2014

Improved solubility and dissolution of BCS class II drug spironolactone by formulating in ternary solid dispersion with carrier Beta-cyclodextrin and adjuvant water soluble vitamin [pyridoxine HCl (vit B6)]

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A Thesis

entitled

Improved Solubility and Dissolution of BCS Class II drug Spironolactone by Formulating in Ternary Solid Dispersion with Carrier β-Cyclodextrin and Adjuvant Water Soluble Vitamin [Pyridoxine HCl (Vit B6)]

by

Amrata Bhonsle

Submitted to the Graduate Faculty as partial fulfillment of the requirements for the

Master of Science Degree in

Pharmaceutical Sciences (Industrial Pharmacy Major)

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August 2014

An Abstract of

Improved Solubility and Dissolution of BCS Class II drug Spironolactone by formulating in ternary solid dispersion with carrier β-cyclodextrin and adjuvant water soluble vitamin [Pyridoxine HCl (Vit B6)]

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The objective of the present study was to show the potential of a water soluble vitamin, Pyridoxine HCl (Pyd) to be used as a pharmaceutical adjuvant in order to increase the solubility and dissolution of BCS Class II dug Spironolactone (Spl). Beta-cyclodextrin (β-CD) was used as the principle carrier. The binary solid dispersion (SD) of β-CD and Spl in the ratio of 1:1 (SD01) and 1:2 (SD02) along with their respective physical mixtures (PM01 and PM02) were obtained and ternary solid dispersions having β-CD, Spl and Pyd in the ratio of 1:1:1 (SD03) and 1:2:1 (SD04) along with their respective physical mixture (PM03 and PM04) were prepared by using the solvent evaporation method. In the ternary solid dispersion system (SD04), Pyd as the adjuvant showed synergism along with the hydrophilic β-CD to enhance the solubility and dissolution of the drug Spl as compared to the binary systems. The SD04 showed a maximum extent of increase in solubility of 362.71± 3.6 µgm/mL as compared to the pure Spl alone at 34.48 ± 1.69 µgm/mL. Drug content in all the SDs was uniform with the lowest being 93.14 ± 3.2% and the highest was 94 ± 2.8%. Solid state characterization, including DSC, FTIR

and PXRD, was performed which indicated that the presence of Pyd, facilitated the formation of an inclusion complex. Inclusion complex formation is considered as the prime mechanism for the enhancement of solubility and dissolution of the drug spironolactone. In-vitro dissolution testing indicated that SD04 showed the maximum drug release of 84.21± 6.3% over the period of time above all the binary SDs, PMs and pure Spl. Evaluation of the dissolution profiles showed that all the systems primarily followed Higuchi Kinetics. Thus, Pyd was found to be efficient as an adjuvant in order to increase the solubility of the BCS Class II model drug spironolactone when formulated in a ternary solid dispersion along with the β-CD.

Dedicated to Mom and Dad

None of this would have been possible without the love and patience of my parents and family. I cannot pen down the thoughts on how blessed and grateful, I am to be a daughter of Mrs. Hemlata Bhonsle and Mr. Shivanand Bhonsle to whom this dissertation is dedicated to.

I would also like to thank my grandmother Mrs. Chandraprabha Dalvi, my Aunts Mrs Sangeeta Shinde, Mrs Kunda Desai, Mrs Kalpana Bhonsle and my Uncles Mr. Mahendra Dalvi, Mr. Ashok Bhonsle, Mr. Ranjit Shinde, my brother Utkarsh and all my loved cousins.

My family has been a constant source of love, concern, support and strength all these years and lifting me uphill to this phase of life. I owe everything to them.

Acknowledgements

This thesis is the end of my journey, but I have not traveled in a vacuum. This thesis has been kept on track and been seen through to completion with the support and encouragement of numerous people including my teachers, my family, well-wishers and my friends.

First and foremost, I must acknowledge and thank God for everything.

I express my profound sense of reverence to my advisor and committee chair Dr. Kenneth Alexander. This work would not have been possible without his guidance.
I am thankful to Dr. Mariann Churchwell and Dr. Caren L. Steinmiller for being on my graduate committee. I am grateful to Dr. Zahoor Shah to be my graduate representative and to Dr. Sai. H. S. Boddu for his practical advices.

I would also like to express my gratitude to Dr. Pannee Burckel for her help in using the PXRD. My sincere thanks to Dr. Joseph Lawrence and Marina to help me with FTIR.

My friends Mudita, Meenal, Aman, Ankur, Sakshi, Nidhi, Soohi, and many more who have helped me stay sane through these difficult years. Their support and care helped me overcome setbacks and stay focused on my graduate studies. I am indebted to many of my friends, UTIP-12, seniors, juniors, University of Toledo Library (Carlson and Mulford Staff) for their valuable help and support.

Finally I greatly appreciate the generosity of former Associate Dean Dr. Wayne Hoss for his financial support for my graduate tuition.

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Chapter 1

Introduction

1.1 Solubility is the property of a solid, liquid, or gaseous chemical substance called a solute to dissolve in a solid, liquid, or gaseous solvent to form a homogeneous solution of the solute in the solvent.[1] The solubility of a substance fundamentally depends on the solvent used as well as on temperature and pressure. The extent of solubility of a substance in a specific solvent is measured as the saturation concentration where adding more solute does not increase its concentration in the solution.[1] The extent of solubility ranges widely, from infinitely soluble (fully miscible) such as ethanol in water, to poorly soluble, such as silver chloride in water. The term insoluble is often applied to poorly or very poorly soluble compounds.[2]

Solubility is defined in quantitative terms as the concentration of the solute in a saturated solution at a certain temperature. In qualitative terms, solubility may be defined as the spontaneous interaction of two or more substances to form a homogeneous molecular dispersion.[3] A saturated solution is one in which the solute is in equilibrium with the solvent. The solubility of a drug may be expressed as parts, percentage, molarity, molality, and volume fraction and mole fraction.[3] Solubility occurs under dynamic equilibrium, which means that solubility results from the simultaneous and opposing processes of dissolution and phase joining (e.g., precipitation of solids). Solubility

equilibrium occurs when the two processes proceed at a constant rate.[4] Under certain conditions equilibrium solubility may be exceeded to give a so-called supersaturated solution, which is metastable.[4]

Solubility is not to be confused with the ability to dissolve or liquefy a substance, since these processes may occur not only because of dissolution but also because of a chemical reaction. For example, zinc is insoluble in hydrochloric acid, but does dissolve in it by chemically reacting into zinc chloride and hydrogen, where zinc chloride is soluble in hydrochloric acid.[4] Solubilization may be defined as the preparation of a thermodynamically stable solution of a substance that is normally insoluble or very slightly soluble in a given solvent, by the introduction of one or more amphiphilic components.[5]

The United States Pharmacopoeia (USP) and British Pharmacopoeia (BP) classify the solubility regardless of the solvent used, only in terms of quantification and have defined the criteria as given below in Table: 1

Table 1: USP solubility Criteria[6]

Descriptive Term	**Parts of Solvent Required for 1 Part of Solute**
Very soluble	Less than 1
Freely soluble	From 1 to 10
Soluble	From 10 to 30
Sparingly soluble	From 30 to 100
Slightly soluble	From 100 to 1000
Very slightly soluble	From 1000 to 10,000
Practically insoluble, or Insoluble	10,000 and over

The International Union of Pure and Applied Chemistry (IUPAC) defines solubility as the analytical composition of a saturated solution expressed as a proportion of a designated solute in a designated solvent.[5]

Solubility is an important determinant in drug liberation and absorption and hence plays a key role in its bioavailability.[5] For a drug to be absorbed, it must be present in the form of an aqueous solution at the site of absorption. Aqueous solubility of the drug can be regarded as a key factor responsible for low oral bioavailability of poor water soluble drugs thereby limiting their therapeutic potential. Other issues related to low oral bioavailability for a sparingly soluble drug are lack of dose proportionality, substantial food effect, and high intra & inter subject variability, gastric irritancy and slow onset of action.[5]

Unfortunately many chemical compounds including NCE (New Chemical Entities) possess very low aqueous solubility at physiological pH. This could be attributed to their high inherent lipophilicity incorporated by drug design in order to ensure good absorption.[5]

Oral ingestion is the most convenient and commonly employed route of drug delivery due to its ease of administration, high patient compliance, cost effectiveness, least sterility constraints, and flexibility in the design of a dosage form. However, the major challenge with the design of oral dosage forms lies with their poor bioavailability. The oral bioavailability depends on several factors including aqueous solubility, drug permeability, dissolution rate, first-pass metabolism, presystemic metabolism, and susceptibility to efflux mechanisms.[7] Water is the solvent of choice for liquid

pharmaceutical formulations. Most of the drugs are either weakly acidic or weakly basic having poor aqueous solubility.[7]

The Biopharmaceutics Classification System (BCS) is a scientific framework for classifying a drug substance based on solubility, permeability, and dissolution criteria[3,8]. According to the BCS, drug substances are classified as follows:

- Class I: high permeability and solubility
- Class II: high permeability and low solubility
- Class III: low permeability and high solubility
- Class IV: low permeability and low solubility

General view on the solubility problem of various BCS Class II drugs can be summarized as-

- The bioavailability of Class I compounds is determined only by delivery of the drug solution to the intestine (Formulation independent).[7,8,9]
- The bioavailability of Class II compounds is limited by drug solubility/dissolution (Formulation dependent). [7,8,9]
- The bioavailability of Class III compounds is limited by intestinal permeability (Dependent on barrier properties). [7,8,9]
- The bioavailability of Class IV compounds is limited both by solubility/dissolution and intestinal permeability (Formulation and barrier properties dependent). [7,8,9]

For BCS Class II compounds, the bioavailability of these products is limited by their solvation rate and dissolution is the rate limiting step for drug absorption. Various approaches have been investigated extensively to improve the aqueous solubility and poor dissolution rate of BCS Class II and IV drugs. [10,11]

The poor solubility and low dissolution rate of poorly water soluble drugs in the aqueous gastrointestinal fluids often cause insufficient bioavailability. This is especially true for Class II (low solubility and high permeability) substances according to the BCS. The bioavailability may be enhanced by increasing the solubility and dissolution rate of the drug in the gastro-intestinal fluids. As for BCS Class II drugs the rate limiting step is drug release from the dosage form and solubility in the gastric fluid and not the absorption, so increasing the solubility in turn increases the bioavailability for BCS Class II drugs. [8, 9]

1.2 Techniques for Solubility Enhancement [12] -

Solubility improvement techniques can be categorized into physical modification chemical modifications of the drug substance, and other techniques.

Physical Modifications —

These methods include particle size reduction such as micronization and nanosuspensions, modification of the crystal habit such as polymorphs, amorphous form and cocrystallization, drug dispersion in carriers such as eutectic mixtures, solid dispersions, solid solutions and cryogenic techniques.

Chemical Modifications —

These include a change in pH, the use of buffers, derivatization, complexation, and salt formation.

Miscellaneous Methods —

These include supercritical fluid processes, use of an adjuvant such as surfactants, solubilizers, cosolvency, hydrotrophy, and novel excipients.

Among the commonly utilized methods, one of the most efficacious is the use of solid dispersions (SDs).

1.3 Solid Dispersion

The concept of solid dispersions was originally proposed by Sekiguchi and Obi [13], who investigated the generation and dissolution performance of eutectic melts of a sulfonamide drug and a water-soluble carrier in the early 1960s. Solid dispersions represent a useful pharmaceutical technique for increasing the dissolution, absorption, and therapeutic efficacy of drugs in dosage forms.[14]

The term solid dispersion refers to a group of solid products consisting of at least two different components, generally a hydrophilic matrix and a hydrophobic drug. The matrix can be either crystalline or amorphous. The drug can be dispersed molecularly, as an amorphous particle (clusters) or as crystalline particles. [14, 15]

When dissolving the solid dispersions, it is believed that the drug substance is released as small discrete units owing to a fast dissolution of the easily soluble carrier. If the drug solubility in the carrier is high enough, a so-called solid solution can be obtained.[16] Such a preparation will then give a system similar to a molecular solution after the carrier has been dissolved. For such systems it has been claimed that the dissolution of the carrier is

the rate limiting step.[16] The formation of the solid solution is therefore normally restricted to relatively low concentrations of drugs. [16]

Coprecipitates and melts are solid dispersions that provide a means of reducing particle size to the molecular level. Sekiguchi and Obi [13] first introduced the concept of using solid dispersions to improve bioavailability of poorly water-soluble drugs in 1961. They demonstrated that the eutectic of sulfathiazole and the physiologically inert water-soluble carrier urea exhibited higher absorption and excretion after oral administration than sulfathiazole alone. [17]

Chiou and Riegelman[18] defined the term solid dispersion as "a dispersion of one or more active ingredients in an inert carrier or matrix in solid state prepared by the melting (fusion), solvent, or melting-solvent method." Dispersions obtained through the fusion process are often called melts, and those obtained by the solvent method are frequently referred to as coprecipitates or coevaporates.

1.4 Classification of Solid Dispersions

Chiou and Riegelman[18] classified solid dispersions into the following six representative types:

1) Simple eutectic mixtures;

2) Solid solutions;

3) Glass solutions and glass suspensions;

4) Amorphous precipitations in a crystalline carrier;

5) Compound or complex formation;

6) Combinations of the previous five types.

1.4.1. Simple Eutectic Mixtures

These are prepared by rapid solidification of the fused melt of two components that show complete liquid miscibility but negligible solid-solid solubility. Thermodynamically, such a system is an intimately blended physical mixture of its two crystalline components[19]. Thus, the x-ray diffraction pattern of a eutectic constitutes an additive composite of the two components. A phase diagram representing a two-component system is given in Fig. 1. Examples of this type include phenacetin-phenobarbital, chloramphenicol-urea, griseofulvin-succinic acid[19], paracetamol-urea, and the dispersions of griseofulvin and tolbutamide in polyethylene glycol-(PEG-2000; [20]).

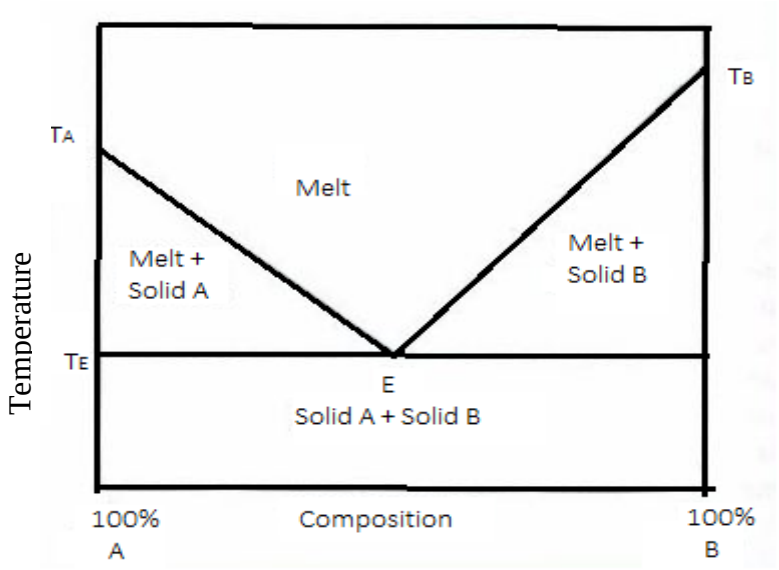

Fig. 1 Representation of a simple binary-phase diagram with eutectic formation. TA is the melting point of pure A; TB is the melting point of pure B; and E is the eutectic point.

1.4.2. Solid Solutions

In a solid solution, the two components crystallize together in a homogeneous one-phase system. The particle size of the drug in the solid solution is reduced to its molecular size.[21] Thus, a solid solution can achieve a faster dissolution rate than its corresponding eutectic mixture. According to the extent of miscibility of the two components, they may be classified as continuous or discontinuous. In continuous solid solutions, the two components are miscible in the solid state in all proportions. Typical phase diagrams for continuous and discontinuous solid solutions are given in Figs. 2 and 3, respectively. Discontinuous solid solutions exist at extremes of composition. In general, some solid-

:ate solubility can be expected for all two-component systems.

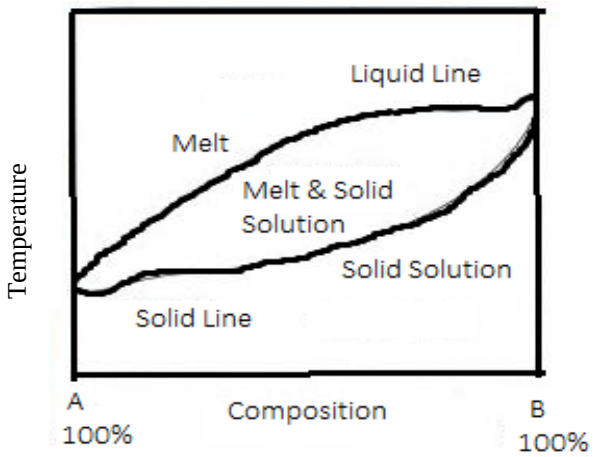

Fig. 2 Representation of a phase diagram of a continuous solid solution for a binary system A and B.

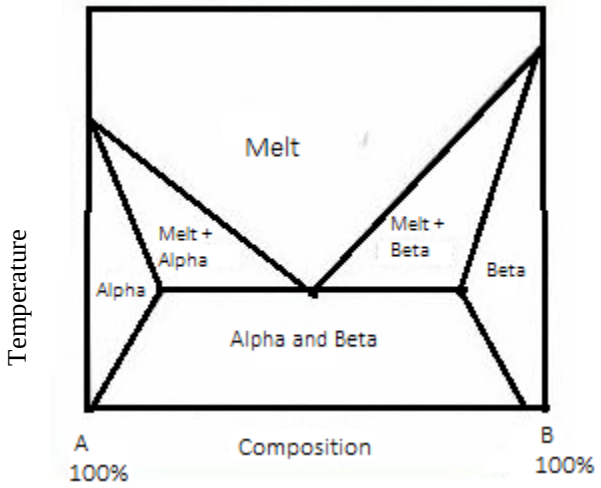

Fig.3 Representation of a typical phase diagram of a discontinuous solid solution for a binary system A and B; α and β are regions of solid solution formation.

According to the criterion for molecular size of the two components, solid solutions are classified as substitutional or interstitial. In the substitutional type, the solute molecule

substitutes for the solvent molecule in the crystal lattice (Fig. 4). The molecular size of the two components should not differ by more than 15%. This class is represented by solid solutions of p-dibromobenzene p-chlorobromobenzene, anthracene-acenaphthene, and ammonium and potassium thiocyanate.[21] An interstitial solid solution is obtained when the solute (guest) molecule occupies the interstitial space (Fig. 4) in the solvent (host) lattice. For this to occur the solute molecule diameter should be less than 0.59 times that of the solvent molecule; therefore, the volume of the solute molecule should be less than 20% of the solvent molecule. Owing to their large molecular size, polymers favor the formation of interstitial solid solutions.[21] They all exhibit a fast rate of dissolution.

