COMPARATIVE EVALUATION OF INCLUSION COMPLEX OF ACECLOFENAC PREPARED BY DIFFERENT TECHNIQUES

A Dissertation Submitted to

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In partial fulfillment for the award of Degree of

MASTER OF PHARMACY

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Submitted by

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MAY 2012

CERTIFICATE

This is to certify that the dissertation entitled **"COMPARATIVE EVALUATION OF INCLUSION COMPLEX OF ACECLOFENAC PREPARED BY DIFFERENT TECHNIQUES"** Submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, in partial fulfillment for the award of the Degree of the Master of Pharmacy was carried out by **P.SATHISH KUMAR (Register No. 26106008)** in the Department of Pharmaceutics under my direct guidance and supervision during the academic year 2011-2012.

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CERTIFICATE

This is to certify that the dissertation entitled **"COMPARATIVE EVALUATION OF INCLUSION COMPLEX OF ACECLOFENAC PREPARED BY DIFFERENT TECHNIQUES"** is the bonafide research work carried out by **P.SATHISH KUMAR (Register No. 26106008)** in the Department of Pharmaceutics, Adhiparasakthi College of Pharmacy, Melmaruvathur which is affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, under the guidance of **Mr. T. AYYAPPAN, M. Pharm.,** Assistant Professor, Department of Pharmaceutics, Adhiparasakthi College of Pharmacy, during the academic year 2011-2012.

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My Heartfelt Dedication To

My Beloved Parents,

&

My beloved ones..."

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P.SATHISH KUMAR

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ABBREVIATIONS

%	---- Percentage
β	---- Beta
γ	---- Gamma
°C	---- Degree Celsius
µ	---- Micro
µg	---- Microgram
ACE	---- Aceclofenac
BCS	---- Biopharmaceutics Classification System
CD	---- Cyclodextrin
cm	---- Centimeter
DE	---- Dissolution Efficiency
DSC	---- Differential Scanning Calorimetry
edn	---- Edition
Fig	---- Figure
FTIR	---- Fourier Transform Infrared Spectroscopy
GIT	---- Gastrointestinal Tract
gm	---- Grams
ICH	---- International Conference on Harmonization
IP	---- Indian Pharmacopoeia
KBr	---- Potassium Bromide

MDT ---- Mean Deviation Time

mg ---- Milligram

min ---- Minute ml

---- Millilitre mM --

-- Millimoles nm -

--- Nanometer

NSAID ---- Non- steroidal anti inflammatory drug

RH ---- Relative Humidity

RPM ---- Revolutions Per Minute

S.No. ---- Serial Number

SD ---- Standard Deviation

t ---- Time

UV ---- Ultra Violet

vol ---- Volume

w/v ---- Weight/Volume

w/w ---- Weight/Weight

XRD ---- X-Ray Diffraction

α ---- Alpha

β-CD ---- Betacyclodextrin

λmax ---- Absorption Maximum

INTRODUCTION

1. INTRODUCTION

(Raymond C.Rowe., 2003)

Cyclodextrins are cyclic oligosaccharides containing at least six D-(+)-glucopyranose units attached by α (1Æ4) glucoside bonds. The three natural cyclodextrins, α, β, and γ differ in their ring size and solubility. They contain 6, 7 or 8 glucose units, respectively.

Cyclodextrins occur as white, practically odourless, fine crystalline powders, having a slightly taste. Some cyclodextrins derivatives occur as amorphous powders. Cyclodextrins were discovered approximately 100 years ago with the foundation of cyclodextrin chemistry being laid down in the first half of this century. In the beginning, only small amounts of relatively impure cyclodextrins could be generated and high production costs prevented their industrial usage. Recent bio-technological advancements have resulted in drastic improvements in the efficiency of manufacture of cyclodextrins, lowering the cost of these materials and making highly purified cyclodextrins and cyclodextrin derivatives available.

Cyclodextrins are used to increase the solubility of water insoluble drugs, through inclusion complexation. Natural cyclodextrins have been used extensively for this purpose. However, they are characterized by relatively low solubility in water, which limits their application. Hence, chemically modified cyclodextrins are gaining considerable interest to improve the physicochemical properties of cyclodextrins. Cyclodextrins are known to form an inclusion complex with many drugs of appropriate

molecular size and polarity in hydrophobic drug molecules. The resulting complex generally leads to an improvement in some of the properties of drugs in terms of solubility, bioavailability and tolerability.

Poorly water soluble drugs are generally associated with slow drug absorption leading eventually to inadequate and variable bioavailability.

Cyclodextrins are 'bucket like' or 'cone like' toroid molecules, with a rigid structure and central cavity, the size of which varies according to the cyclodextrin type. The internal surface of the cavity is hydrophobic and the outside of the torus is hydrophilic; this is due to arrangement of hydroxyl groups within the molecule. This arrangement permits the cyclodextrin to accommodate a guest molecule within the cavity, forming an inclusion complex.

The interaction of guest molecules with cyclodextrins may induce useful modifications of the chemical and physical properties of guest molecules, which may lead to improve stability, solubility in aqueous medium and bioavailability. Poorly water soluble drugs therefore can be orally administered in the complex form by taking advantage of the well established low toxicity of the cyclodextrins by the oral route. β-Cyclodextrin appears to be the most useful complexing agent because of its unique cavity size, and ease with which it can be obtained on the industrial scale leading to reasonably cheaper price of the compound. Pharmaceutical applications of cyclodextrins as additive and drug complexing agents have been rapidly growing.

The behavior of the drug in the body involves the fate of the drug during it's transmit as well as its fate while in the biophase. The drug potentially interacts with a

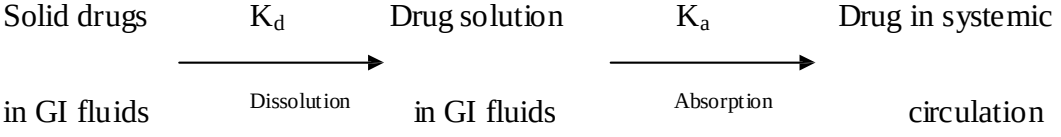
variety of substances leading to undesired drug loss as loss desired drug of absorption. In every instance the degree of this loss or absorption is a function of the drug and the type of the dosage form.

1.1. Biopharmaceutics and Pharmacokinetics:

(Brahmankar D.M and Sunil B. Jaiswal., 2009)

Biopharmaceutics is the study of physiologic and pharmaceutical factors influencing drug release and absorption from dosage forms. The optimal delivery of the active moiety to the site of action depends on an understanding of specific interaction between formulation variables and biological variables. Absorption is defined as the amount of drug that reaches the general circulation in unchanged form from the site of administration.

The drug efficacy can be severely limited by poor aqueous solubility. An insoluble or sparingly soluble drug, if administered the rate of absorption and extent of bioavailability is controlled by dissolution rate in gastrointestinal fluid. The therapeutic effectiveness of a drug whose absorption is dissolution rate limited is hampered by its poor dissolution in an aqueous medium. The absorption of solid drugs administered orally can be depicted by the following chart.



where K_d and K_a are the rate constants for dissolution and absorption process respectively.

1.2. Methods for Enhancement of Bioavailability:

(Brahmankar D.M and Sunil B. Jaiswal., 2009)

As far as the definition of bioavailability is concerned, a drug with poor bioavailability is the one with

1. Poor aqueous solubility and / or slow dissolution rate in the biological fluids.
2. Poor stability of the dissolved drug at the physiologic pH.
3. Inadequate partition coefficient and thus poor permeation through the biomembrane.
4. Extensive presystemic metabolism.

The three major approaches in overcoming the bioavailability problems due to such causes are:

a. The Pharmaceutic Approach which involves modification of formulation, manufacturing process or the physicochemical properties of the drug without changing the chemical structure.

b. The pharmacokinetic approach in which the pharmacokinetics of the drug is altered by modifying its chemical structure.

c. The Biologic approach whereby the route of drug administration may be changed such as changing from oral to parenteral route.

The second approach of chemical structure modification has a number of drawbacks of being very expensive and time consuming, requires repetition of clinical studies and long time for regulatory approval. Moreover, the new chemical entity may suffer from another pharmacokinetic disorder or bear the risk of precipitating adverse effects. Only the pharmacokinetic approach will be dealt herewith.

The attempts, whether optimizing the formulation, manufacturing process or physicochemical properties of the drug, are mainly aimed at enhancement of dissolution rate as it is the major rate limiting step in the absorption of most drugs. There are several ways in which the dissolution rate of a drug can be enhanced. Some of the widely used methods, most of which are aimed at increasing the effective surface area of the dugs will be discussed briefly.

1.2.1. Techniques of Solubility Enhancement:

(Mohanachandran P.S et al., 2010; Brahmankar D.M and Sunil B. Jaiswal., 2009)

There are various techniques available to improve the solubility of poorly soluble drugs. Some of the approaches to improve the solubility are,

a) Physical Modifications:

Particle size reduction:

- Micronization
- Nanosuspension
- Sonocrystalisation
- Supercritical fluid process

Modification of the crystal habit:

- Polymorphs
- Pseudopolymorphs

Drug dispersion in carriers:

- Eutectic mixtures
- Solid dispersions
- Solid solutions

Complexation:

- Use of complexing agents

Solubilization by surfactants:

- Microemulsion
- Self microemulsifying drug delivery systems

b) Chemical Modifications:

Physical Modifications:

Particle size reduction: Particle size reduction can be achieved by micronisation and nanosuspension. Each technique utilizes different equipments for reduction of the particle size.

Micronization: The solubility of drug is often intrinsically related to drug particle size. By reducing the particle size, the increased surface area improves the

dissolution properties of the drug. Conventional methods of particle size reduction, such as communication and spray drying, rely upon mechanical stress to disaggregate the active compound.

Nanosuspension: Nanosuspensions are sub-micron colloidal dispersion of pure particles of drug, which are stabilized by surfactants. The advantages offered by nanosuspension is increased dissolution rate is due to larger surface are exposed, while absence of Ostwald ripening is due to the uniform and narrow particle size range obtained, which eliminates the concentration gradient factor.

Sonocrystallisation: Recrystallization of poorly soluble materials using liquid solvents and antisolvents has also been employed successfully to reduce particle size. The novel approach for particle size reduction on the basis of crystallization by using ultrasound is Sonocrystallisation. Sonocrystallisation utilizes ultrasound power characterized by a frequency range of 20-100 kHz for inducing crystallization. It's not only enhances the nucleation rate but also an effective means of size reduction and controlling size distribution of the active pharmaceutical ingredients. Most applications use ultrasound in the range 20 kHz-5 MHz.

Modification of the crystal habit: Polymorphism is the ability of an element or compound to crystallize in more than one crystalline form. Different polymorphs of drugs are chemically identical, but they exhibit different physicochemical properties including solubility, melting point, density, texture and stability. Broadly polymorphs can be classified as enantiotropes and monotropes based on thermodynamic properties.

In the case of an enantiotropic system, one polymorphs form can change reversibly into another at a definite transition temperature below the melting point, while no reversible transition is possible for monotropes. Once the drug has been characterized under one of this category, further study involves the detection of metastable form of crystal. Metastable forms are associated with higher energy and thus higher solubility.

Drug dispersion in carriers: The solid dispersion approach to reduce particle size and therefore increase the dissolution rate and absorption of drugs was first recognized in 1961. The term "solid dispersions" refers to the dispersion of one or more active ingredients in an inert carrier in a solid state, frequently prepared by the melting method, solvent method, or fusion solvent- method. Novel additional preparation techniques have included rapid precipitation by freeze drying and using supercritical fluids and spray drying, often in the presence of amorphous hydrophilic polymers and also using methods such as melt extrusion.

Complexation: Complexation is the association between two or more molecules to form a nonbonded entity with a well defined stoichiometry. Complexation relies on relatively weak forces such as London forces, hydrogen bonding and hydrophobic interactions.

Staching complexation: Staching complexes are formed by the overlap of the planar regions of aromatic molecules, Nonpolar moieties tend to be squeezed out of water by the strong hydrogen bonding interactions of water. This causes some molecules to minimize the contact with water by aggregation of their hydrocarbon moieties. This

aggregation is favored by large planar nonpolar regions in the molecule. Stached complexes can by homogeneous or mixed. The former is known as self association and latter as complexation.

