have very low water solubility. An alternative solution to this problem is chemical modification or complexation, which increases the solubility but often, reduces the pharmacological activity \(^{4-5}\). In the present work hydrotropy has been used to increase the solubility of ibuprofen, and nicotinamide has been selected as the suitable hydrotrope.

The concept of hydrotropy was first introduced in 1916 by Neuberg\(^{6}\). According to his definition hydrotropes are metal salts of organic acids, which at fairly high concentration increase the solubility of poorly water soluble compounds. Saleh et al\(^{7}\) in 1985 extended the definition of a hydrotrope and said that it can be cationic, anionic or a neutral molecule provided it has a hydrophobic as well as a hydrophilic group. The mechanism of hydrotropy is not understood very clearly, but it has been found to be very effective in some cases where multi-fold solubility enhancement has been shown to
occur. A 36-fold solubility increase has been shown for Riboflavin at 2 M concentration of nicotinamide \cite{8}. In this work also, a 45-fold solubility enhancement has been observed at 0.1065 M (13.3mg/ml) concentration of nicotinamide in the suspension.

Most commonly used hydrotropic agents include sodium benzoate, sodium salicylate, urea, caffeine as well as nicotinamide and its analogues\cite{8-10}. Among these hydrotropes, nicotinamide or vitamin B₃, has two advantages which make it a very attractive agent when used as a hydrotrope; it has FDA approval status, and very low toxicity \cite{8}. Nicotinamide has been used to improve the solubility of anti-cancer, anti-tumor and anti-viral drugs, including nucleoside analogues, and antimitabolites\cite{9,10}.

The most commonly proposed and accepted mechanism of solubilization for nicotinamide is complexation which occurs through a $\pi$–donor $\pi$–acceptor mechanism \cite{10,11}. It has also been proposed that the stacking of the pyridine ring, due to its planarity, might promote the complexation\cite{12}. An alternate proposal by Kenley et al\cite{13} suggests that the hydrophobicity of ligands is more important in complexation than aromaticity. Further research is required to be done in this direction to reach any concrete conclusion, but self-association in solution is exhibited by all the hydrotropes\cite{3}. A pilot study has suggested that nicotinamide, if given in high enough doses, might be beneficial to some patients with osteoarthritis, granuloma annulare, Type 1 diabetes and cancer, although the mechanism of the putative activity is unknown\cite{14}. Nicotinamide overdose is not reported in the literature and there is an indication that the body is capable of tolerating high doses. Unlike nicotinic acid, nicotinamide does not cause flushing and has very rarely been associated with any diabetogenic effects\cite{14}. Liver damage is reported at doses of 10g/day or greater\cite{14}. 
In the present work an Ibuprofen suspension has been made which has 45 times more solubilized drug in the aqueous phase as compared to two of the most popular suspensions available in the market. Ibuprofen is a non-steroidal anti-inflammatory (NSAID) drug belonging to the class of propionic acid derivatives. It has been rated as the safest NSAID by the spontaneous adverse drug reaction reporting system in the U.K\textsuperscript{[15]}. The major mechanism for their anti-inflammatory action is by blocking prostaglandin generation. This is manifested by the inhibition of the enzyme cyclooxygenase. NSAIDs are also used as antipyretics since they block the action of pyrogens\textsuperscript{[15]}. It has been shown recently that it also acts by preventing the translocation of nuclear factor-κB (NF-κB) from the cytoplasm into the nucleus which further prevents the up-regulations of many pro-inflammatory genes like tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1 β)\textsuperscript{[16]}. Ibuprofen has already been recommended in the relief from pain associated with osteoarthritis, lumbago, headaches, menstrual cramps and muscular aches and pains\textsuperscript{[17,18]}. A review of the literature shows that it can also be used in post-operative dental pain and menstrual migraine\textsuperscript{[19,20]}. The literature also recommends Ibuprofen as the first line analgesic for the short term management of painful conditions.
because of the poor tolerability of aspirin and the potential risks of paracetamol overdose\textsuperscript{[21]}.  

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{ibuprofen_structure.png}
\caption{Molecular structure for ibuprofen}
\end{figure}

The primary objective of this study was to examine the nature of the solid-state interaction between ibuprofen and nicotinamide using thermal, spectroscopic and microscopic techniques. A secondary objective was to determine the usefulness of nicotinamide as a solubilizing agent and to develop a suitable efficacious formulation. A suspension was found to be the suitable choice for product formulation since it provided the required concentration of the hydrotropic agent to be effective as a solubilizer.
CHAPTER TWO
HYDROTROPY

2.1 Introduction

Hydrotropy is the nonstoichiometric solubilization of an insoluble solute by a partially water miscible aromatic solute— for example, solubilization of caffeine or anthracene by sodium benzoate. Hydrotropy, categorized as a complexation, micellization, or cosolvency is not well understood from a mechanistic point of view\cite{1}. The concept of hydrotropy was first introduced in 1916 by Neuberg\cite{2}. According to his definition hydrotropes are metal salts of organic acids, which at fairly high concentration increase the solubility of poorly water soluble compounds. Saleh et al\cite{3} in 1985 extended the definition of a hydrotrope and said that it can be cationic, anionic or a neutral molecule provided it has a hydrophobic as well as a hydrophilic group. Therefore, any aromatic solubilizing agent used in high concentration (i.e. in nonstoichiometric excess) can be termed a hydrotrope. Although the mechanism of hydrotropy is not understood very clearly, it has been found to be very effective in some cases where multi-fold solubility enhancement has been shown to occur. A 36-fold solubility increase has been shown for Riboflavin at 2 M concentration of nicotinamide\cite{4}. If these aromatic solubilizing agents are analogous to surfactants, then their aggregates are analogous to micelles. Some workers report a critical hydrotrope concentration, which is analogous to the critical micelle concentration for surfactants\cite{5}.
If the solubilizing molecules interact independently with the solute, hydrotropy is simply a nonstoichiometric complexation. Then, the difference between hydrotropy and complexation is largely semantic and deals with whether or not the solubilizing agent self-associates and whether or not there is definite stoichiometry. If the solubilization versus complexant curve is linear, there is a clear indication of a 1:1 complexation. However, if the curve is concave up, the characterization becomes more complicated. The figure below depicts the difference between the curves for hydrotropy and complexation. Based on solubility data, it is difficult to distinguish between a combination of 1:1 and 1:2 complexes that have low stability constants and a nonstoichiometric structure. The structure of the hydrotropes and the stacking ligands are identical and it is very difficult to differentiate between these two types of interactions.

![Hydrotropy vs 1:1 Complexation](image)

**Fig.2.1**: The graphical depiction of the solubility curves to differentiate between hydrotropy and complexation
The most reliable generalization regarding complexation and structure is that just about any aromatic molecule can stack to self-associate or form a complex with just about any other aromatic molecule in water. This is because aromatic molecules are rigid and planar and can significantly reduce their area of contact with water by forming stacked structures. For pairs of different molecules that form stacked complexes, it is not always meaningful to distinguish between the solubilizing agent and the solubilizate from the structural perspective. For example, sodium benzoate can be used to solubilize caffeine, and caffeine can be used to solubilize an alkyl benzoate. Usually the compound designated as the solubilizate is the less soluble of the two. The solute and the ligand are identical for self-associating molecules. If a pair of planar molecules can form a 1:1 stacked complex, it is likely that they can also form a 1:2 sandwich complex. Higher order complexes can form from the interaction of a monomer with an already formed complex or from the interaction of two 1:1 complexes. Other than having a planar aromatic region, there are no specific structural requirements for a complexing molecule or hydrotrope. Although every aromatic compound can interact to a certain extent with itself or any other aromatic compound, some combinations interact more strongly than others. Several generalizations and models have been developed to aid in predicting the dependence of complexation on chemical structure. Although none of them are totally satisfactory, they each offer some insight into a very complicated situation\cite{1}.

Common hydrotropic agents that are used include sodium benzoate, sodium salicylate, urea, caffeine as well as nicotinamide and its analogues\cite{4-6}. Among these hydrotropes, nicotinamide or vitamin B₃, has two advantages which make it a very attractive agent when used as a hydrotrope. It has FDA approval status and has a very
low toxicity\textsuperscript{[4]}. Nicotinamide has been used to improve the solubility of anti-cancer, anti-tumor and anti-viral drugs, including nucleoside analogues, and antimetabolites\textsuperscript{[6,7]}.

The most common proposed and accepted mechanism for solubilization with nicotinamide is complexation which occurs through a $\pi$ donor $\pi$ acceptor mechanism\textsuperscript{[7,8]}. It has also been proposed that the stacking of the pyridine ring, due to its planarity, might promote the complexation\textsuperscript{[9]}. Although there are a number of models that are offered for explanation of this phenomenon, there is no unified theory as such. Further research is required in this direction to reach any concrete conclusion but self-association in solution is exhibited by all the hydrotropes.

2.2 Models

2.2a Maximum aromatic overlap model

Purines and pyrimidines interact with each other in both aqueous and nonaqueous media. However, although there is a high degree of specificity of interactions that occur in organic media, there is very little specificity regarding aqueous media. It has been shown that the complexation constants for a wide variety of purine and pyrimidines derivatives with adenine and deoxyguanosine fall within only one order of magnitude and that the size of the $\pi$ electron system is the single most important factor in determining their values\textsuperscript{[10]}. If associating molecules have similar dipole regions (as in self-association), these regions are likely to be kept as far apart as possible so as to minimize their contact with each other and maximize their contact with the polar aqueous phase. For example, a dimer of benzoic acid in water would be expected to have the carboxyl
groups facing in opposite directions allowing the rings to overlap. This can be seen in the figure below.

![Diagram of hypothetical benzoic acid complexes](image)

**Fig. 2.2: Hypothetical benzoic acid complexes: (a) in water; (b) in benzene or in a cyclodextrin cavity**

On the basis of crystallographic data, it was seen that only partial ring overlap occurs and that the molecules are arranged so that each dipole is in maximum contact with the polarizable aromatic ring of the other molecule\textsuperscript{[11]}. Although this arrangement may be most stable in the crystal, it is not likely to be stable in an aqueous environment where water molecules compete with aromatic rings for interaction with polar moieties. The importance of the environment in determining the type of intermolecular interaction can be appreciated by considering the interaction of benzoic acid molecules to form a cyclic dimer in nonpolar media or within a cyclodextrin cavity.

If they have polar regions that can interact attractively, the electron donor and acceptor regions will overlap as much as possible, maximizing the attractive interactions. These interactions tend to be the most stable stacked complexes because the hydrophobic effect (i.e. the overlap of the aromatic portions of the molecules that are squeezed out of the water) is stabilized by electrostatic attractions and vice versa. Researchers have studied the complexation of a number of pairs of largely planar aromatic compounds and found that the free energy of complex formation correlates with their estimate of the maximal overlap area of the complex\textsuperscript{[12,13]}. Although the estimates of overlap are
somewhat subjective, their work indicates the importance of the size of the aromatic moieties in complex stability. There is little doubt that the size of the aromatic systems of the complexing species and their maximum degree of overlap are major factors in determining the magnitude of the complexation constant. However, these factors alone cannot explain the specificity that is often observed in complexation.

2.2b Two-Class models

Stability constants for a large number of stacking complexes have been determined\cite{14}. It was observed that the magnitude of the complexation constants showed an interesting dependence on the chemical structure. All potential stacking agents were divided into two classes: class A and class B. Compounds that contain aromatic nitrogen and conjugated cyclic amide groups (including pyrimidines, purines and xanthines) were designated as class A which is shown below.

![Fig. 2.3 General category of Class A compounds](image)

It was found that 1,3,5,7-tetramethylpyrimido(5,4-g)pteridine-2,4,6,8-tetrone (TMPPT) has a very strong class A complexing ability. The most common encountered examples of class A compounds are caffeine and theophylline.
Fig. 2.4 Examples of Class A compounds

Aromatic acids and aromatic amides are designated as class B. These include benzoic acid, ferulic acid, cinnamide, and their derivatives. Many anti-inflammatory agents also fall into this category.

Fig. 2.5 General category of Class B compounds

Complexes composed of Class A and Class B molecules consistently have higher stability constants than those formed from only Class A or only Class B molecules. Because of their relatively high water solubility, the hydroxybenzoic acids, their anions and their amides are particularly useful complexing agents for Class A compounds. Gentisic acid (2,4-dihydroxybenzoic acid) is an excellent complexing agent for hexamethylmelamine and for acronine. Picric acid (2,4,6-trinitrophenol) and the xanthines are also useful for complexation with Class B compounds, such as the alkyl benzoates. Molecules that are not completely planar can be solubilized by hydrotropy or stacking complexation, provided that they have a substantial planar region. Chartreusin...
and etoposide are examples of partially planar molecules that are solubilized by planar aromatic compounds. The following are some of the limitations of this model\cite{1}:

1) Some compounds such as imidazole or antipyrine, complex poorly with both TMPPT (a Class A compound) and cinnamide (a Class B compound).

2) Many compounds form complexes with both Class A and Class B compounds, with comparable stability, including tetracycline analogs and volatile flavor compounds.

3) The complexation constants for TMPPT with the Class A compounds (caffeine and theophylline) are larger than for its association with Class B compounds (sodium benzoate and theophylline acetate).

4) Self-association has been reported for both Class A and Class B compounds, including both caffeine and other alkyl xanthines, and benzoates and benzenesulfonates.

5) It is often difficult to classify a compound in either Class A or Class B on the basis of its structure. For example, the anions of caffeine and theophylline which complex strongly with TMPPT are assigned to Class B although they are structurally similar to Class A compounds.