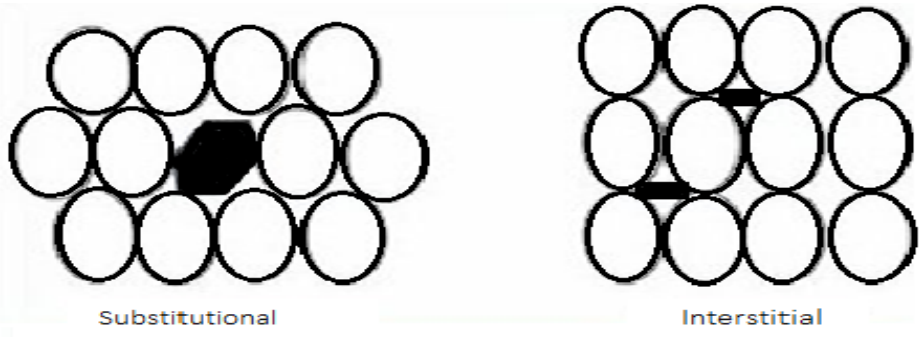

Fig.4 A schematic representation of substitution and interstitial solid solutions. The dark symbols represent solute atoms or molecules; open symbols indicate solvent atoms or molecules.

1.4.3. Glass Solutions and Suspensions

A glass solution is a homogeneous glassy system in which a solute dissolves in the glassy carrier.[21] A glass suspension refers to a mixture in which precipitated particles are suspended in a glassy solvent. The glassy state is characterized by transparency and brittleness below the glass transition temperature. Glasses do not have sharp melting

points. Instead, they soften progressively on heating. The lattice energy, which represents a barrier to rapid dissolution, is much lower in glass solutions than in solid solutions. Fig. 5 shows the volume changes associated with glass formation when a melt is cooled down. Examples of carriers that form glass solutions and suspensions include citric acid, sugars such as dextrose, sucrose, and galactose; PVP; urea; and PEG. [22, 23, 24]

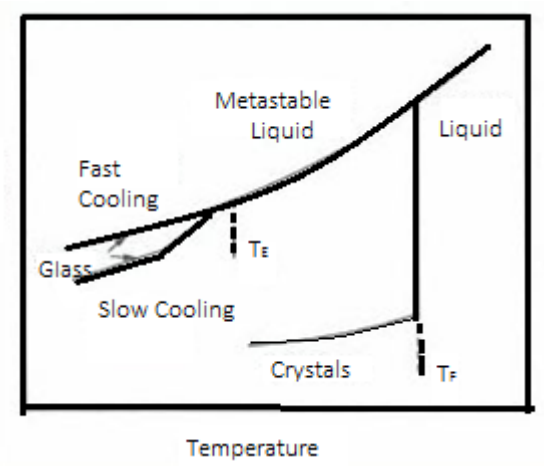

Fig.5 Representation of the volume changes associated with the cooling of a melt: The Tg is the glass transition temperature and T_f is the melting point of the material.

1.4.4. Amorphous Precipitations in a Crystalline Carrier

This type of solid dispersion is distinguished from a simple eutectic mixture by the fact that the drug is precipitated out in an amorphous form.[21] In a simple eutectic mixture, the drug is precipitated out in a crystalline form.[21] It is postulated that a drug with a propensity to super cooling has more tendency to solidify as an amorphous form in the presence of a carrier.[21]

1.4.5. Compound and Complex Formation

When two substances form a molecular compound, it usually gives rise to a maximum in the phase diagram. It is difficult to generalize the influence that complex formation has on dissolution. A complex between digoxin and hydroquinone exhibited a high dissolution rate [20, 21], whereas the insoluble complex between phenobarbital and PEG was shown to reduce both the rates of dissolution and the permeation of phenobarbital through rat gut. [19, 21]

1.4.5. a Complexation and Cyclodextrins

Complexation is one of several ways to favorably enhance the physicochemical properties of pharmaceutical compounds. It may loosely be defined as the reversible association of a substrate and ligand to form a new species.[21] Although the classification of complexes is somewhat arbitrary, the differentiation is usually based on the types of interactions and species involved, e.g., metal complexes, molecular complexes, inclusion complexes, and ion-exchange compounds. Cyclodextrins (CDs) are classic examples of compounds that form inclusion complexes. These complexes are formed when a "guest" molecule is partially or fully included inside a "host" molecule e.g. CD with no covalent bonding. When inclusion complexes are formed, the physicochemical parameters of the guest molecule are disguised or altered and improvements in the molecule's solubility, stability, taste, safety, bioavailability, etc., are commonly seen.[22] Limitations in the pharmaceutical utility of the CDs were becoming known and derivatives were prepared with the goal of improving characteristics such as complexing ability, solubility, and safety. [22]

Scientific articles have established the research applications for CDs, however it is the patents that have shown an increasing interest in the commercial protection of CDs in pharmaceutical products.[22] Increasing numbers of pharmaceutical products are reaching the market place as CD formulations and research studies exploring their applications are growing exponentially.[22] Nevertheless, the routine use of CDs in formulations is still questioned. The reluctance to develop a CD formulation is mainly due to the uncertain regulatory acceptance of a formulation containing a "nonstandard" inactive ingredient. [22]

Inclusion Complexation and CD

CDs are cyclic oligosaccharides containing 6, 7, or 8 glucopyranose units, referred to as α, β, or γ-CD, respectively. Each glucose unit contains two secondary alcohols at C-2 and C-3 and a primary alcohol at the C-6 position, providing 18-24 sites for chemical modification and derivatization. [22]

1.4.5.a.i Factors Affecting Complexation

- **Steric effects**:

Cyclodextrins are capable of forming inclusion complexes with compounds having a size compatible with the dimensions of the cavity. Complex formation with molecules significantly larger than the cavity may also be possible in such a way that only certain groups or side chains penetrate into the carbohydrate channel. The three natural CDs namely α, β, and γ have different internal diameters and are able to accommodate molecules of different size. The presence of bulky groups can sterically block entrance to the CD cavity. Some groups, depending on their number, flexibility, and position of

attachment, may actually act to extend the cavity and provide for better complexation. [22, 24, 25, 26].

- **Electronic effects**: Electronic effects seem to be more of a factor than steric effects. The ionic substituents too close to the CD cavity adversely disrupt the thermodynamics driving the inclusion complexation. [26, 27, 28]
- **Micellaneous**: The effect of proximity of charge to CD cavity: Moving the charge away from the cavity re-establishes the complexation characteristics but this is dependent on the charge density in the structure. The effect of charge density and the effect of charge state of the CD and drug are important considerations. [28]

Temperature, additives, and co-solvent effects: In most cases, as the temperature increases, the binding constant will decrease.

1.4.5.a.ii Release from the Complex:

Complexation of drugs by CDs improves their delivery characteristics and does not interfere with their activity because complexation is a rapidly reversible process. In aqueous solution, drug:CD complexes are continually forming and dissociating. [29]

Although slower kinetics of dissociation are seen with stronger binding, the rates are still fast and essentially instantaneous. After administration, the drug is released from the complex upon dilution, and in some cases with contributions from competitive displacement with endogenous lipophiles, as well as binding to plasma and tissue components. Drug uptake into tissues is not available to the complex, and rapid elimination of the CD occurs. [30, 31]

1.4.5.b. Complexation and Non-Cyclodextrins [32]

Complexation processes, also known simply as complexation, are based on the ability of many well-known drugs to interact and to form new complex drugs with altered properties in comparison with a drug alone.[32] The pharmaceutical technology and the pharmaceutical industry have long considered research and development in the area of complexation a priority.[32] The complexation process offers new possibilities for the improvement of existing drugs (side effects, therapeutical activity, and solubility).[32] Such drug complexes with optimized characteristics can be prepared by complexation as a result of various interactions such as drug-metal ion, drug-drug, drug-excipient(s), etc. The non-cyclodextrins complexes can be broadly categorized as:

- Complexes formed by interactions with metal ions,
- Complexes formed by interactions with excipients, and
- Complexes formed by drug-drug interactions.

1.5 Mechanism of increased Solubility and dissolution rate:

The enhancement in dissolution rate as a result of solid dispersion formation, relative to pure drug, varies from as high as 400-fold[33] to less than twofold. Corrigan[34] reviewed the current understanding of the mechanism of release from solid dispersions. The increase in dissolution rate for solid dispersions can be attributed to a number of factors. It is very difficult to show experimentally that anyone particular factor is more important than another. The main reasons postulated for the observed improvements in dissolution of these systems are as follows:

1. Reduction of particle size. In the case of glass, solid solutions, and amorphous dispersions, particle size is reduced to a minimum level. This can result in an enhanced dissolution rate due to an increase in both the surface area and solubilization.

2. Solubilization effect. The carrier material, as it dissolves, may have a solubilization effect on the drug. This was shown to be the case for acetaminophen and chlorpropamide in urea, as well as for numerous other drugs [35]

3. Wetability and dispersibility. The carrier material may also have an enhancing effect on the wetability and dispersibility of the drug in the dissolution media. This should retard any agglomeration or aggregation of the particles, which can slow the dissolution process.

4. Metastable forms. Formation of metastable dispersions with reduced lattice energy would result in faster dissolution rates.

1.6 Methods of Preparation for Solid Dispersion

The fusion and solvent process are the most common methods used to prepare solid dispersions. General methods employed to prepare the solid dispersion are as follows:

1.6.1 Fusion Process

In the fusion method of preparation, the carrier is heated to a temperature just above its melting point and the drug is incorporated into the matrix. The mixture is cooled with constant stirring to homogeneously disperse the drug throughout the matrix. Several mechanisms could operate during the process of dispersion. If the drug has a high degree of solubility in the carrier, the drug could remain “dissolved” in the solid state, yielding what is known as a solid solution.

Particle size reduction under these conditions proceeds to the ultimate level leading to molecular dispersion of the drug in the carrier matrix. These systems show very high drug dissolution rates compared to control samples. If, on the other hand, the solubility of the drug in solid state is not so high, crystallites of the drug become dispersed in the matrix. Such systems show only moderate increases in dissolution rates.

A third mechanism is the conversion of a drug to an amorphous form in the presence of the matrix, again exhibiting different dissolution rates and solubility. Other factors that may play a role include solubilizing effect conferred by the carrier itself, improved wetting or decreased surface hydrophobicity, complexation, and crystallization of the drug in a metastable polymorphic form of altered thermodynamic properties.

An important limitation of the fusion method of preparation is the exposure of drugs to elevated temperatures, particularly if the carrier is a high-melting solid and the drug is heat-sensitive[36].

Advantages:

This method is very suitable for drugs and carrier that are miscible in the molten state, making melting of the ingredients very easy to accomplish. Preparing solid dispersion by the melt method is not time consuming. Hence, many batches of the product can be prepared in a very short period of time. The method is also advantageous for compounds, which do not undergo significant thermal degradation.

Disadvantages:

The main disadvantages of the melt method include thermal degradation, sublimation, and polymeric transformation. These can affect the physicochemical properties of the drug including its rate of dissolution. The decomposition or thermal degradation is often

composition dependent and affected by melting time and the rate of cooling. In order to reduce decomposition to acceptable levels, melting may be carried out at a temperature just above the highest melting component of the dispersion, which completely melts both drug and the carrier. [37, 38]

The temperature at which the dispersion solidifies affects crystallization rates and may alter both the size of the crystals and the hardness of the dispersion. This may result in tacky or glassy and unmanageable dispersions, which will require storage at elevated temperature to facilitate hardening. Upon communutionof such dispersions, crystallization may be induced resulting in the modification of dissolution characteristics[36].

1.6.2 Solvent Method:

In the solvent method of preparation, the carrier and the active ingredient are dissolved in a suitable organic solvent. This solvent is evaporated at an elevated temperature or under vacuum. 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, there is a possibility of the formation of a solvate within the crystal lattice. This presents a problem in terms of pharmaceutical acceptance since most of the solvents used are non-aqueous (organic) and toxic. Today there is a trend to move away for organic solvents to hydrophilic solvents if possible. Hence, removal of even trace amounts of the solvent is implied. Highly sensitive techniques such as differential

scanning calorimetry (DSC), differential thermal analysis (DTA), thermogravimetric analysis (TGA), and less sensitive procedures such as gravimetry and spectroscopy can be used to demonstrate complete solvent removal. [39]

Selection of Solvent:

The choice of solvent and its removal rate are critical to the quality of the dispersion. Since the chosen carriers are generally hydrophilic and the drugs are hydrophobic, the selection of a common solvent is difficult and its complete removal, necessitated by its potential toxic nature, is imperative. Certain solvents may plasticize polymeric carriers, e.g., Polyvinylpyrrolidone (PVP), making their complete removal even more difficult. Careful control of the temperature and rate of evaporation of solvents is essential in controlling the particle size of the drug, and although low temperature cannot always be avoided. The Tolbutamide- PVP dispersion showed an instability that varied with the evaporatingtemperature[36].

Advantages:

The procedure is suitable for drugs that are thermolabile; reduced pressure and lower temperatures can be used to evaporate solvent. For aqueous systems, frozen temperatures can be used to evaporate the solvent, which can enhance the integrity of the drug. [39, 40]

Disadvantages:

Finding a suitable solvent that will dissolve both the drug and the carrier is very difficult and sometimes impossible. This is due to the fact of that most of the carriers are hydrophilic, whereas most of the drugs are hydrophobic organic substances. This may be further complicated by the fact that different polymorphic forms of the same drug may be

obtained if different solvents are used. Spironolactone dispersions in polyvinylpyrollidone were evaporated from solutions of ethanol, acetonitrile, and chloroform, respectively.[41] The highest dissolution rate was provided by ethanolic dispersions, whereas the chloroform dispersion provided the lowest dissolution rate. After a suitable solvent has been found, the rate of its removal is very critical in some solid dispersion, and complete removal of the solvent is even more difficult to accomplish. Plasticization of some polymers such as polyvinylpyrrolidone has occurred with the use of some solvents.[42] This made removal of the solvent extremely difficult. The volume of organic solvent needed to dissolve a suitable amount of drug and carrier may be very large in some cases, and the recovery of the solvent may be economically prohibitive.

1.6.3. Fusion – Solvent Method:

In the fusion method a carrier(s) is/are melted and the drug(s) is/are incorporated in the form of a solution. If the carrier is capable of holding a certain proportion of liquid yet maintaining its solid properties, and if the liquid is innocuous, the need for solvent removal may be eliminated. Otherwise, this method faces the same criticism of solvent retention described before. This method is particularly useful for drugs that have high melting points or that are thermolabile. The feasibility of the method has been demonstrated for spironolactone and griseofulvin dispersions in polyethylene glycol 6000[39].

1.6.4. Spray Drying:

In this type of preparation, the carrier and the active ingredient are dissolved or suspended in a suitable solvent. This solvent is evaporated by drying by applying a

stream of heated air to remove the solvent [43]. Due to the large surface area of the droplets, the solvent rapidly evaporates and the solid dispersion is formed quickly.

1.6.5 Lyophilization (Spray Freeze Drying Method):

This method is used to avoid heating during the preparation of thermosensitive drugs; Spray freeze drying (SFD) has been successfully developed to prepare solid dispersions at ambient temperature. The SFD technology involves the atomization of a feed liquid containing poorly water-soluble or insoluble active pharmaceutical ingredients (APIs) and excipients directly into a cryogenic liquid at ambient temperature to produce a frozen micronized powder that is subsequently dried. This process offers a variety of advantages compared to traditional technologies for solid dispersions, including amorphous structure and high surface area[44, 45, 46].

1.6.6. Hot-melt Extrusion:

It is a very common method used in the polymer industry. However Speiser[47, 48] and Huttenrach[49] were the first persons who used this technology for pharmaceutical purposes. A melt extrusion consists of the following required processing equipment:

- An opening to feed raw materials,
- A heated barrel that consists of extruder screws to convey and mix the fed materials,
- And an exit port, which consists of an optional die to shape the extruding mass.

The active ingredients and the carrier are fed into the heated barrel of the extruder at a constant rate. When the mixture of active ingredient and the carrier is conveyed through the heated screws, it is transformed into its “fluid like state”. This state allows intimate

and homogeneous mixing by the high shear of the extruder screws. An exit port, which consists of an optional die, shapes the melt in the required form such as granules, pellets, films, or powder. An important advantage of the hot melt extrusion method is that the drug/carrier mix is only subjected to an elevated temperature for about one minute, which enables drugs that are somewhat thermo labile to be processed.

1.6.7. Electrostatic Spinning Method:

The electrostatic spinning method is a straight-forward process for generating nanofibers. The popularity of this system is due to its ease of implementation, capability of being used with a variety of materials, convenience in obtaining composites of multiple components, and with secondary microstructures (such as core-sheath, side-by-side, and island-in-sea). The applications for electro spun products are expanding, especially in areas relating to tissue engineering and drug delivery.[50-54]. The fast-drying electrospinning process is able to “freeze” drug molecules randomly in the solid polymer fiber matrix into a state comparable with that in a liquid form. This is very useful for preventing phase separation, eg, recrystallization of either drug or matrix during removal of solvents [55-60.]

1.6.8. Supercritical Fluid Technology:

Supercritical fluid technology (SCFT) is a new method to produce fine drug particles and is valuable for product quality. In the pharmaceutical field, the supercritical fluid technology was industrially applied in the early 1980’s.[60]

In this technique the active ingredient and the carrier are dissolved in a common solvent that is introduced into a particle formation vessel through a nozzle, simultaneously with CO_2. When the solution is sprayed, the solvent is rapidly extracted by the supercritical

fluid, resulting in the precipitation of solid dispersion particles on the walls and bottom of the vessel [61.] A supercritical fluid exists as a single phase above its critical temperature and pressure [62]. The most commonly used supercritical fluids include supercritical fluid carbon dioxide (SC-CO_2), nitrous oxide, water, methanol, ethanol, ethane, propane, n-hexane and ammonia-18. SC-CO_2 is a popular solvent or anti-solvent since it is safe, inexpensive, readily available, and an ideal substitute for many hazardous and toxic solvents. The SC-CO_2 exists when both the temperature and pressure equals or exceeds the critical point of 31°C and 73 atm and has both gas-like and liquid-like qualities. It is this dual characteristic of supercritical fluids that provides the ideal conditions for extracting compounds with a high degree of recovery in a short period of time.

By controlling the level of pressure/temperature /modifier, SC-CO_2 can dissolve a broad range of compounds, both polar and non-polar. At present, carbon dioxide technology is one of the fastest growing new process technologies being adopted by the pharmaceutical industry. [63] Supercritical water is a unique medium for safe destruction of dangerous waste by total oxidation due to its special physicochemical properties. Various supercritical fluid technologies used in pharmaceutical processing include:

- Rapid expansion of supercritical solutions (RESS),
- Supercritical antisolvent (SAS) precipitation technique
- Particles from Gas Saturated Solutions (PGSS),
- Gas antisolvent system (GAS),
- Precipitation using compressed antisolvent (PCA),
- Aerosol solvent extraction system (ASES),
- Solution enhanced dispersion by supercritical fluids (SEDS),

• Supercritical antisolvent system with enhanced mass transfer (SAS-EM).