Inclusion complexation: Inclusion complexes are formed by the insertion of the nonpolar molecule or the nonpolar region of one molecule (known as guest) into the cavity of another molecule or group of molecules (known as host). The major structural requirement for inclusion complexation is a snug fit of the guest into the cavity of host molecule. The cavity of host must be large enough to accommodate the guest and small enough to eliminate water, so that the total contact between the water and the nonpolar regions of the host and the guest is reduced. Three naturally occurring CDs are α-Cyclodextrin, β -Cyclodextrin, and γ – Cyclodextrin. The complexation with cyclodextrins is used for enhancement of solubility. Cyclodextrin inclusion is a molecular phenomenon in which usually only one guest molecule interacts with the cavity of a cyclodextrin molecule to become entrapped and form a stable association. The internal surface of cavity is hydrophobic and external is hydrophilic; this is due to the arrangement of hydroxyl group within the molecule. The kinetics of cyclodextrin inclusion complexation has been usually analyzed in terms of a one – step reaction or a consecutive two – step reaction involving intracomplex structural transformation as a second step. Cyclodextrins is to enhance aqueous solubility of drugs through inclusion complexation. It was found that cyclodextrins increased the paclitaxel solubility by 950 fold, complex formation of rofecoxib, celecoxib, clofibrate, melarsoprol, taxol, cyclosporine A etc., with cyclodextrins improves the solutility of particular drugs.

Approaches for Making Inclusion Complexes:

Physical blending method: A solid physical mixture of drug and CDs are prepared simply by mechanical trituration. In laboratory scale CDs and drug are mixed together thoroughly by trituration in a mortar and passes through appropriate sieve to get the desired particle size in the final product.

Kneading method: This method is based on impregnating the CDs with little amount of water or hydroalcoholic solutions to converted into a paste. The drug is then added to the above paste and kneaded for a specified time. The kneaded mixture is then dried and passed through sieve if required.

Co – precipitation technique: This method involves the co – precipitation of drug and CDs in a complex. In this method, required amount of drug is added to the solution of CDs. The system is kept under magnetic agitation with controlled process parameters and the content is protected from the light. The formed precipitate is separated by vacuum filtration and dried at room temperature in order to avoid the loss of the structure water from the inclusion complex.

Solution / solvent evaporation method: This method involves dissolving of the drug and CDs separately in to two mutually miscible solvents, mixing of both solutions to get molecular dispersion of drug and complexing agents and finally evaporating the solvent under vacuum to obtain solid powdered inclusion compound. Generally, the aqueous solution of CDs is simply added to the alcoholic solution of drugs. The resulting mixture is stirred for 24 hours and evaporated under vacuum at 45° C. The dried mass was pulverized and passed through a 60 – mesh sieve. This method is

quite simple and economic both on laboratory and large scale production and is considered alternative to the spray drying technique.

Neutralization precipitation method: This method is based on the precipitation of inclusion compounds by neutralization technique and consists of dissolving the drug in alkaline solutions like sodium / ammonium hydroxide and mixing with an aqueous solution of CDs. The resultant clear solution is then neutralized under agitation using HCI solution till reaching the equivalence point. A white precipitate is being formed at this moment, corresponding to the formation of the inclusion compound. This precipitate is filtered and dried.

Milling / Co – grinding technique: A solid binary inclusion compounds can be prepared by grinding and milling of the drug and CDs with the help of mechanical devices. Drug and CDs are mixed intimately and the physical mixture is introduced in an oscillatory mill and grinded for suitable time. Alternatively, the ball milling process can also be utilized for preparation of the drug – CD binary system. The ball mill containing balls of varied size is operated at a specified speed for a predetermined time, and then it is unloaded, sieved through a 60 – mesh sieve. This technique is superior to other approaches from economic as well as environmental stand point in that unlike similar methods it does not require any toxic organic solvents. This method differs from the physical mixture method where simple blending is sufficient and in co-grinding it requires to achieve extensive combined attrition and impact effect on powder blend.

Atomization / spray drying method: Spray – drying is a common technique used in pharmaceuticals to produce a dry powder from a liquid phase. Another application is its use as a preservation method, increasing the storage stability due to the water elimination. This method represents one of the most employed methods to produce the inclusion complex starting from a solution. The mixture pass to a fast elimination system propitiate solvent and shows a high efficiency in forming complex. Besides, the product obtained by this method yield the particles in the controlled manner which in turn improves the dissolution rate of drug in complex form.

Lyophilization / Freeze drying technique: In order to get a porous, amorphous powder with high degree of interaction between drug & CD, lyophilization / freeze drying technique is considered as a suitable. In this technique, the solvent system from the solution is eliminated through a primary freezing and subsequent drying of the solution containing both drug & CD at reduced pressure. Thermolabile substances can be successfully made into complex form by this method. The limitations of the technique are long time process and yield poor flowing powdered product. Lyophilization /freeze drying technique are considered as an alternative to solvent evaporation and involve molecular mixing of drug and carrier in a common solvent.

Microwave irradiation method: This technique involves the microwave irradiation reaction between drug and complexing agent using a microwave oven. The drug and CD in definite molar ratio are dissolved in a mixture of water and organic solvent in a specified proportion into a round bottom flask. The mixture is reacted for short time of about one to two minutes at 60° C in the microwave oven. After the reaction

completes, adequate amount of solvent mixture is added to the above reaction mixture to remove the residual, uncomplexed free drug and CD. The precipitate so obtained is separated using whatman filter paper, and dried in vacuum oven at 40° C from 48 hrs.

Supercritical antisolvent technique: In this technique, carbon dioxide is used as anti- solvent for the solute but as a solvent with respect to the organic solvent. The use of supercritical carbon dioxide is advantageous as its low critical temperature and pressure makes it attractive for processing heat – labile pharmaceuticals. It is also non – toxic, nonflammable, inexpensive and is much easier to remove from the polymeric materials when the process is complete, even through small amount of carbon dioxide remains trapped inside the polymer, it poses no danger to the consumer. Supercritical particle generation processes are new and efficient route for improving bioavailability of pharmaceutically active compounds. In addition, supercritical fluid processes were recently proposed as a new alternative method for the preparation of drug cyclodextrin complexes. Supercritical carbon dioxide is suggested as a new complexation medium due to its properties of improved mass transfer and increased solvating power. This method constitutes one of the most innovators methods to prepare the inclusion complex of drug with CD in solid state. This is a non – toxic method as it is not utilizing any organic solvent, fast process, maintenance cost is low with promising results, but it requires a quite high initial cost.

In this technique, first, drug and CD are dissolved in a good solvent then the solution is fed into a pressure vessel under supercritical conditions, through a nozzle (i.e. sprayed into supercritical fluid anti – solvent). When the solution is sprayed into

supercritical fluid anti – solvent, the anti – solvent rapidly diffuses into that liquid solvent as the carrier liquid solvent counter diffuses into the anti– solvent. Because of the supercritical fluid expanded solvent has lower solvent power than the pure solvent, the mixture becomes supersaturated resulting in the precipitation of the solute and the solvent is carried away with the supercritical fluid flow.

Solubilization by surfactants:

Surfactants are molecules with distinct polar and nonpolar regions. Most surfactants consist of a hydrocarbon segment connected to a polar group. The polar group can be anionic, cationic, zwitterionic or nonionic. When small apolar molecules are added they can accumulate in the hydrophobic core of the micelles.

This process of solubilization is very important in industrial and biological processes. The presence of surfactants may lower the surface tension and increase the solubility of the drug within an organic solvent.

Micro emulsions: The term microemulsion was first used by Jack H. Shulman in 1959. A microemulsion is a four component system composed of external phase, internal phase, surfactant and cosurfactant. The addition of surfactant, which is predominately soluble in the internal phase unlike the cosurfactant, results in the formation of an optically clear, isotropic, thermodynamically stable emulsion. It is termed as microemulsion because of the internal or dispersed phase is <0.1 μ droplet diameter. The formation of microemulsion is spontaneous and does not involve the input of external energy as in case of coarse emulsions. The surfactant and the cosurfactant alternate each other and form a mixed film at the interface, which

contributes to the stability of the microemulsions. Non – ionic surfactants, such as Tweens and Labrafil with high hyrophile – lipophile balances are often used to ensure immediate formation of oil – in – water droplets during production. Advantages of microemulsion over coarse emulsion include its ease of preparation due to spontaneous formation, thermodynamic stability, transparent and elegant appearance, increased drug loading, enhanced penetration through the biological membranes, increased bioavailability, and less inter-and intra – individual variability in drug pharmacokinetics.

Chemical Modifications:

For organic solutes that are ionizable, changing the pH of the system may be simplest and most effective means of increasing aqueous solubility. Under the proper conditions, the solubility of an ionizable drug can increase exponentially by adjusting the pH of the solution. A drug that can be efficiently solubilized by pH control should be either weak acid with a low pKa or a weak base with a high pKa. Similar to the lack of effect of heat on the solubility of non – polar substances, there is little effect of pH on nonionizable substances. Nonionizable, hydrophobic substances can have improved solubility by changing the dielectric constant of the solvent by the use of co-solvents rather than the pH of the solvent. The use of salt forms is a well known technique to enhanced dissolution profiles. Salt formation is the most common and effective method of increasing solubility and dissolution rates of acidic and basic drugs. An alkaloid base is, generally, slightly soluble in water, but if the pH of medium is reduced by addition of acid, and the solubility of the base is increased as the pH continues to be reduced. The reason for this increase in solubility is that the

base is converted to a salt, which is relatively soluble in water. The solubility of slightly soluble acid increased as the pH is increased by addition of alkali, the reason being that a salt is formed.

1.3. Monomolecular Inclusion Complexes:

(Martin's., 2006)

Monomolecular inclusion compounds involve the entrapment of a single guest molecule in the cavity of one host molecule. Most of the host molecules are cyclodextrins. Cyclodextrins are cyclic oligo-saccharides containing a minimum of six D-(+) glucopyranose units attached by alpha-1,4 linkage. Three types of cyclodextrins namely α, β, γ consist of 6, 7 and 8 units of glucose, respectively. Alpha cyclodextrin has the smallest cavity (internal diameter almost 5 A). Beta and gamma cyclodextrins have larger internal diameter (6 and 8 A. respectively) and are useful for pharmaceutical technology.

The structures of cyclodextrins assume a truncated cone and can accommodate a wide varity of compounds. The interior of the cavity is relatively hydrophobic, whereas the entrance of the cavity is hydrophilic in nature.

Applications: These types of complexes are extensively studied for their possible uses in the design of dosage forms.

Enhanced solubility: The solubility of retinonic acid (0.5 mg/litre) is increased to 160 mg/litre by complexation with β -cyclodextrin.

Enhanced dissolution: The dissolution rate of drugs such as famotidine and tolbutamide is enhanced by complexation with β -cyclodextrin.

Enhanced stability: The stability of drugs such as aspirin, benzocaine, ephedrine and testosterone is improved when complexed with β cyclodextrin. The inclusion complexes protect the drugs by preventing the exposure of the functional groups to the exterior of the environment.

Sustained release: Ethylated β -cyclodextrin retards the release of drugs such as diltiazem and isosorbide dinitrate, when complexed. The drug releases slowly for prolonged periods and provides sustained effect.

1.4. Cyclodextrin:

(Loftsson T. et al., 1996; Gerold Mosher et al., 2007; Szejtli J.,1990)

Cyclodextrin or cycloamylases which have recently been recognized as useful pharmaceutical excipient and comprise a family of cyclic oligosaccharides produced from starch by enzymatic degradation. The enzyme cyclodextrin-glucosyl transferase produced by Bacillus macerans acts on partially hydrolyzed starch and produces a mixture of cyclic and acyclic dextrins from which cyclodextrin are isolated.

The β-cyclodextrin appears to be the most useful complexing agent because of its unique cavity size and ease with which it can be obtained on industrial scale. β-Cyclodextrin displays a water solubility of 1.8 g/100 ml. Recently derivatives of β-cyclodextrin have been received considerable attention because of their higher water solubility (>50 g/100 ml). Partial methylation of some of the hydroxyl (OH) groups in cyclodextrin reduces the inter-molecular hydrogen bonding, leaving some hydroxyl groups free to interact with water, thus increasing the aqueous solubility of cyclodextrins. They belong to methylated β-cyclodextrins (dimethyl β-CD and trimethyl β-CD). Hydroxy alkylation of the hydroxyl groups of the cyclodextrin

produced hydroxy alkylated β-cyclodextrins (2 hydroxy ethyl β-CD, 2 hydroxypropyl β-CD). This modification results in greater solubility, of hydroxypropyl β-cyclodextrin and its complexes compared to β-cyclodextrin. The hydroxy groups and the hydroxypropyl groups are on the exterior of the molecule and interact with water to provide the increased aqueous solubility of the hydroxypropyl β-cyclodextrin.