2.2c Partition coefficient models

Stability constants for the interaction of acyclovir and a related guanine derivative with a variety of ligands have been determined\cite{15}. The results were then compared with hydrophobicity (as measured by the logarithm of the octanol-water partition coefficient) and with donor-acceptor strength (as measured by oxidation potentials and by various
molecular orbital calculations). The best correlation with the logarithm of the stability constant was obtained with hydrophobicity\textsuperscript{[15]}. They propose that solute and ligand hydrophobicity are the driving force for the complex formation and that almost 80% of the data are described by the correlation with log $K_{\text{ow}}$. In spite of its obviousness, hydrophobicity is not very good as a sole indicator for stacking complexation. Hydrophobicity alone cannot explain the two-class models. In fact, in some cases, it is completely contradictory to the supposition that nonpolar compounds are the strongest complexing agents.

2.2d The $\pi$-donor and $\pi$-acceptor orbital overlap model

Attempts were made to rationalize the strengths of the stability constants for complexation between nicotinamide and several quinazoline and phthalazine drugs on the basis of Hückel frontier molecular orbital calculations\textsuperscript{[16]}. A linear relationship was found between the stability constants and the interaction energy of overlapping $\pi$-donor and $\pi$-acceptor orbitals. Although these calculations are difficult to perform and are based on a number of assumptions, they offer the possibility of accounting for the subtle effects of slight structural modifications on complexation. This approach could be useful in understanding why complexing molecules tend to fall into the two broad Classes A and B.

2.3 Overview

In spite of considerable effort, no single theory or model has been developed that can successfully describe all of the dependences of stacking complexation on chemical
structure. The primary driving force for nonbonded complexation in aqueous solution is the decrease in the hydrocarbon water surface area that accompanies stacking. This is illustrated by the fact that TMPPT complexes have larger stability constants than the corresponding caffeine or theophylline complexes. The most efficient stacking of similar structures often leads to minimum repulsion of bond dipoles, whereas the stacking of dissimilar class molecules may allow for more favorable attractive dipolar interactions. In all cases, aromatic surface area is a qualitative indicator of complexing ability. Octanol-water partition coefficients provide a convenient secondary parameter for complexation. Thus, for a given aromatic surface area, the solute that has the larger partition coefficient is generally the stronger complexing agent. This is not unreasonable because both partitioning and complexation are the result of the solute being “squeezed out” of water. Unfortunately, this relationship is not quantitative and depends on a number of factors. It can also be concluded that it is not absolutely necessary that the complexing species be aromatic. Nicotinamide which can self-associate in water, has been shown to enhance the water solubility of diazepam, griseofulvin, and three steroids (estradiol, testosterone, and progesterone), all of which are largely non-aromatic. It was also showed that nipecotinamide, the fully saturated analog of nicotinamide increases the solubility of these drugs, as does dimethylacetamide which is neither aromatic nor planar. In these studies, however, the amides were present in nearly a thousandfold molar excess and may act more like cosolvents than complexing agents[7]. It can also be concluded that if a solute can be solubilized by either of the two ligands, the more water soluble ligand normally produces the more water soluble complex. The presence of an additional component in the solute phase can have two effects. It will lower the concentration of the
pure solute in the solute phase if it is a liquid, and it will lower the melting point if it is a solid. Many mixtures that form complexes in solution also form eutectics in the absence of the solvent. Liquefaction in the organic phase results in increased solubility of either component because of the loss of the crystal term in the general solubility equation[1]
3.1 Differential Scanning Calorimetry

3.1a Introduction

Differential scanning calorimetry (DSC) is a thermal technique in which differences in heat flow into a substance and a reference are measured as a function of sample temperature while the two are subjected to a controlled temperature program. The basic difference between differential scanning calorimetry and differential thermal analysis is that the former is a calorimetric method in which differences in energy are measured. In contrast, in differential thermal analysis, differences in temperature are recorded. The temperature programs for the two methods are similar. Differential scanning calorimetry has by now become the most widely used of all thermal methods[1]. When a material undergoes a change in its physical state, or when it reacts chemically, heat is either absorbed or liberated. This corresponds to endothermic or exothermic changes respectively. It should be emphasized that thermal effects can originate from either physical or chemical changes[2].

Two types of DSC units are recognized[3]:

1. **Power-compensated DSC**, where the sample and reference are heated by separate, individual heaters and the temperature difference is kept close to zero, while the
2. difference in electric power needed to maintain equal temperatures ($\Delta P = d(\Delta Q)/dt$) is measured; and

3. *Heat flux DSC*, where the sample and reference are heated from the same source and the temperature difference $\Delta T$ is measured. This signal is converted to a power difference $\Delta P$ using the calorimetric sensitivity.

Differential Scanning Calorimetry (DSC) is the most widely used thermal technique. Numerous reviews have discussed the relevance of the DSC technique\(^ {\text{[4-7]}}\) and a general chapter on DSC is documented in the United States Pharmacopoeia\(^ {\text{[8]}}\). The following diagram presents a schematic illustration of results obtained from both types of DSC units.

![Figure 3.1: Representation of the results of DSC scan of a typical polymeric material\(^ {\text{[1]}}\)](image-url)
3.1b Instrumentation

Power compensated DSC

The diagram shown below depicts the design of a power compensated calorimeter for performing DSC measurements. The instrument has two independent furnaces, one for heating the sample and the other for heating the reference. In a commercial model based upon this design, the furnaces are small, weighing about a gram each, a feature that leads to rapid rates of heating, cooling and equilibration. The furnaces are embedded in a large temperature-controlled heat sink. Above the furnaces are the sample and reference holders, which have platinum resistance thermometers embedded in them to monitor the temperature of the two materials continuously.

![Diagram of DSC sample holder and furnaces for power compensation](image)

Two control circuits are employed in obtaining differential thermograms, one for average temperature control and one for differential temperature control. In the average temperature control circuit, a programmer provides an electrical signal that is proportional to the desired average temperature of the sample and reference holders as a
function of time. This signal is compared in a computer with the average of the signals from the sample and reference detectors embedded in the sample and reference holders. Any difference between the programmer signal and the average platinum sensor signal is used to adjust the average temperature of the sample and reference. The average temperature then serves as the abscissa for the thermogram. In the differential-temperature circuit, sample and reference signals from the platinum resistance sensors are fed into a differential amplifier via a comparator circuit that determines which is greater. The amplifier output then adjusts the power input to the two furnaces in such a way that their temperatures are kept identical. Throughout the experiment the sample and reference are isothermal. A signal that is proportional to the difference in power input to the two furnaces is also transmitted to the data acquisition system. This difference in power, usually in milliwatts, is the information most frequently plotted as a function of sample temperature\[1\].

**Heat Flux DSC**

The figure below is a schematic of a commercially available heat flux DSC cell. Heat flows into both the sample and reference material via an electrically heated constantan thermoelectric disk. Small aluminum sample and reference pans sit on raised platforms formed on the constantan disk. Heat is transferred through the disks and up into the sample and reference via the two pans. The differential heat flow to the sample and reference is monitored by a chromel/constantan area thermocouple formed by the junction between the constantan platform and chromel disks attached to the underside of the platforms. It can be shown that the differential heat flow into the two pans is directly
proportional to the difference in output of the two thermocouple junctions. The sample temperature is estimated by means of the chromel/alumel junction under the sample disk\textsuperscript{[1]}.

**Fig. 3.3: Schematic of a heat flux DSC\textsuperscript{[3]}**

3.1c Pans and crucibles

Pans and crucibles of many materials have been employed, but the majority of low-temperature instruments use aluminum pans and lids, provided they are not attacked by the samples and are only used well below the melting point of aluminum which is 660 °C. For more aggressive environments, platinum or ceramic crucibles may be used. The conductivity of the crucibles and their contact with the sensors affect the thermal analysis curves obtained. A standard experiment might involve a sample of about 10-20 mg of powder, or a disc punched from a polymer film, or a bundle of fibres being placed in a weighed, lidded aluminum crucible, and the total mass recorded. If vaporization is not a
problem, small pinholes can be punched in the lid to allow escape of gas products and reaction with the surrounding purge gas.

Special crucibles have been designed for special purposes like high pressure, mixing liquids or for observing the sample during heating. Liquids may be injected into the crucible using a syringe. The technique of pressure DSC is used for very high pressures (70 atm.)\textsuperscript{[3]}.

### 3.1d Sample size

When a sample is undergoing a reaction, there is a temperature gradient within it, as heat is given out or absorbed. To obtain meaningful calorimetric data, it is imperative that the sample size be kept to the minimum in order to reduce such gradients. The sample size is normally kept between 3-5 mg. However, if the sample consists of a number of materials, as in a study of reaction process, then it is usually difficult to obtain a small, representative sample. In such a situation, the ideal solution is to use a larger sample with the thermocouple embedded in it and to recognize that the apparatus is then only qualitative when used in this way\textsuperscript{[3]}.

### 3.1e Calibration

For accurate work, it is essential to calibrate the temperature scale, and for DSC instruments the apparatus must also be calibrated for calorimetric sensitivity. Indium and zinc are the most popular calibration materials. The table below gives the list of a standard set of substances for which calibration has been approved by ICTAC\textsuperscript{[3]}. 

Table 3.1: Calibration materials for DTA and DSC\textsuperscript{[3]}

<table>
<thead>
<tr>
<th>Material</th>
<th>Temperature (°C)</th>
<th>Enthalpy (J/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclohexane</td>
<td>(t)</td>
<td>-83</td>
</tr>
<tr>
<td>(m)</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>1,2-Dichloroethane</td>
<td>(m)</td>
<td>-32</td>
</tr>
<tr>
<td>Phenyl ether</td>
<td>(m)</td>
<td>30</td>
</tr>
<tr>
<td>Biphenyl</td>
<td>(m)</td>
<td>69.3</td>
</tr>
<tr>
<td>o-Terphenyl</td>
<td>(m)</td>
<td>58</td>
</tr>
<tr>
<td>Polystyrene</td>
<td>($T_d$)</td>
<td>105</td>
</tr>
<tr>
<td>Potassium nitrate</td>
<td>(t)</td>
<td>128</td>
</tr>
<tr>
<td>Indium</td>
<td>(m)</td>
<td>156.6</td>
</tr>
<tr>
<td>(m)</td>
<td>231.9</td>
<td></td>
</tr>
<tr>
<td>Tin</td>
<td>(m)</td>
<td>300</td>
</tr>
<tr>
<td>Potassium perchlorate</td>
<td>(t)</td>
<td>419.4</td>
</tr>
<tr>
<td>Zinc</td>
<td>(m)</td>
<td>430</td>
</tr>
<tr>
<td>Silver sulphate</td>
<td>(t)</td>
<td>573</td>
</tr>
<tr>
<td>Quartz</td>
<td>(t)</td>
<td>583</td>
</tr>
<tr>
<td>Potassium sulphate</td>
<td>(t)</td>
<td>665</td>
</tr>
<tr>
<td>Potassium chromate</td>
<td>(t)</td>
<td>810</td>
</tr>
<tr>
<td>Barium carbonate</td>
<td>(t)</td>
<td>925</td>
</tr>
</tbody>
</table>

3.1f Applications

There are a very large number and variety of applications of DTA and DSC. The applications may be divided roughly into two categories\textsuperscript{[3]}:

1) *Physical changes and measurements*, such as melting, crystalline phase changes, changes in liquid and liquid crystalline states and in polymers, phase diagrams, heat capacity, and glass transitions, thermal conductivity and diffusivity and emissivity.

2) *Chemical reactions* such as dehydration, decompositions, polymer curing, glass formation and oxidative attack.

The numerous applications for DSC have been clearly cited in a number of reviews and research articles\textsuperscript{[4-21]}. 
3.2 X-ray Powder Diffraction Spectroscopy

3.2a Introduction

Although the crystallographic structure provides the greatest understanding of a crystal, the necessity for obtaining suitable single crystals is the cause of its unpopularity. The degree of complexity associated with the data analysis precludes this technique from being used on a routine basis for batch characterization. In fact, most drug substances are obtained as microcrystalline powders that do not contain crystallographically adequate crystals. During the most common evaluation of the drug substances it is usually sufficient only to establish the polymorphic identity of the solid and to verify that the isolated compound is indeed of the desired structure. The XRPD technique is exceedingly important in pharmaceutical physics because it is the easiest and fastest method to obtain fundamental information about the structure of a crystalline substance. Structural analysis by single-crystal x-ray diffraction provides the largest amount of information but is significantly harder to obtain and requires a suitable crystal. Since the majority of drug substances are obtained as crystalline powders, researchers often use the powder pattern of these substances as a readily obtainable fingerprint to determine structural type. In fact, it is only by chance that the two compounds might form crystals for which the ensemble of molecular planes happen to be identical in all space. One such example is the trihydrate phases of ampicillin and amoxicillin, but such instances are uncommon.

Bragg explained the diffraction of x-rays by crystals using a model in which the atoms of a crystal are regularly arranged in space and can be regarded as lying in parallel sheets separated by a definite and defined distance (d). He then showed that the scattering centers arranged in a plane act like a mirror to an x-ray incident on them, so that
constructive interference occurs for the direction of specular reflection. Within a given family of planes, defined by a Miller index\textsuperscript{[22]} of (hkl), each plane produces a specular reflectance of the incident beam. If the incident x-rays are monochromatic having wavelength (\(\lambda\)), then for an arbitrary glancing angle (\(\theta\)), the reflections from successive planes are out of phase with one another. This yields destructive interference in the scattered beams. However, by varying \(\theta\), a set of values for \(\theta\) can be found so that the path difference between the x-rays reflected by successive planes will be an integral number (\(n\)) of wavelengths, and constructive interference occurs. The equation is then obtained which is called Bragg’s law\textsuperscript{[22]}, which explains the phenomenon:

\[
2d\sin\theta = n\lambda
\]

Eqn. 3.1

Fig. 3.4: Resultant reflection of x-rays by single lattice array (above) and two different, intermeshed lattice arrays (below)\textsuperscript{[24]}
Unlike the diffraction of light by a ruled grating, the diffraction of x-rays by a crystalline solid leads to constructive interference (such as reflection at only the critical Bragg angles. When reflection does occur, it is stated that the plane in question is reflecting in the nth order or that one observes the nth order diffraction for that particular crystal plane. Therefore, one will observe an x-ray scattering response for every plane defined by a unique Miller index of (hkl). To measure a powder pattern, one would prepare a randomly oriented powdered sample to expose all possible planes of a crystalline powder. One can measure the scattering angle, (θ), for each family of crystal planes by slowly rotating the sample and measuring the angle of diffracted x-rays with respect to the angle of the incident beam. Alternatively, the angle between the sample and source can be fixed while the detector is moved to determine the angles of the scattered radiation. Knowing the wavelength of the incident beam, one can calculate the spacing between the planes (d-spacings) using Bragg’s law.[22]

### 3.2b Instrumentation

The figure below shows the essential components of an x-ray spectrometer. The monochromator consists of a pair of beam collimators, which serve the same purpose as the slits in an optical instrument, and a dispersing element. The latter is a single crystal mounted on a goniometer or rotatable table that permits variation and precise determination of the angle θ between the crystal face and the collimated incident beam. From Bragg’s law, it is evident that at any given angular setting, of goniometer, only a few wavelengths are diffracted (λ, λ2, λ3,…… λn where λ = 2dsinθ). In order to derive a spectrum, it is necessary that the exit beam collimator and the detector be mounted on a
second table that rotates at twice the rate of the first; that is, as the crystal rotates through
an angle $\theta$, the detector must simultaneously move through an angle $2\theta$. Clearly, the
interplanar spacing ($d$) must be known precisely\[1\].