1.6.9. Coating on Sugar Beads Using Fluidized Bed-Coating System:

In this method a fluidized bed-coating concept is involved. The drug and the active ingredient solution is sprayed onto the granular surface of excipients or sugar spheres to produce either granule ready for tableting or drug-coated pellets for encapsulation in one step. The method can be applied for both controlled- and immediate-release solid dispersions.

1.6. CHARACTERIZATION OF SOLID DISPERSIONS:

Many methods are available that can contribute information regarding the physical nature of solid dispersion system. A combination of two or more methods is required to study its complete picture and may include the following:

- Thermal analysis (Modulated temperature differential scanning calorimetry).
- Spectroscopic method.
- X-ray diffraction method.
- Dissolution rate method and Dissolution testing
- Microscopic method. (Scanning Electron Microscopy and Transmission Electron Microscopy)
- Thermodynamic method.

Chapter 2

Material and Methods

2.1 Materials

Spironolactone USP micro (Lot no. 1120570) and Pyridoxine Hydrochloride USP (Lot No. 1212120045) was obtained from Letco MEDICAL. Cyclodextrin (Beta) Reagent (Lot no. C148359) was procured from PCCA(Professional Compunding Center of America.). All reagents and solvent were of analytical grade and were used as received without further treatment.

2.1.1 Spironolactone(Spl):

Spironolactone is a potassium sparing diuretic. Spironolactone is a synthetic steroid that competes for the cytoplasmic aldosterone receptor. In adults it is used for edema, hypertension, hypokalemia, primary hyperaldosteronism, diagnosis, hirsutism, congestive heart failure, and primary hyperaldosteronism. In pediatric use it treats hypertension, and primary hyperaldosteronism diagnosis.

Chemical Nature: It is 7α-acetylthio-3-oxo-17α-pregn-4-ene-21, 17-carbolactone or 17-hydroxy-7α-mercapto-3-oxo-17α-pregn-4-ene-21-carboxylic acid, γ-lactone acetate. Spironolactone contains not less than 97.0 percent and not more than 103.0 percent of $C_{24}H_{32}O_4S$.

IUPAC Name: S-[(7R, 8R, 9S, 10R, 13S, 14S, 17R)-10,13-dimethyl-3,5'dioxospiro [2,6,7,8,9,11,12,14,15,16-decahydro-1H-cyclopenta[a]phenanthrene-17,2'-oxolane]-7-yl] ethanethioate.

Physical Properties:

Solubility: Spironolactone is practically insoluble in water. Soluble in most organic solvents including ethyl acetate, in benzene and in chloroform and ethanol. It is slightly soluble in methanol.

Molecular Formula: $C_{24}H_{32}O_4S$

Average mass: 416.573486 Da

Monoisotopic mass: 416.202118 Da

Molar Refractivity: 112.7±0.4 cm^3

Molar Volume: 335.8±5.0 cm^3

Surface Tension: 50.9±5.0 dyne/cm

UV max: 238 nm

Flash Point: 302.3±18.1 °C

Density: 1.2±0.1 g/cm^3

Polarizability: 44.7±0.5 10-24cm3

Enthalpy of Vaporization: 88.9±3.0 kJ/mol

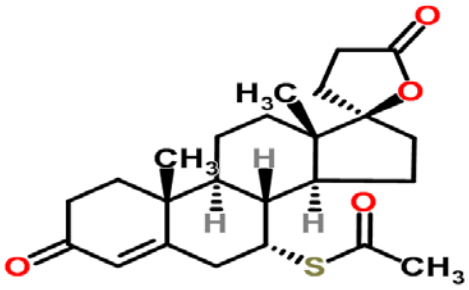

Fig:6 Chemical Structure of Spironolactone

2.1.2 Cyclodextrin

Schardinger's dextrins or cyclohexaamylose, cyclomaltohexaose or cyclo-α(1->4)-glucohexaoside, cyclo[D-Glcpα(1->4)][6].

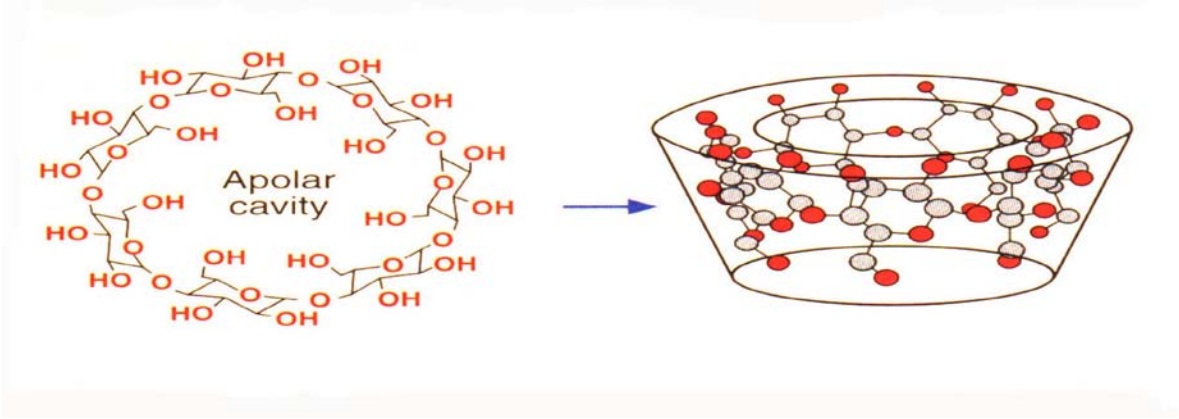

Fig.7 Chemical Structure of Cyclodextrin

Chemically they are cyclic oligosaccharides containing at least 6 D-(+) glucopyranose units attached by α-(1, 4) glucosidic bonds. CDs, α-, β-, and γ-CDs (with 6, 7, or 8 glucose units respectively), differ in their ring size and solubility (Table. 1)

Table. 2: Type of CD, cavity diameter, molecular weight and aqueous solubility

Type of CD	Cavity Diameter(A)	Molecular Weight	Solubility(g/100ml)
A-CD	4.7-5.3	972	14.5
B-CD	6.0-6.5	1135	1.85
Gamma-CD	7.5-8.3	1297	23.2
Delta-CD	10.3-11.2	1459	8.19

CD- Cyclodextrins

Due to the chair conformation of the glucopyranose units, the cyclodextrins are shaped like a truncated cone rather than perfect cylinders. The hydroxyl functions are orientated to the cone exterior with the primary hydroxyl groups of the sugar residues at the narrow edge of the cone and the secondary hydroxyl groups at the wider edge. The central cavity is lined by the skeletal carbons and ethereal oxygens of the glucose residues, which gives it a lipophilic character. The polarity of the cavity has been estimated to be similar to that of an aqueous ethanolic solution.[1]

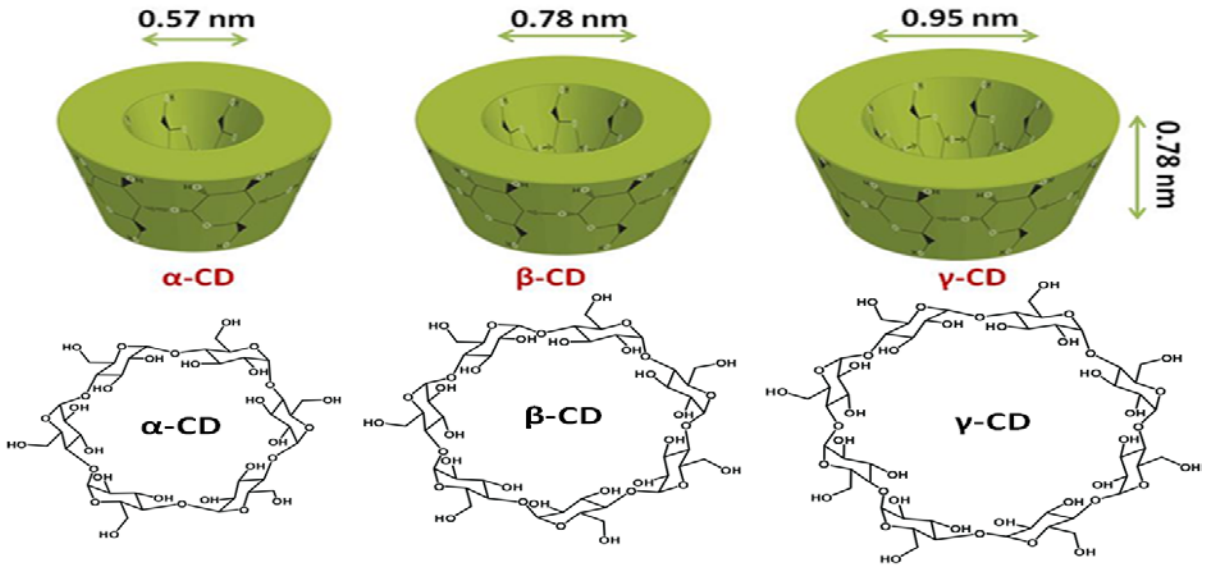

Fig.8 Chemical Structure of Types of Cyclodextrins.

Cyclodextrins (CDs), with lipophilic inner cavities and hydrophilic outer surfaces, are capable of interacting with a large variety of guest molecules to form non-covalent inclusion complexes. These noncovalent complexes offer a variety of physicochemical

advantages over the unmanipulated drugs including the possibility for increased water solubility and solution stability. The driving forces for the complex formation include release of enthalpy-rich water molecules from the cavity, electrostatic interactions, van der Waals interactions, hydrophobic interactions, hydrogen bonding, release of conformational strain and charge-transfer interactions Cyclodextrins are frequently regarded as a new group of pharmaceutical excipients. Beta-cyclodextrins are excellent solubilizers owing to their amorphous character which can be transferred to crystalline drugs in solid combinations. They have low toxicity and are biodegradable.

2.1.3 Pyridoxine Hydrochloride (Pyd):

Chemical Name: 3, 4-Pyridinedimethanol, 5-hydroxy-6-methyl-, hydrochloride.

IUPAC Name: 4, 5-bis (hydroxymethyl)-2-methylpyridin-3-ol

Molecular Formula: $C_8H_{11}NO_3$

Molecular Weight: 169.17784

Physical Properties: Colorless or white crystals or white crystalline powder.

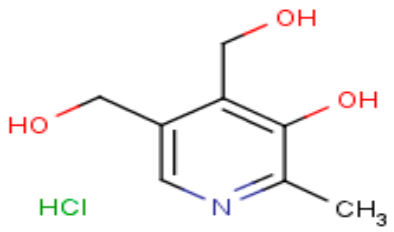

Fig. 9 Chemical Structure of Pyridoxine Hcl

Pyridoxine is also known as vitamin B6. It is based on a pyridine ring, with hydroxyl, methyl, and hydroxymethyl substituents. Pyridoxine is one of the

compounds that can be called vitamin B6, along with pyridoxal and pyridoxamine.[2] It differs from pyridoxamine by the substituent at the '4' position. Its hydrochloride salt pyridoxine hydrochloride is most often used. It is also used in counteracting the neurotoxic effects of Isoniazid and as an antidote to Cycloserine. It functions as a coenzyme essential for the synthesis and breakdown of amino acids, the conversion of tryptophan to niacin, the breakdown of glycogen to glucose 1-phosphate, the production of antibodies, and the formation of heme in hemoglobin.

2.2 Methods

2.2.1 Spectral Characteristics of Spl and Pyd

Solutions of Spl and Pyd (10 µg/ml) were prepared by appropriate dilution of a standard 100 µg/ml stock solution in methanol. The solution was scanned in spectrum mode from 200-400 nm. The iso-absorptive point was detected.

2.2.2 Preparation of calibration curve and simultaneous estimation:

Appropriate dilutions from the standard stock solution of 100 µg/ml were prepared for both Spl and Pyd separately and the absorption spectra of all solutions were recorded between 200-400 nm. The absorbance was measured at 242 nm (λ_{max} for Spl), 285 nm (λ_{max} for Pyd) and 263 nm (iso-absorptive point). Ethanol was used as blank. The Beer Lambert's range for Spl and Pyd were selected and working calibration curves were plotted separately. Along with the Spl another drug Azithromycin was also initially considered for the study but it was rejected since it was determined to be a BCS Class III

drug, and our study was focused on the BCS Class II drugs. Further only Spl was considered for all the formulation and studies.

Simultaneous Estimation of drugs and excipients:

Since no spectrophotometric method is reported for simultaneous estimation of Spironolactone and Pyridoxne HCl , in the present work, a successful attempt has been made to estimate both these drugs simultaneously by Q-Absorbance ratio method[9].

From the following set of equations the concentration of each component in sample can be calculated:

$$Cx = (Qm - Qy \div Qx - Qy) \times A \times a \quad \text{Eq 1}$$

$$Cx = (Qm - Qy \div Qy - Qx) \times A \times a \quad \text{Eq 2}$$

Where, Cx = Concentration of Spl

Cy = Concentration of Pyd

A = Absobance of sample at iso-absorptive wavelength.

a = Mean absorptivity of Spl and Pyd at iso-absorptive Wavelength

Qm= Ratio of sample solution at 242nm and isoabsoptive point

Qx= Absorptivity ratio of Pyd at 242nm and isoabsorptive point.

Qy= Absoptivity of Pyd at 285nm and isoabsorptive point

2.2.3 Method of preparation for Solid Dispersion and Physical Mixture.

Preliminary aqueous phase solubility studies to determine which ratio of beta-cyclodextrin and drug spironolactone should be used for future formulation was performed. Spl and β-cyclodextrin were taken in the ratio of 1:1, 1:2, 1:3, 1:4, 1:5, 1:6

and 1:7. Phase solubility for the ratios 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7 was found out to be 35.38 ±1.2 µg/ml, 46.48 ± 3.2 µg/ml, 45.68 ± 3.1 µg/ml, 46.33 ± 3.6 µg/ml, 46.45 ± 2.7 µg/ml, 47.3 ± 1.7 µg/ml, and 47.1± 1.8 µg/ml respectively. Spl alone which showed solubility of 28 µg/ml. On the basis of suitable solubility shown, 1:1 and 1:2 ratio of Spl : β-CD was considered for further preparation of binary and ternary formulation system as there was no significant increase in solubility shown by subsequent higher ratio. Solid dispersions were prepared using the solvent evaporation method containing Spl, β-cyclodextrin in the ratios of 1:1 (SD01) and 1:2 (SD02) w/w. Further solid dispersions comprising of Spl, β-cyclodextrin and Pyd called ternary dispersions in the ratios of 1:1:1 (SD03) and 1:2:1 (SD04) (w/w/w) were also obtained. The drug, carrier and adjuvant were dissolved in methanol (50%w/v), and the solvent was subsequently allowed to evaporate while stirring at room temperature. The products were then stored in an oven at 40°C for 24 hours to ensure complete methanol evaporation. Drying was confirmed by obtaining constant weight of the residual solid mass consistently for three weighings.

The physical mixtures (PM) comprising of Spl and β-cyclodextrin in the ratios of 1:1 (PM01) and 1:2 (PM02) w/w and consisting of Spl, β-cyclodextrin and Pyd in the ratios 1:1:1 (PM03) and 1:2:1 (PM04) (w/w/w) were obtained by triturating in a glass mortar and carefully mixing accurately weighed ratios of drug and carriers together. Solid dispersions and physical mixtures were then pulverized and passed through an 80 mesh sieve. Preparations were then protected against humidity and light for further analysis by packing them in dark plastic wrapped dessicators.

2.2.4 Solid Dispersion and Physical Mixture Characterization (Liquid State)

2.2.4. a Saturation Solubility or Aqueous solubility-

To evaluate the increase in solubility of the drug, both physical mixtures and solid dispersions, saturation solubility was screened. The known excess of drug, physical mixture and solid dispersion (10 mg) were added to 10 ml of phosphate buffer with 7.4 pH in screw capped vials. Samples were shaken at 37± 0.5 °Celsius at 25 rpm for 48 hours. Samples were then filtered through a 0.45 µm Millipore filter. The filtrate was then suitably diluted with medium used for solubility analysis in a UV-spectrophotometer (Genesys-6® UV Spectrophotometer from Thermo Scientific Inc). Each experiment was performed in triplicate.

Solutions containing only Spl (i.e. PM01, PM02, SD01, and SD02) were analyzed at 242 nm in UV-spectrophotometer. The solutions containing of both the Spl and Pyd (PM03, PM04, SD03 and SD04) were analyzed at both the wavelength 242 nm (λ_{max} for Spl) and 285nm(λ_{max} for Pyd) and estimated using the simultaneous estimation method as previously described. Each experiment was performed in triplicate.

2.2.4. b Drug Content: (Percentage drug content):

Solid dispersions and physical mixtures equivalent to 10 mg of spironolactone were weighed accurately and dissolved in 100 ml of methanol by shaking at 37± 0.5 °Celsius at 25 rpm for 48 hours. The solutions were filtered and the drug content was evaluated by UV spectrophotometer after suitable dilution. The percentage of dug content was calculated using the formulae:

$$\text{Percent drug content} = \frac{\text{Practical drug content in solid dispersions}}{\text{Theoretical drug content in solid dispersion}} \times 100 \quad \text{...Eq 3}$$

2.2.4. c Determination of Percent Yield: The percent yield of spironolactone solid dispersions was determined by using the following formula:

$$\text{Percent Yield} = \frac{\text{Weight of prepared solid dispersion}}{\text{Weight of drug + carriers}} \text{x}100. \qquad \text{....Eq 4}$$

2.2.5. Solid Dispersion and Physical Mixture Characterization (Solid State)

2.2.5. a. Differential Scanning Calorimetry (DSC)-

DSC analysis was performed using a DSC 822^e with a robotic sampler from Mettler-Toledo and thermal characteristics of the drug, matrix, physical mixtures and solid dispersions were determined. The instrument was calibrated using indium as the standard. Approximately 5 mg of each sample was placed in 100 µL aluminum pans which were sealed and heated from 25°C to 300°C at a rate of 10°C/min under nitrogen flow of 30 ml/min. Data analysis was done using STAR^e software.

2.2.5. b Fourier transform infrared spectroscopy (FTIR) analysis-

FTIR was employed for all the solid dispersions and physical mixture preparations containing drug and matrix using a Varian Excalibur Series FTIR so as to characterize drug-carrier interactions. FTIR spectra were recorded on samples prepared in potassium bromide (KBr) pellets using the Varian Excalibur spectrophotometer Series FTIR. The resolution of 4 cm^{-1} with 256 scans was co-added for each spectrum over the scanning frequency range of 400 to 4000 cm^{-1}. Varian software was used for data analysis.