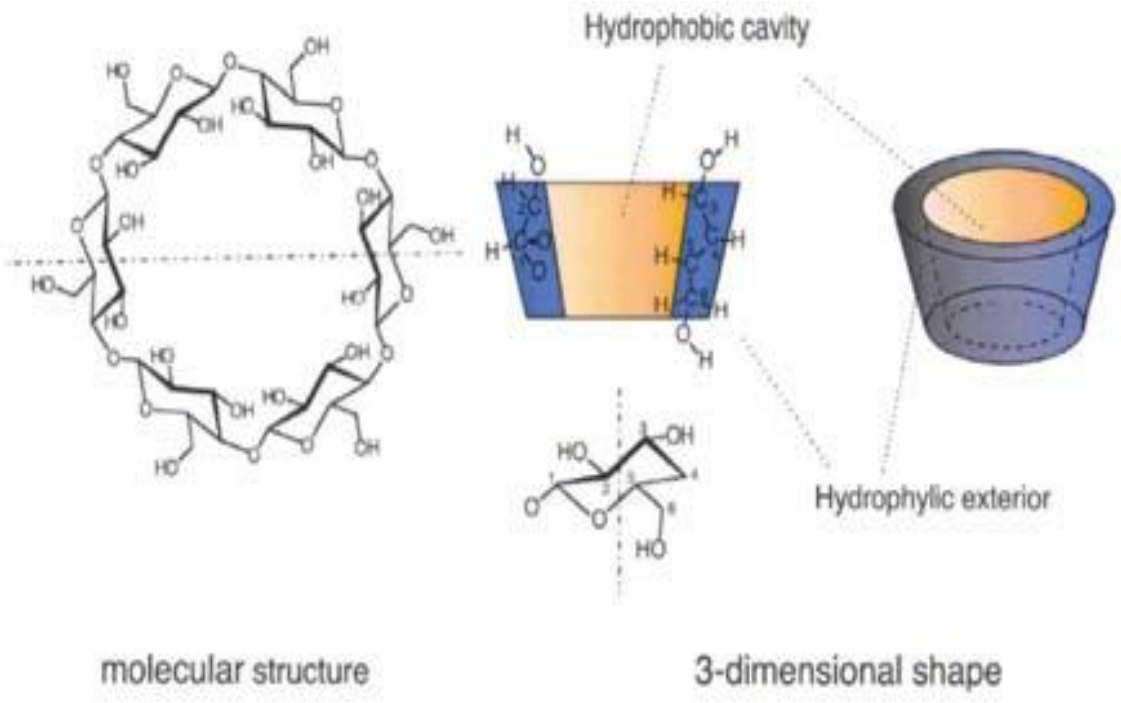


Fig. 1.1: Structure of cyclodextrins

The most stable three-dimensional structure of cyclodextrin is a toroid with the larger and smaller openings presenting hydroxyl groups to the external environment and mostly hydrophobic functionality lining the interior and the cavity.

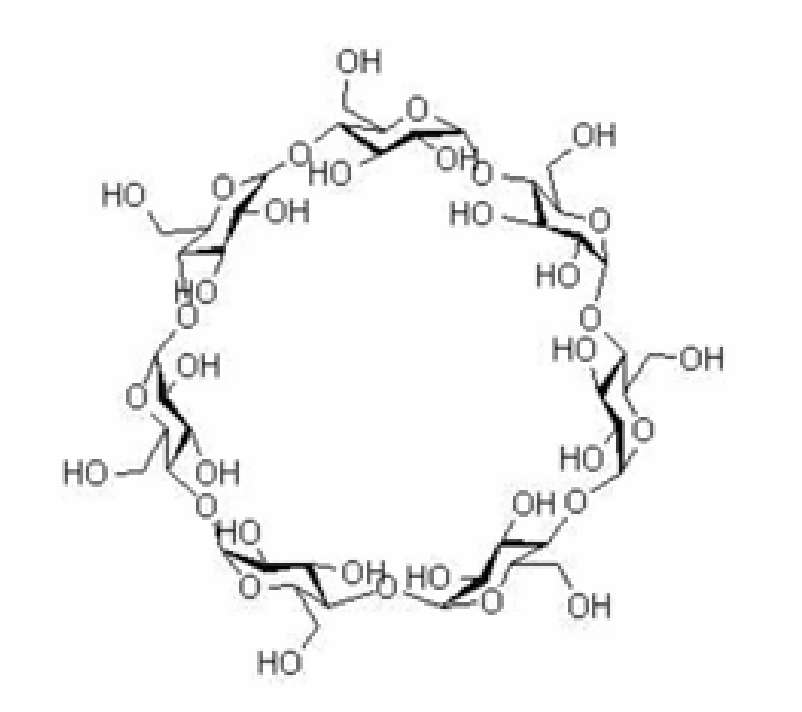


Fig. 1.2: Molecular structure of β-cyclodextrins

It is the unique configuration that gives cyclodextrins their interesting properties and creates the thermodynamic driving force needed to form host guest complexes with a polar molecules and functional groups.

1.4.1. Chemistry of Cyclodextrin:

(Raymond C.Rowe., 2003)

Cyclodextrin are cyclic, non-reducing, water-soluble oligosaccharides. Three different forms of cyclodextrin known are α, β and γ. Cyclodextrins are also called Schardinger dextrins, cycloglucans or cycloamylases are α-1, 4 linked cyclic oligosaccharides obtained from enzymatic conversion of starch. The parent or natural cyclodextrins contain 6, 7 or 8 glucopyranose units.

Table 1.1: Characteristics of cyclodextrins

	α	**β**	γ
No. of glucose units	6	7	8
Molecular weight	972	1135	1297
Cavity diameter	4.7 – 5.3	6.0 – 6.5	7.5 – 8.3
Water solubility (g/100 ml)	14.50	1.85	23.2
Content (dry basis)	>98 %	>98 %	>98 %
Specific rotation in aqueous solution $(\alpha)_{D,20}$	+ 147 to +150°	+160 to +164°	+174 to +180°
Water	<10 %	<14 %	<11 %
Heavy metals	<5 ppm	<5 ppm	<5 ppm
Cavity height (A°)	7.9	7.9	7.9
Cavity volume $(A°)^3$	174	262	427

In addition, all three cyclodextrins exhibit good flow properties and handling characteristics and are:

• Thermally stable (<200° C)

• Using stable alkaline solution (pH < 14).

• Stable in acidic solution (pp>3)

• Biocompatible.

1.4.2. Method of Manufacture:

Cyclodextrins are manufactured by the enzymatic degradation of starch using specialized bacteria (Bacillus macerans). The insoluble β-CD organic solvent complex is separated from the non-cyclic starch, and the organic solvent removed in vacuole so that less than 1 ppm of solvent remains in the β-CD. The cyclodextrin is then carbon treated and crystallized from water, dried and collected.

Hydroxyethyl-β-CD is made by reacting β-CD with ethylene oxide, while hydroxypropyl-β-CD is made by reacting with propylene oxide.

1.4.3. Complexation of Cyclodextrins:

(Martin's., 2006)

The ability to form inclusion compounds in aqueous solution is due to the typical arrangement of glucose units in aqueous solutions. Cyclodextrins form complexes with many drugs through a process in which the water molecules located in the central cavity are replaced by either the whole drug molecule or more frequently by some lipophilic portion of the drug structure.

The interior of the cyclodextrin cavity is relatively hydrophobic because of the presence of the skeletal carbons and ethereal oxygen, which comprise the cavity, whereas the cavity entrances are hydrophilic owing to the presence of the primary and secondary hydroxyl groups. As the water molecules located inside the cavity cannot satisfy their hydrogen bonding potential, they are having high enthalpy than bulk

water molecules located in the solution. Water inside the cavity tends to be squeezed out and to be replaced by more hydrophobic species. Thus, molecules of appropriate size and stereochemistry can be included in the cyclodextrin by hydrophobic interactions.

Water is preferred solvent for complexation. The guest or proton of the which complexes with the cavity of cyclodextrin as a non-polar (hydrophobic) and prefers the non-polar environment of the cavity of cyclodextrin rather than polar aqueous environment as a result water provides a driving force for complexation reaction in addition to dissolving the guest and cyclodextrin.

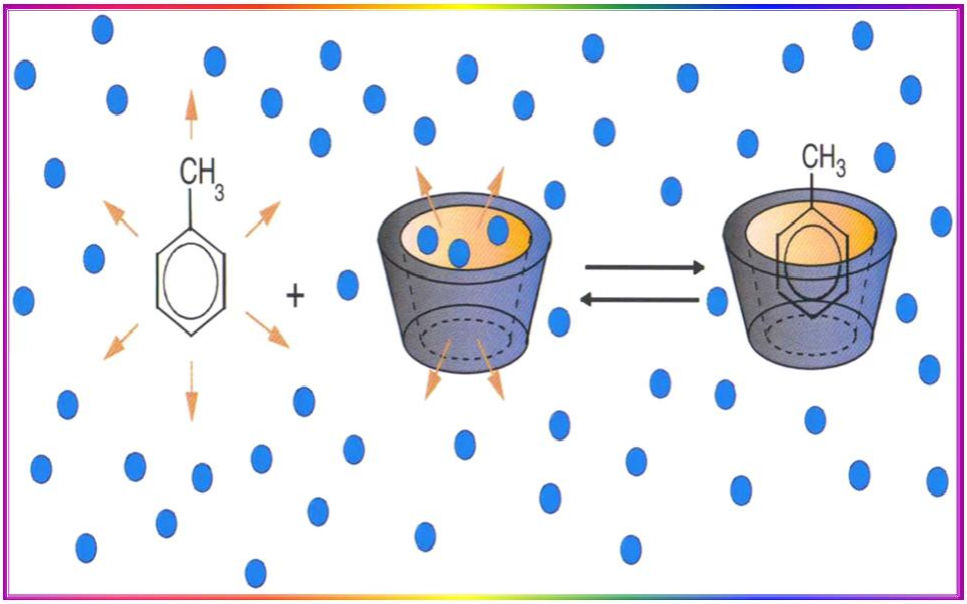


Fig. 1.3: Complexation of cyclodextrin

The above figure provides a schematic representation of the equilibrium involved in forming an inclusion complex between cyclodextrin and toluene in the

presence of a small amount of water. In general, there are four energetically favorable interactions that help shift the equilibrium to right:

- The displacement of polar water molecules from the a polar cyclodextrin cavity.
- The increased number of hydrogen bonds formed as the displaced water returns to the larger pool.
- A reduction of the repulsive interactions between the hydrophobic guest and the aqueous environment.
- An increase in the hydrophobic interactions as the guest inserts itself into the a polar cyclodextrin cavity.

While this initial equilibrium to form the complex is very rapid (often within minutes), the final equilibrium can take much longer to reach. Once inside the cyclodextrin cavity, the guest molecule makes conformational adjustments to take maximum advantage of the weak Vander Waals' forces that exist.

1.5. Preparation of Complexes:

(Sanoferjan A.M et al., 2000; Chowdary K.P.R et al., 2000)

Cyclodextrin complexes are prepared by the following methods:

a) Kneading method
b) Common solvent method
c) Physical mixture
d) Co-precipitate method

a) Kneading method:

β-Cyclodextrin was taken in a glass mortar and little water was added and mixed to obtain a homogenous paste. Drug was then added slowly while grinding. The mixture was ground for 1 h during this process appropriate quantity of water was added to maintain suitable consistency. The paste was dried in an oven at 40° C for 48 hr. The dried complex was taken for study.

b) Common solvent method:

Drug and β-Cyclodextrin were dissolved in 25 % ammonia and the solvent was allowed to evaporate overnight at room temperature. The complex so prepared was pulverized and sifted through sieve no.80.

c) Physical mixture:

Previously weighted drug and Cyclodextrin mixture was blended in glass mortar for about an hour and passed through sieve no.85 to get physical mixture and stored in desiccator over fused calcium chloride.

d) Co-precipitate Method:

Drug and cyclodextrin in different molar ratio are dissolved in different solvent. The solution of the drug is incorporated into solution of cyclodextrin drop wise with continuous stirring at room temperature for 1 hour and then slowly evaporated on a boiling water bath. The inclusion complex precipitated as a crystalline powder, which is then pulverized, sieved and stored in a desiccator till free from any traces of the organic solvent.