![Fig. 3. 5: X-ray monochromator and detector\[1\]](image)

### 3.2c Applications

Typical applications of x-ray powder diffraction methodology include the
evaluation of polymorphism and solvatomorphism, the study of phase transitions, and
evaluation of degrees of crystallinity. More recently researchers have made advances in
the use of powder diffraction as a means to obtain solved crystal structures. A very useful
compliment to ordinary XRPD is variable-temperature x-ray diffraction. In this method
the sample is contained on a stage that can be heated to any desired temperature. The
method is extremely useful for the study of thermally induced phenomena and can be a
vital complement in thermal methods of analysis. These applications are discussed as follows:

1) Phase identity of materials: The 1995 USP 23/NF 18 general chapter on x-ray diffraction states that ‘identity is established if the scattering angles of the 10 strongest reflections obtained for an analyte agree to within ± 0.20 degrees 2-θ with that of the reference material and if the relative intensities of these reflections do not vary by more than 20%’. The intensity rule was effectively removed in USP 24/NF 19, which states that relative intensities may vary considerably from that of the reference standard.

2) Degree of Crystallinity: When reference samples of the pure amorphous and the pure crystalline phases of a substance are available, calibration samples of known degrees of crystallinity can be prepared by mixing these. By establishing a calibration curve for the XRPD response versus the degree of crystallinity, unknown samples can be evaluated.

3) Polymorphism and solvatomorphism: One of the most important uses of XRPD in pharmaceuticals is its application as the primary determinant of polymorphic or solvatomorphic identity. Because polymorphism and solvatomorphism are the results of purely crystallographic phenomenon, x-ray diffraction represents the essential method of determination. Because of its ease of data acquisition, XRPD is particularly useful as a screening technique for batch characterization, and the criteria already described for phase identification can serve to differentiate polymorphs or solvatomorphs. It is prudent, however, to verify the results of an XRPD study with a confirmatory technique such as polarizing light microscopy,
differential scanning calorimetry, solid-state vibrational spectroscopy or solid-state nuclear magnetic resonance.

4) Phase compositions of mixtures: The antiulcer drug ranitidine hydrochloride is known to crystallize in two anhydrous polymorphs. For various reasons, most researchers are extremely interested in determining the quantity of Form II in a bulk Form I sample.

5) Crystallographic properties: A given sample consists of crystallographically pure material if that sample’s powder pattern can be successfully indexed (for example a Miller index can be assigned to each and every peak). This is much easier when done using the synchrotron radiation as the source. The researchers have obtained powder patterns at high resolution (<0.03 degrees 2-θ). These high-quality patterns can be processed using indexing programs to deduce unit cell dimensions for the sample being studied. The data can be used as input for molecular – modeling programs and refined with Rietveld analysis to deduce crystal structures from the powder data.

6) Structural interpretation for thermal transitions: The assignment of the origin of thermal events detected during the conduct of differential thermal analysis or differential scanning calorimetry (DSC) is not always straightforward, and the use of supplementary technology is frequently desirable. By performing XRPD studies on a heatable stage, one can bring the system to positions where a DSC thermogram indicates the existence of an interesting point of thermal equilibrium. For instance, after the dehydration of a hydrate phase, one may obtain either a crystalline anhydrate phase or an amorphous phase. The XRPD
pattern of a dehydrate hydrate will clearly indicate the difference. In addition, should one encounter an equivalence in powder patterns between the hydrate phase and it’s dehydrated form, this would indicate the existence of channel-type water (as opposed to genuine lattice type water).

7) Accelerated stability studies: The physical quality of a drug substance during the conduct of stability studies must be well established, and XRPD is certainly capable of being validated to the status of a stability-indicating assay. The crystallographic stability of a drug substance can be studied using XRPD as part of the protocol, which is especially important when one chooses a metastable or an amorphous form of the drug substance for development. One may conduct such work either on samples that have been stored at various conditions and pulled at designated time points or on substances that are maintained isothermally and the XRPD periodically measured.

3.3 Fourier transform infrared spectroscopy

3.3a Introduction

The infrared region of the spectrum encompasses radiation with wavenumbers ranging from about 12,800 to 10 cm\(^{-1}\) or wavelengths from 0.78 to 1000 µm. From the standpoint of both application and instrumentation, the infrared spectrum is conveniently divided into near-, mid- and far-infrared radiation; the figure below compares the infrared energy with other forms of radiation\[^1\].
Generally, infrared radiation is not energetic enough to bring about the kinds of electronic transitions that we encounter in ultraviolet and visible radiation. Absorption of infrared radiation is thus confined largely to molecular species for which small energy differences exist between various vibrational and rotational states. In order to absorb infrared radiation, a molecule must undergo a net change in dipole moment as a consequence of its vibrational or rotational motion. Only under these circumstances can the alternating electrical field of the radiation interact with the molecule and cause changes in the amplitude of one of its motion. If the frequency of the radiation exactly matches a natural vibrational frequency of the molecule, a net transfer of energy takes place that results in a change in the amplitude of the molecular vibration; absorption of the radiation is the consequence. Similarly, the rotation of asymmetric molecules around their centers of mass results in a periodic dipole fluctuation that can interact with radiation. No net change in dipole moment occurs during the vibration or rotation of
homonuclear species such as O₂, N₂ or Cl₂; consequently, such compounds cannot absorb in the infrared. The two forms of transitions are discussed below:

1. Rotational transitions: The energy required to cause a change in rotational level is minute and corresponds to radiation of 100 cm⁻¹ or smaller (>100 μm). Because rotational levels are quantized, absorption by gases in this far-infrared region is characterized by discrete, well-defined lines. In liquids or solids, intramolecular collisions and interaction cause broadening of the lines into a continuum.

2. Vibrational transitions: Vibrational energy levels are also quantized, and for most molecules the energy differences between quantum states correspond to the mid-infrared region. The infrared spectrum of a gas usually consists of a series of closely spaced lines, because there are several rotational energy states for each vibrational state. On the other hand, rotation is highly restricted in liquids and solids; in such samples, discrete vibrational/rotational lines disappear, leaving only somewhat broadened vibrational peaks. The figure below depicts the types of molecular vibrations.
A mechanical model consisting of two masses connected by a spring can approximate the characteristics of an atomic stretching vibration. A disturbance of one of these masses along the axis of the spring results in a vibration called a simple harmonic motion. The natural frequency of the oscillation is then given by:

$$\nu_m = \frac{1}{2\pi} \sqrt{\frac{k}{\mu}} \quad \text{Eqn. 3.2}$$

$$\mu = \frac{m_1 m_2}{m_1 + m_2} \quad \text{Eqn. 3.3}$$

**Fig. 3.7:** Types of molecular vibrations; + indicates motion from the plane towards the reader; - indicates motion from the reader [1]

(a) Stretching vibrations

Symmetric

Asymmetric

In-plane rocking

In-plane scissoring

Out-of-plane wagging

Out-of-plane twisting

(b) Bending vibrations
Where $ν_m$ is the natural frequency of the molecular vibration, $k$ is the force constant of the chemical bond; and $\mu$ is the reduced mass of the system connected by two atoms of masses $m_1$ and $m_2$.

The number and possible vibrations in a polyatomic molecule can be calculated based on the fact that whether it is a linear or a non-linear molecule. For a non-linear molecule, the number of possible vibrations are $(3N-6)$ and that for a linear molecule are $(3N-5)$. This is usually referred as normal mode. Four factors tend to produce fewer peaks then would be expected from the calculated number of normal modes. A lesser number of peaks is found when (1) the symmetry of the molecule is such that no change in dipole results from a particular vibration; (2) the energies of two or more vibrations are identical or nearly identical; (3) the absorption intensity is so low as to be undetectable by ordinary means; or (4) the vibrational energy is in a wavelength region beyond the range of the instrument[1].

3.3b Instrumentation

The figure below shows schematically the arrangements of components in a typical infrared spectrophotometer. Like most inexpensive dispersive infrared instruments it is a null type, in which the power of the reference beam is reduced, or attenuated, to match that of the beam passing through the sample. There are three types of linkages that can be seen below: (1) a radiation linkage indicated by dashed lines; (2) a mechanical linkage, shown by thick dark lines; (3) an electrical linkage, shown by narrow solid lines. Radiation from the source is split into two beams, half passing into the sample-cell compartment and the other half into the reference area. The reference beam
then passes through the attenuator and onto a chopper. The chopper consists of a motor-driven disk that alternately reflects the reference or transmits the sample beam into the monochromator. After dispersion by a grating, the alternating beams fall on a detector and are converted to an electrical signal. The signal is amplified and passed to the synchronous rectifier, a device that is mechanically or electrically coupled to the chopper to cause the rectifier switch and the beam leaving the chopper to change simultaneously. If the two beams are identical in power, the signal from the rectifier is an unfluctuating direct current. If, on the other hand, the two beams differ in power, a fluctuating or alternate current is produced, the phase of which is determined by which beam is more intense. The current from the rectifier is filtered and further amplified to drive a synchronous motor in one direction or the other, depending on the phase of the input current. The synchronous motor is mechanically linked to both the attenuator and the pen drive of the recorder and causes both to move until a null is achieved. A second synchronous motor drives the chart and varies the wavelength simultaneously. There is frequently a mechanical linkage between the wavelength and slit drives so that the radiant power reaching the detector is kept approximately constant by variation in the slit width.

Fourier transform spectrometers are attached to the FTIR instrument due to numerous advantages. Fourier transform instruments appear to have signal-to-noise ratios that are better than those of a good-quality dispersive instrument by more than an order of magnitude. The enhanced signal-to-noise ratio can be a trade off for rapid scanning, with good spectra being attainable in a few seconds in most cases. Interferometric instruments are also characterized by high resolutions (< 0.1 cm⁻¹) and highly accurate and
reproducible frequency determinations. The latter property is particularly helpful when spectra are to be subtracted for background correction\textsuperscript{1}.

![Diagram of a double-beam spectrophotometer](image)

**Fig. 3.8: Schematic of a double-beam spectrophotometer\textsuperscript{1}**

### 3.3c Application

To date, the majority of analytical applications have been confined to a portion of the mid-infrared region extending from 4000 to 400 $\text{cm}^{-1}$ (2.5 to 25 $\mu\text{m}$). However, an ever-growing number of applications of near- and far-infrared spectroscopy are finding their way into the literature. Infrared spectroscopy finds widespread application to qualitative and quantitative analyses. Its single most important use has been for the identification of organic compounds whose mid-infrared spectra are generally complex.
and provide numerous maxima and minima that are useful for comparison purposes. Indeed, in most instances, the mid-infrared spectrum of an organic compound provides a unique fingerprint, which is readily distinguished from the absorption patterns of all other compounds; only optical isomers absorb in exactly the same way. In addition to its application as a qualitative analytical tool, infrared measurements are finding increasing use for quantitative analysis as well. Here, the high selectivity of the method often makes possible the quantitative estimation of an analyte in a complex mixture with little or no prior separation steps. The most important analyses of this type have been of atmospheric pollutants from industrial processes. Another important use of infrared absorption spectroscopy is as a detector for gas chromatography, where it’s power for identifying compounds is coupled with the remarkable ability of gas chromatography to separate the components of complex mixtures. This application has been fostered by the development of high-speed Fourier transform spectrometers\[1\].

Since Fourier transform infrared (FTIR) spectrometers have become commercially available, infrared (IR) spectrometry has undergone a renaissance. Owing to the wide variety of sampling accessories currently available, virtually every sample presented to the infrared spectroscopist, irrespective of physical form or composition, can now be analysed and meaningful data obtained. A recent development in sample analysis is the use of the FTIR microscope. This allows the scientist to examine visually small samples and select the region(s) of interest for optical and IR analysis. Within the pharmaceutical industry, the requirement to obtain increasingly more information about impurities and degradation products has meant that scientists must perform much of their work using microscopical amounts of materials. The studies reported here illustrate the
potential of FTIR microspectrometry to solve pharmaceutically related problems. The following are some of the applications for FTIR microspectrometry:\[30\]:

1) Fibre Analysis: The technique has been used to determine the identity of fibres present as contaminants in a number of pharmaceutical materials. Fibres and particulates are the most common contaminants in the pharmaceuticals. By using FTIR microspectrometry, good transmission spectra of these materials can be obtained. As a consequence, a library has been created containing IR spectra of natural, synthetic, inorganic and organic fibres, to be used in the identification of pharmaceutically related contaminants.

2) Particulate analysis: The FTIR microscope is also ideally suited to the identification of particulates, especially those which are organic.

3) Tandem FTIR Thermomicroscopy: Using a hot stage on the FTIR microscope, a technique known as tandem FTIR microscopy, it was possible to observe spectral changes occurring on heating, to microscopic amounts of the compound.