2.2.5. c Power X-ray Diffraction (PXRD)-

The powder x-ray diffraction (PXRD) method was performed and the PXRD pattern was studied using PANalytical X'Pert Pro MPD, operating at a voltage of 45 kV, and a current of 40 mA with a scintillation counter. The instrument was operated in the continuous

scanning speed of 4°/min over a range of 5° to 45° 2Θ. Analysis programs include JADE which was the available version of the PANalytical X'Pert software.

2.2.6 Dissolution Studies-

In order to increase the oral absorption of poorly water soluble drugs, it is very important to improve the drug solubility in the gastrointestinal tract. In-vitro drug release studies were performed using 0.1 N HCl at 37 ± 0.5°C, using a 6 station unit SOTAX AT-7 Dissolution testing apparatus with the paddle rotating at 50 rpm. Accurately weighed drug, physical mixture or solid dispersion equivalent to 100 mg of drug (Spl) was placed into 900 mL of dissolution medium. Samples (5ml) were withdrawn at predetermined times, and replaced by fresh media, Withdrawn samples were filtered through a 0.45 µm membrane filter, and spectrophotometrically assayed for drug content. Samples containing only Spl were estimated at 242 nm. Samples containing both the Spl and Pyd were analyzed at both wavelengths 242nm (λ_{max} for Spl) and 285nm (λ_{max} for Pyd) and estimated for drug content using simultaneous estimation method previously described using a UV-VIS spectrophotometer. The dissolution profiles were evaluated and data was analyzed by model dependent techniques.

Kinetic behavior assessment of the dissolution profiles for Spl, PM and SD were also done. The dissolution data was fitted to commonly used drug release models such as zero order, first order, Higuchi and Korsmeyer-Peppas in order to determine the pattern and mechanism of drug release.

The linear regression coefficient for the entire four release pattern was reported.

Chapter 3

Results and Discussion

3.1 Spectral Characteristics of Spl and Pyd :

The wavelengths or absorption maxima selected for analysis by the simultaneous equation method were 242 nm (λ_{max} of Spl) and 285 nm (λ_{max} of Pyd).

3.2 Preparation of the calibration curves :

The Beer Lambert range was found to be in the concentration range of 2 to 28 µg/ml for Spl (Table 3) and 5 to 25 µg/ml for Pyd (Table 4). Standard curves obtained depicted the regression coefficient (R^2) of 0.9992 for Spl and 0.9967 for Pyd which indicates a definitude of linearity. Absorptivity coefficients were calculated for both the drug and adjuvant at selected wavelengths for determining the concentration of Spl and Pyd into its solution state using the simultaneous estimation method.

Table. 3 Standard Calibration curve (Estimation of Spironolactone).

S. No.	Concentration (µg/ml)	Absorbance
1	4	0.13249
2	6	0.21483
3	8	0.29098
4	10	0.36155
5	12	0.43746
6	14	0.50591
7	16	0.58331
8	18	0.65336
9	20	0.70476

10	22	0.76752
11	24	0.85885
12	26	0.92721
13	28	0.99564

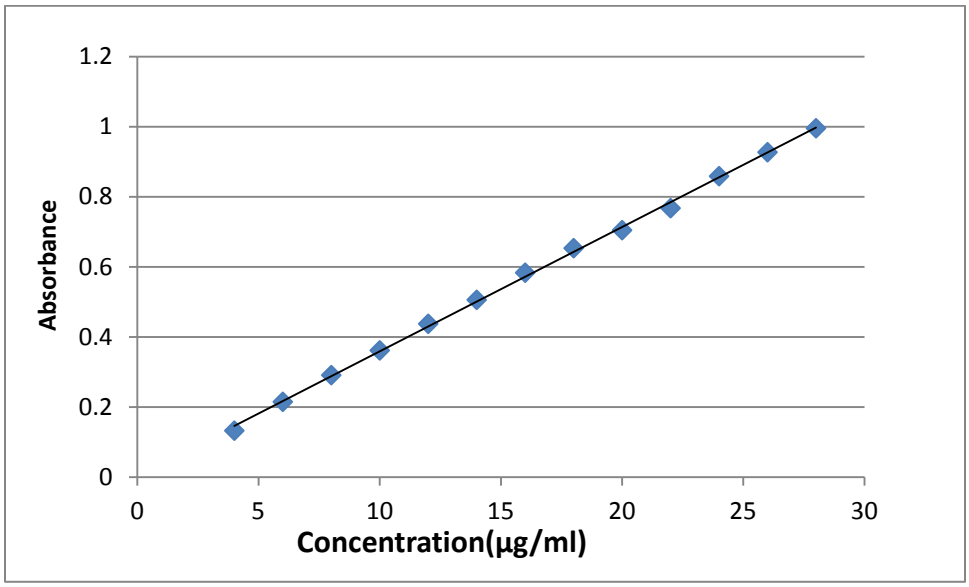

Fig. 10 Standard Calibration curve for Spironolactone when y = 0.0355x + 0.0043 and R^2 = 0.999

Table.4 Standard calibration curve (Estimation of Pyridoxine Hcl)

Sample No.	Concentration (µg/ml)	Absorbance
1	3	0.1491
2	5	0.1958
3	7	0.2872
4	9	0.3746
5	11	0.46607
6	13	0.5243
7	15	0.61355
8	17	0.69406
9	19	0.7602
10	21	0.8485
11	23	0.8993

12	25	0.9621

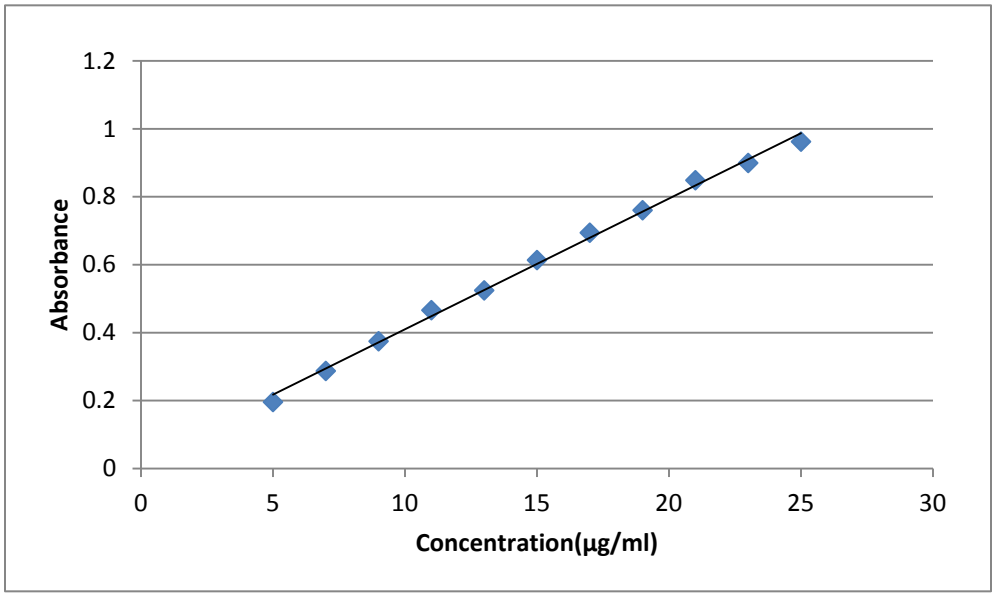

Fig.11 Standard Calibration curve of Pyridoxine HCl when y = 0.0385x + 0.0255 and R^2 = 0.9967

3.3 Solid Dispersion and Physical Mixture Characterization (Liquid State)

3.3.1 Saturation Solubility:

Saturation solubility for the raw spironolactone at standard room temperature of 25 °C was 34.48 ± 1.69 µgm/mL. The maximum solubility for Spl was determined to be in the SD04 and was 362.71± 3.6 µgm/mL. All the solid dispersions showed increased the drug solubility over that of the drug powder itself. Solid dispersions prepared by the solvent-evaporation method with the incorporation of Pyd along with β-CD had a synergistic effect for increasing the solubility significantly. In all the solid dispersions higher solubility than the pure Spl and corresponding physical mixtures were reported.

The higher solubility of spironolactone in the solutions of the carriers suggests greater miscibility of spironolactone into the carrier and thus there was a higher probability of solid solution formation during the formulation of the solid dispersion.

3.3.2 Drug content and % Yield:

The drug content was found to be uniform. Drug content in the prepared samples of the solid dispersions ranged from 93.75± 4.32% to 95.53± 3.15%. SDs of the drug exhibit uniform content uniformity and thus can be employed for the development of a quality formulation.

Table.5 Saturation solubility for the different spironolactone solid dispersions along with pure spironolactone and the physical mixtures.

Formulation	Saturation solubility of Spl (µgm/mL)	% Drug Content (%)	Percentage Yield (%)
Spl	34.48 ± 1.69	-	-
PM01	89.64± 6.2	96.34± 3.65	-
PM02	105± 3.7	97.62± 3.32	-
PM03	94± 4.8	96.24± 3.13	-
PM04	114± 4.8	95.72± 4.34	-
SD01	176.47± 5.2	94.17± 3.14	93.14 ± 3.2
SD02	274.205± 4.6	93.75± 4.32	94 ± 2.8
SD03	194.09±3.33	95.53± 3.15	93.82 ± 2.6
SD04	362.71± 3.6	94.47± 3.64	93.56 ± 2.87

3.4 Solid Dispersion and Physical Mixture Characterization (Solid State)-

3.4.1 Differential Scanning Calorimetry Evaluation:

Differential scanning calorimetry (DSC) is frequently the pharmaceutical thermal analysis technique of choice because of its ability to provide detailed information about both the physical and energetic properties of a substance. DSC enables the quantitative detection of all processes in which energy is required or produced (i.e., endothermic or exothermic phase transformations). Spl has an endothermic peak at 207°C corresponding to its melting point and also indicating its typical crystalline and anhydrous nature. The β-CD showed broad endothermic peaks at 60 to100°C and 204 to 210°C associated with crystal water loss. The solid dispersions SD01 and SD03 showed smaller peaks indicating that there is good interaction and reduction in crystallinity implying that not all the drug has undergone complexation. The SD01 and SD02 showed three endothermic events for Spl, drug and β-CD. Whereas in the SD02 and SD04 all the peaks disappeared suggesting that the drug was present in the amorphous state. There is also a strong interaction and formation of a true inclusion complex. The loss of crystallinity can be attributed to two effects: interactions, such as hydrogen bonding between the drug and the carrier and the entrapment of the drug molecules (i.e. formation of inclusion complexes) in the carrier during the solvent evaporation method. Such an interaction can be interpreted by assuming that the hydrogen bonds of the crystalline drug embedded in the amorphous cyclodextrin matrix are weakened and other hydrogen bonds involving water molecules and hydroxyl groups of the carrier may be established. The corresponding physical mixture showed comparatively weak but clearly distinguishable endothermic peaks for the drug and excipients. This indicates that in such systems the drug has basically maintained its original crystallinity. Thermograms of PMs also show the broad endothermic effect due to the cyclodextrins dehydration process.

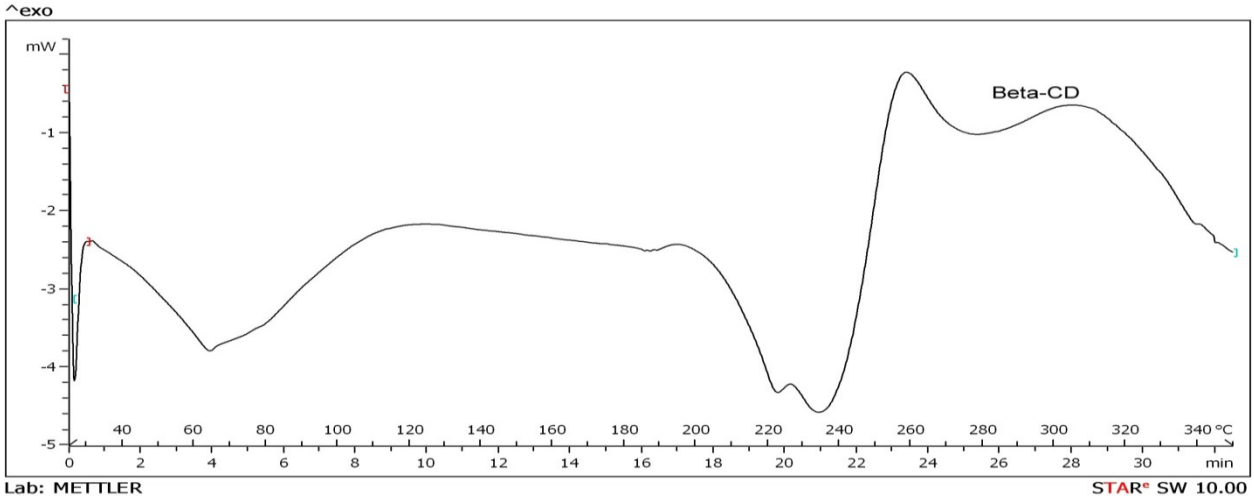

Fig.12 DSC Thermogram for Beta- Cyclodextrin (β-CD).

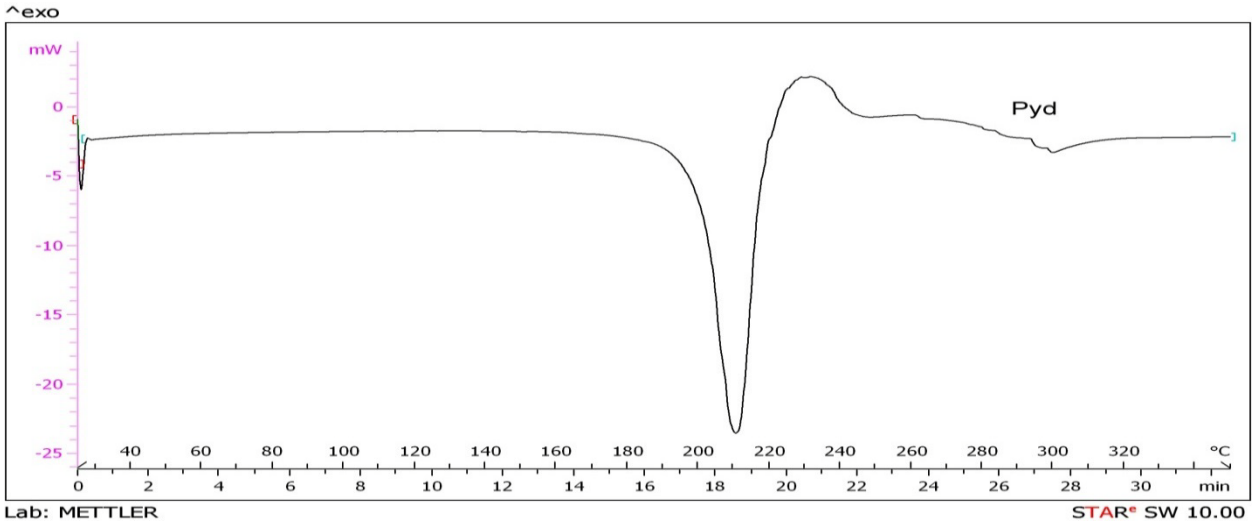

Fig. 13 DSC Thermogram for Pyridoxine HCl (Pyd).

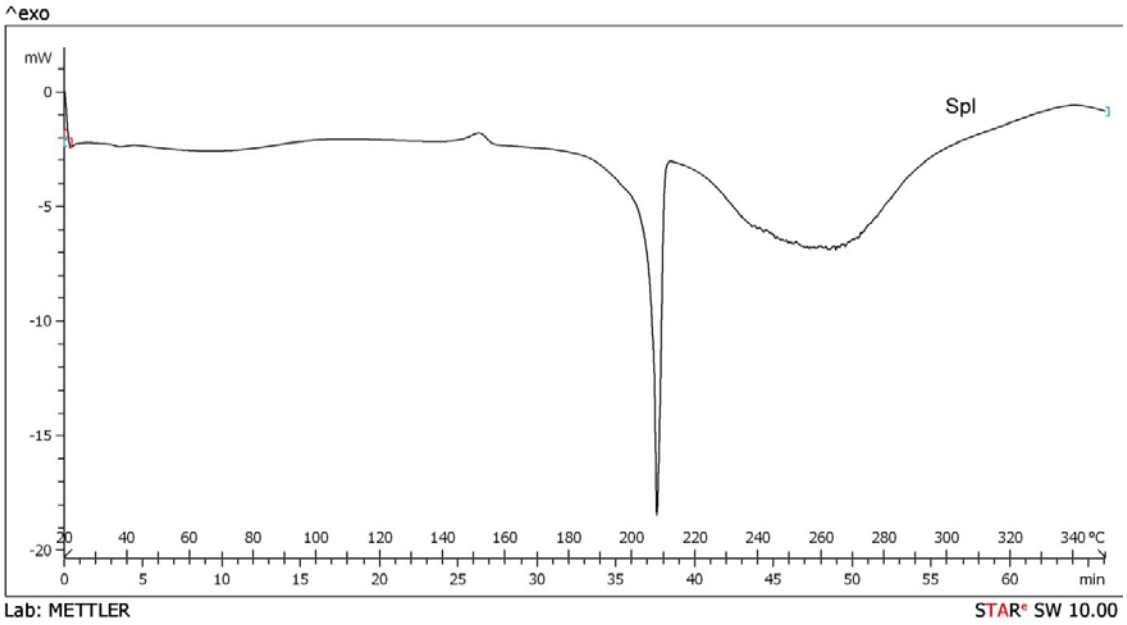

Fig. 14 DSC Thermogram for Spironolactone (Spl).

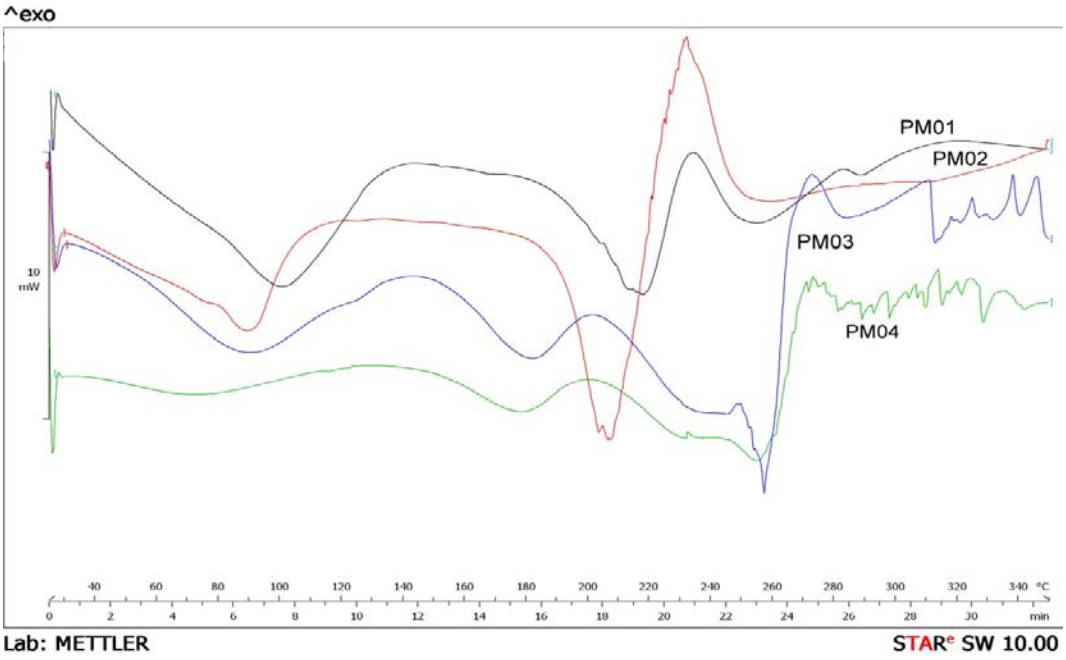

Fig.15 Overlay of the DSC Thermograms for all the Physical Mixtures PM01, PM02, PM03, PM04.