1.6. Phase Solubility Technique: *(Higuchi T. and Connors K.A., 1965)*

"T. Higuchi and K.A. Connors" developed the phase solubility technique. It is based on research related to how complexes of different complexing agents such as cyclodextrin, caffeine, polyvinyl pyrrolidone and some aromatic acids affect the aqueous solubility of drug.

1.6.1. Phase Solubility Diagrams:

Phase solubility diagrams are plots of drug solubility versus cyclodextrin concentration. The stoichiometry of drug/cyclodextrin complexes and the numerical values of their stability constants are frequently obtained from phase solubility diagrams.

The physicochemical properties of free drug molecules are different from those bound to the cyclodextrin molecules. Likewise, the physicochemical properties of free cyclodextrin molecules are different from those in the complex. In theory, any methodology that can be used to observe these changes in addition physicochemical properties may be utilized to determine the stoichiometry of the complexes formed and numerical values of their stability constants. These include changes in solubility, changes in chemical reactivity, changes in UV/Vis absorbance, changes in fluorescence, NMR chemical shift, changes in drug retention (e.g., in liquid chromatography), changes in pka values, potentiometric measurement, changes in chemical stability and effects on drug permeability through artificial membranes. Furthermore, since complexation will influence the physicochemical properties of the aqueous complexation media, methods that monitor these media changes can be applied to study the complexation.

For example, measurements of conductivity changes, determination of freezing point depression, viscosity measurements and calorimetric titration's. However, only few of these methods can be applied to obtain structural information on drug/ cyclodextrin complexes.

1.7. Stability Studies:

(Lachmann L. and Herbert A. Libberman., 1991)

Stability of a drug can also be defined as the time from the date of manufacture and packaging of the formulation until its chemical or biological activity is not less than a predetermined level of labeled potency and its physical characteristics have not changed appreciably or deleteriously. The environmental factors, ingredients used and the nature of the container can affect stability.

Loss of potency usually occurs from a chemical change, the most common reaction being hydrolysis, oxidation and reduction. Potency is determined by means of assay procedure that differentiated between the intact drug and its degradation product.

Accelerating the decomposing process and extrapolating the result to normal storage conditions may make a prediction of the life of the product. Acceleration of chemical decomposition is achieved by raising the temperature of the preparations. Application of the principles of chemical kinetics to the results of accelerated storage test carried out at three or more elevated temperatures enable prediction to be made of the effective life of the preparation at normal temperature.

Plotting the appropriate function of concentration against time and obtaining a linear relationship determine the order of the reaction for the decomposition process.

The reaction velocity constant k for the decomposition at each of the elevated temperature can be calculated from the slope of the line. The most satisfactory method for expressing the influence of temperature on reaction velocity is the quantitative relation proposed by Arrhenius.

Where, $K = Ae^{-Ea/RT}$

K = Specific rate of degradation.

R = Gas Constant (1.987 cals/deg/mol)

T = Absolute temperature.

A = Frequency factor.

E_a = Energy of activation.

The Arrhenius relationship is then employed to determine the 'K' value for decomposition at room temperature. This is obtained from the linear plot of the logarithm of 'k' value against reciprocal of absolute temperature, which is then extrapolated to room temperature (25° C). The value of 'K' at 25° C may be then substituted in the appropriate rate equation and an estimate obtained of the time during which the product will maintain the required quality or potency (shelf-life).

The table below indicates maximum time and minimum time at which potency must be at least 90 % of label claim at the temperature indicated in order to predict a shelf-life of two years at room temperature.

Table 1.2: Stability requirement for maintenance of shelf-life

Temperature° C	Maximum time	Minimum time
37° C	12 months	6.4 months.
45° C	8.3 months	2.9 months.
60° C	4.1 months	3 weeks.
85° C	6 weeks	2.5 days

If the assay is over 90 % of original potency at the minimum time (with activation energy 20 K cals/mol) at the respective temperature, in all probability the assays will be over 90 % after two years at room temperature. If the assay remain over 90 % at the maximum time shown (with activation energy 10 K cals/mol) it is certain that a potency of over 90 % will be maintained after two years at room temperature.

1.8. Applications of Cyclodextrins:

(Higuchi T. and Connors K.A., 1965; Rao B.P et al., 2007; Suresh S et al., 2004)

™ β-Cyclodextrin Inclusion Complexes:

Bioavailability Enhancement: Drugs with poor bioavailability typically have low water solubility and/or tend to be highly crystalline. As cyclodextrins is water soluble

and form inclusion complexes with apolar molecules or functional groups in water insoluble compounds.

The resulting complex hides most of the hydrophobic functionality in the interior cavity of the cyclodextrin while the hydrophilic hydroxyl groups on its external surface remain exposed to the environment. The net effect being a water-soluble cyclodextrin-drug complex. In addition to improving solubility, cyclodextrins also prevent crystallization of active ingredients by complexing individual drug molecules so that they can no longer self-assemble into a crystal lattice.

Active Stabilization: For an active molecule to degrade upon exposure to radiation, heat, oxygen or water, chemical reactions must take place. When a molecule is constrained within the cyclodextrin cavity, it is difficult for reactants (water or oxygen) to diffuse into the cavity and react with the protected guest. In the case of thermal or radiation induced degradation, the active must undergo molecular rearrangements. Again, due to the steric constraints on the guest molecule within the cavity, it is difficult for the active to fragment upon exposure to heat or light or if it does fragment, the fragments do not have the mobility needed to separate and react before a simple recombination takes place.

Odour or Taste Masking: Through encapsulation within the cyclodextrin cavity, molecules or specific functional groups that cause unpleasant tastes or odours are hidden from the sensory receptors. The resulting formulations have no or little taste or odour and are much more agreeable to the patient.

Compatibility Improvement: Often one would like to combine multiple ingredients or drug actives within a single formulation due to the potential for synergistic benefits. However, different drugs are often incompatible with each other or another inactive ingredient within a formulation. Encapsulating one of the incompatible ingredients within a cyclodextrin molecule stabilizes the formulation by physically separating the components in order to prevent chemical interaction.

Material Handling Benefits: Active ingredients that are oils/ liquids or are volatile materials can be difficult to handle and formulate into stable solid dosage forms. Encapsulating these types of substances in a cyclodextrin converts them to a solid powder that has good flow properties and can be conveniently formulated into a tablet by conventional production processes and equipment.

Irritation Reduction: Active ingredients that irritate the stomach, skin or eye can be encapsulated within a cyclodextrin to reduce their irritancy. The formation of an inclusion complex reduces the local concentration of free active ingredient below the irritancy threshold. As the complex gradually disassociates, the active ingredient is absorbed into the body for therapeutic benefits, but its local free concentration remains below levels that might be irritating.

Oral Drug Delivery System: Rapid dissolving complexes with cyclodextrin have also been formulated for buccal and sublingual administration in this type of drug delivery system a rapid increase in the systemic drug concentration takes place along with the avoidance of systemic and hepatic first pass metabolism such as enhanced solubility of the ofloxacin drug.

Industrial Applications: Cyclodextrin change the physical and chemical properties of organic compounds. By this unique characteristic, cyclodextrins are used extensively in pharmaceutical, food and other industries for the following purpose:

- Suppression of volatility of volatile compounds.
- Stabilization of labile components decomposed and denatured by oxidation, UV-irradiation, hydration, heating, freezing and drying.
- Drying assistance of humid or liquid food extracts, seasoning and beverages.
- Reduction of bad taste and odour.
- Emulsification of oily material, solubilization water-in-soluble substances and removal of non-volatile compounds from food.

Potential Applications of β-Cyclodextrin: Cyclodextrin iodine complexes (CDS) by Nippon Chemical Co. Ltd., Japan are antibacterial deodorants, wrapping iodine in cyclodextrin cavity. CDIs possess powerful antibacterial activity with broad spectrum β-cyclodextrin iodine complexes are used in powder formulation.

LITERATURE SURVEY

2. LITERATURE SURVEY

2.1. Literature Review:

Recent advancements in Inclusion complexes:

1. **Akbari B.V *et al*., (2011)** had developed cyclodextrin complexes to increase the solubility, and dissolution rate of Rosuvastatin Calcium (RST), a poorly water- soluble 3-hydroxy3-methyl glut, aryl (CoA (HMG-CoA) Reductase inhibitor through inclusion complexation with β–cyclodextrin (β-CD). The inclusion complexes were prepared by three different methods viz. physical, kneading, Co- evaporation and precipitation method. The inclusion complex prepared with β–CD by kneading method exhibited greatest enhancement in solubility and fastest dissolution (98.96 % RST release in 30 min) of RST.

2. **Vavia P.R *et al*., (1999)** focused on inclusion complexation of ketoprofen with β– cyclodextrin (β-CD) and hydroxypropyl β–cyclodextrin (HP-β-CD). The solid complexes were prepared by various methodologies such as physical mixture co-precipitation, kneading and freeze drying. The drug and cyclodextrins were used in molar ratio of 1:1. Freeze drying method was found to be the method of choice for successful inclusion complexation of ketoprofen with β-CD and HP-β-CD.

3. **Bushetti S.S *et al*., (2009)** the work was to improve the solubility, dissolution rate and antibacterial activity of drug by formulating its inclusion complexes with β- cyclodextrin and hydroxyl propyl β-cyclodextrin by kneading and physical mixture methods. Drug release profile was studied in 7.2 pH phosphate buffer.

The results showed that an improvement in the antibacterial activity of the drug in its inclusion complex with hydroxyl propyl β-cyclodextrin in 1:3 molar ratio.

4. **Patil J.S. *et al.*, (2010)** studied about orally administered drugs completely absorb only when they show fair solubility in gastric medium and such drugs shows good bioavailability. Solid dispersion, solvent deposition, micronization are some vital approaches routinely employed to enhance the solubility of poorly water soluble drugs. Cyclodextrins, the unique cyclic carbohydrates are successfully utilized as the potential complexing agents which form inclusion complex with insoluble drugs of various techniques have been investigated to explain the methods for preparation of inclusion complexes. In the present review, an attempt was made to discuss various complexation techniques and tried to briefly highlight the potential applications, technical and economical limitations associated with these approaches.

5. **Ramink singh *et al.*, (2010)** A review conducted on CD, cyclodextrins are cyclic oligosaccharides which have recently been recognized as useful pharmaceutical excipients. The molecular structure of these glucose derivatives generates a hydrophilic exterior surface and a non polar cavity interior. Such cyclodextrins can interact with appropriate size drug molecules which lead to the formation of inclusion complexes. The characterization of inclusion complexes was done with a purpose to determine the interaction of drug molecules with cyclodextrins which confirm the formation of inclusion complexes.

6. **Swaroop S. *et al.*, (2007)** were formulated the inclusion complex of curcumin with β-cyclodextrin (β-CD) has been characterized by absorption and fluorescence

spectroscopy. From temperature-dependent fluorescence measurements, the thermodynamic parameters ΔH and ΔS, for the complexation were estimated. Kinetics of binding was studied by stopped flow technique, from which the binding constant for inclusion complex was estimated. Further, influence of such inclusion complex on change in superoxide radical scavenging property of curcumin was examined using xanthine/xanthine oxidase assay.

7. **Guowang Diao *et al*., (2006)** A research work on CD, β-Cyclodextrin can react with nitrobenzene to form an inclusion complex which is characterized by 'H NMR and power X-ray diffractometry. The ratio of β-CD to NB in inclusion complex is determined as 1:1. At 25° C, the dissociated constant, K_D, of the inclusion complex is measured as 6.5 x 10^{-3} M in neutral solution (pH=7.0), but in alkali (pH=13.5), K_D is 3.2 x 10^{-2} M which is much larger than that measured in neutral.

8. **Shewale B.D *et al*., (2008)** were examined the effect of pH and concentrations of hydroxyl propyl β-cycloedextrin on the solubility of carvedilol as it shows pH-dependent solubility. But inclusion in the cavity of hydroxyl propyl-β-cyclodextrin might depend upon charge state of the molecule. So it can be concluded that solubility of carvedilol, can be increased either by the addition of hydroxypropyl-β-cyclodextrin or by adding pH lowering agents. But both these methods if are to be used together, pH should be selected carefully.

9. **Indap M.A *et al*., (2002)** had suggested hydroxyl propyl beta cyclodextrin complex was able to protect bone marrow cells from lethal effect of radiation. When the cytotoxicities of quercetin and its complexes were compared on erythrocytes of rat

and rabbits, no significant differences were observed. The ability to selectively target quercetin via its cyclodextrin inclusion complex against cancer growth could improve the therapeutic effectiveness of cyclodextrin preparations as well as reduce adverse side effects associated with quercetin. The new cyclodextrin inclusion complex appears to have high potential for the treatment of leukemia's and possibly also for solid tumors.