3.4 Polarized light microscopy

3.4a Introduction

Polarized light microscopes have a high degree of sensitivity and can be utilized for both quantitative and qualitative studies targeted at a wide range of anisotropic specimens. Qualitative polarizing microscopy is very popular in practice, with numerous volumes dedicated to the subject. In contrast, the quantitative aspects of polarized light
microscopy, which is primarily employed in crystallography, represent a far more difficult subject that is usually restricted to geologists, mineralogists, and chemists. Natural sunlight and most forms of artificial illumination transmit light waves whose electric field vectors vibrate in all perpendicular planes with respect to the direction of propagation. When the electric field vectors are restricted to a single plane by filtration then the light is said to be polarized with respect to the direction of propagation and all waves vibrate in the same plane. The polarized light microscope is designed to observe and photograph specimens that are visible primarily due to their optically anisotropic character. In order to accomplish this task, the microscope must be equipped with both a polarizer, positioned in the light path somewhere before the specimen, and an analyzer (a second polarizer), placed in the optical pathway between the objective rear aperture and the observation tubes or camera port. Image contrast arises from the interaction of plane-polarized light with a birefringent (or doubly-refracting) specimen to produce two individual wave components that are each polarized in mutually perpendicular planes. The velocities of these components are different and vary with the propagation direction through the specimen. After exiting the specimen, the light components become out of phase, but are recombined with constructive and destructive interference when they pass through the analyzer. Polarized light is a contrast-enhancing technique that improves the quality of the image obtained with birefringent materials when compared to other techniques such as darkfield and brightfield illumination, differential interference contrast, phase contrast, Hoffman modulation contrast, and fluorescence [24].

Crystallographic properties are among those physical properties which may affect the performance of a substance used in a process or a product. Thus, they are particularly
important to the industrial microscopist. Polymorphism is a phenomenon observable with most, and probably all, elements and compounds. A compound showing polymorphism can crystallize with different internal lattices, thereby giving corresponding different external crystal morphology and internal physical properties. The crystal systems of the two or more modifications of the compound are usually, but not always, different. Polymorphism is frequently seen in pharmaceuticals and polarized light microscopy is very useful in differentiating between the polymorphs and solvatomorphs\textsuperscript{25}.

3.4b Instrumentation

Although similar to the common brightfield microscope, the polarized light microscope contains additional components that are unique to instruments of this class. These include a polarizer and analyzer, strain-free objectives and condenser, a circular graduated stage capable of 360-degree rotation, Bertrand lens, and an opening in the microscope body or intermediate tube for compensators, such as a full-wave retardation plate, quartz wedge, or quarter-wavelength plate. Removal of the polarizer and analyzer (while other components remain in place) from the light path renders the instrument equal to a typical brightfield microscope with respect to the optical characteristics. Below is the depiction of the various parts and working of a polarizing microscope.
3.4c Applications

Although much neglected and undervalued as an investigative tool, polarized light microscopy provides all the benefits of brightfield microscopy and yet offers a wealth of information, which is simply not available with any other optical microscopy technique. As well as providing information on absorption color and boundaries between minerals of differing refractive indices obtainable in brightfield microscopy, polarized light microscopy can distinguish between isotropic and anisotropic materials. The technique exploits optical properties of anisotropy to reveal detailed information about the structure and composition of materials, which are invaluable for identification and diagnostic purposes. Polarized light microscopy is perhaps best known for its geological applications--primarily for the study of minerals in rock thin sections, but it can also be
used to study many other materials. These include both natural and industrial minerals whether refined, extracted or manufactured, composites such as cements, ceramics, mineral fibers and polymers, and crystalline or highly ordered biological molecules such as DNA, starch, wood and urea. The technique can be used both qualitatively and quantitatively and is an outstanding tool for materials science, geology, chemistry, biology, metallurgy and even medicine[^26].

Fig. 3.10 Polarized light Photomicrographs of Ranitidine Hydrochloride Form I (top) and Form II (bottom)[^27]
Hot stage methods have extended the applications of polarized light microscopy. The following are some of the applications for polarized light microscopy in pharmaceuticals and chemistry\cite{25}:

1. Determination of purity

2. Analysis of mixtures

3. Characterization and identification of fusible compounds and mixtures

4. Determination of composition diagrams
   a. Two-component systems
   b. Three-component systems

5. Investigations of polymorphism

6. Measurement of physical properties
   a. Molecular weight
   b. Rates of crystal growth
   c. Crystal morphology
   d. Crystal optics

7. Study of boundary migration

8. Study of kinetics of crystal growth

9. Correlation of physical behavior with crystal properties
4.1 High performance liquid chromatography (HPLC)

4.1a Introduction

Chromatography is a method in which the components of a mixture are separated on an adsorbent column in a flowing system\cite{1}. The adsorbent material, or stationary phase, described by Tswett\cite{2} in 1906, has taken many forms over the years that include paper, thin layers of solids attached to glass plates, immobilized liquids, gels, and solid particles packed in columns. The flowing component of the system is either a liquid or a gas, giving it the respective names, liquid chromatography and gas chromatography. Liquid chromatography can be applied to wider variety of samples than any other separation technique and is applicable to extremely complex mixtures that scientists encounter in chemical and biological systems\cite{3}. Modern chromatography is defined as the separation of the components of a mixture due to differences in the equilibrium distribution of components between two phases, the mobile phase and the stationary phase\cite{4}. High performance liquid chromatography (HPLC) is the term used to describe liquid chromatography in which the liquid mobile phase is mechanically pumped through a column that contains the stationary phase. As a result of significant development during the past two decades, which have brought significant improvements to instrumentation and column packing, HPLC has emerged as the preferred method for the separation and quantitative analysis for a wide range of samples\cite{3}. 
4.1b Basic concepts of HPLC

Chromatography is described and measured in terms of four major concepts: capacity, efficiency, selectivity, and resolution\textsuperscript{[2]}. The capacity and selectivity of the column are variables that are controlled largely by the column manufacturers whereas efficiency and resolution can be controlled to some extent by the chromatographer. To obtain the best possible separation, the efficiency of the system must be optimized in order to minimize band broadening\textsuperscript{[5]}. The capacity factor (k\textsubscript{R}’) of a column is a direct measure of the interaction of the sample with the packing material and is defined by the expression\textsuperscript{[2]}

\[ k_{R}' = \frac{(t_R - t_0)}{t_0} = \frac{(V_R - V_0)}{V_0} \]  

Eqn. 4.1

where,

\( t_R \) = time taken for a specific solute to reach the detector (retention time)

\( t_0 \) = time taken for non-retained species to reach the detector (holdup time)

\( V_R \) = Volume of solvent that is pumped through the detector before a specific peak is eluted (retention volume)

\( V_0 \) = Volume of solvent that is pumped through the detector between the time of injection and appearance of the peak of the non-retained species (void volume)

The higher the capacity factor, the greater is the ability of the column to retain solutes. If the value of \( k_{R}' \) is 2-5, it indicates a good balance between analysis time and retention. The resolution (R\textsubscript{S}) of a column provides a quantitative measure of its ability to separate two analytes\textsuperscript{[6]}. It is affected by the selectivity\( (\alpha) \), efficiency \( (N) \) and capacity \( (k'_{R}) \) of the column.
\[ R_S = \Delta Z/(W_A/2 + W_B/2) = 2\Delta Z/(W_A + W_B) = 2[(t_R)_B - (t_R)_A]/(W_A + W_B) \]  
Eqn. 4.2

Where,

- \( W_A, W_B \) = Base widths of peaks A and B
- \((t_R)_B, (t_R)_A\) = Retention times of peaks A and B; and
- \( R_S \) = Resolution.

As shown in Fig. 4.1, two peaks are better separated as the value of \((R_S)\) increases.

**Fig. 4.1 Resolution of two peaks at Rs values of 0.75, 1.0, and 1.5, respectively**[^6]

The selectivity (\( \alpha \)) of a chromatographic column is the function of the thermodynamics of the mass transfer process and can be measured in terms of the relative separation of peaks as given below[^7]:
\[ \alpha = \frac{[(t_R)_2 - (t_0)]}{[(t_R)_2 - (t_0)]} = \frac{(V_2 - V_0)}{(V_1 - V_0)} = \frac{(k'_2)}{(k'_1)} \quad \text{Eqn. 4.3} \]

The values of \( \alpha \) range from \( 1 - \infty \). At \( \alpha = 1 \), the two components of the mixture have the same retention time. The most powerful approach to changing \( \alpha \) is changing the composition of the mobile phase. The broader a chromatographic peak is relative to its retention time, the less efficient the column it is eluting from. Efficiency of a column is most readily assessed from the width of the peak at half its height \( W_{1/2} \) and its retention time using the following equation\(^8\):

\[ N = 5.54 \left( \frac{t_R}{W_{1/2}} \right)^2 \quad \text{Eqn. 4.4} \]

Where, \( N \) = number of theoretical plates

The plate theory is attributed to Martin and Synge\(^9\). Plate theory models a chromatographic column as a series of narrow, discrete sections called theoretical plates\(^10\). The theoretical plate concept is used widely to characterize the quality of a chromatographic column. A large plate number results in narrow peaks and is thus indicative of a good column\(^7\).

### 4.1c Instrumentation

The basic components of an HPLC system consist of a solvent (also termed the mobile phase or eluent) in a reservoir, a solvent delivery system (or pump), a sample introduction device (injector) which may be operated either manually or automatically, a column, one or more detectors, and a data system\(^15\). The primary function of the solvent delivery system or the pump is to pump the mobile phase as reproducibly as possible. The majority of the HPLC pumps deliver the solvent at a constant flow-rate, although some
pumps are also designed to deliver the mobile phase at a constant pressure. However, constant pressure mode is not generally used for pressure chromatography \[16\]. The figure below depicts the basic components of a modern HPLC system.

![Schematic of a modern HPLC system](image)

Fig. 4.2 Schematic of a modern HPLC system\[28\]

The function of the HPLC sample introductory device (injector) is to introduce the sample into the flowing solvent stream prior to the column so that it may be carried to the column and subsequently separated. The injector may be operated either manually via a hand-held microsyringe or automatically to enable unattended injection\[15\]. It should
also allow the sample to be introduced without overly disturbing the solvent flow as this may result in significant baseline disturbances in flow-sensitive detectors, such as refractive index or conductivity. In some cases the sample injector may also need to withstand high temperatures (up to 150°C) as certain polymer analyses require that the injector be heated in order to keep the sample soluble[21].

The majority of HPLC columns today are packed with spherical, microparticulate (3-10 µm) materials which result in stable, high-efficiency columns which can be used for relatively large sample loadings. Column packing alternatives include the use of rigid solids (most commonly silica), resins (usually polystyrene divinyl benzene), and soft gels[15]. Rigid solids based on silica are the most common HPLC packings used today. They can withstand the high pressures generated when 10-30 cm columns packed with 3-10 µm particles are used. Resin based packings are commonly used in gel-permeation chromatography (GPC) and ion-exchange chromatography (IEC). They can be used over a wide pH range although most resin types are limited to moderate operating pressures (1000-2000 p.s.i.). Soft gels, such as, agarose and cephadex, are almost used exclusively for the separation of aqueous proteins; however they cannot tolerate very high backpressure. The most commonly used columns are 250-300 mm in length with a diameter of 3.9-4.6 mm, although 150 mm columns are becoming increasingly popular[15].

A wide variety of HPLC detectors are available in the market and the most appropriate detector for a given problem is determined by the requirement. For instance, a detector with a wide, linear dynamic range and low detection limit can be used for the determination of trace components in addition to main components in the sample.
Conversely, the use of a selective detector is convenient if trace components belong to a particular class of substance or bear a common functional group. Some of the common detectors include flame ionization, thermal conductivity, electron capture, alkali flame ionization, flame photometric, ultraviolet, refractive index, and electrochemical \[15\]. Absorbance detectors are nondestructive and respond only to substances that absorb radiation at the wavelength of the source light\[2\]. They are classified as selective or solute property detectors because the mobile phase is chosen such that it exhibits little or no absorption at the wavelength of interest. Detectors that measure only in the wavelength 190-350 nm are termed as ultraviolet (UV) absorbance detectors, while those that measure in the range 350-700 nm are termed as visible (Vis) detectors. Detectors that span the range 190-700 nm are termed as UV/Vis detectors. The UV/Vis detectors are the most widely used, not only because of the relative insensitivity of the detector to temperature and gradient changes but also because of the great number of compounds that absorb radiation in the UV range.

4.1d Applications

High-performance liquid chromatography is unquestionably the most widely used of all the analytical separation techniques, with annual sales of HPLC equipment approaching the billion dollar mark. The reasons for the popularity of the method are its sensitivity, its ready adaptability to accurate quantitative determinations, its suitability for separating nonvolatile species or thermally fragile ones, and above all, its widespread applicability to substances that are of prime interest to industry, to many fields of science, and to the public. Examples of such materials include amino acids, proteins, nucleic
acids, hydrocarbons, carbohydrates, drugs, terpenoids, pesticides, antibiotics, steroids, metal-organic species, and a variety of inorganic substances. Various liquid chromatographic procedures tend to be complementary insofar as their areas of application are concerned. Thus for solutes having molecular weights greater than 10,000, exclusion chromatography is often used, although it is now becoming possible to handle such compounds by reversed-phase partition chromatography as well. For low molecular weight ionic species, ion exchange chromatography is widely used. Small polar but non-ionic species are best handled by partition methods. In addition, this procedure is frequently useful for separating members of a homologous series. Adsorption chromatography is often chosen for separating nonpolar species, structural isomers, and compound classes such as aliphatic hydrocarbons from aliphatic alcohols.