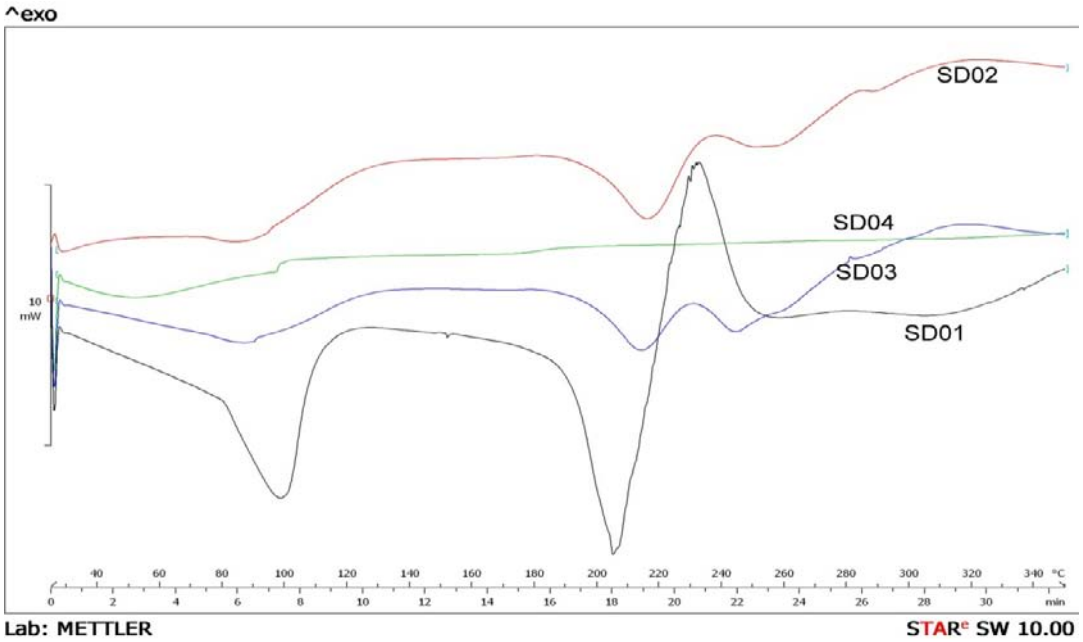

Fig. 16 Overlay of the DSC Thermogram of all the Solid Dispersion SD01, SD02, SD03, SD04.

3.4.2 Fourier Transform Infra Red Evaluation:

Furthermore evidences for the complex formation were obtained from the FTIR study. The FTIR spectra for spironolactone showed characteristic peaks at 1735, 1710, 1630 and 1605 cm^{-1} corresponding to –C=O stretching of lactone ring, –C=O stretching of thioacetyl group, –C=O stretching of α, β-unsaturated ring and –C=C stretching of α, β-unsaturated ring respectively. The frequencies for β-Cyd observed at 3345.71 cm^{-1}, 2931.62 cm^{-1}, 1187.34 cm^{-1}, and 1029.24 cm^{-1} which correspond to the symmetric and antisymmetric stretching of C-OH, CH_2, C–C and bending vibration of O–H respectively. The sharp and intense band at 1560 cm^{-1} may be assigned to the in-plane pyridine ring stretching vibration and C-N^+-H stretching vibration of nitrogen-protonated pyridine ring of pyridoxine HCl. The frequencies for β-Cyd observed at 3345.71 cm^{-1}, 2931.62 cm^{-1},

1187.34 cm^{-1}, and 1029.24 cm^{-1} which corresponds to the symmetric and antisymmetric stretching of ν [OH], ν [CH_2], ν[C–C] and bending vibration of ν [O–H], respectively

The FTIR spectra for the physical mixtures seemed to be only a summation of the drug and carriers spectra. The carbonyl groups appeared as sharp bands in the PMs and were less intense in the SDs. The spectrum for the SD02 and SD04 complex looks almost similar to the pure β-cyclodextrin. However SD04 has even more decreased intensity as compared to SD02. The decrease in the frequency between the inclusion complex and its constituent molecule is due to the changes in the microenvironment which lead to the formation of hydrogen bonding and the presence of van der Waals forces during their interaction to form the inclusion complex. The broad hydroxyl band for the pure β-cyclodextrin at 3370.72 cm^{-1} was found to be narrowed in the FTIR spectrum of the inclusion complex SD02 and SD04 which is a good indication of the formation of the inclusion complex. In the case of SD01 and SD03 there were some minor changes in the peaks comparatively indicating weak interaction and incomplete inclusion in the complex.

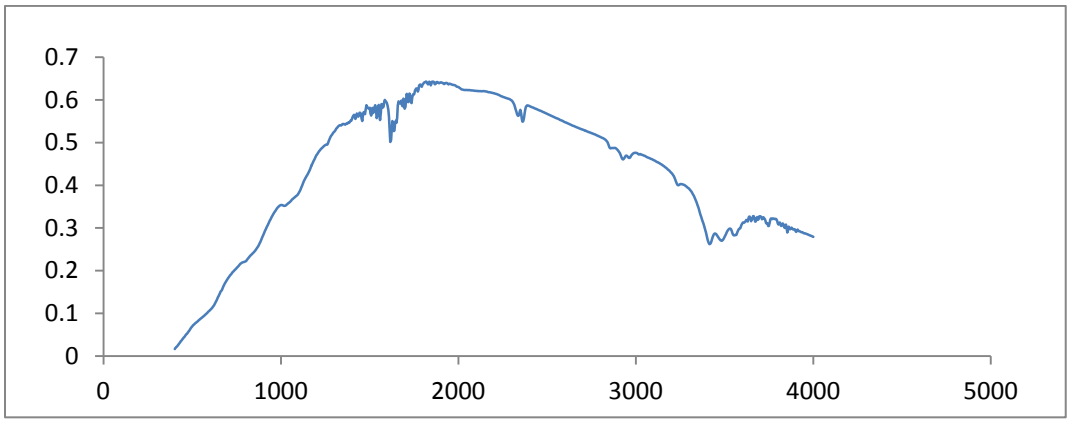

Fig. 17 FTIR Spectrum for KBR (Reference)