10. **Chowdary K.P.R *et al.*, (2002)** the complex formation of nifedipine with β-cyclodextrin and hydroxyl propyl β-cyclodextrin was studied. The phase solubility studies indicated the formation of a nifedipine-β-cyclodextrin (1:1 M) and nifedipine-hydroxypropyl β-cyclodextrin (1:1 M) inclusion complexes with a stability constant of121.9M^{-1} and 253.7M^{-1} respectively.

11. **Chowdary K.P.R *et al.*, (2006)** tested the solubility and dissolution rate of celecoxib were markedly enhanced by complex with β-cyclodextrin and hydroxyl propyl-β-cyclodextrin. Celecoxib-hydroxypropyl-β-cyclodextrin (1:2) inclusive complex gave a 36.57-fold increase in the dissolution rate of celecoxib. Addition of polyvinylpyrrolidone results in higher complexation efficiency and markedly enhanced solubilizing efficiency of β-cyclodextrin and hydroxypropyl-β-cyclodextrin.

12. **Jagtap Rajesh S. *et al.*, (2009)** enhanced the solubility and impart a controlled release in a single formulation. Glipizide, an oral hypoglycemic agent which belongs to Class II of BCS with relatively short elimination half life (2-4 hour) was complexed with β-cyclodextrin. The dissolution study of kneading complex shows

significant increase in the drug release from kneading complex than pure drug and physical mixture. The formulation F3 having Similar dissolution profile like marketed preparation (GLUCOTROL XL) which having 50 mg of PEO. Finally it can be concluded from the study that molecular complex with β-CD play a vital to obtain a uniform, controlled and complete drug release of a poorly soluble drug from the swellable / erodible matrix tablet.

13. **Sanoferjan A.M *et al.*, (2000)** had formulated the cyclodextrin complexation of tenoxican was attempted to enhance the solubility features of the drug. The complex prepared in 1:1 M ratio by various techniques was evaluated for its dissolution profile, thermal stability and photostability. The complex prepared by neutralization method was found to yield very reliable and best results over that of the common solvent and kneading method.

14. **Ramana M.V *et al.*, (2008)** showed that enhanced dissolution rate of rofecoxib in both media from betacyclodextrin complex and the further enhancement of dissolution was found in presence of ascorbic acid and citric acid and thus betacyclodextrin enhances the solubility by creating the acidic environment around the drug molecule, solubility of rofecoxib could be enhance further, which in turn would enhance the absorption of rofecoxib and produced greater pharmacological activity.

15. **Deshmukh S.S *et al.*, (2007)** made an attempt to prepare and characterized inclusion complexes of ziprasidone HCl with β-cyclodextrin and HP-β-cyclodextrin. The phase solubility analysis indicated that the formation of 1:1 molar inclusion complex

of a drug with β-cyclodextrin and HP-β-cyclodextrin. The inclusion complexes were prepared co-precipitation, kneading and microwave method. The prepared complexes were characterized using solubility study, DSC and XRD. The inclusion complexes prepared with HP-β-cyclodextrin by microwave method exhibited greatest enhancement in solubility and fastest dissolution of ziprasidone HCl.

16. **Sarasija Suresh *et al*., (2006)** made an attempt to enhance the solubility features of the drug. Carbamazepine β-cyclodextrin complex prepared by kneading method was used to produce dispersible tablets. A 2^3 factorial design was employed to investigate the effect of factors such as amount of binder, hardness and type of disintegrant on the tablet disintegration time and dissolution rate. The three main factors studied had a significant influence on both response parameters.

17. **Sanjula Baboota *et al*., (2005)** have prepared inclusion complexes of rofecoxib by using dimethyl β-cyclodextrin. Complexes were prepared by physical, kneading and spray drying methods. The release profile of the drug from complexes were studied in pH 1.2 and pH 7.4 and it was found that the marketed preparations showed lesser release characteristic as compared to the complex prepared by kneading method.

18. **Aithal K.S *et al*., (2005)** had prepared the complexes of the norfloxacin with β-CD and enhanced UV absorption (a=3 times) and fluorescence emission (k=4 times) was observed. The 1:1 M neutralization complex gave maximum values of both UV absorption and fluorescence emission compared to physical mixture or kneading complex.

19. **Dhaneshwar S.R *et al*., (2004)** had prepared the methotrexate pro-drugs of α and γ - cyclodextrin. The primary hydroxyl group of α and γ-cyclodextrin block the acidic group and the hydrolysis of cyclodextrin conjugates in colon is confirmed by hydrolysis kinetic studies in rat fecal material and the conjugate showed good masking ulcerogenic potential of free drug.

20. **Nagesh Bandi *et al*., (2004)** had determined whether budesonide and indomethacin HPβ-CD complexes could be formed using a supercritical fluid process. The process involved the exposure of drug-HP-β-CD mixtures to supercritical carbon dioxide. The ability of supercritical fluid process to form complexes was assessed by determining drug dissolution, drug crystallinity and drug excipients interactions. Thus budesonide and indomethacin HP-β-CD complexes with enhanced dissolution can be formed using a single step organic solvent free SCF process.

21. **Sanjula Baboota *et al*., (2005)** had developed the inclusion complexes of meloxicam with β-cyclodextrin by various methods like grinding, kneading, solid dispersion and freeze-drying. The in-vitro dissolution rate of drug β-cyclodextrin complex was faster compared to the drug alone.

22. **Saha RK *et al*., (2002)** had prepared inclusion complexes of nimesulide with β-cyclodextrin by solvent evaporation method and solid dispersion of nimesulide and ibuprofen by using various hydrophilic excipients (PEG-6000, sorbitol, PVPK-30, MCC). Solid dispersion of nimesulide with PEG-6000 enhanced the solubility of nimesulide more than 1000 %. The dispersion of ibuprofen in sorbitol showed

maximum enhancement of solubility up to 75 %. The inclusion complexes of nimesulide in β-cyclodextrin also increase the solubility by 663 %.

23. **Dalmora M.E *et al.*, (2001)** evaluated the topical formulations of piroxicam by determination of their *in vitro* release and *in vivo* anti-inflammatory. Piroxicam was incorporated in ME (micro emulsion) and in the combined system β-cyclodextrin / ME, respectively relative to the buffered piroxicam (42.2 %). These results demonstrated that micro emulsion induced prolonged effects, providing inhibition of the inflammation for 9 days after a single dose administration.

Literature Review on Selected drug Aceclofenac:

24. **Thiyagarajan Ayyappan *et al.*, (2009)** had formulated the solid dispersion tablet formulation of Aceclofenac, a novel non steroidal anti inflammatory drug mainly used for rheumatoid arthritis osteoarthritis and ankylosing spondylitis. Aceclofenac solid dispersion with crosspovidone showed maximum drug release and hence, the tablet formulation containing Aceclofenac, crosspovidone and polyvinyl pyrolidone K-30 solid dispersion, was prepared with a view to improve its water solubility. The drug profile was studied in 2 % w/v sodium lauryl sulphate in Distilled water. F-III gave far better dissolution than other laboratory developed formulations. The formulation was found to be stable for 30 days at 40° C± RH 75 % as per ICH guidelines, with insignificant change in the hardness, disintegration time, and *in vitro* drug release pattern.

25. **Kamal Dua *et al.*, (2011)** had developed and compared the stability of the β-CD dimer-Aceclofenac complex with β-CD monomer-Aceclofenac. Such molecular-

modeling studies can be employed as an additional tool to support the formation of stable molecular inclusion complexation of any water insoluble drug complexed with cyclodextrins.

26. **Dahiya S. *et al.*, (2006)** was prepared and characterized the binary systems of Acelofenac (AC) with hydroxypropyl beta-cyclodextrin (HP-β-CD) in equimolar ratio. Solid binary systems of Aceclofenac with HP-β-CD were prepared using co grinding, kneading, and co evaporating methods, and a physical mixture was prepared for comparison. All the binary systems showed superior dissolution and lower dose: solubility ratio (D: S ratio) as compared to pure AC. It was suggested that complexation of Aceclofenac with HP-β-CD may be used as an approach to change the drug from Biopharmaceutics Classification System BCS Class II to BCS Class I without changing its intrinsic permeability.

27. **Gajendran P. *et al.*, (2010)** tested the phase solubility profile of Aceclofenac with beta-cyclodextrin (β-CD) prepared by kneading method and those were characterized by X-ray diffraction and FTIR spectra. The complexes were prepared as tablets with and without sodium starch glycolate (2 %) (SSG) as super disintegrants. Precompression parameters like tapped density, Carr's index, bulk density and angle of repose were determined. The order of faster dissolution rate observed in various ratios with SSG was 1:2 complex > 1:1 complex >2:1 complex > pure drug, in acidic medium contains 1 % SLS. Another batch was also prepared in the same ratio with β-CD, this batch have higher solubility of Aceclofenac when 2 % SSG added; the release was 30 min faster in 1:2 pH medium containing 1 % sodium lauryl sulphate (SLS) from the above result we conclude the inclusion complex of

drugs and β-CD ratio 1:2 with SSG as superdisintegrate could be used as an effective technique for immediate release of Aceclofenac.

28. **Kulkarni N.S *et al*., (2010)** carried out research work by developing the inclusion complexation of Aceclofenac with beta-cyclodextrin by grinding, microwave and spray drying techniques. The samples were subjected to *in vitro* dissolution studies, fourier transform infra-red spectroscopy, DSC, NMR and X-ray diffraction studies. They were reported *in vitro* dissolution of Aceclofenac-hydroxy propyl-beta-cyclodextrin complex was faster as compared to the Aceclofenac-beta-cyclodextrin complex and Aceclofenac alone.

2.2. DRUG PROFILE

(IP., 2007; BP., 2009; Kabir., et al., 2009; Merck index., 2006)

ACECLOFENAC:

Aceclofenac is a potent non-steroidal anti-inflammatory drug. Due to its preferential cox-2 blockade it has better safety than conventional NSAIDs with respect to adverse effects on gastro intestinal and cardiovascular system.

IUPAC Name **:** 2-[(2, 6-Dichlorophenylamino) phenyl] acetoxy acetic acid.

Description **:** A white to almost white crystalline powder.

Structure **:**

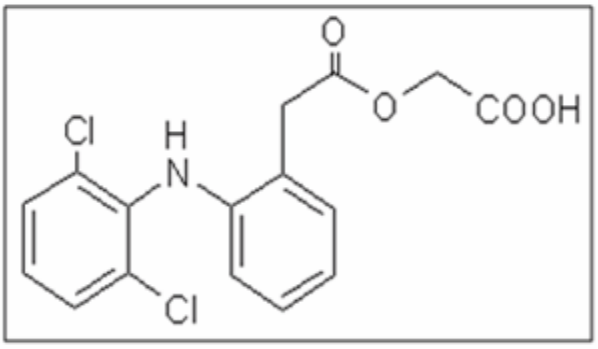


Fig. 2.1: Structure of Aceclofenac

Molecular formula **:** $C_{16}H_{13}C_{l2}NO_4$

Molecular weight **:** 354.2

Category **:** Non-steroidal anti inflammatory drug.

Solubility **:** It is practically insoluble in water; soluble in alcohol and methyl alcohol; It is freely soluble in acetone and dimethyl formamide.

Melting point **:** 149 - 153° C

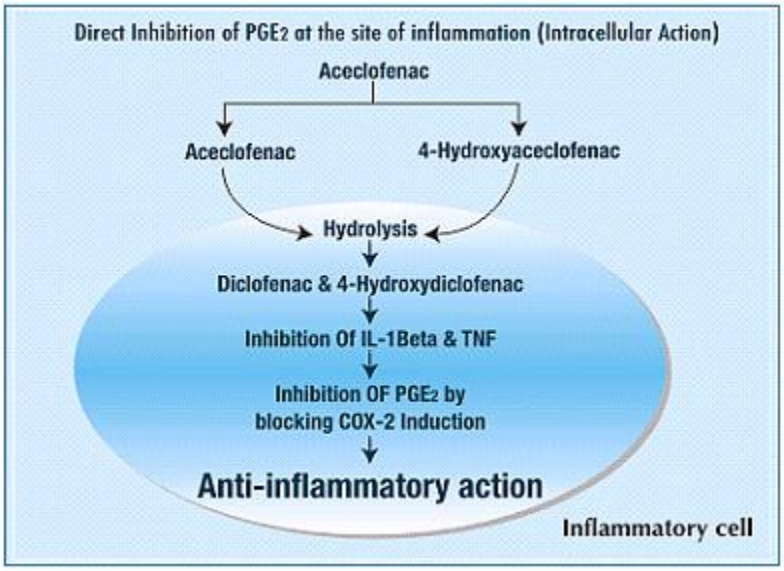
Pharmacology:

1. Aceclofenac directly blocks PGE2 secretion at the sight of inflammation by inhibiting IL- Beta TNF in the inflammatory cells. (Intracellular action)

2. Aceclofenac stimulates the synthesis of the extra cellular matrix of the human articular cartilages.

3. Inhibits neutrophil adhesion and accumulation at the inflammatory sites in the early phase and thus blocks the pro-inflammatory action of neutrophil.