4.2 Nuclear magnetic resonance spectroscopy

4.2a Introduction

Nuclear magnetic resonance spectroscopy (NMR) is based upon the measurement of absorption of electromagnetic radiation in the radio-frequency region of roughly 4 to 600 MHz. In contrast to ultraviolet, visible, and infrared absorption, nuclei of atoms are involved in the absorption process. Furthermore, in order to cause nuclei to develop energy states required for absorption to occur, it is necessary to place the analyte in an intense magnetic field. It is one of the most powerful tools available to the chemist and biochemist for elucidating the structure of both organic and inorganic species. It has also proved very useful for the quantitative determination of absorbing species. The theoretical basis for nuclear magnetic resonance spectroscopy was proposed in 1924 by
W. Pauli\textsuperscript{[6]}, who suggested that certain atomic nuclei should have the properties of spin and magnetic moment and that, as a consequence, exposure to the magnetic field would lead to splitting of the energy levels. During the next decade, experimental verification of these postulates was obtained. It was not until 1946, however, that it was demonstrated that nuclei absorb electromagnetic radiation in a strong magnetic field as a consequence of the energy level splitting induced by the magnetic field. The two physicists shared the 1952 Nobel Prize for their work. In 1953, the first high-resolution NMR spectrometer designed for chemical structural studies was marketed by Varian Associates. Two general types of nuclear magnetic resonance spectrometers are currently in use, continuous wave (cw) and pulsed, or Fourier transform (FT NMR). Nearly all NMR instruments produced at the present time are of the Fourier transform type and the use of the continuous wave is limited to special routine applications\textsuperscript{[6]}.

The phenomenon of magnetic resonance results from the interaction of the magnetic moment of an atomic nucleus ($\mu$) with an external magnetic field. The cause of this magnetic moment is the quantum mechanical angular momentum (spin angular momentum) of all nuclei that are no (g,g) nuclei (even number of protons and neutrons). In order to understand this, imagine the nucleus as a small charged particle which is spinning around its own axis thus representing an electric current. Due to this current the atomic nucleus behaves as a small electromagnet.

The spin is quantized according to the following:

$$J = \frac{\hbar}{2\pi} \times \sqrt{(I(I+1))}$$

Eqn. 4.5
with $J$ being the spin angular momentum, $I$ the spin quantum number (which can have values of $I=0, 1/2, 1, 3/2, ..., 6$). By convention it is simply called 'spin'. and $h$ the Planck's constant. The angular momentum and the magnetic moment are directly proportional:

$$\mu = \gamma J = \gamma \frac{h}{2\pi} \sqrt{I(I+1)} \quad \text{Eqn. 4.6}$$

The constant $\gamma$ is characteristic for each isotope and is called the gyromagnetic ratio. The sensitivity of a nucleus in NMR depends on $\gamma$ and higher the value of $\gamma$ higher is the sensitivity.

In an external magnetic field the magnetic moment orients according to:

$$J_z = -m \frac{h}{2\pi} \rightarrow \mu_z = m \gamma \frac{h}{2\pi} \quad \text{Eqn. 4.7}$$

The magnetic quantum number $m$ can be an integer number between $-I$ and $+I$. Thus, the external field leads to a splitting of the energy levels. For spin 1/2 nuclei (e.g. protons) two energy levels exist according to a parallel or antiparallel orientation of the magnetic moment with respect to the magnetic field:

Fig. 4.3 The effect of external magnetic field on spin quantum state of nuclei
The energy of these levels is given by the classical formula for a magnetic dipole in a homogenous magnetic field of the strength $B_0$:

$$E = - \mu_z x B_0 = - m x \gamma x h/(2\pi) x B_0$$  \hspace{1cm} \text{Eqn. 4.8}

The magnetic moment of each nucleus precesses around $B_0$. The frequency of this precession is the Larmor frequency ($w_0$) which is equivalent to the resonance frequency of the nucleus and the energy difference between the two levels.

$$\gamma x h/(2\pi) x B_0 = \Delta E = h x \mu = h/(2\pi) x w_0$$  \hspace{1cm} \text{Eqn. 4.9}

$$=> w_0 = \gamma * B_0$$  \hspace{1cm} \text{Eqn. 4.10}

The Larmor frequency depends on the gyromagnetic ratio and the strength of the magnetic field and is different for each isotope. At a magnetic field of 18.7 T the Larmor frequency of protons is 800 MHz$^{[30]}$.

Fig. 4.4 The effect of gyromagnetic ratio and external magnetic field on larmor frequency
4.2b Instrumentation

The figure below is a simplified block diagram of a typical FT-NMR. The central component of the instrument is a highly stable magnet in which the sample is placed. The sample is surrounded by a transmitter/receiver coil. The excitation pulses are produced by a crystal-controlled continuous oscillator having an output frequency of $\nu_c$. This signal passes into a pulser switch and power amplifier, which creates an intense and reproducible pulse of radio-frequency radiation, which passes into the transmitter coil. The length of the pulse, and sometimes its amplitude, shape and phase can be adjusted by the operator. In the figure the pulse is shown to be 5µs in length. The resultant FID signal is picked up by the same coil (which now serves as a receiver), is amplified, and is transmitted to a detector. The phase sensitive detector computes the difference between the nuclear signals ($\nu_n$) and the crystal oscillator output ($\nu_c$), which leads to the low-frequency time domain signal shown on the right of the figure. This signal is digitized and collected in a computer for frequency analysis by a Fourier transform program. The output from this program is plotted giving a frequency domain spectrum$^{[6]}$. 
Proton and $^{13}$C NMR spectroscopy are the most widely used ones and have been used for the identification and structural elucidation of organic, metal-organic, and biochemical molecules. An NMR spectrum, in conjunction with other observations such as elemental analysis, as well as ultraviolet, infrared, and mass spectra, is a major tool for the characterization of pure compounds. A unique aspect of NMR spectra is the direct proportionality between peak areas and the number of nuclei responsible for the peak. As
a consequence, a quantitative determination of a specific compound does not require pure samples for calibration. Thus, if an identifiable peak for one of the constituents of a sample does not overlap the peaks of the other constituents, the area of this peak can be employed to establish the concentration of the species directly, provided only that the signal area per proton is known. This latter parameter can be obtained conveniently from a known concentration of an internal standard.

Methods for the analysis of many multicomponent mixtures have been reported. Methods for the determination of aspirin, phenacetin, and caffeine in commercial analgesic preparations requires 20 min. and relative errors are in the range of 1-3%. A procedure for the rapid analysis of benzene, heptane, ethylene glycol, and water in mixtures is also described with a precision of 0.5%. Another application of NMR has been the determination of functional groups. NMR spectroscopy can also be used to determine the total concentration of a given kind of magnetic nucleus in a sample. Although $^{13}$C NMR is about 6000 times less sensitive than proton NMR, it provides useful information about the backbone of the molecule. With the advent of decoupling methods, the $^{13}$C spectra is much easily interpreted and is simple\[6\].

Following are some of the other applications for NMR in drug development, drug research and analysis\[31\]:

1) Identification and quantification of impurities in drugs
2) Decomposition reactions
3) Quantification of drugs in dosage form
4) Analysis of complex mixtures, e.g. Excipients
5) pH-dependent measurements such as determination of the site of protonation, dissociation constants and stability constants

6) Complexation behavior of drugs such as selfassociation and interaction with other components of formulation as well as cations.

7) Determination of the isomeric composition of drugs

8) NMR of body fluids such as urine for the metabolism studies

9) Protein structure determination and protein-ligand interaction studies

10) Study of drug-ligand Complexation and calculation of stoichiometry

11) Study of ligand-membrane interactions

12) Use of solid-state NMR for finding solid state conformation of drugs and biologically active molecules and also to study polymorphism and pseudopolymorphism of drugs

**4.3 Ultraviolet/Visible absorption spectroscopy**

**4.3a Introduction**

The information concerning the structure of an organic molecule which may be gained from an ultraviolet-visible spectrum is more limited than in the case of IR, NMR and mass spectra. The principle features which may be detected are multiply bonded systems, conjugated systems and aromatic (and hetero-aromatic) nuclei. The electronic transitions in these systems which give rise to absorption in the 200-700 nm region are $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ and these are of diagnostic value. The $n \rightarrow \sigma^*$ electronic transition which arises in saturated compounds containing the heteroatom sulfur, nitrogen, bromine and iodine is of less importance since it leads to absorption just below 200 nm. The
corresponding transition in compounds containing oxygen and chlorine leads to absorption at somewhat shorter wavelengths; indeed, the transparency of alcohols and chloroalkanes in the region 200-700nm makes them ideal solvents for UV spectral determination. The $\sigma \rightarrow \sigma^*$ electronic transition in saturated hydrocarbons leads to absorption in the far-ultraviolet$^{[33]}$. The diagram below depicts the electronic transitions.

![Electronic transitions diagram](image)

**Fig. 4.6 A hypothetical energy diagram depicting the energy transitions**$^{[34]}$

Many molecules absorb ultraviolet or visible light. The absorbance of a solution increases as attenuation of the beam increases. Absorbance is directly proportional to the path length, $(b)$, and the concentration, $(c)$, of the absorbing species. Beer's Law states that:

$$A = \varepsilon bc = \log(P_o/P) = -\log T$$

Eqn. 4.11

where $(\varepsilon)$ is a constant of proportionality, called the absorptivity, $(b)$ is the path length of the cell, $(c)$ is the concentration of solute, $(P_o)$ is the power of the input beam, $(P)$ is the power of the attenuated beam and $(T)$ is transmittance. Different molecules absorb
radiation of different wavelengths. An absorption spectrum will show a number of absorption bands corresponding to structural groups within the molecule. For example, the absorption that is observed in the UV region for the carbonyl group in acetone is of the same wavelength as the absorption from the carbonyl group in diethyl ketone.\[^{34}\]

Beer’s law also applies to a solution containing more than one kind of absorbing species. Provided there is no interaction among the various species, the total absorbance for a multicomponent system is given by:

\[
A_{\text{total}} = A_1 + A_2 + \ldots + A_n 
\]

\[
= \varepsilon_1bc_1 + \varepsilon_2bc_2 + \ldots + \varepsilon_nbc_n 
\]

**4.3b Instrumentation**

Single beam and double beam spectrophotometers are available from commercial sources. The figure below shows the construction details of a manual double-beam ultraviolet/visible spectrophotometer. In this instrument, the radiation is dispersed by a concave grating, which also focuses the beam on a rotating sector mirror.
Fig. 4.7 Schematic of a typical manual double-beam spectrophotometer for the ultraviolet/visible region

4.3c Applications

Ultraviolet and visible spectrophotometry have somewhat limited application for qualitative analysis, because the number of absorption maxima and minima is relatively small. Thus, unambiguous identification is frequently impossible. An absorption spectrum in the visible and the ultraviolet regions is nevertheless useful for detecting the presence of certain functional groups that act as chromophores. For example, a weak absorption band in the region of 280 to 290 nm, which is displaced towards the shorter wavelength with increasing solvent polarity, strongly indicates the presence of the carbonyl group. A weak absorption band at about 260 nm with indications of vibrational fine structure constitutes evidence for the existence of an aromatic ring. Confirmation of the presence of an aromatic amine or a phenolic structure may be obtained by comparing the effects of pH on the spectra of solutions containing the sample with those for phenol and aniline.
Absorption spectroscopy is one of the most useful and widely used tools available to the chemist for quantitative analysis. Important characteristics of spectrophotometric and photometric methods include: (1) wide applicability to both organic and inorganic; (2) typical sensitivities of $10^{-4}$ to $10^{-5}$ M (this range can often be extended to $10^{-6}$ to $10^{-7}$ M by certain modifications); (3) moderate to high selectivity; (4) good accuracy (typically, relative uncertainties of 1-3% are encountered although with special precautions, errors can be reduced to a few tenths of a percent); and (5) ease and convenience of data acquisition.

Photometric or spectrophotometric measurements can be employed to advantage in locating the equivalence point of a titration, provided the analyte, the reagent, or the titration product absorbs radiation. Alternatively, an absorbing indicator can provide the absorbance change necessary for location of equivalence.
CHAPTER FIVE
FORMULATION INGREDIENTS – IBUPROFEN-NICOTINAMIDE COMPLEX AND EXCIPIENTS

5.1 Ibuprofen

5.1a Introduction

Chemical Name: $(\pm)$-2-(p -isobutylphenyl)propionic acid;

$(\pm)$-p-Isobutylhydratropic acid

Empirical Formula: $C_{13}H_{18}O_{2}$

Molecular weight: 206.28

5.1b Structural formula

Fig. 5.1: Structure of Ibuprofen

5.1c Physicochemical properties

Dissociation constant: $pK_a = 5.2$
Appearance, color, odor, taste: White to off-white, crystalline powder; slight characteristic odor and taste

Melting Point: 75 °C

Solubility: Very slightly soluble in water, very soluble in alcohol and other organic solvents\[1\].

5.1d Mechanism of action and use

Ibuprofen is a non-steroidal anti-inflammatory (NSAID) drug belonging to the class of propionic acid derivatives. It has been rated as the safest NSAID by the spontaneous adverse drug reaction reporting system in the U.K\[6\]. The major mechanism for NSAID anti-inflammatory action is by blocking prostaglandin generation. This is manifested by the inhibition of the enzyme cyclooxygenase. NSAIDs are also used as antipyretics since they block the action of pyrogens\[6\]. It has been shown recently that it also acts by preventing the translocation of nuclear factor-κB (NF-κB) from the cytoplasm into the nucleus which further prevents the up-regulations of many pro-inflammatory genes like tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1 β)\[7\]. Ibuprofen has already been recommended in the relief from pain associated with osteoarthritis, lumbago, headaches, menstrual cramps and muscular aches and pains. A review of the literature shows that it can also be used in post-operative dental pain and menstrual migraine\[8,9\]. The literature also recommends Ibuprofen as the first line analgesic for the short term management of painful conditions because of the poor tolerability of aspirin and the potential risks of paracetamol overdose\[10\].
5.1e Available dosage forms and dose\textsuperscript{[1,11,12]}

Adult: Tablets, Caplets and Gelcaps are the available dosage forms for the adults.