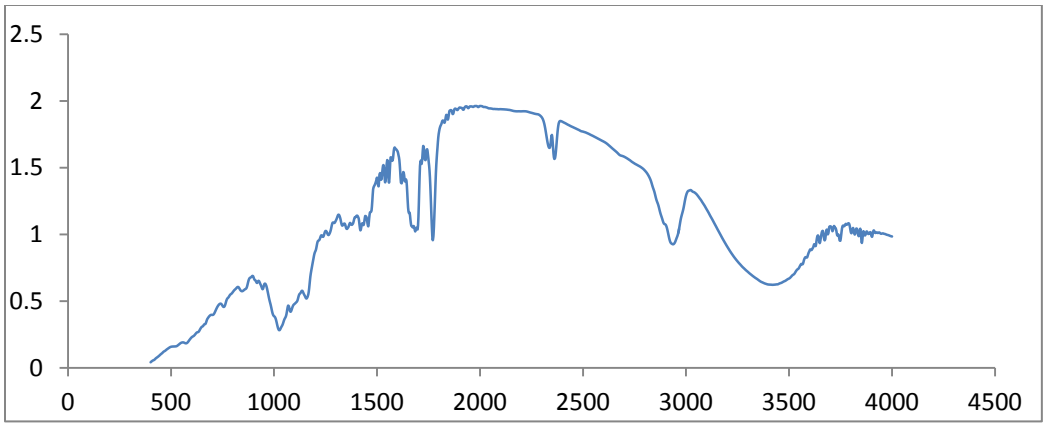

Fig.18 FTIR Spectrum for B-CD

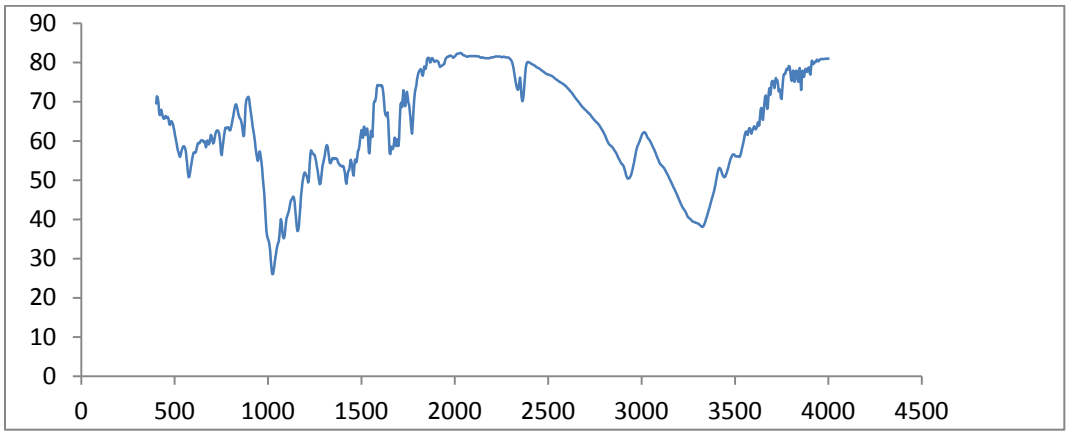

Fig.19 FTIR Spectrum for Spl

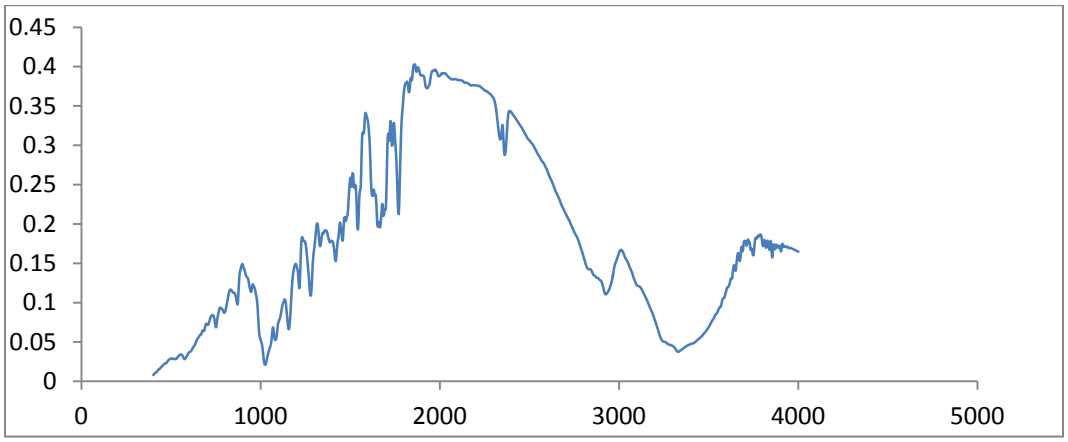

Fig. 20 FTIR Spectrum for Pyd

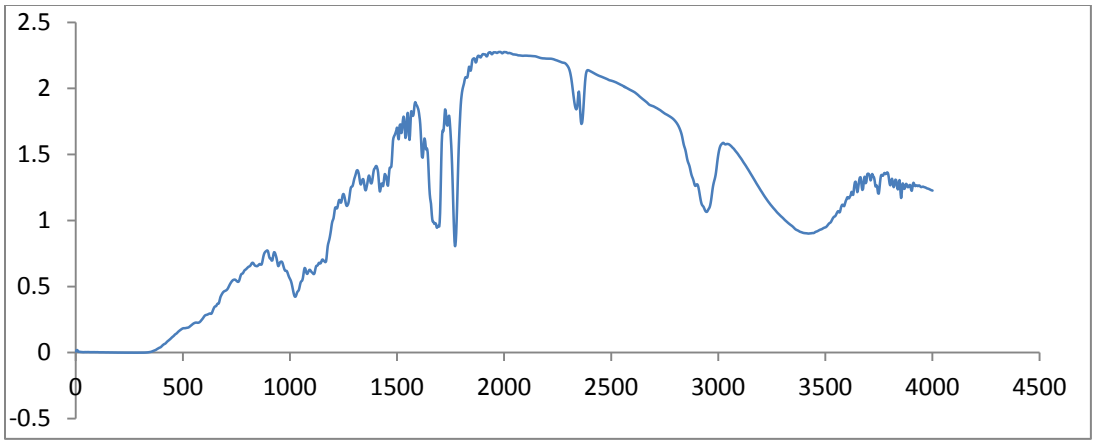

Fig. 21 FTIR Spectrum for PM01

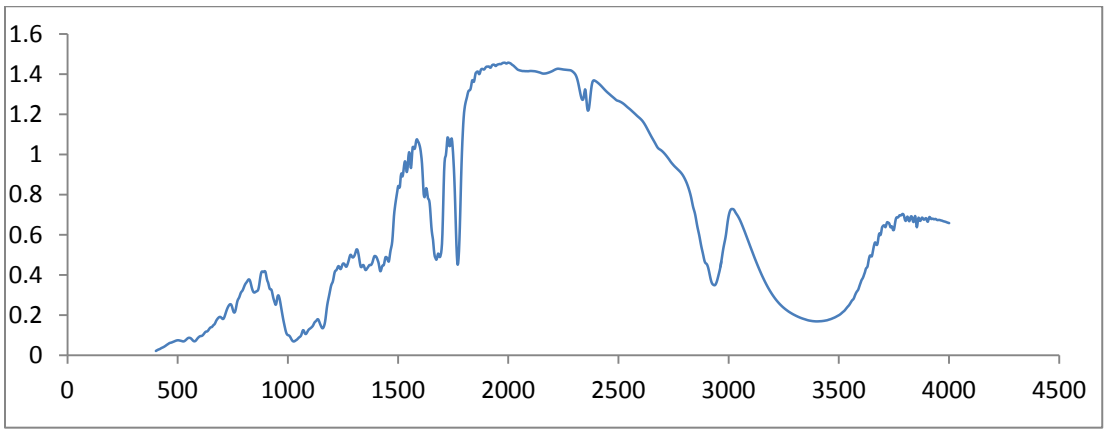

Fig. 22 FTIR Spectrum for PM02

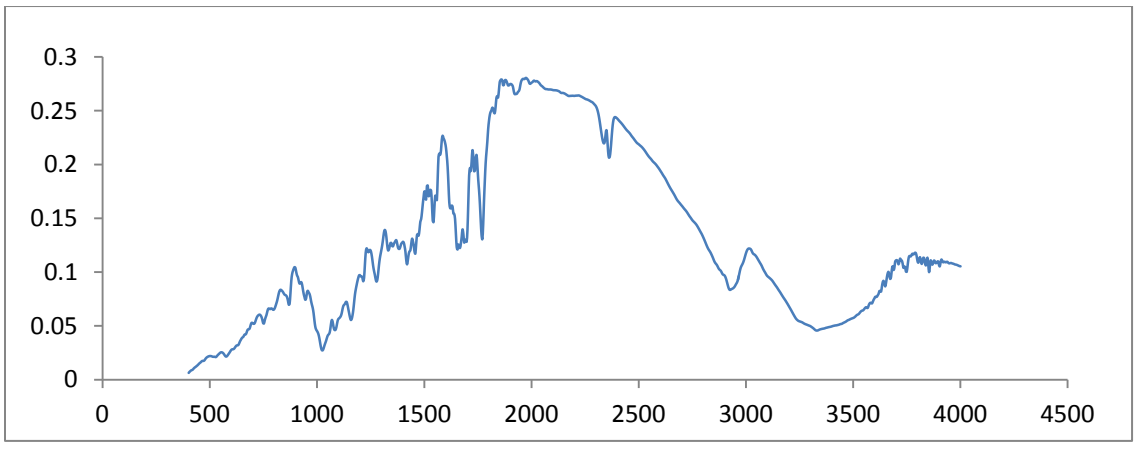

Fig.23 FTIR Spectrum for PM03

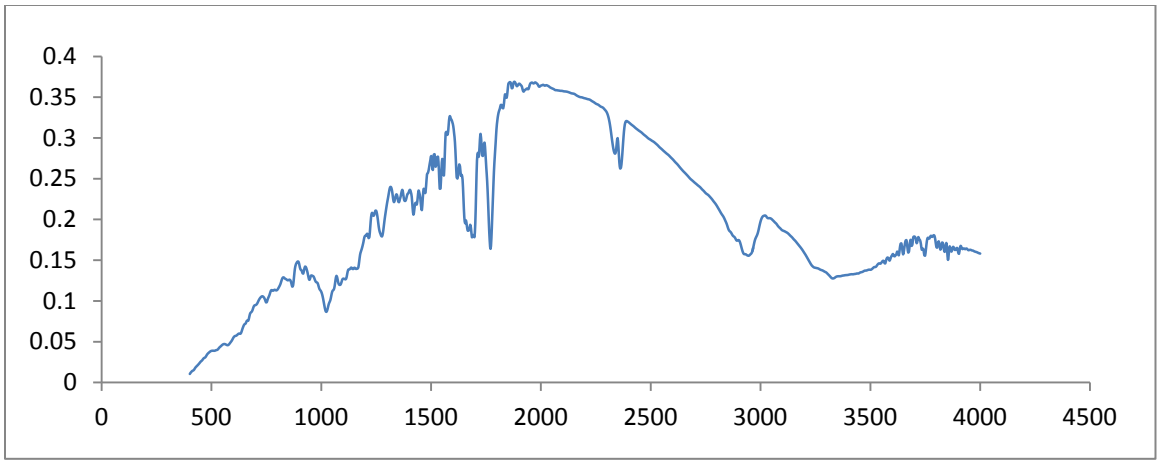

Fig. 24 FTIR Spectrum for PM04

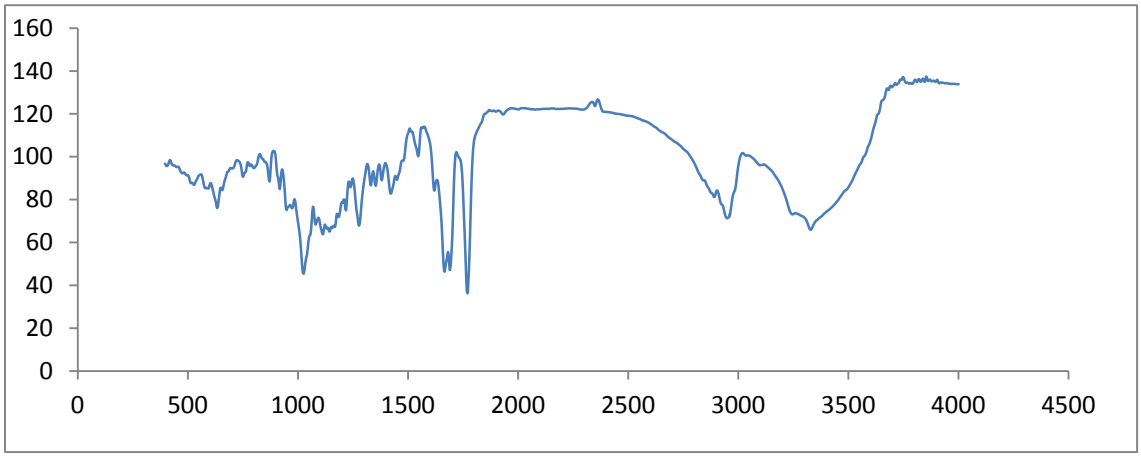

Fig. 25 FTIR Spectrum for SD01

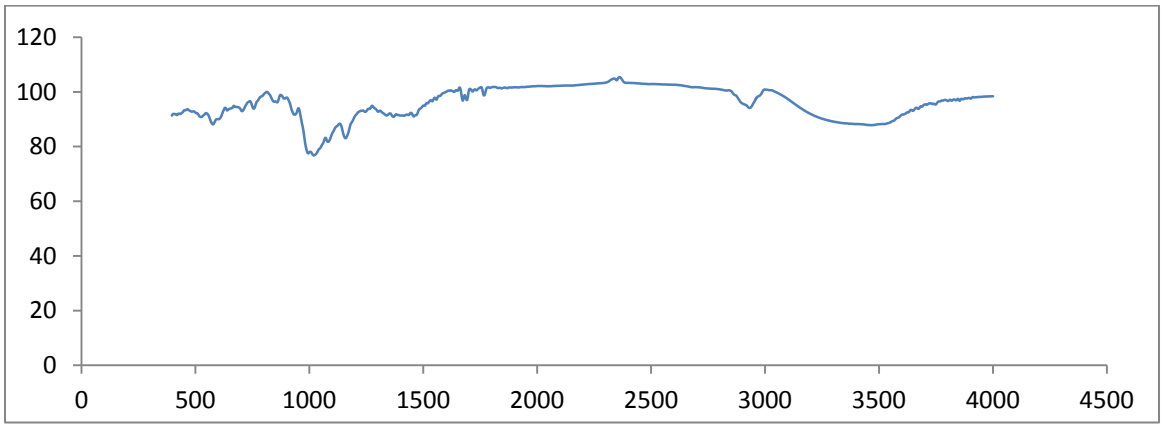

Fig. 26 FTIR Spectrum for SD02

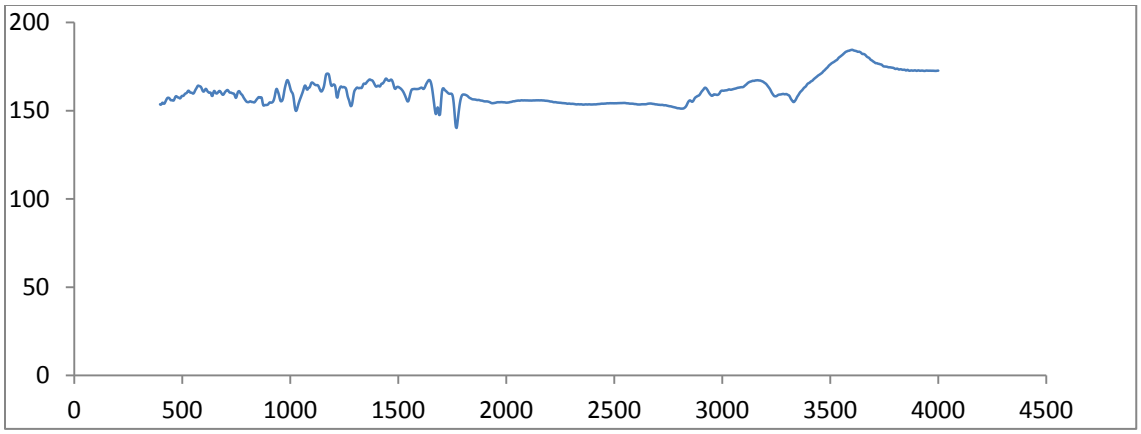

Fig. 27 FTIR Spectrum of SD03

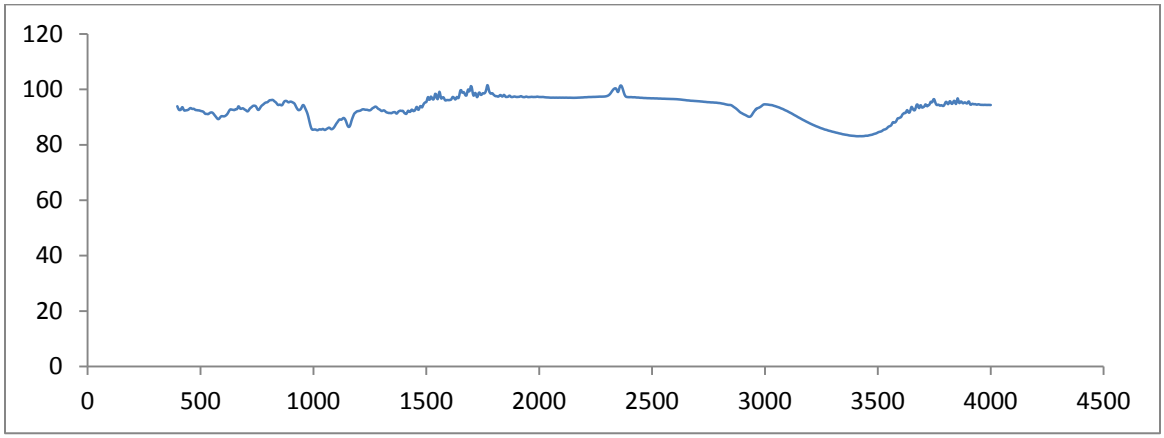

Fig. 28 FTIR Spectrum of SD04

3.4.3 Power X-Ray Diffraction Evaluation:

The pure drug Spl exhibited its characteristics diffraction peaks in the 2Θ range of 10° to 25° (9˚, 11.2˚, 12˚, 13.3˚, 15.8˚, 16.2˚, 17˚, 18˚ and 20˚) indicating the presence of drug in its highest crystalline state. The X-ray diffraction study for PM showed the peak corresponding to crystalline drug molecules present in the mixture. In the case of the PM, the PXRD spectrum is simply the superposition of those of the single components. Some changes in the peak positions and reduction of crystalline nature of spironolactone to some extent was observed for SD01 and SD03 indicating a small amount of complex formation or presence of drug in its semi-crystalline state. The pattern was different for

the SDs. In the SDs containing drug, peaks attributed to the drug at 6.1, 11.38, 16.14, 19.6, and 20.32 can be identified, but they are broadened compared with the pure drug or with the PM.

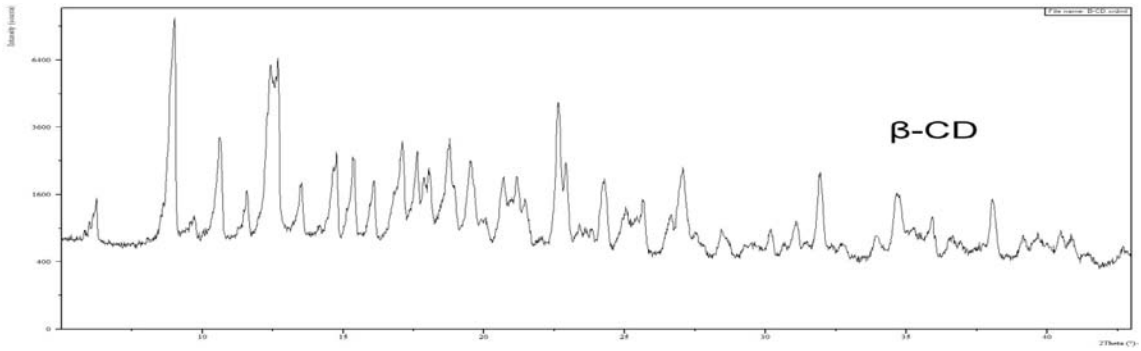

Fig. 29 X-ray diffraction (PXRD) patterns for Beta-Cyclodextrins.