4. Aceclofenac has higher COX2 specificity than diclofenac sodium.



. **Fig. 2.2:** Pathway describing general anti-inflammatory action of Aceclofenac

Pharmacokinetics:

Aceclofenac is rapidly and completely absorbed after oral administration, peak plasma concentration is reached 1 to 3 hours after an oral dose. The drug is highly protein bound. The presence of food does not alter the extent of absorption of Aceclofenac but the absorption rate is reduced. It is metabolized to a major metabolite 4-hydroxyAceclofenac and to a number of other metabolites including 5–Hydroxy Aceclofenac, 4-Hyrodxydiclofenac, Diclofenac and 5-Hydroxydiclofenac. Renal excretion is the main route of elimination of Aceclofenac with 70 to 80 % of an administered dose found in the urine, mainly as the glucuronides of Aceclofenac and its metabolites of each dose of Aceclofenac, 20 % is excreted in the faeces. The plasma elimination half life of the drug is approximately 4 hours.

Drug interactions:

Aceclofenac may increase plasma concentrations of lithium, digoxin and methotrexate, increase the activity of anticoagulant, inhibits the activity of diuretics, enhance cyclosporine nephrotoxicity and precipitate convulsions when co-administered with quinolone antibiotics. Furthermore, hypo or hyperglycemia may result from the concomitant administration of Aceclofenac and antidiabetic drugs, although this is rare. The co-administration of Aceclofenac with other NSAIDs results in increased frequency of adverse event.

Adverse drug interactions:

Aceclofenac is well tolerated with, most adverse events being minor and reversible and affecting mainly the G.I system. Most common events include dyspepsia

(7.5 %), abdominal pain (6.2 %), nausea (1.5 %), diarrohoea (1.5 %), flatulence (0.8 %), gastritis (0.6 %), constipation (0.5 %), vomiting (0.5 %), and pancreatitis (0.1 %). Other adverse effects which is not common such as dizziness (1 %), vertigo (0.3 %), and rare cases of paraesthesia and tremor.

Dosage and administration : The usual dose of Aceclofenac is 100 mg given twice daily by mouth, one tablet in the morning and one in the evening.

Storage : In an air tight container, protected from light.

Uses : Aceclofenac is used in the treatment of Osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, dental pain, post operative pain, dysmenorrhoea, acute lumbago, musculoskeletal trauma and gonalgia (knee pain).

2.3. β-CYCLODEXTRIN PROFILE

(Raymond C.Rowe., 2003)

β-CYCLODEXTRIN:

Synonyms **:** Beta-cycloamylose; betadex; beta-dextrin; cyclohepta amylase; Cycloheptaglucan: cyclomaltoheptose; kleptose.

Functional category **:** Solubilizing agent and stabilizing agent.

Empirical Formula **:** $C_{42}\ H_{70}O_{35}$

Molecular Weight **:** 1135.

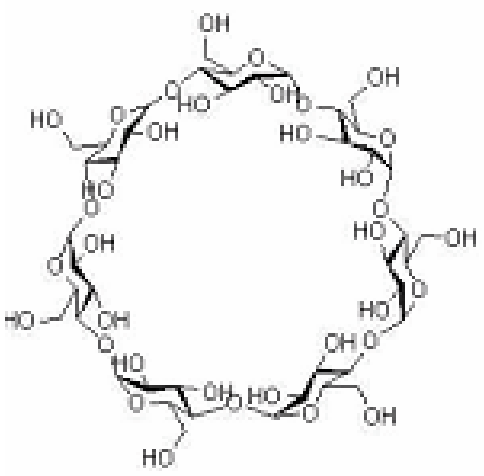


Fig.2.3: Structure of β-Cyclodextrin

Description **:** White, practically odorless, amorphous powder, having a slightly sweet taste.

Solubility	**:**	Soluble in 1 in 200 part of propylene glycol, 1 in 50 of water at 20° C, 1 in 20 at 50° C.
Stability and storage conditions:		Stable in the solid state if protected from high humidity, should be stored in a tightly sealed container in cool, dry place.
Safety	**:**	Used in oral pharmaceutical formulations.
Handling Precaution	**:**	It should be handled in a well-ventilated environment efforts should be made to limit the generation of dust, which can be explosive.

AIM

AND

OBJECTIVE

3. AIM AND OBJECTIVE

The poor aqueous solubility and dissolution of relatively insoluble drugs has been a major problem for a long time in the formulation of oral dosage form. So in the recent years much attention has been focused on the problem of drug bioavailability. The dissolution rate of a drug from its dosage form now considered as an important parameter in bioavailability, dissolution is the rate limiting step in the absorption of the drug from solid dosage forms, especially when the drug is poorly water soluble. Among the various approaches to improve the dissolution of the drugs, the preparation of cyclodextrin inclusion complexes has often proven to be highly successful.

It has short biological half-life (4 to 4.3 hours), and the usual oral dosage regimen is 100 mg taken 2 times a day. The basic goal of therapy is to achieve a steady state blood or tissue level that is therapeutically effective and non-toxic for the treatment period of time.

Aceclofenac is Nonsteroidal anti-inflammatory drug (NSAID). It is phenyl acetic acid derivative showing effective anti-inflammatory, analgesic and anti-pyretic properties mainly used in osteoarthritis and rheumatoid arthritis.

Aceclofenac is practically insoluble in water. Its aqueous solubility is reported as 0.53 mg/ml. The aqueous solubility of drug is improved by various techniques like solid dispersions, micronization, solvent deposition, use of surfactants and inclusion complexation etc.

In the present work, was aimed to prepare and characterize the inclusion complexes of Aceclofenac with cyclodextrins in different molar ratios by different methods such as physical, kneading and common solvent method.

Preparation of inclusion complex using cyclodextrin for its ready water-solubility, significant potential for solubilization and good safety profile.

The potential applications of Aceclofenac inclusion complexes:

- ¾ May minimize the frequent dosing intervals.
- ¾ Prolong the pharmacological effect.
- ¾ May help to improve patient compliance by reducing the gastrointestinal side effects like peptic ulcer etc.,

PLAN OF WORK

4. PLAN OF WORK

- **LITERATURE SURVEY**
- **SELECTION OF DRUG AND β-CYCLODEXTRIN**
- **PROCUREMENT OF DRUG AND β-CYCLODEXTRIN**
- **EXPERIMENTAL WORK**
  - ✓ **Preformulation Study**
    - ➢ **Identification of Drug**
      - Organoleptic Properties
      - Determination of Melting Point
      - Solubility Study
      - Loss on Drying
      - FTIR
      - UV Spectrophotometric Study in Methanol
      - UV Spectrophotometric Study in Distilled Water
      - Assay of Aceclofenac
  - ✓ **Formulation of complexes with β-Cyclodextrin**
    - Kneading method
    - Common Solvent method
    - Physical mixtures
  - ✓ **Evaluation of inclusion Complexes**

    Phase Solubility Studies
    - UV Spectrophotometric Study of Prepared Inclusion Complexes
    - Fourier Transform Infrared (FTIR) Spectroscopy
    - Differential Scanning Calorimetry (DSC) Analysis

- X-ray Diffraction Studies
- Drug Content Estimation
- *In vitro* Dissolution Studies
- Stability Studies

- **RESULTS AND DISCUSSION**
- **SUMMARY AND CONCLUSION**
- **FUTURE PROSPECTUS**
- **BIBLIOGRAPHY**

MATERIALS AND EQUIPMENTS

5. MATERIALS AND EQUIPMENTS

5.1. Materials Used:

Table 5.1: List of raw materials with source

S. No	Name of Ingredients	Name of supplier
1	Aceclofenac	Tristar Pharmaceuticals, Puduchery.
2	β - Cyclo dextrin	Alkem laboratories pvt ltd, Mumbai.
3	25 % ammonia	Qualigens fine chemicals, Mumbai.
4	Calcium chloride	Qualigens fine chemicals, Mumbai.
5	Methanol	Qualigens fine chemicals, Mumbai.

5.2. Equipments Used:

Table.5.2: List of equipments with model/make

S. No	Equipment	Model/ Make
1	Electronic balance	Shimadzu BL-220H.
2	Hot air oven	Chemi Equipments, Bombay.
3	USP tablet dissolution apparatus Type I	Veego scientific VDA-8DR.
4	UV spectrophotometer	Shimadzu-1700 Pharmaspec.
5	FTIR spectrophotometer	JASCO FT/IR-5300
6	Differential scanning calorimeter	Shimadzu DSC 60, Japan.
7	Rotary Shaker	Remi Electrical
8	pH Meter	Elico Li-120
9	Melting Point Apparatus	Guna Enterprises, Chennai.
10	Standard sieve (40 #)	Jayant scientific, IND.
11	X-ray Diffractometer	X-ray Diffractometer PW-1710

PREFORMULATION STUDIES

6. PREFORMULATION STUDY

6.1. Identification of Drug:

The preliminary studies were carried out by testing of different physical and chemical properties of drug as follows.

6.1.1. Organoleptic properties: *(Lachman L. et al., 1991)*

The Organoleptic properties like physical state, colour, odour etc., of the drug was reported with help of the descriptive terminology. It helps to identify the drug.

6.1.2. Melting point:

(IP., 2007)

It is the easy way to identify the drug. The melting point of Aceclofenac was tested by use of a laboratory melting point apparatus with a procedure given in the Indian Pharmacopeia 2007.

6.1.3. Solubility study:

(IP., 2007)

The solubility of Aceclofenac was determined by micropipette method in various solvents in order to meet the official standards.

6.1.4. Loss on drying:

(IP., 2007)

Loss on drying is the loss of weight expressed as percentage w/w resulting from water and volatile matter of any kind that can be driven off under specified condition. The test can be carried out on the well mixed sample of the substance.

$$\text{Loss on drying} = \frac{\text{Initial weight of substance} - \text{Final weight of substance}}{\text{Initial weight of substance}} \times 100$$

6.1.5. FTIR spectroscopy:

The infrared spectrum was generally used as an identification parameter to know the chemical structure of drugs. For the FTIR spectrum of Aceclofenac, FTIR spectrophotometer was used.

A small quantity of sample was mixed with sufficient potassium bromide and compressed into a pellet by applying a 10 tons pressure with help of a hand operated press. This pellet was kept in a sample holder and scanned from 4000 to 400 cm^{-1}.

6.1.6. UV spectrometric study in Methanol:

¾ **Determination of λ_{max}:**

Weighed amount of Aceclofenac was dissolved in Methanol to obtain a 1000 mcg/ml solution. This solution was subjected to scanning between 200-400 nm and absorption maximum was determined. The effect of dilution on absorption maxima was studied by diluting the above solution to 15 mcg /ml and scanned from 200-400 nm. The wave length of maximum absorbance was found to be 275 nm.

¾ **Preparation of standard curve of Aceclofenac in Methanol:**

(IP., 2007)

The standard curve was prepared by dissolving 50 mg of Aceclofenac 50 ml of Methanol. In the stock solution 1 ml was withdrawn and diluted to 25 ml of Methanol. It was further diluted with Methanol to get the solution in the concentration range of 4-20 µg/ml. The absorbance values were determined at 275 nm. A graph of absorbance Vs concentration was plotted.

6.1.7. UV Spectrometric study in Distilled Water:

¾ **Preparation of standard curve of Aceclofenac in Distilled Water:**

(Rohit Shah et al., 2008)

The standard curve was prepared by dissolving 50 mg of Aceclofenac in 50 ml of Methanol. In the stock solution 1 ml was withdrawn and diluted to 25 ml of Methanol. It was further diluted with Distilled Water to get the solution in the concentration range of 4-20 µg/ml. The absorbance values were determined at 274 nm. A graph of absorbance Vs concentration was plotted.