Analgesia - 200 to 400 mg every 4-6 hours as needed

Rheumatoid arthritis and osteoarthritis - 300 to 400 mg every 6-8 hours

Maximum total daily dosage - 2400 mg

Pediatric: Tablets and suspensions and are the available dosage forms for pediatrics.

Pain and fever – 100 mg every 6-8 hours

Infant (6-23 months): Drops are available for infants.

Pain and fever – 50 mg every 6-8 hours

5.2 Nicotinamide

5.2a Introduction

Chemical Name: Pyridine-3-carboxamide

Empirical Formula: C\textsubscript{6}H\textsubscript{5}N\textsubscript{2}O

Molecular weight: 122.13

5.2b Structural formula

Fig. 5.2: Structure of Nicotinamide
5.2c Physicochemical properties

Appearance, color, odor, taste: White crystals or crystalline powder; odorless or has a slight odor; bitter in taste.

Melting Point: 128-131°C

Solubility: 1g in 1.5 ml water, 5.5 ml alcohol, and 10 ml glycerin[^6].

5.2d Mechanism of action and use

Nicotinamide or vitamin B₃ is an important constituent of coenzymes nicotinamide-adenine dinucleotide (NAD) and nicotinamide-adenine dinucleotide phosphate (NADP) that occur in a wide variety of enzyme systems involved in the anaerobic oxidation of carbohydrates. The coenzyme acts as a hydrogen acceptor in the oxidation of the substrate. These enzymes are present in all living cells and take part in many reactions of biological oxidation[^1].

5.2e Available dosage forms

Tablets and injections are available for prophylactic and therapeutic use.

Prophylactic: 10-20 mg once a day

Therapeutic: Parenteral – 25-50 mg 2 to 10 times a day

Oral – 50 mg 3 to 10 times a day

5.3 Carboxymethylcellulose sodium

Carboxymethylcellulose sodium is widely used in oral and topical pharmaceutical formulations primarily for its viscosity-increasing properties. Viscous aqueous solutions
are used to suspend powders intended for either topical application or oral or parenteral administration\textsuperscript{[13]}. It is also used as a tablet binder and disintegrants\textsuperscript{[14]} and is also used to stabilize emulsions\textsuperscript{[15]}.

Higher concentrations around 3-6\% of the medium viscosity grade are used to produce gels. It is also one of the major ingredients of self-adhesive ostomy, wound care, and dermatological patches where it is used to absorb wound exudate or transepidermal water and sweat. As an emulsifying agent it is used in the concentration of 0.25-1\%. Aqueous solutions are stable between pH 2-10; below pH 2 precipitation can occur, while above pH 10 viscosity of the solution rapidly increases. Sterilization of solutions by autoclaving or by gamma-radiation results in a decrease in viscosity. Aqueous solutions stored for prolonged periods should contain an antimicrobial preservative. Under high humidity conditions it can absorb more than 50\% water, which can lead to a decrease in tablet hardness. CMC is incompatible with strongly acidic solutions and with the soluble salts of iron, aluminium, mercury, and zinc. It can precipitate when mixed with ethanol (95\%). It forms complex coacervates with gelatin and pectin. It additionally forms a complex with collagen and is capable of precipitating certain positively charged proteins.

5.4 Veegum

Veegum is not a “gum”, rather it is a montmorillonite mineral called “smectite”\textsuperscript{[16]}. It is a complex colloidal and extremely plastic magnesium aluminum silicate. It is an off-white insoluble flake magnesium aluminum silicate that swells to many times its original volume when added to water. Its aqueous dispersions are thus high viscosity thixotropic gels at low solids. Veegum is not subject to attack by
microorganisms. Various grades are classified according to viscosity and ratio of aluminum to magnesium content.

It is practically insoluble in ethanol, glycerin, propan-2-ol, and water\textsuperscript{16}. In water it swells up to about 12 times its original volume to form viscous homogeneous suspensions, sols, or gels depending upon the concentration. Sols and gels may be conveniently prepared by sprinkling the bentonite on the surface of hot water and allowing it to stand for 24 hours, stirring occasionally. Its viscosity is 75-225 mPa for a 5.5% w/v aqueous suspension at 25\(^0\)C. It is primarily used in the formation of suspensions, gels, and sols for topical pharmaceutical application. It is also used to suspend powders in aqueous preparations and to prepare cream bases containing oil-in-water emulsifying agents. In oral preparations it is used to absorb cationic drugs and retard their release. Veegum HS or bentonite aqueous suspensions retain their viscosity above pH 6 but are precipitated by acids\textsuperscript{16}.

5.5 Sorbitol Solution 70%

Sorbitol solution is a water solution containing in each 100g, 69-71g of total solids consisting essentially of D-sorbitol and mannitol and other isomeric polyhydric alcohols. The content of D-sorbitol \([\text{C}_6\text{H}_8(\text{OH})_6]\) in each 100g is not less than 64g\textsuperscript{14}. It is a clear, colorless, odorless viscous liquid having a sweet taste.

Density: 1.293g/cm\(^3\)

Viscosity: 110.0 mPas at 25\(^0\)C

Refractive index: 1.458

Pharmaceutical category: Sweetening agent
5.6 Saccharin Sodium

Chemical name: 1,2-Benzisothiazol-3(2H)-one 1,1 dioxide sodium salt

It exists in the anhydrous and dihydrate forms.

![Structure of sodium saccharin](image)

**Fig. 5.3 Structure of sodium saccharin**

Appearance: White, odorless, faintly aromatic, efflorescent crystalline powder

Acidity/alkalinity: 10% w/v aqueous solutions have a pH = 6.6

Density: 0.86 g/cm³ (bulk), 1.70 g/cm³ (particle), 0.96 g/cm³ (tapped)

Melting point: Decomposes upon heating

Moisture Content: Sodium saccharin 84% contains 5.5% w/w water

Solubility: 1g in 1.5 ml water, 50ml alcohol[^6]

Sodium saccharin is a sweetening agent used in tablets, powders, medicated confectionary, gels, suspensions, and liquids. It is also used in vitamin preparations. Its sweetening power is approximately 300 times that of sucrose. Sodium saccharin decomposes only when exposed to a high temperature (125°C) at a low pH (pH 2) for over 1 hour[^16].
5.7 Disodium edetate (Disodium EDTA)

Chemical name: Disodium ethylenediamine tetraacetate

Appearance: Odorless, white crystalline powder with a slightly acidic taste

Acidity/alkalinity: pH=4.3-4.7 for a 1% w/v solution

Melting point: Decomposition at 252\(^{0}\)C for the dihydrate

Refractive Index: 1.335 for a 1% w/v solution

Solubility: Soluble 1 in 11 parts of water, slightly soluble in ethanol (95%)\(^{[17]}\)

Specific gravity: 1.004 for a 1% w/v solution

Viscosity (kinematic): 1.03 mm\(^{2}\)/s for a 1% w/v solution

In pharmaceutical formulations disodium edetate is typically used as a chelating agent at concentrations between 0.005-0.1% w/v. It is primarily used as an antioxidant synergist by sequestering trace amounts of metal ions\(^{[5]}\).
 chapter six
materials and methods

6.1 Reagents and Chemicals

Ibuprofen USP, Upjohn Co., (Lot#1194 F)

Ibuprofen oral suspensions, Advil®, (Lot#A34587), Madison, NJ and Motrin®, (Lot#HPM031), Fort Washington, PA

Nicotinamide, Sigma Chemical Co., (Lot#37H0859), St. Louis, MO

200 proof Ethyl Alcohol – Pharmco Products Inc.

CMC sodium, Amend Drug and Chemical Co., (Lot#W42042G04), Irvington, NJ

Sorbitol solution 70% USP, Amend Drug and Chemical Co., (Lot#Y48134M16), Irvington, NJ

Veegum, Dist: Lapine Scientific Co., Blue Island, IL

Disodium EDTA, Ruger Chemical Co., (Lot#38091), Irvington, NJ

Saccharin Sodium, Ruger Chemical Co., (Lot#X1581J22), Irvington, NJ

Simple syrup 70% w/v, The University of Toledo, College of Pharmacy prepared

6.2 Methods and Instruments

6.2a Solvent evaporation method

Solvent evaporation method, also known as coprecipitation or coevaporation, was used to obtain homogeneous mixtures of Ibuprofen and Nicotinamide. In this method, the
carrier(s) and the active ingredient(s) are dissolved in a suitable organic solvent and the solvent is evaporated at an elevated temperature or under vacuum\textsuperscript{[1]}. As the solvent is being removed, supersaturation occurs followed by simultaneous precipitation of the constituents resulting in a solid residue. The coprecipitate is then dried under vacuum to drive out any solvent freely adhering to the particle surface. However, the possibility of formation of a solvate with any of the constituents cannot be ruled out. This can take place when the solvent molecules are held within the crystal lattice. The formation of a solvate was ruled out in this study by the use of DSC. A series of Ibuprofen:Nicotinamide mixtures were made with the use of ethanol as solvent. It was then kept on a hot plate at low temperature until most of the solvent evaporated. The sample was then vacuum dried for 12 hours before it was used for study.

6.2b Differential scanning calorimetry studies

For the DSC studies, an MDSC 2910 unit from TA Instruments (P/N: 924500.501; S/N: 5000-2C0053) was used. Universal Analysis 2000 software was used for the analysis of data. For this study, open aluminum crucibles (100µl) were used. The heating range used was 25°C to 150°C and the heating rate employed was 5°C per minute. The DSC was calibrated with Indium (5-10 mg., 99.999% pure, onset 156.6°C and heat of fusion of 28.42 J/g) and Zinc (2-5 mg., 99.999% pure, onset 419.6°C and heat of fusion of 107.5 J/g). Mettler MT 5 microbalance was used for weighing the samples and the nitrogen flow used was 50 ml per minute.
6.2c Powder x-ray diffraction (PXRD) studies

PXRD studies were performed on XDS 2000 unit from Scintag Corp. and the samples were exposed to CuKα radiation (45 KV, 40 mA, λ = 1.540600 Å) and scanned from 2θ = 0 to 70° at a scan rate of 2° per minute. A plastic sample holder (1x1 inch) was used for the study.

6.2d Fourier transform infrared (FTIR) studies

Nicolett SX-60 unit was used for these studies. Potassium Bromide (KBr) was used to make the pellets which were used for the study.

6.2e Microscopic studies

Nikon SMZ 1500 microscope was used with Nikon coolpix 990 (3.34MPixel) digital camera attached over it. The samples were mounted on a glass slide and dispersed in liquid paraffin before they were seen under the microscope. The samples were also viewed under polarized light to get a feel of surface topography of the particles.

6.2f Ultraviolet-Visible spectroscopy (UV-Vis) studies

Beckman DU® 640 Spectrophotometer was used for these studies and the solvent used was ethanol. The dilutions were prepared in order to get the absorbance values less than unity.
6.2g Nuclear magnetic resonance spectroscopy (NMR) studies

Varian 400 MHz FTNMR was employed for these studies and DMSO-d6 was used as a solvent at ambient temperature. Trimethyl silane was used as a reference standard.

6.2h High performance liquid chromatography (HPLC) studies

Waters HPLC system – Phenomenex®, Jupiter C18 column 2.0 x 250 mm (S.no.: 195986-6) – particle size 5 µm, Waters 501 HPLC pump, 100µl loop injector, Waters 712 WISP autosampler, Waters 486 absorbance detector, Millennium 2010 Chromatography Manager – Version 3.2, Bedford, MA.

PH211 Microprocessor pH meter from Hanna Instruments, Woonsocket, RI and Electric laboratory stirrer, Phipps & Bird, Inc., Richmond, VA were the additional instruments used.

Mobile phase preparation: The mobile phase consisted of a mixture of 95% buffer and 5% acetonitrile delivered at 0.5 ml/min. The buffer was composed of 1% (w/v) chloroacetic acid. The pH of buffer was adjusted to 3.0 with ammonium hydroxide. Both solutions were filtered through 0.45µm FP Vericel Membrane Filter, HPLC certified, Lot#2092010 supplied by Gelman Sciences, Ann Arbor, MI, with the help of a Millipore filter holder, part#4, obtained from Millipore Filter Corporation, Bedford, MA, and degassed using argon gas (AGA gas, Toledo, OH). The column was maintained at room temperature for the entire analytical procedure. The UV detector was set at 254 nm because ibuprofen and nicotinamide have their UV absorption maxima at this
wavelength. The sample processor was adjusted to inject 20 µl of the sample into the column.