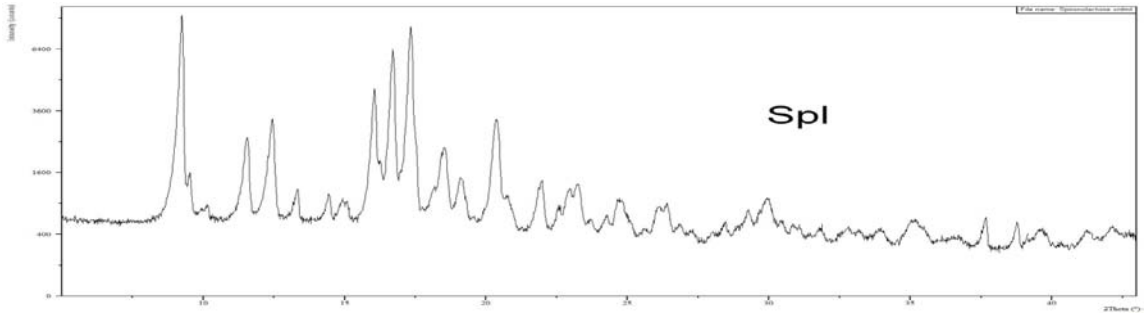

Fig. 30 X-ray diffraction (PXRD) patterns for Pure Spironolactone

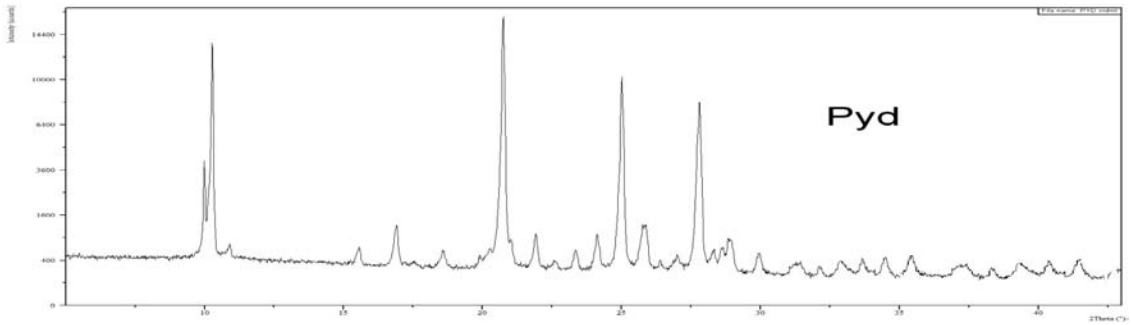

Fig 31 X-ray diffraction (PXRD) patterns for Pyridoxine

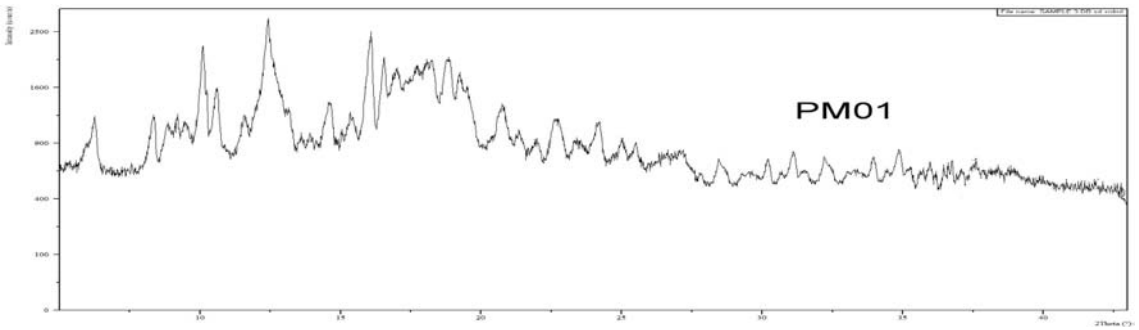

Fig. 32 X-ray diffraction (PXRD) patterns for Physical Mixture PM01

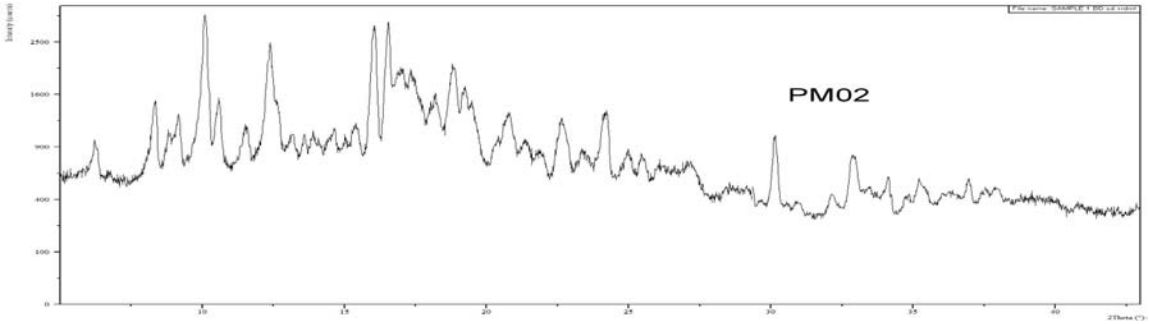

Fig. 33 X-ray diffraction (PXRD) patterns for Physical Mixture PM02

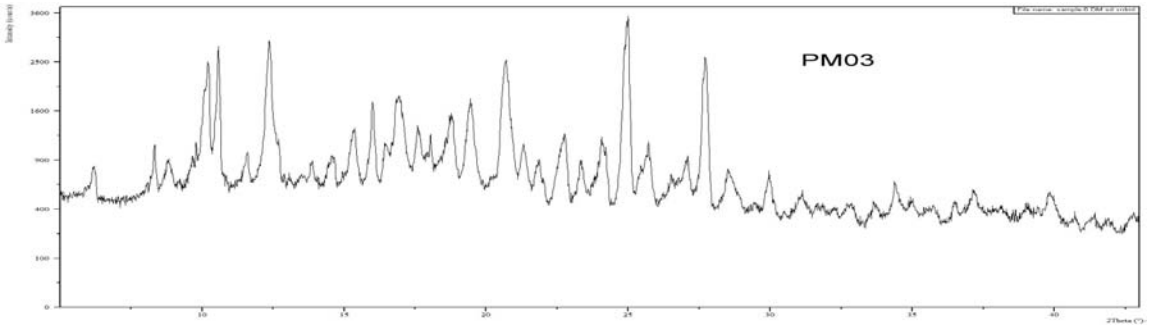

Fig. 34 X-ray diffraction (PXRD) patterns for Physical Mixture PM03

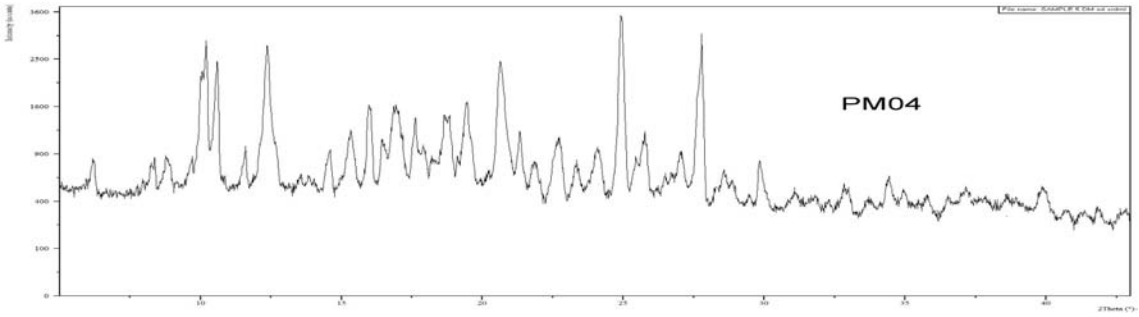

Fig. 35 X-ray diffraction (PXRD) patterns for Physical Mixture PM04

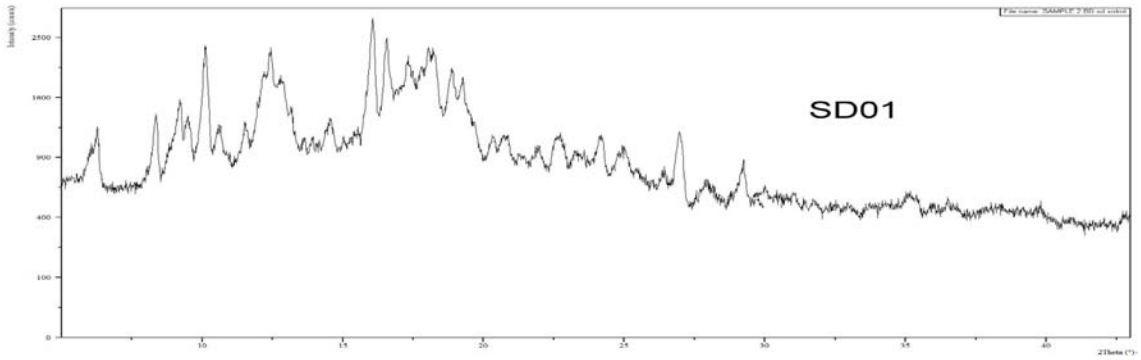

Fig. 36 X-ray diffraction (PXRD) patterns for Physical Mixture SD01

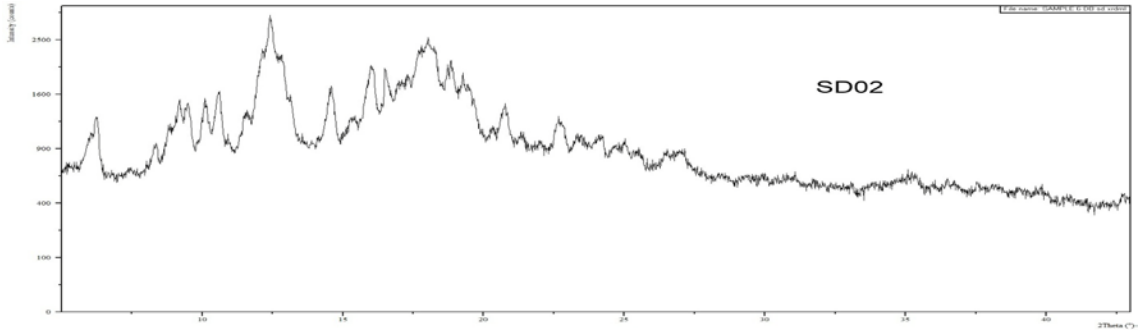

Fig. 37 X-ray diffraction (PXRD) patterns o for SD02

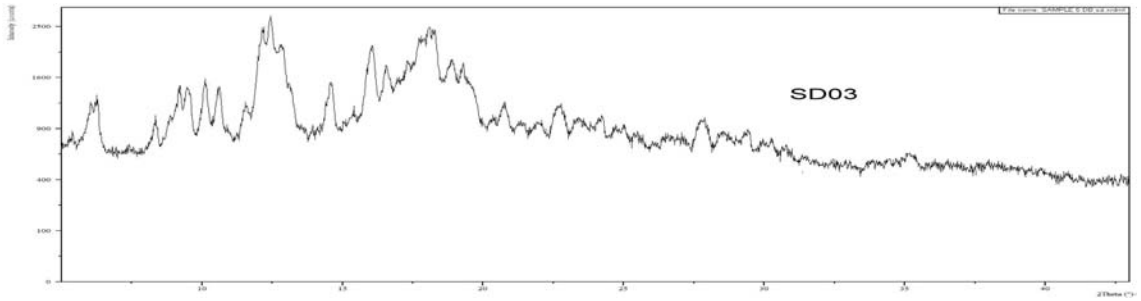

Fig. 38 X-ray diffraction (PXRD) patterns for SD03

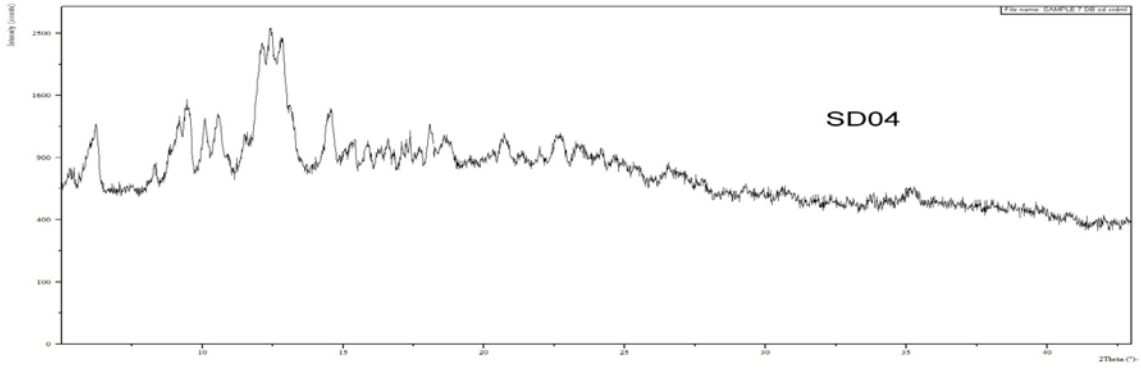

Fig. 39 X-ray diffraction (PXRD) patterns for SD04

However the diffraction pattern for SD02 and SD04 showed an absence of Spl crystalline diffraction peaks which indicates that drug amorphization occurs to a greater extent, that is, the drug is principally existing in its amorphous inclusion complex. These results obtained from PXRD are in accordance with the higher solubility observed for these SD complexes in comparison with the Spl and PM since crystallinity and amorphicity are important factors that must be related to the compounds' solubility and dissolution. However, they are useful to monitor the compounds' crystallinity changes upon any host-guest interactions.

3.5 In-vitro Dissolution Studies

Dissolution profiles for the original drug crystals and drug carrier binary and ternary systems are presented in Figure 40. It is clearly seen that the solid dispersion (SD) technique has improved the dissolution rate for Spl to a great extent.

Ternary solid dispersions were found to be more effective to increase the dissolution than binary solid dispersions. Furthermore, these results also suggest that all solid dispersions of spironolactone exhibited increased dissolution, significantly as compared to their respective physical mixtures. Also, spironolactone dissolution from all the solid dispersions reached a plateau at around 120 min to 180 min for dissolution and complete spironolactone dissolution was not obtained in any formulation at the end of the 180 min study times.

Table.6 Percentage cumulative release from Physical Mixture as compared to drug.

Time (in min)	Spl	SD	PM01	SD	PM02	SD	PM03	SD	PM04	SD
10	1.9	±2.2	4.32	±2.3	4.85	±2.2	5.32	±3.1	4.56	±2.3
20	2.34	±2.1	5.89	±1.4	6.72	±3.2	6.73	±3.3	7.41	±1.9
30	2.76	±2.6	7.32	±3.2	8.31	±3.9	11.83	±3.4	10.35	±3.1
40	3.68	±1.8	9.14	±2.4	12.58	±2.3	14.38	±3.1	12.31	±3.5
50	6.26	±3.4	13.2	±2.1	17.63	±2.4	19.67	±2.9	15.21	±2.9
60	7.26	±3.5	17.63	±3.5	21.33	±2.1	22.56	±3.4	19.34	±3.5
75	8.03	±3.5	21.34	±3.8	26.23	±2.9	23.69	±3.7	22.14	±4.1
90	8.91	±2.8	24.17	±4.9	27.26	±2.3	25.85	±3.6	27.76	±3.2
105	9.72	±3.1	32.83	±4.2	29.37	±2.1	29.71	±2.9	32.87	±2.6
120	12.54	±1.4	33.9	±4.4	32.83	±3.9	33.76	±3.5	39.14	±3.6
150	13.26	±2.1	35.74	±3.6	39.17	±2.7	37.82	±3.6	44.31	±3.5
180	14.2	±3.5	36.3	±3.4	40.71	±2.6	41.59	±3.2	45.62	±3.3

Table.7 Percentage cumulative release from Solid Dispersion as compared to drug

Time (in min)	Spl	SD	SD01	SD	SD02	SD	SD03	SD	SD04	SD
10	1.9	±2.2	9.12	±3.4	11.62	±4.1	14.01	±5.2	14.87	±5.2
20	2.34	±2.1	15.85	±3.1	16.73	±3.4	18.96	±5.5	18.91	±5.5
30	2.76	±2.6	21.34	±3.2	24.59	±3.2	28.93	±5.6	30.25	±3.8
40	3.68	±1.8	27.34	±3.4	31.23	±2.9	34.27	±4.7	41.24	±4.6
50	6.26	±3.4	33.56	±2.9	37.1	±3.8	39.24	±4.1	47.32	±4.4
60	7.26	±3.5	41.45	±2.4	42.3	±2.7	48.14	±4.2	51.4	±5.5
75	8.03	±3.5	47.52	±2.4	46.2	±2.6	52.08	±3	59.15	±3.6
90	8.91	±2.8	50.76	±2.6	50.2	±4.2	56.91	±5.2	69.7	±3.7
105	9.72	±3.1	54.21	±2.9	54.21	±3.2	59.36	±4.3	72.16	±4.6
120	12.54	±1.4	56.71	±3.5	60.25	±3.3	61.34	±5.7	79.24	±4.9
150	13.26	±2.1	60.59	±3.6	64.3	±3.5	62.1	±5.1	82.36	±5.8
180	14.2	±3.5	62.59	±3.2	66.52	±3.2	64.74	±5.9	84.21	±6.3

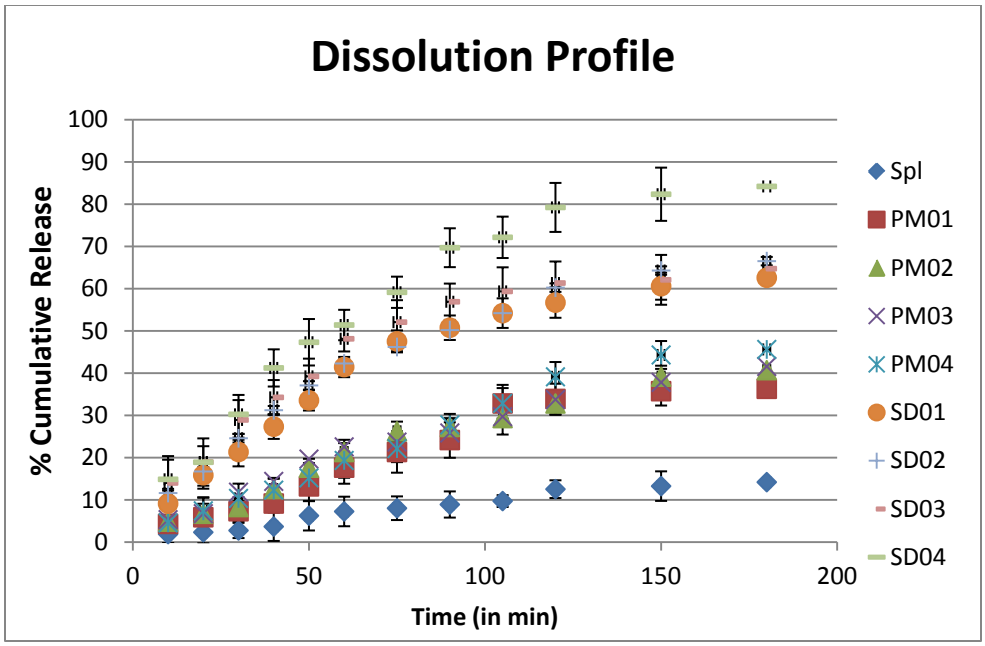

Fig 40 Dissolution profile of all the solid dispersion with respect to their physical mixture and drug

The values given in Table 7 indicate that SD04 shows maximum enhancement in the dissolution rate. This may be due to the greater hygroscopic nature of Pyd which assures enhanced wettability for Spl. Both of these polymers definitely showed a synergistic increase in the Spl release profile from the ternary system. However, SD02 also produces comparable results in terms of dissolution efficiency. Physical mixtures (PM) also improve dissolution rate by a significant extent as compared with drug alone.

This enhancement of dissolution of Spl from drug carrier systems can be ascribed to several factors. The mechanism for the dissolution rate improvement from solid dispersion has been reviewed. Lack of crystallinity (ie, amorphization) increased wettability and dispersibility and particle size reduction, encapsulation of the drug inside

the β-CD cavity can also be considered as important factors for the dissolution rate enhancement.

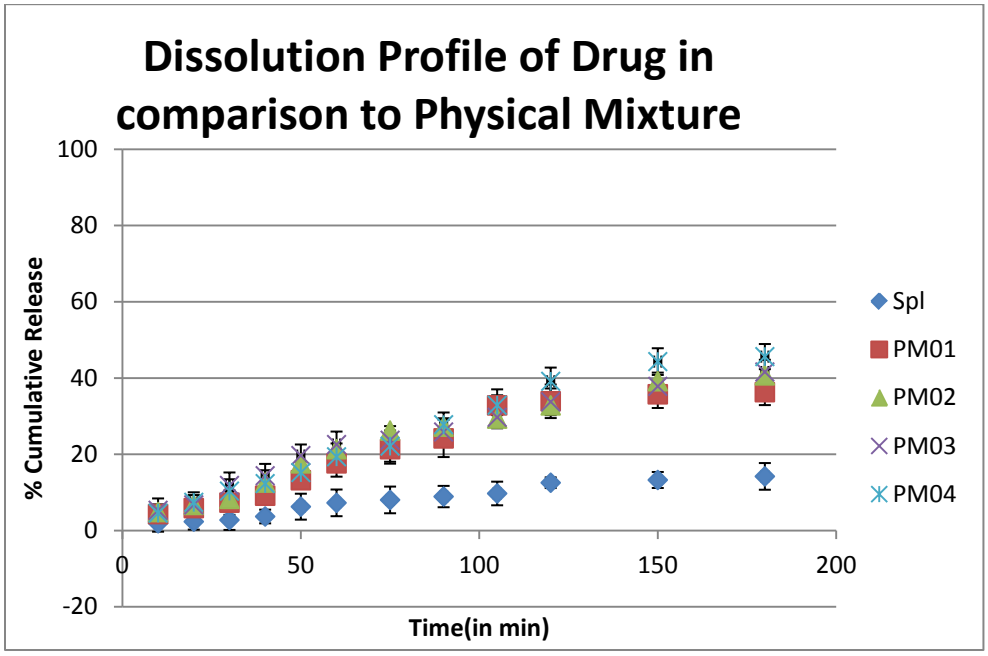

Fig 41 Dissolution profile for all the Physical Mixture with respect to drug Spl

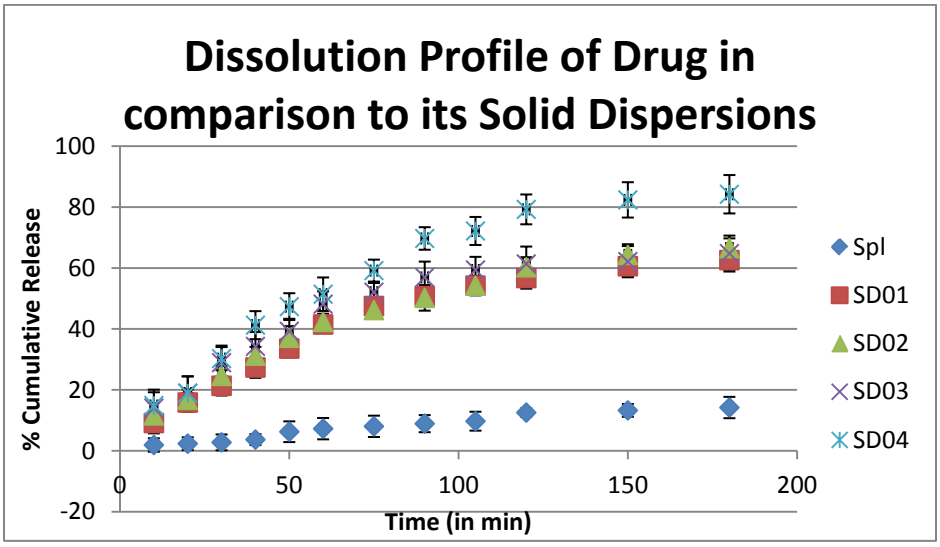

Fig 42 Dissolution profile for all the Solid Dispersion with respect to drug Spl.

As indicated from the dissolution data for the physical mixtures, improvement could be attributed to the higher wettability and dispersibility. Dry mixing of the drug with a hydrophilic carrier results in greater wetting and increases surface available for dissolution by reducing interfacial tension between the hydrophobic drug and dissolution media.

During the dissolution studies, it was noted that the drug carrier systems sink immediately, whereas pure drug keeps floating on the surface for a longer time interval. The Y-equation (Y = aX+b) and its correlation co-efficient (R^2) for selected binary solid dispersion and all ternary solid dispersion system are given in Table 8(A) and Table 8 (B)

Table 8(A). Release Kinetics Evaluation [Y-equation (Y = aX+b) and correlation co-efficient (R^2)]

Type	Zero Order		First order		Higuchi Model		Koresmeyer-Peppas	
	Y equation	R^2	Y equation	R^2	Y equation	R^2	Y equation	R^2
Spl	0.5614x + 9.8124	0.9529	-0.0102x + 2.2652	0.883	9.5555x - 25.72	0.967	0.785x + 0.494	0.886
PM01	0.6128x + 8.0166	0.923	-0.0126x + 2.333	0.933	10.486x - 31.23	0.946	0.809x + 0.419	0.928
PM02	0.5575x + 11.449	0.9501	-0.0105x + 2.267	0.89	0.1025x + 2.671	0.980	0.8369x + 0.453	0.929
SD01	0.5147x + 24.158	0.8772	-0.011x + 2.170	0.9739	0.1056x + 1.508	0.961	0.801x + 0.630	0.999
SD02	0.4933x + 25.063	0.9118	-0.0107x + 2.174	0.947	0.0558x + 2.787	0.986	0.7367x + 0.736	0.985

Table 8(B). Release Kinetics Evaluation [Y-equation (Y = aX+b) and correlation co-efficient (R^2)]

Type	Zero Order		First order		Higuchi Model		Koresmeyer-Peppas	
	Y equation	R^2	Y equation	R^2	Y equation	R^2	Y equation	R^2
PM03	0.5213x + 14.28	0.960	-0.0095x + 2.220	0.850	0.1107x + 2.221	0.989	0.1107x + 2.2214	0.989
PM04	0.5857x + 5.9386	0.974	-0.0111x + 2.326	0.885	0.0982x + 3.229	0.969	0.8158x + 0.3742	0.992
SD03	0.4644x + 33.531	0.825	-0.0109x + 2.074	0.984	0.1118x + 0.502	0.931	0.6669x + 0.8649	0.977
SD04	0.5122x + 24.709	0.889	-0.0116x + 2.191	0.960	0.1072x + 1.371	0.966	0.7606x + 0.6921	0.948

The data shows that Spl, as well as all the physical mixtures (PM01, PM02, PM03, PM04), SD02 and SD03 fit the Higuchi release model, whereas even though the difference in regression values were marginal, formulation SD01 and SD04 followed first order release kinetics.

Chapter 4

Conclusion and Future-Work

Conclusions

The objective of the present study was to show the potential for the water soluble vitamin, Pyridoxine HCl (Pyd) to be used as a pharmaceutical adjuvant in order to increase the solubility and dissolution of the BCS Class II dug Spironolactone (Spl). Beta-cyclodextrin (β-CD) was used as the principle carrier. Binary solid dispersions (SD) of β-CD and Spl in the ratio of 1:1 (SD01) and 1:2 (SD02) along with their respective physical mixtures (PM01 and PM02) were obtained and ternary solid dispersions having β-CD, Spl and Pyd in the ratio of 1:1:1 (SD03) and 1:2:1 (SD04) along with their respective physical mixtures (PM03 and PM04) were prepared by using the solvent evaporation method. In the ternary solid dispersion system SD04, Pyd as the adjuvant showed synergism along with hydrophilic β-CD to enhance the solubility and dissolution of the drug Spl as compared to the binary systems. The SD04 showed a maximum extent of increase in solubility of 362.71± 3.6 µgm/mL as compared to pure Spl alone at 34.48 ± 1.69 µgm/mL. Drug content in all the SD was uniform with the lowest being 93.14 ± 3.2% and the highest 94 ± 2.8%.

Ternary solid dispersions of Spl with carrier (Beta-Cyclodextrin) and adjuvant (Pyd) gave higher intrinsic dissolution rates and solubility. In contrast to the physical mixtures and

binary solid dispersions, Spl in the solid dispersions was present in its amorphous form and was found to interact with the matrix by inclusion complexation suggesting greater stability for the drug.

The results obtained utilizing DSC, FTIR and PXRD showed that a stable ternary inclusion complex of Spl:β-CD: Pyd in the w/w ratio of 1:2:1 could be prepared which is more effective than there binary solid dispersion in terms of enhancing solubility and dissolution rate.