6.1.8. Assay of Aceclofenac:

(IP., 2007)

Accurately weighed the powder equivalent to 100 mg of Aceclofenac was transferred to 100 ml volumetric flask and the volume was made up to the mark with Methanol. The mixture was filtered through whatmann filter paper No.41 and then 5 ml of filtrate was transferred to 50 ml volumetric flask and made up to the mark with Methanol.1 ml aliquots of the sample were transferred and diluted to 10 ml with Methanol to obtain strength as 10 µg/ml and then the absorbance was measured at 275 nm against Methanol as blank. The percentage purity of the drug was calculated by using calibration graph method.

FORMULATION OF INCLUSION COMPLEXES

7. FORMULATION OF INCLUSION COMPLEXES

Table 7.1: Composition of inclusion complexes of Aceclofenac

Method	Formulation Code	Drug to carrier	Drug to carrier ratio	Drug (g)	β-Cyclodextrin (g)
Kneading method	F1	ACE : β-CD	1:1	1	1
	F2	ACE : β-CD	1:2	1	2
	F3	ACE : β-CD	1:3	1	3
Common solvent method	F4	ACE : β-CD	1:1	1	1
	F5	ACE : β-CD	1:2	1	2
	F6	ACE : β-CD	1:3	1	3
Physical method	F7	ACE : β-CD	1:1	1	1
	F8	ACE : β-CD	1:2	1	2
	F9	ACE : β-CD	1:3	1	3

Where,

ACE - Aceclofenac

β-CD - Beta-Cyclodextrin

7.1. Methods of Preparation of Inclusion Complexes:

(Sanoferjan A.M., 2000; Chowdary K.P.R., 2000)

Kneading method:

β-Cyclodextrin was taken in a glass mortar and little water was added and mixed to obtain a homogenous paste. Aceclofenac was then added slowly while grinding. The mixture was ground for 1h during this process appropriate quantity of water was added to maintain suitable consistency. The paste was dried in an oven at 40º C for 48 hr. The dried complex was taken for study.

Common solvent method:

Aceclofenac and β-Cyclodextrin were dissolved in 25 % ammonia and the solvent was allowed to evaporate overnight at room temperature. The complex so prepared was pulverized and sifted through sieve no.80.

Physical mixture:

Previously weighted drug and β- cyclodextrin mixture was blended in glass mortar for about an hour and passed through sieve no.85 to get physical mixture and stored in desiccator over fused calcium chloride.

EVALUATION OF INCLUSION COMPLEXES

8. EVALUATION OF INCLUSION COMPLEXES

8.1 Phase Solubility Studies:

(Chowdary K.P.R., 2006 and Babu R.J., 2004)

Phase solubility studies for Aceclofenac (ACE) complexes were performed to determine how the complexes of cyclodextrin affect the solubility of the Aceclofenac. These studies also determine the stoichiometry of drug:cyclodextrin complexes and numerical values of their stability constants.

Phase solubility of Aceclofenac (ACE) with beta-cyclodextrin (β-CD)

Procedure:

For phase solubility studies of ACE, an excess of drug (200 mg) was added to 20 ml portions of Distilled water, each containing variable amount of β-cyclodextrin such as 0, 1, 3, 6, 9, 12, and 15 x 10^{-3} moles/liter. All the above solutions with variable amount of β-cyclodextrins were shaken for 72 hours. After shaking, the solutions were filtered and their absorbance was noted at 275 nm. The solubility of the ACE in every β-cyclodextrin solution was calculated and phase solubility diagram was drawn between the solubility of ACE and different concentrations of β-cyclodextrin.

The stability constant of ACE β-cyclodextrin complex was calculated using Higuchi and Connor's equation.

$$K\ (1{:}1) = \frac{\text{Slope}}{S_0\ (1\text{-Slope})}$$

S_0 = Intrinsic solubility of ACE in aqueous complexation media (Distilled water) "slope" was calculated from phase solubility diagram.

8.2. UV Spectrometric Study of Prepared Inclusion Complexes:

(Rohit shah et al., 2008)

Weighed accurately the quantity of inclusion complex equivalent to 50 mg was transferred to 50 ml volumetric flask and volume was made up to the mark with Methanol. The mixture was filtered through whatmann filter paper No.41 and then 5 ml of filtrate was transferred to 50 ml volumetric flask and made up to the mark with Methanol. From the resulting solution 1ml was taken and diluted with 10 ml of Methanol to get a final concentration of 10 µg/ml. The sample solutions were scanned at 200-400 nm.

8.3. Fourier Transform Infrared (FTIR) Spectroscopy:

(Gajendran P. et al., 2010)

Infrared spectroscopy is one of the most powerful analytical techniques that offer the possibility of chemical identification. The IR spectra of Aceclofenac, β-cyclodextrin and inclusion complex were obtained by KBr pellet method by JASCO FT/IR-5300 spectrometer.

8.4. Differential Scanning Calorimetry (DSC) Analysis:

(Gajendran P. et al., 2010)

The thermal behavior of Aceclofenac, β-cyclodextrin and inclusion complex was studied using differential scanning calorimetry in order to confirm the formation of the solid complex. When guest molecule are incorporated in the cyclodextrin cavity or in the crystal lattice, their melting, boiling and sublimation points are usually shifted to a different temperature or disappear within the temperature range, where the cyclodextrin lattice is decomposed.

8.5. X-ray Diffraction Studies:

(Srinivas Mutalik et al., 2008)

The X-ray diffraction patterns of Aceclofenac, β-cyclodextrin and inclusion complex were reordered using Philips X-ray diffractometer (Model: PW 1710) with copper target. The conditions were: voltage -30kV; current -30mA; scanning speed -1°/min; temperature of acquisition; room temperature; detector: scintillation counter detector; sample holder: non-rotating holder.

8.6. Drug Content Estimation:

(IP., 2007)

Accurately weighed the quantity of inclusion complex equivalent to 100 mg of drug was transferred to 100 ml volumetric flask and the volume was made up to the mark with Methanol. The mixture was filtered through whatmann filter paper No.41 and then 5 ml of filtrate was transferred to 50 ml volumetric flask and made up to the mark with Methanol.1 ml aliquots of the sample were transferred and diluted to 10 ml with Methanol to obtain strength as 10 µg/ml and then the absorbance was measured at 275 nm against Methanol as blank.

8.7. *In vitro* Dissolution studies:

(IP., 2007)

In vitro dissolution of Aceclofenac inclusion complex was studied in USP XXIV dissolution apparatus employing an apparatus I, 900 ml of Distilled water was used as dissolution medium. The speed of rotation was set at 50 rpm. The temperature of dissolution media was previously warmed to 37±0.5° C and was maintained throughout the experiment. 1 ml of sample of dissolution medium were withdrawn at known intervals of time, filtered the solution and analyzed for the drug release by measuring the absorbance at 274 nm after suitable dilution with Distilled

Water. Then the volume withdrawn at each time interval was replaced with the fresh Distilled Water. The percentage amount of Aceclofenac released was calculated and plotted against time.

Table 8.1: Parameters were used for the dissolution study

Apparatus	**USP Dissolution apparatus (Type I)**
Dissolution medium	Distilled Water
Temperature	37 ± 0.5° C
Volume	900 ml
Speed	50 rpm
Sample withdrawn	1 ml
Running Time	60 min

8.8 Stability Studies:

(Sanoferjan AM et al., 2000)

The formulation F2 was packed in dessicator, which were tightly plugged with cotton and capped. They were then stored at 25° C at 60 % and 40° C at 75 % RH for a period of three months and the samples were withdrawn at every month for the estimation of drug content and *in vitro* dissolution studies.

RESULTS AND DISCUSSION

9. RESULTS AND DISCUSSION

9.1. Identification of Drug:

9.1.1. Organoleptic Properties:

Colour : White or almost white powder

Odour : Odourless

Appearance : White crystalline powder

9.1.2. Melting Point:

Melting point of Aceclofenac was found to be 150.3±21° C. The official melting point range for Aceclofenac is between 149-153° C. Hence, results were complied the limits specified in official Book.

9.1.3. Solubility Study:

Table 9.1: Solubility of Aceclofenac in various solvents

S. No.	Solvents	Extent of Solubility	Inference
1	Distilled Water	10 mg in more than 10 ml	Insoluble
2	Ethanol	10 mg in 160 µl	Soluble
3	Methanol	10 mg in 200 µl	Soluble
4	0.1N HCl	10 mg in more than 10 ml	Insoluble
5	0.1N NaOH	10 mg in more than 10 ml	Insoluble
6	Acetone	10 mg in 70 µl	Freely soluble
7	pH buffer 7.4	10 mg in more than 10 ml	Insoluble
8	Chloroform	10 mg in 400 µl	Sparingly soluble

The results were complied with the official book.

9.1.4. Loss on Drying:

The percentage loss on drying after three hours was found to be 0.25 %.

Table 9.2: Percentage loss on drying of Aceclofenac

S. No.	Percentage LOD	Average percentage LOD
1	0.25	0.25 ± 0.01
2	0.26	
3	0.24	

The sample passes the test for loss on drying as per limit specified in IP 2007 (NMT 1 %).

9.1.5. FTIR spectroscopy:

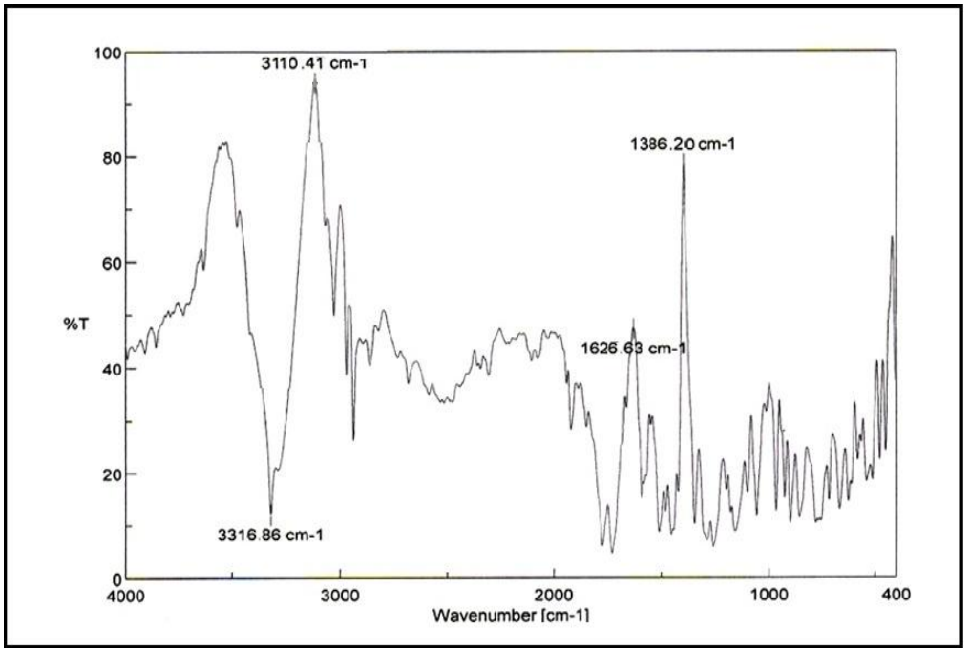


Fig 9.1: FTIR spectrum of Aceclofenac

Table 9.3: Characteristic frequencies in FTIR spectrum of Aceclofenac

S. No	Wave number(cm^{-1})	Inference
1	3316.41	N-H stretching
2	3110.41	C-H stretching
3	1626.63	C=O stretching
4	1386.20	Aromatic C-H stretching

The drug was confirmed as Aceclofenac with results obtained from FTIR spectrum analysis.