6.2i Preparation of ibuprofen suspension

The formula outlined in Table 6.1 was utilized for preparing the suspension of ibuprofen. Carboxymethylcellulose sodium was weighed and dispersed in 200ml warm water, mixed with a magnetic stirrer, and left overnight to increase hydration. Veegum was weighed and hydrated in 200 ml water overnight. One liter of simple syrup USP (85% w/v) was prepared by adding 850 g of sucrose to 450 ml of RO water and heating it to dissolve and then making up the volume with RO water. To prepare the 20 mg/ml ibuprofen suspension, 20.0g of ibuprofen was weighed and placed in a mortar. The hydrated carboxymethylcellulose sodium was then added slowly to the powder with constant trituration. The flavor was then added and triturated. The contents of the mortar were then transferred to a 2000 ml beaker. The mortar was washed with RO water and the washings added to the 2000 ml beaker. An electric laboratory stirrer was used to disperse and mix the suspension. Simple syrup USP and sorbitol solution 70% (w/v) were added, mixed and dispersed. Then 1.5 g of saccharin were dissolved in 15 ml boiling water and added to the dispersion. Following this, 0.1 g of EDTA was dissolved in 5 ml water and added. Then, 10 ml of the paraben concentrate were added and the volume made up with RO water to 1000 ml. The contents were mixed with electric stirrer. The pH of the suspension was found to be 5.82.
Table 6.1 Formula for the preparation of Ibuprofen suspension

<table>
<thead>
<tr>
<th>Amount Prepared: 1000 ml</th>
<th>Ingredient</th>
<th>% Concentration</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ibuprofen</td>
<td>2.0</td>
<td>20 g</td>
</tr>
<tr>
<td></td>
<td>Carboxymethylcellulose sodium</td>
<td>0.75</td>
<td>7.5 g</td>
</tr>
<tr>
<td></td>
<td>Veegum</td>
<td>0.75</td>
<td>7.5 g</td>
</tr>
<tr>
<td></td>
<td>Simple syrup USP</td>
<td>25.0</td>
<td>250 ml</td>
</tr>
<tr>
<td></td>
<td>Sorbitol solution 70% w/v</td>
<td>25.0</td>
<td>250 ml</td>
</tr>
<tr>
<td></td>
<td>Saccharin</td>
<td>0.15</td>
<td>1.5 g</td>
</tr>
<tr>
<td></td>
<td>Disodium EDTA</td>
<td>0.01</td>
<td>0.1 g</td>
</tr>
<tr>
<td></td>
<td>Paraben concentrate (10% ethyl paraben and 2% propyl paraben in propylene glycol q.s. 1000ml)</td>
<td>1.0</td>
<td>10 ml</td>
</tr>
<tr>
<td></td>
<td>Flavor (Orange and raspberry)</td>
<td>0.1</td>
<td>1 ml of 1:1 mixture</td>
</tr>
<tr>
<td></td>
<td>RO water q.s.</td>
<td>100.0</td>
<td>q.s. 1000 ml</td>
</tr>
</tbody>
</table>

6.2j Sample preparation for the analysis of ibuprofen suspensions

Samples for the ibuprofen suspension for HPLC assay were prepared as follows. The suspensions – Motrin®, Advil® and the one prepared in this study, were shaken well and filtered through Watman filter paper and 20µl portion of this solution was injected into the HPLC system for analysis. In case of ibuprofen suspension made from the complex, the filterate was diluted ten times before 20µl of it were injected into the HPLC system.
7.1 DSC Studies

The DSC studies clearly showed some interaction between ibuprofen and nicotinamide. In almost all of the scans for the binary mixtures, a peak at a temperature of around 88°C was seen which was intermediate between the melting endotherms for the individual components. The heat of fusion and sharpness of the endotherm for the complex kept on increasing as the percentage of Ibuprofen was increased until 60% after which it again decreased. Had this endotherm been at a lower temperature than the melting endotherms of the pure components, it would have been a eutectic. This clearly indicated a possibility for a complexation reaction that results in a single compound giving a melting endotherm at an intermediate temperature. At a composition of 60% ibuprofen and 40% nicotinamide, only a sharp single endotherm was observed. This composition corresponds to approximately a 1:1 molar ratio for the two components, which could be the stoichiometry of the possible complex in the solid state. Since the melting point of the drug has been reduced in the complexed form, it can be predicted that the drug might have more solubility in the complexed form as compared to the pure form\[^1\]. Figure 1 shows the overlay of the DSC scans for the various binary mixtures of ibuprofen and nicotinamide.
Figure 7.1 Overlay of the DSC scans for the ibuprofen and nicotinamide binary mixtures

7.2 UV-Vis Spectroscopy

The wavelength scan for the three compounds were performed from 600-200 nm in 200 proof ethyl alcohol to observe any change in λ_max for the probable complex. The solutions were sufficiently diluted to get their absorbance values less than unity. The probable complex had λ-values that were quite close to that observed for nicotinamide. Table 2 gives the λ_1 and λ_2 values for the three compounds.

Table 7.1: Results from UV scans

<table>
<thead>
<tr>
<th>Compound</th>
<th>λ_1</th>
<th>λ_2</th>
<th>Molecular Weight</th>
<th>A (Observed)</th>
<th>A (Calculated)</th>
<th>ε</th>
<th>Concentration (µmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ibuprofen</td>
<td>219.0</td>
<td>---</td>
<td>206.27</td>
<td>0.4325</td>
<td>---</td>
<td>8103.8</td>
<td>0.04848</td>
</tr>
<tr>
<td>Nicotinamide</td>
<td>213.0</td>
<td>261.0</td>
<td>122.12</td>
<td>0.7636</td>
<td>---</td>
<td>8817.5</td>
<td>0.08188</td>
</tr>
<tr>
<td>Complex</td>
<td>214.0</td>
<td>260.5</td>
<td>328.39</td>
<td>0.5325</td>
<td>0.5308</td>
<td>17279.4</td>
<td>0.03045</td>
</tr>
</tbody>
</table>
The absorbance of the complex was calculated by assuming that there is no interaction and the two components are independently present in the solution. Since the calculated and the observed absorbance for the assumed complex are quite close, we can conclude that either the value of the complexation constant is not large enough or there is no complex formation. Also, the epsilon value for the complex is quite close to the sum of the epsilon values for the individual components which further indicates a lack of any interaction. In the calculation of the epsilon value of the complex it was assumed that the molecular weight of the complex is sum of molecular weights of the two compounds. No conclusive information regarding the nature of the interaction could be obtained by UV-Vis spectroscopy.
7.3 FTIR Spectroscopy

The FTIR spectrum of the complex showed a disappearance of the broad peak for hydrogen bonding that was observed from 2300–3485 cm$^{-1}$ in the spectrum for Ibuprofen. This suggests that the molecules of nicotinamide occupy the spaces between the molecules of ibuprofen and prevent the formation of hydrogen bonding.

Figure 7.3: FTIR spectrum for ibuprofen

Figure 7.4: FTIR spectrum for nicotinamide
Table 7.2: Summary of the FTIR group frequencies

<table>
<thead>
<tr>
<th>Group</th>
<th>Wavenumber (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ibuprofen</td>
</tr>
<tr>
<td>C=O</td>
<td>1728.9</td>
</tr>
<tr>
<td>O-H</td>
<td>2300-3485</td>
</tr>
<tr>
<td>N-H</td>
<td>---</td>
</tr>
<tr>
<td>Aliphatic C-H</td>
<td>2961.9</td>
</tr>
<tr>
<td>Aromatic C-H</td>
<td>Hidden</td>
</tr>
</tbody>
</table>

7.4 $^1$H NMR Spectroscopy

The $^1$H NMR imaging was done in deuterated DMSO for all the three samples. The imaging for the solid solution behaved as a simple mixture and appearance of the
carboxylic proton shows that there is no chemical interaction between ibuprofen and nicotinamide.

Figure 7.6: $^1$H NMR imaging for ibuprofen

Figure 7.7: $^1$H NMR imaging for nicotinamide
Figure 7.8: $^1$H NMR imaging for the complex

7.5 Powder X-Ray Diffraction Studies

It was observed from the X-Ray diffraction studies that the crystallinity of the solid solution is remarkably reduced as compared to the two individual components. The reduction of the crystallinity of the complex is the most probable cause of the solubility enhancement. The peaks that were observed in the scans for ibuprofen and nicotinamide were in good agreement with the standard x-ray diffraction peaks for the two components[2-4]. Since it was difficult to identify a single peak in the complex which could be identified in the spectra of the pure components, it was not possible to calculate the reduction in the crystalline behavior in terms of percentage[5,6].
7.6 Microscopic Studies

These studies were done in normal light as well as polarized light in order to get more visual information about the complex and the nature of the interaction. The results are shown in the form of pictures obtained by the digital camera. It was observed under normal light that the shape of the Ibuprofen crystals were irregular and globular and that of nicotinamide were rod-like. Both the pure components were transparent to normal light. The complex on the other hand had reduced and uniform particle size and was quite opaque to normal light. Under polarized light, the topography and three dimensional view of the samples could be seen more clearly. It was concluded that the complex was less crystalline and less transparent and it is in agreement with the results obtained from the PXRD studies.
Figure 7.10: The microscopic picture for ibuprofen

Figure 7.11: The microscopic picture for nicotinamide

Figure 7.12: The microscopic picture for the complex
Figure 7.13: The microscopic picture of ibuprofen in polarized light

Figure 7.14: The microscopic picture of nicotinamide in polarized light

Figure 7.15: The microscopic picture of complex in polarized light
7.7 High Performance Liquid Chromatography Studies

The HPLC studies were performed to calculate the solubility enhancement achieved from the complexed ibuprofen as compared to the pure drug. The first part of the study was to compare the solubility of ibuprofen in saturated Ibuprofen solution and complexed ibuprofen solution. The second part of the study was to compare the extent of solubilized ibuprofen in Advil®, Motrin® and the suspension made with the Ibuprofen:Nicotinamide complex. Figure 7.16 is a representative HPLC chromatogram showing separation and solubility enhancement of ibuprofen in the presence of Nicotinamide.

![HPLC Chromatogram](image)

**Figure 7.16 HPLC chromatogram showing the peak separation and solubility enhancement for ibuprofen (A) in the presence of nicotinamide (B)**
7.7a Solubility comparison of saturated solutions of ibuprofen and complexed ibuprofen

For this study, one gram each of the pure drug and the complex were dissolved separately in 50 ml of reverse osmosis water and shaken overnight on a magnetic stirrer. HPLC studies were then performed to calculate the solubility enhancement. Solubility enhancement was simply calculated by dividing the average area of the peaks of Ibuprofen obtained in both the cases.
Solubility Enhancement  =  \frac{23881220}{465983} = 51

Thus, it was seen that when the concentration of nicotinamide is 8 mg/ml, the solubility of ibuprofen in the complex solution was enhanced 51 times. Table 2 gives the peak area and retention times for the ibuprofen in both solutions.

Table 7.3 Comparison of the solubility of pure ibuprofen and complexed ibuprofen

<table>
<thead>
<tr>
<th>Saturated Ibuprofen</th>
<th>Complexed Ibuprofen (10 times diluted)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention Time (Min)</td>
<td>Peak Area</td>
</tr>
<tr>
<td>3.347</td>
<td>499172</td>
</tr>
<tr>
<td>3.341</td>
<td>488748</td>
</tr>
<tr>
<td>3.344</td>
<td>428096</td>
</tr>
<tr>
<td>3.343</td>
<td>401080</td>
</tr>
<tr>
<td>3.334</td>
<td>491915</td>
</tr>
<tr>
<td>3.339</td>
<td>466225</td>
</tr>
<tr>
<td>3.352</td>
<td>486643</td>
</tr>
</tbody>
</table>

7.7b Comparison of the extent of solubilized ibuprofen in various suspensions

These studies were performed to see if the suspension prepared in this work was in any way better than those already in the market. The purpose of this study was therefore to see if the extent of solubilization of Ibuprofen in the aqueous phase of the suspension was greater than that of Advil® and Motrin®. For this study, the suspensions were well shaken and filtered through Watman filter paper. Since the suspensions were very viscous, the filtration system was kept overnight to get substantial amount of filtrate.
The filtrate was then injected in HPLC system for analysis. Table 3 gives the results that were obtained from this study.

**Table 7.4 Comparison of the solubility of ibuprofen in various suspensions studies**

<table>
<thead>
<tr>
<th>Retention Time (Min)</th>
<th>Peak Area</th>
<th>Retention Time (Min)</th>
<th>Peak Area</th>
<th>Retention Time (Min)</th>
<th>Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Advil</td>
<td>Motrin</td>
<td>Complexed Ibuprofen suspension (10 times diluted)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.269</td>
<td>676044</td>
<td>4.267</td>
<td>1084633</td>
<td>3.350</td>
<td>2944395</td>
</tr>
<tr>
<td>4.289</td>
<td>679491</td>
<td>4.264</td>
<td>1142314</td>
<td>3.351</td>
<td>2911735</td>
</tr>
<tr>
<td>4.296</td>
<td>677424</td>
<td>4.268</td>
<td>1115738</td>
<td>3.352</td>
<td>2898632</td>
</tr>
<tr>
<td>4.300</td>
<td>674446</td>
<td>4.263</td>
<td>1139110</td>
<td>3.351</td>
<td>2874668</td>
</tr>
<tr>
<td>4.292</td>
<td>668028</td>
<td>4.270</td>
<td>1083034</td>
<td>3.351</td>
<td>2870760</td>
</tr>
<tr>
<td>4.289</td>
<td>665241</td>
<td>4.262</td>
<td>1030182</td>
<td>3.349</td>
<td>3007957</td>
</tr>
</tbody>
</table>

The results obtained in this study were used to calculate the enhancement in the extent of solubilized Ibuprofen. This data is summarized in Table 5. Table 6 lists some of the physical characteristics of the suspensions used.