Solid state characterization, such as DSC, FTIR and PXRD, were performed which indicated that the presence of Pyd, facilitated the formation of an inclusion complex. Inclusion complex formation is considered as the prime mechanism for the enhancement of solubility and dissolution for the drug spironolactone. In-vitro dissolution testing indicated that SD04 showed the maximum drug release (84.21± 6.3) over the period of time above all the binary SDs, PMs and Spl. Evaluation of dissolution profiles showed that all the systems primarily followed Higuchi Kinetics. Thus Pyd was found to be efficient as an adjuvant in order to increase the solubility of the BCS Class II model drug spironolactone when formulated in a ternary solid dispersion along with the β-CD.

Future Work

Formulating solid dispersions into a solid or liquid dosage form and studying its characteristics could be the next step for this project.

References

Chapter 1

1. Lachman L, Lieberman H, Kanig JL. The Theory And Practise of Industrial Pharmacy. 3rd edition. Lea & Febiger; 1986
2. Clugston M, Fleming R. Advanced Chemistry. 1st edition. Oxford, UK: Oxford Publishing; 2000
3. Myrdal PB, Yalkowsky SH. Solubilization of drugs in aqueous media. In: Swarbrick J, editor. Encyclopedia of Pharmaceutical Technology. 3rd edition. New York, NY, USA, : Informa Health Care; 2007. p. p. 3311.
4. Martin A. Solubility and Distribution Phenomena. 6th edition. Lippincott Williams and Wilkins; 2011. (Physical Pharmacy and Pharmaceutical Sciences).
5. Aulton M. Dissolution and solubility. In: Aulton ME, editor. Pharmaceutics: The Science of Dosage form Design. 2nd edition. Churchill Livingstone; 2002. p. p. 15.
6. The United States Pharmacopeia, USP 30-NF 25, 2007.
7. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv. Drug Deliv. Rev. 1997, 23, 3-25.

8. Amidon GL, Lennernaes H, Shah VP, Crison JR. A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. Pharm. Res. 1995, 12, 413-420.
9. The Biopharmaceutics Classification System (BCS) Guidance, accessed on 25 may 2009,http://www.fda.gov/AboutFDA/CentersOffices/CDER/ucm128219.htm
10. Sharma D, Soni M, Kumar S, Gupta GD. Solubility enhancement—eminent role in poorly soluble drugs. Research Journal of Pharmacy and Technology. 2009;2(2):220–224.
11. 14. Kumar A, Sahoo SK, Padhee K, Kochar PS, Sathapathy A, Pathak N. Review on solubility enhancement techniques for hydrophobic drugs. Pharmacie Globale. 2011;3(3):001–007.
12. Ketan T. Savjani, Anuradha K. Gajjar, and Jignasa K. Savjani Drug Solubility: Importance and Enhancement Techniques, ISRN Pharm. 2012, 195727.
13. Sekiguchi K, Obi N. Studies on absorption of eutectic mixtures. I.A. comparison of the behaviour of eutectic mixtures of sulphathiazole and that of ordinary sulphathiazole in man. Chemical and Pharmaceutical Bulletin. 1961;9:866–872.
14. Gupta P, Kakumanu VK, Bansal AK. Stability and solubility of celecoxib-PVP amorphous dispersions: a molecular perspective. Pharmaceutical Research. 2004;21(10):1762–1769.
15. 20. Abdul-Fattah AM, Bhargava HN. Preparation and in vitro evaluation of solid dispersions of halofantrine. International Journal of Pharmaceutics. 2002;235(1-2):17–33.

16. 21. Sinha S, Ali M, Baboota S, Ahuja A, Kumar A, Ali J. Solid dispersion as an approach for bioavailability enhancement of poorly water-soluble drug ritonavir. AAPS PharmSciTech. 2010;11(2):518–527.
17. Atkinsons, R.M.; Bedford, c.. Child, K.J.; Tomich, E. Antibiot. Chemother. 1962, 12, 232-238.
18. Chiou, W.L.; Riegelman, S. J. Pharm. Sci. 1971, 60, 1281-1302.
19. Bochner, F.; Huffman, D.H.; Shen, D.D.; Azarnoff, D.L. J. Pharm. Sci. 1977,66,644-647.
20. Guillory, J.K; Hwang, S.C.; Lach, J.L. J. Pharm. Sci. 1969, 58, 301-308.
21. Singh, P.; Guillory, J.K; Sokolosky, T.D.; Benet, L.Z.; Bhatia, V.N. J. Pharm. Sci. 1966, 55, 63-68.
22. Myrdal PB, Yalkowsky S,H. Complexation and Cyclodextrin. In: Swarbrick J, editor. Encyclopedia of Pharmaceutical Technology. 3rd edition. New York, NY, USA, : Informa Health Care; 2007. p. p. 531-552.
23. Thompson, D.O. Cyclodextrins-EnablingExcipients:their Present and Future Use in Pharmaceuticals. Crit, Rev Ther. Drug Carrier Syst. 1997, 14 (I), 1-104.
24. Abdel-Rahman, A.A.; Saleh, S.l.; Nakai, Y.; Aboutaleb, A.E.; Ahmed, M.D. Investigation of the Interaction of Bromazepam with Cyclodextrins in Solutions and in Ground Mixtures. J. Pharm. Belg. 1994,.149 (1), 23-32.
25. Ahmed, M.D.; Nakai, Y.; Aboutaleb, A.E.S.; Yamamoto, K.; Abdel Rahman, A.A.Z.; Saleh, S.l. Complex Formation of Nitrazepam in Coprecipitating and in Co-grinding with Methylated Beta-Cyclodextrins. Chern. Pharm. Bull. 1990, 38 (12), 2423-2427.

26. Muller, B.W.; Brauns, U. Solubilization of Drugs by Modified Beta-Cyclodextrins. Int. J. Pharm. 1985, 26 (112), 77-88.
27. Exhibiting Enhanced Aqueous Solubility and the Use Thereof. US Patent 5,134,127, 1992.
28. Gerloczy, A.; Hoshino, T.; Pitha, J. Safety of Oral Cyclodextrins: Effects of (Hydroxypropyl) Cyclodextrins, Cyclodextrin Sulfates and Cationic Cyclodextrins on Steroid Balance in Rats. J. Pharm. Sci. 1994, 83 (2), 193-196.
29. TUITo,N.I.; Okubo, T.; Chung, c.J. Analysis of Static and Dynamic Host-Guest Associations of Detergents with Cyclodextrins via Photoluminescence Methods. J. Am. Chern. Soc. 1982, 104 (7), 1789-1794.
30. Hashimoto, S.; Thomas, J.K. Fluorescence Study of Pyrene and Naphthalene in Cyclodextrin-Amphiphile Complex Systems. J. Am. Chern. Soc. 1985, 107 (16), 4655-4662.
31. Stella, V.J.; Rao, V.M.; Zannou, E.A.; Zia, V. Mechanisms of Drug Release from Cyclodextrin Complexes. Adv. Drug Del. Rev. 1999, 36 (1), 3-16.
32. Myrdal PB, Yalkowsky S,H. Complexation : Non-Cyclodextrin. In: Swarbrick J, editor. Encyclopedia of Pharmaceutical Technology. 3rd edition. New York, NY, USA: Informa Health Care; 2007. p. p. 553-555.
33. Koenigbauer, M.J. Pharmaceutical Applications of Microcalorimetry. Pharm. Res. 1994, 11 (6), 777- 783.
34. Mwakibete, H.; Cristantino, R.; Bloor, D.M.; Wyn-Jones, E.; Holzwarth, J.F. Reliability of the Experimental Methods to Determine Equilibrium Constants for SurfactantlzCyclodextrin Inclusion Complexes. LangmuiIr 1995,11 (1),57-60.

35. Dharmawardana, U.R.; Christian, S.D.; Tucker, E.E.; Taylor, R.W.; Scamehorn, J.F. A Surface Tension Method for Detennining Binding Constants for Cyclodextrin Inclusion Complexes of Ionic Surfactants. Langmuir 1993, 9 (9), 2258-2263.
36. Habib, M.J., Pharmaceutical solid dispersion Technology, Technomic Publishing Company, Inc. Lancaster, Pennsylvania (U.S.A.). 2001, pp. 1-36.
37. Martin, M. T., Margarit, M.V., Salcedo, G.E., 2002. Characterization and solubility study of solid dispersions of flunarizine and plyvinyl pyrrolidone. Farmaco. , 2002, 57, 723-727.
38. Dubois, J.L., and Ford, J.L., 1985. Similarities in the release rate of different drugs from polyethylene glycol 6000 solid dispersion. J.Phram. Pharmacol, 37, 494-496.
39. Higuchi, T., Ikeda, M. “Rapidly dissolving forms of digoxin: hydroquinone complex”. J. Pharm. Sci. 1974, 63(5), 809-811.
40. Yamashia, K., Nakate, T., Okimoto, K., Ohike, A., Tokunaga, Y., Ibuki, R., Higaki, K., Kimura, T.,. Establishment of new preparation method for solid dispersion formulation of tacrolimus. Int. J. Pharm. 2003, 267, 79-91.
41. Nelson, E., Knoechel, E.L., Hamlin, W.E., Wagner, J.G.,. Influence of the absorption rate of tolbutamide on the rate of decline of blood sugar levels in normal humans. Int. J. Pharm. 1962, 51, 509-514.
42. Lin, S.L., Lachman, L., Swartz, C.J., Heubner, C.F.,. Preformulation investigation I. Rslation of salt forms and biological activity of an experimental antihypertensive. J. Pharm. Sci. 1972, 61(9), 1418-1422

43. T. Vasconcelos, B. Sarmento, and P. Costa, “Solid dispersions as strategy to improve oral bioavailability of poor water soluble drugs,” Drug Discov. Today, vol. 12 issues 23-24, 2007, pp. 1068-1075,
44. Rogers TL, Hu JH, Yu ZS, Johnston KP, Williams RO., III A novel particle engineering technology: spray-freezing into liquid. Int J Pharm. 2002, 242, 93-100.
45. Rogers TL, Nelsen AC, Hu JH, Brown JN, Sarkari M, Young TJ, et al. A novel particle engineering technology to enhance dissolution of poorly water soluble drugs: spray-freezing into liquid. Eur J Pharm Biopharm. 2002, 54, 271–280
46. Hu JH, Rogers TL, Brown J, Young T, Johnston KP, Williams RO. Improvement of dissolution rates of poorly water soluble APIs using novel spray freezing into liquid technology. Pharm Res. 2002, 19, 1278-1284
47. Speiser P. Galenische Aspecte der Arzneimittelwirkung. Pharm Acta Helv. 1966, 41, 321-342.
48. Adel EI-Egakey M, Soliva M, Speiser P. Hot extruded dosage forms. Pharm Acta Helv. 1971, 46, 31-52.
49. Huttenrauch R. Spritzgieβverfahren zur Herstellung peroraler Retardpraperate. Pharmazie. 1974, 29, 297-302.
50. Seil JT, Webster TJ. Spray deposition of live cells throughout the electrospinning process produces nanofibrous three-dimensional tissue scaffolds. Int J Nanomedicine. 2011, 6, 1095-1099.
51. Jannesari M, Varshosaz J, Morshed M, Zamani M. Composite poly(vinyl alcohol)/poly(vinyl acetate) electrospun nanofibrous mats as a novel wound

dressing matrix for controlled release of drugs. Int J Nanomedicine. 2011, 6, 993-1003

52. Neves NM, Campos R, Pedro A, Cunha J, Macedo F, Reis RL. Patterning of polymer nanofiber meshes by electrospinning for biomedical applications. Int J Nanomedicine. 2007, 2, 433-448.
53. Wang Y, Zhang C, Zhang Q, Li P. Composite electrospun nano-membranes of fish scale collagen peptides/chito-oligosaccharides: antibacterial properties and potential for wound dressing. Int J Nanomedicine. 2011, 6, 667-676
54. Wang Y, Zhang C, Zhang Q, Li P. Composite electrospun nano-membranes of fish scale collagen peptides/chito-oligosaccharides: antibacterial properties and potential for wound dressing. Int J Nanomedicine. 2011, 6, 667-676.
55. Vasconcelos T, Sarmento B, Costa P. Solid dispersions as strategy to improve oral bioavailability of poor water soluble drugs. Drug Discov Today. 2007, 12, 1068-1075.
56. Yu DG, Gao LD, White K, Brandford-White C, Lu WY, Zhu LM. Multicomponent amorphous nanofibers electrospun from hot aqueous solutions of a poorly soluble drug. Pharm Res. 2010, 27, 2466-2477
57. Yu DG, Yang JM, Branford-White C, Lu P, Zhang L, Zhu LM. Third generation solid dispersions of ferulic acid in electrospun composite nanofibers. Int J Pharm. 2010, 400, 158-164.
58. Yu DG, Shen XX, Brandford-White C, White K, Zhu LM, Bligh SWA. Oral fast-dissolving drug delivery membranes prepared from electrospun polyvinylpyrrolidone ultrafine fibers. Nanotechnology. 2009,

59. Yu DG, Branford-White C, White K, Li XL, Zhu LM. Dissolution improvement of electrospun nanofiber-based solid dispersions for acetaminophen. AAPS Pharm Sci Tech. 2010, 11, 809-817.

60. Yu DG, Branford-White C, Shen XX, Zhang XF, Zhu LM. Solid dispersions of ketoprofen in drug-loaded electrospun nanofibers. J Dispersion Sci Tech. 2010, 31, 902-908.

61. T. Vasconcelos, B. Sarmento, and P. Costa, “Solid dispersions as strategy to improve oral bioavailability of poor water soluble drugs,” Drug Discov. Today. 2007, vol. 12 issues 23-24, pp. 1068

62. F. V. T. Thakkar, T. G. Soni, M. C. Gohel, and T. R. Gandhi, “Supercritical fluid technology: A promising approach to enhance the drug solubility,” J. Pharm. Sci. Res., 2009, vol. 1, issue 4, pp. 1-14.

63. H. Karanth, V. S. Shenoy, and R. R. Murthy, “Industrially feasible alternative approaches in the manufacture of solid dispersions: A technical report,” AAPS PharmSciTech., 2006, vol. 7 issue 4, Article 87, E31-38.

Chapter 2

1. M.J.O'Neil. The Merck Index, 14th ed. Merck & Co. USA; 2006. p.583 & 1373.
2. Challa, R.; Ahuja, A.; Ali, J.; Khar, R.K. Cyclodextrins in drug delivery: An updated review. AAPS Pharm. Sci. Tech. 2005, 6, E329-E357.
3. Loftsson, T.; Duchêne, D. Cyclodextrins and their pharmaceutical applications. Int. J. Pharm. 2007, 329, 1-11.
4. Wong S.M., Kellaway I.W., Murdan S., Enhancement of the dissolution rate and oral absorption of a poorly water soluble drug by formation of surfactant-containing microparticles, Int. J Pharm., 2006, 317, 61– 68
5. T. Higuchi and K.A. Connors: Adv. Anal. Chem. Instrum. 4, 117 (1965).
6. International Conference on Harmonization. Draft Guideline on Validation of Analytical Procedures: 1995. Definitions and Terminology, Federal Register. 60, 11260.
7. Pramod L. Ingale et al Development and validation of UV-spectrophotometric methods for simultaneous estimation of amlodipine besylate and clopidogrel bisulfate in bulk and tablet dosage form, Der Pharma Chemica, 2013, 5 (4):282-287
8. Elsayed M, Belal S, Elwalily A, Abdine H. Spectrophotometric determination of tolbutamide, thiamine hydrochloride and pyridoxine Hydrochloride in combination products. J Pharm Sci 1979; 68(6): 739–741.

9. Aberasturi F, Jimenez A, Arias J, Jimenez F. Simultaneous spectrometric determination of folic acid, pyridoxine riboflavin, and thiamine by partial least-squares Regression. Anal Lett 2002; 35(10):1677-1691
10. Allen PV, Rahn PD, Sarapu AC, Vanderwielen AJ. Physical characterization of erythromycin: anhydrate, monohydrate, and dihydrate crystalline solids. Journal of Pharmaceutical Sciences. 1978;67(8):1087–1093
11. Mura P, Adragna E, Rabasco A, Moyano J, Perez-Martinez J, Arias M, Gines J. 1999. Effect of the host cavity size and the preparation method on the physicochemical properties of ibuproxam– cyclodextrin. Drug Dev Ind Pharm 25: 279–287.
12. J. Peeters, P. Neeskens, J.P. Tollenaere, P. Van Remoortere, M. Brewster, Characterization of the interaction of 2-hydroxypropyl-bcyclodextrin with itraconazole at pH 2, 4 and 7, J. Pharm. Sci. 91 (2002) 1414–1422
13. X. Wang, A. Michoel, G. Van den Mooter, Solid state characteristics of ternary solid dispersions composed of PVPVA 64, Myrj 52 and Itraconazole, Int. J. Pharm. 303 (2005) 54–61.
14. T. Roisnel, J. Rodriguez Carvajal, WinPLOTR: a Windows tool for powder diffraction pattern analysis, Materials Science Forum (EPDIC 7) (2000) 118–123.
15. El-Gazayerly ON. Characterization and evaluation of tenoxicam coprecipitates. Drug Dev Ind Pharm. 2000;26:925Y930.

Chapter 3

1. Lutka A. Investigation of interaction of promethazine with cyclodextrins in aqueous solution. Acta Pol Pharm. 2002;59:
2. Marsac PJ, Shamblin SL, Taylor LS. 2006. Theoretical and practical approaches for prediction of drug-polymer miscibility and solubility. Pharm Res 23:2417–2426.
3. Diaz D, Escobar Llanos CM, Bernad MJB. Study of the binding in an aqueous medium of inclusion complexes of several cyclodextrins involving fenoprofen calcium. Drug Dev Ind Pharm. 1999;25.
4. Nagase Y, Hirata M, Wada K, et al. Improvement of some pharmaceutical properties of DY-9760e by sulfobutyl ether beta-cyclodextrin. Int J Pharm. 2001; 229:
5. Kaukonem, A.M.; Lennernas, H.; Mannerman, J.P. Water-soluble β-cyclodextrins in paediatric oral solutions of spironolactone: Preclinical evaluation of spironolactone bioavailability from solutions of β-cyclodextrin derivatives in rats. J. Pharm. Pharmacol. 1998, 50, 611-619
6. Zhanga, G.G.Z.; Lawa, D.; Schmittb, E.A.; Qiub, Y. Phase transformation considerations during process development and manufacture of solid oral dosage forms. Adv. Drug Deliv. Rev. 2004, 56, 371-390.
7. Lu, Y.; Zang, X.; Lai, J.; Yin, Z.; Wu, W. Physical characterization of meloxican-β-cyclodextrininclusion complex prepared by a fluid-bed method. Particuology 2009, 7, 1-8.