**Table 7.5 Comparison of ibuprofen solubility in various suspensions as compared to saturated ibuprofen solution**

<table>
<thead>
<tr>
<th></th>
<th>Average peak area</th>
<th>Factor by which solubility is enhanced</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated Ibuprofen</td>
<td>465983</td>
<td>1.0</td>
</tr>
<tr>
<td>Advil</td>
<td>673446</td>
<td>1.5</td>
</tr>
<tr>
<td>Motrin</td>
<td>1099168</td>
<td>2.4</td>
</tr>
<tr>
<td>Complexed Ibuprofen suspension</td>
<td>29180245</td>
<td>62.6</td>
</tr>
</tbody>
</table>
Table 7.6 Characteristics of various suspensions studied

<table>
<thead>
<tr>
<th></th>
<th>Advil</th>
<th>Motrin</th>
<th>Complexed Ibuprofen suspension</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>4.3</td>
<td>3.9</td>
<td>5.8</td>
</tr>
<tr>
<td>Conc. of Ibuprofen</td>
<td>20mg/ml</td>
<td>20mg/ml</td>
<td>20mg/ml</td>
</tr>
<tr>
<td>Conc. of Nicotinamide</td>
<td>---</td>
<td>---</td>
<td>13.3mg/ml</td>
</tr>
<tr>
<td>Flavor</td>
<td>Blue Raspberry</td>
<td>Bubblegum</td>
<td>Orange &amp; Raspberry</td>
</tr>
</tbody>
</table>

It can also be seen that a much higher solubilization is achieved in the complexed ibuprofen suspension even at relatively higher pH value of 5.8, keeping in mind the fact that Ibuprofen being a weak acid is more soluble at lower pH values. These studies show that the extent of solubilization can be further enhanced by reducing the pH of the formulation and increasing the concentration of nicotinamide in the suspension. The suspension prepared in this work has 62 times more solubilized Ibuprofen in the aqueous phase as compared to saturated Ibuprofen solution. The concentration of nicotinamide in this suspension was 13.3 mg/ml.
CHAPTER EIGHT
CONCLUSIONS

The concept of hydrotropy has been successfully used in this work to increase the water solubility of a very poorly soluble drug – ibuprofen. In the present work, an ibuprofen suspension has been made which has 62 times more drug solubility in the aqueous phase as compared to two of the most popular suspensions available on the market. Ibuprofen has been rated as the safest NSAID by the spontaneous adverse drug reaction reporting system in the U.K\textsuperscript{[1]}. Ibuprofen has already been recommended for the relief from pain associated with osteoarthritis, lumbago, headaches, menstrual cramps and muscular aches and pains\textsuperscript{[2,3]} and a review of the literature shows that it can also be used in post-operative dental pain and menstrual migraine\textsuperscript{[4,5]}. The literature also recommends ibuprofen as the first line analgesic for the short term management of painful conditions because of the poor tolerability of aspirin and the potential risks of paracetamol overdose\textsuperscript{[6]}. Because of these benefits for ibuprofen over other pain relieving drugs, it was chosen for the study. There is always a need of faster action of drugs used for pain relief. In this work, nicotinamide has been successfully used as a hydrotrope to increase the solubility of ibuprofen. Usually high concentrations of the hydrotropic agent are required to achieve any substantial improvement in the drug solubility. It is for this
reason that the concept has not become popular. In this regard, nicotinamide has proven to be a successful hydrotrope since it is a non-toxic vitamin which can be used in higher concentrations. A pilot study has suggested that nicotinamide, if given in high enough doses, might be beneficial to some patients with osteoarthritis, granuloma annulare, Type 1 diabetes and cancer, although the mechanism of the putative activity is unknown\cite{7}.

The primary objective of this study was to examine the nature of the solid-state interaction between ibuprofen and nicotinamide using thermal, spectroscopic and microscopic techniques. A secondary objective was to determine the usefulness of nicotinamide as a solubilizing agent and to develop a suitable efficacious formulation. A suspension was found to be the suitable choice for product formulation since it provided the required concentration of the hydrotropic agent to be effective as a solubilizer.

DSC studies showed that a complex was formed in the solid state between ibuprofen and nicotinamide. The stoichiometry of the complex was found to be 1:1. The results also predicted higher solubility for the drug in the complexed form since its melting point has been reduced. UV-Visible spectroscopy was not adequate to explain the type of interaction since the inter-molecular forces responsible for the complex formation were too weak to sustain the integrity of complex in solution. Disappearance of the broad hydrogen bonding peak in the FTIR spectrum for the complex suggests that the molecules of nicotinamide occupy the intermolecular spaces between the molecules of ibuprofen and prevent the formation of hydrogen bonding. FTIR studies also indicated greater solubility for the complex since the strong forces of inter-molecular hydrogen bonding were absent in the complex. Proton NMR studies ruled out the possibility of any chemical interaction between the two compounds since the number and position of the
peaks in the spectrum for the complex were not different from those of the individual compounds. Since the solvent used was deuterated DMSO, the only way the appearance of a carboxylic proton in the spectrum for the complex can be explained is when there is only a physical interaction between the two components. PXRD studies showed that the crystallinity of the complex is remarkably reduced which again predicted greater solubility for the complex. Microscopic studies supported the results obtained from previous studies. The pictures showed remarkable reduction in the particle size for the complex sample. The HPLC studies were performed to calculate the solubility enhancement for the complex in solution as well as in various suspensions. It was observed that the solubility enhancement increased with increasing concentration of nicotinamide. The solubility of ibuprofen was enhanced 51 times in solution when the concentration of nicotinamide was 8 mg/mL. In a similar manner the solubility was enhanced 62 times in the suspension when the concentration of nicotinamide was 13.3 mg/mL.

**Recommendations for future work**

Although the solid state studies that were performed in this work were good enough to study the solid state interaction, solid state NMR could give more insight and information about the type of interaction. The solution state interaction was not studied in this work. HPLC could be used to study the interaction between ibuprofen and nicotinamide in solution. This would give us the effect of nicotinamide concentration on solubility enhancement of ibuprofen, the stoichiometry and the formation constant of the complex in solution. The concept of hydrotropy could be better understood when these studies are performed.
References for Chapter one

1. Chen A.X., Zito S.W., Nash R.A.; Solubility enhancement of nucleosides and structurally related compounds by complex formation; Pharm. Res.; 11(3); (1994); 398-401.

2. Fukokova E., Makita M., Yamamura S; Glassy state of pharmaceuticals II. Bioinequivalence of glassy and crystalline indomethacin; Chem. Pharm. Bull; 35; (1987); 2943-2948.

3. Yalkowsky S.H.; Techniques of solubilization of drugs; Marcel Dekker; 1981; U.S.A.


5. Stenberg P., Bergstrom C.A.S., Luthman K., Artursson P.; Theoretical predictions of drug absorption in drug discovery and development; Clin. Pharmacokinetics; 41(11); (2002); 877-899.

6. Neuberg C.; Hydrotropy; Biochemische Zeitschrift; 76, (1916); 107-176.


12. Rasool A.A., Hussain A.A., Dittert L.W.; Solubility enhancement of some water-insoluble drugs in the presence of nicotinamide and related compounds; J. Pharm. Sci.; 80(4); (1991); 387-393.


17. Earl R.T., Jenkins R., Munro A.J.; A double-masked comparison of the efficacy of once-daily sustained-release ibuprofen and once-daily piroxicam for 24-hour control of arthralgia due to osteoarthritis in the elderly; Current Therap. Res.; 57(10); (1996); 811-821.

19. Moschiano F., Grazzi L., D’Amico D., Schieroni F., Bussone G.; Menstrual Migraine; J. Headache Pain; 2; (2001); S117-S119.


References for Chapter two

1. Yalkowsky S.H.; Solubility and solubilization in aqueous media; Oxford University Press; 1999; U.S.A.;

2. Neuberg C.; Hydrotropy; Biochemische Zeitschrift; 76, (1916); 107-176.


10. Nakano N.I., Igarashi S.J.; Molecular interactions of pyrimidines, purines, and some other heteroaromatic compounds in aqueous media; Biochemistry; 9; (1970); 577-583.


14. Higuchi T., Kristiansen H.; Binding specificity between small organic solutes in aqueous solution: Classification of some solutes into two groups according to binding tendencies; J. Pharm. Sci.; 59; (1970); 1601-1608.


18. Donbrow M., Ben-Shalom H.; Molecular interactions of caffeine with o-, m- and p-iodobenzoic acids and o-, m- and p-fluorobenzoic acids; J. Pharm. Pharmac.; 19; (1967); 495-501.

19. Yalkowsky S.H.; Techniques of solubilization of drugs; Marcel Dekker; 1981; U.S.A.

20. Chen A.X., Zito S.W., Nash R.A.; Solubility enhancement of nucleosides and structurally related compounds by complex formation; Pharm. Res.; 11(3); (1994); 398-401.

21. Hussain M.A., Diluccio R.C., Maurin M.B.; Complexation of Moricizine with nicotinamide and evaluation of the complexation constants by various methods; J. Pharm. Sci.; 82(1); (1993); 77-79.

22. Ford J.L., Rubinstein M. H.; Phase equilibria and stability characteristics of chlorpropamide-urea solid dispersions; J. Pharm. Pharmac.; 29; (1977); 209-211.

23. Verma M.M., Kumar M.T., Balasubramaniam J., Pandit J.K.; Bioavailability studies on fast release formulations of indomethacin; Boll. Chim. Farmac; 141(3); (2002); 176-180.


25. Eshra A.G., Naggar V.F., Boraie N.A.; A study of indomethacin-nicotinamide solid dispersion; Pharm. Ind.; 48(12); (1986); 1557-1560.

27. Ammar H.O., Khalil R.M.; Effect of aromatic hydrotropes on the solubility of oxamniquine; Pharmazie; 51(7); (1996); 490-493.


References for Chapter three


2. Dodd J.W., Tonge K.H.; Thermal methods; Wiley; 1987; Great Britain.

3. Haines P.J.; Thermal methods of analysis; Blackie academic & professional; 1995; New Zealand.

4. Townsend I.; Basic strategy for the thermal stability assessment of pharmaceutical synthetic intermediates and products; J. Therm. Anal.; 37; (1991); 2031.

5. Barnes A.F., Hardy M.J., Lever T.J.; A review of the applications of thermal methods in the pharmaceutical industry; J. Therm. Anal.; 40; (1993); 499.

6. Ford J.L., Timmins P.; Pharmaceutical thermal analysis; Ellis Horwood; Chicheste; 1989.

7. Clas S.D., Dalton C.R., Hancock B.C.; Differential scanning calorimetry: applications in drug development; PSTT; 2(8); (1999); 311-320.


22. Brittain H.G.; X-ray diffraction III: Pharmaceutical applications of x-ray powder diffraction; Spectroscopy; 16(7); (2001); 14-18.

23. Ranter C.J.; Applications of X-ray diffractometric techniques in the analysis of drugs; J.Pharm. Biomedical Anal.; 4(6); (1986); 747-754.


30. Clark D.A., Nichols G.; Pharmaceutical applications of FTIR microspectrometry; Analytical Proceedings; Vol. 27; (1990); 19-21.
References for Chapter four

3. Bidlingmeyer, B.A.; Practical HPLC Methodology and applications; John Wiley & Sons Inc.; (1992); U.S.A.
4. Cope, M.J., Davidson, I.E.; Some aspects of pharmaceutical analysis using high performance liquid chromatography; Journal of Physics E: Scientific Instruments; 19(10); (1986); p763-775
5. Haddard, P.R., Jackson, P.E.; Journal of Chromatographic Library; 46(2); (1990)
7. Szepesi, G.; How to use reverse phase HPLC; VCH Publishers; (1992); U.S.A.
8. Watson, D.G., Pharmaceutical Analysis – A textbook for pharmacy students and pharmaceutical chemists; Churchill Livingstone; (1999); U.S.A.
9. Martin, A.J.P., Synghe, R.L.M.; Biochemical Journal; 35; (1941); 351-358
10. Robards, Haddards, Jackson; Theory of Chromatography; 49

15. Robards, K., Haddards, P.R., Jackson, P.E., Principles and Practice of modern chromatographic methods, 1994, Academic Press Limited


17. Franklin, G., Lab News, October 50, 1985


22. Spruce, B., Bakalayar, S.R., Troubleshooting guide for HPLC injection problem, 2\textsuperscript{nd} edn., Rheodyne incorporated, Cotati

23. Millipore Corporation, Waters 717 Autosampler, Service manual, 1993


27. Dal Nogare, S., Juvet, R.S., Gas Liquid Chromatography, Wiley Interscience, New York, p315


29. Fong G.W., Lam S.K.; HPLC in the pharmaceuticals industry; Marcel Dekker Inc.; 1991; U.S.A.


31. Holzgrabe U., Wawer I., Diehl B.; NMR spectroscopy in drug development and analysis; Wiley –vch; 1999; Germany

32. Jaroszewski J.W., Schaumburg K., Kofod H.; NMR spectroscopy in drug research; Munksgaard; 1988; Denmark


34. http://www.chem.ucla.edu/~bacher/UV-vis/uv_vis_tetracyclone.html.html

35. http://www.shu.ac.uk/schools/sci/chem/tutorials/molspec/uvvisab1.htm
References for Chapter five


2. Ibid, p1024

3. Ibid, p1016

4. Ibid, p1290

5. Ibid, p838


8. Moschiano F., Grazzi L., D’Amico D., Schieroni F., Bussone G.; Menstrual Migraine; J. Headache Pain; 2; (2001); S117-S119.


14. Khan K.A., Rhodes C.T.; Evaluation of different viscosity grades of sodium carboxymethylcellulose as tablet disintegrants; Pharm Acta Helvetica; 50; (1975); 99-102

15. Oza K.P., Frank S.G.; Microcrystalline cellulose stabilized emulsions; J. Disper. Sci. Technol.; 7(5); (1986); 543-561


18. British pharmacopoeia; London Her Majesty’s stationary office; (1973); 401
References for Chapter six

1. Habib M.J.; Pharmaceutical Solid Dispersion Technology; Technomic Publishing Co. Inc.; 2001; US.A; pp22
References for Chapter seven

1. Haines P.J.; Thermal methods of analysis; Blackie academic & professional; 1995; New Zealand.

2. Passerini N., Albertini B., Gonzalez-Rodriguez M.L., Cavallari C., Rodriguez L.; Preparation and characterisation of ibuprofen–poloxamer 188 granules obtained by melt granulation; European. J. Pharm. Sci.; 15; (2002); 71–78

3. Phadnis N.V., Suryanarayanan R.; Simultaneous quantification of an enantiomer and the racemic compound of ibuprofen by X-ray powder diffraction; 14(9); (1997); 1176-1180.


5. Brittain H.G.; X-ray diffraction III: Pharmaceutical applications of x-ray powder diffraction; Spectroscopy; 16(7); (2001); 14-18.

6. Ranter C.J.; Applications of X-ray diffractometric techniques in the analysis of drugs; J.Pharm. Biomedical Anal.; 4(6); (1986); 747-754.
References for Chapter eight


2. Earl R.T., Jenkins R., Munro A.J.; A double-masked comparison of the efficacy of once-daily sustained-release ibuprofen and once-daily piroxicam for 24-hour control of arthralgia due to osteoarthritis in the elderly; Current Therap. Res.; 57(10); (1996); 811-821.


4. Moschiano F., Grazzi L., D’Amico D., Schieroni F., Bussone G.; Menstrual Migraine; J. Headache Pain; 2; (2001); S117-S119.