9.1.6. UV spectrophotometric study:

- The absorption maximum for Aceclofenac in Methanol was found to be 275 nm

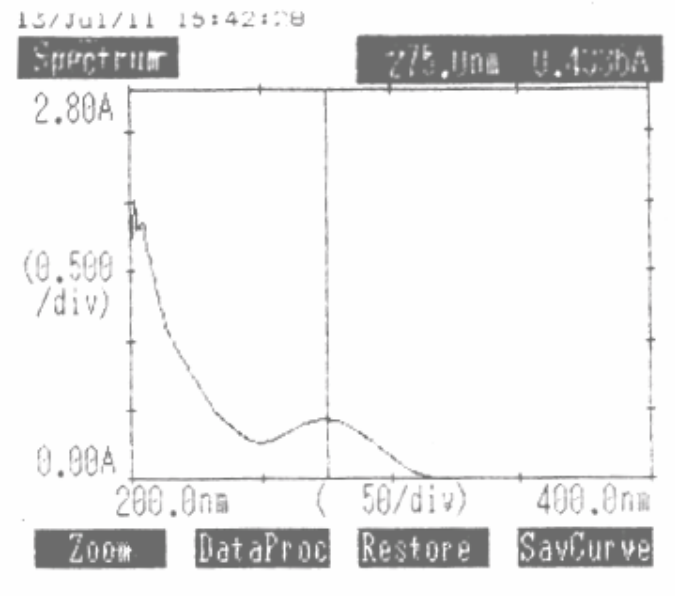


Fig. 9.2: λ_{max} of Aceclofenac in Methanol

¾ The absorption maximum for Aceclofenac in Distilled Water was found to be 274 nm

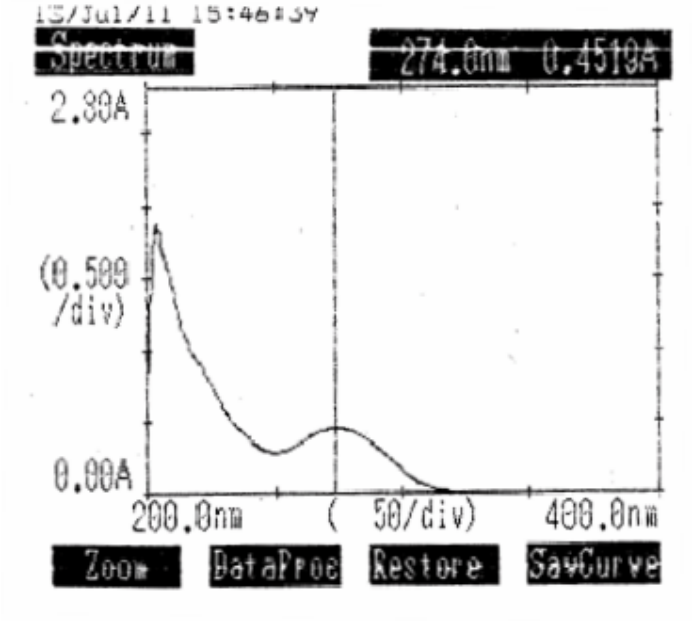


Fig. 9.3: λ_{max} of Aceclofenac in Distilled Water

9.1.7. Calibration curve of Aceclofenac in Methanol:

UV absorption spectrum of Aceclofenac in Methanol shows λ max at 275 nm. Absorbance obtained for various concentrations of Aceclofenac are given in Table 9.4. The graph of absorbance vs concentration for Aceclofenac was found to be linear in the concentration range of 4 – 20 µg /ml.

Table 9.4: Data of concentration and absorbance for Aceclofenac in Methanol

S. No.	Concentration (µg/ml)	Absorbance(nm)
1	0	0.000
2	4	0.110
3	8	0.209
4	12	0.327
5	16	0.414
6	20	0.484

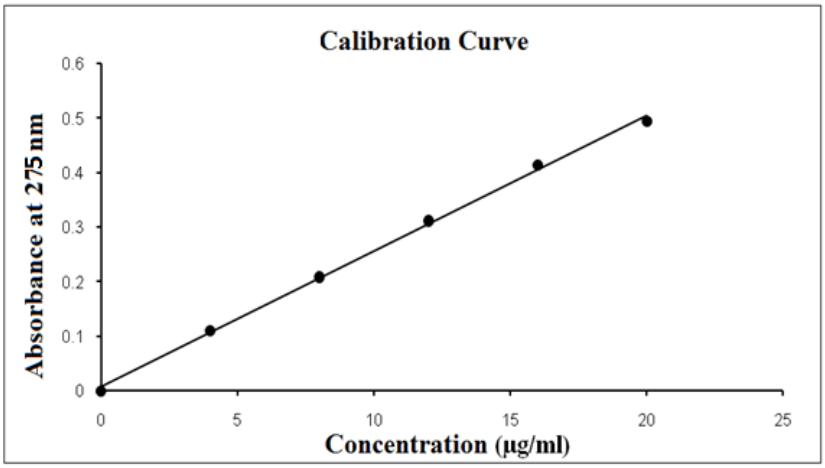


Fig. 9.4: Standard graph of Aceclofenac in Methanol

Table 9.5: Data for calibration curve parameter of Methanol

S. No.	Parameters	Values
1	Correlation coefficient (r)	0.999
2	Slope	0.008
3	Intercept	0.034

9.1.8. Calibration curve of Aceclofenac in Distilled Water:

UV absorption spectrum of Aceclofenac in Distilled Water shows λ max at 274 nm. Absorbances obtained from various concentrations of Aceclofenac in Distilled Water are given in Table 9.6. The graph of absorbance *vs* concentration for Aceclofenac was found to be linear in the concentration range of 4 – 20 µg/ml.

Table 9.6: Data of concentration and absorbance for Aceclofenac in Distilled Water

S. No.	Concentration (µg/ml)	Absorbance (nm)
1	0	0.000
2	4	0.051
3	8	0.112
4	12	0.152
5	16	0.195
6	20	0.241

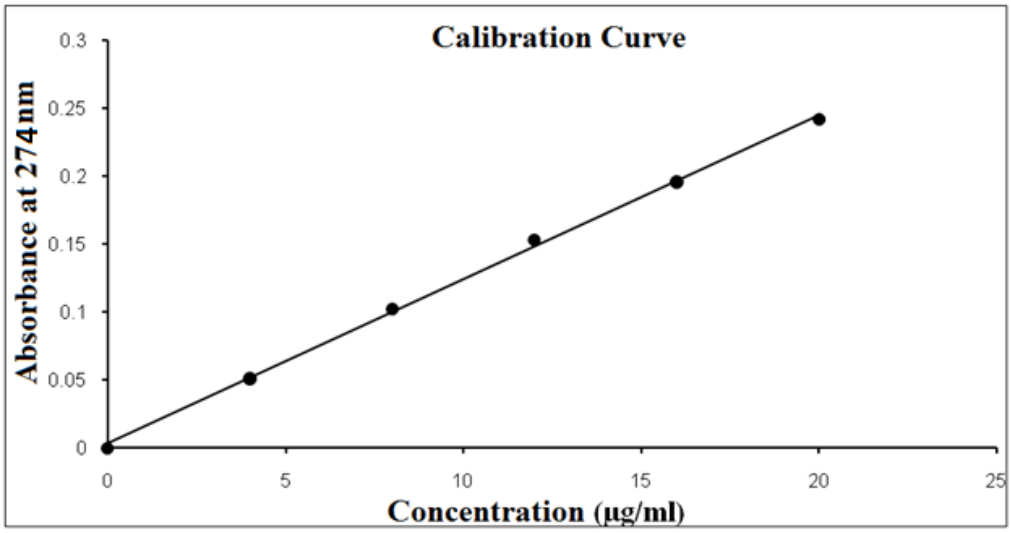


Fig. 9.5: Standard graph of Aceclofenac in Distilled Water

Table 9.7: Data for calibration curve Parameter of Distilled Water

S. No.	Parameters	Values
1	Correlation coefficient (r)	0.998
2	Slope (m)	0.014
3	Intercept(c)	0.064

9.1.9. Assay of Aceclofenac:

The percentage purity of drug was calculated by using calibration graph method (least square method).

Table 9.8: Assay of Aceclofenac

S. No.	Percentage purity (%)	Avg. percentage purity (%)
1	98.32	99.69 ± 1.2095
2	100.16	
3	100.60	

*All the values are expressed as mean ± SD, n = 3.

The percentage purity of raw material Aceclofenac was found to be 99.69 %. Hence, the sample declared as pure.

9.2. Phase Solubility Studies:

Table 9.9: Phase solubility studies of Aceclofenac - β-cyclodextrin complexes

Concentration of β-CD (mM)	Concentrations of Aceclofenac* (mM)
0	0.651 ± 0.026
1	0.675 ± 0.045
3	0.701 ± 0.062
6	0.784 ± 0.027
9	0.869 ± 0.035
12	0.948 ± 0.042
15	1.056 ± 0.039

*All the values are expressed as mean ± SD, n=3.

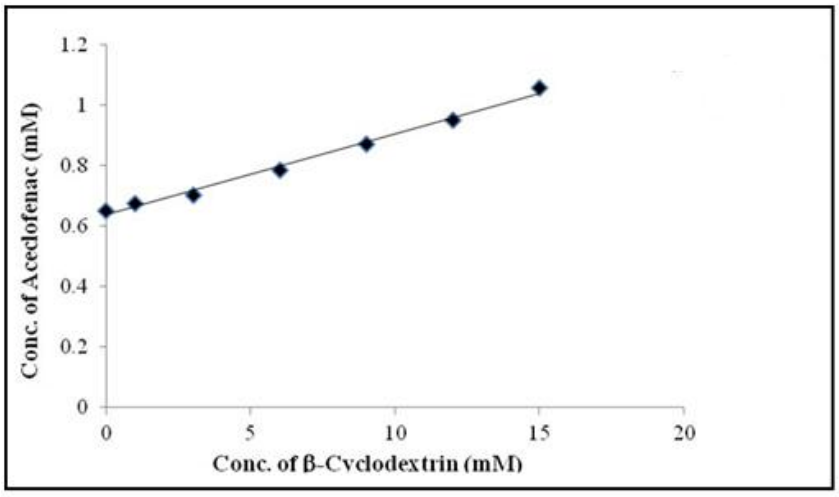


Fig. 9.6: Phase solubility studies of Aceclofenac - β-cyclodextrin complexes

r^2 - value = 0.991

B- value (slope) = 0.026

A- value (intercept) = 0.636

Stability constant – $K_{(1:1)}$ = $\frac{\text{Slope}}{S_0 (1 - \text{slope})}$

$= 0.026 / 0.636 \times 10^{-3} (1-0.026)$

$= 42.003.$

The phase solubility revealed a linear relationship between aqueous drug solubility with increase in β-cyclodextrin (r^2= 0.991). The phase solubility results were shown in Table 9.9 and Fig. 9.6.

9.3. UV Spectrometric Study of Prepared Complexes:

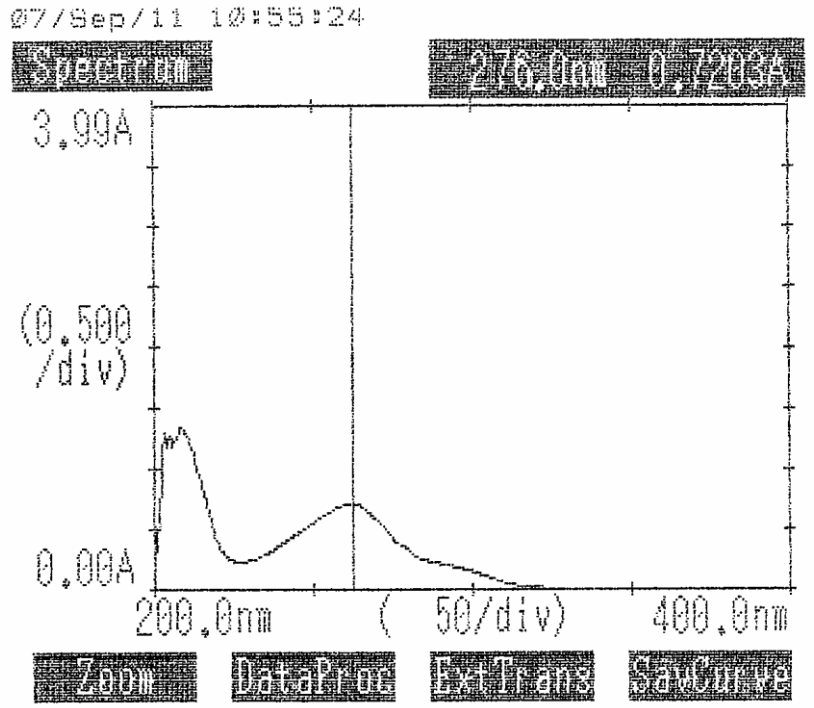


Fig. 9.7: λ_{max} of prepared complexes

The λ_{max} of Aceclofenac in Methanol shows 275 nm and prepared complex (formulation F2) shows 276 nm. This slight change is due to formation of inclusion complex.

9.4. Fourier Transform Infrared (FTIR) Spectroscopy:

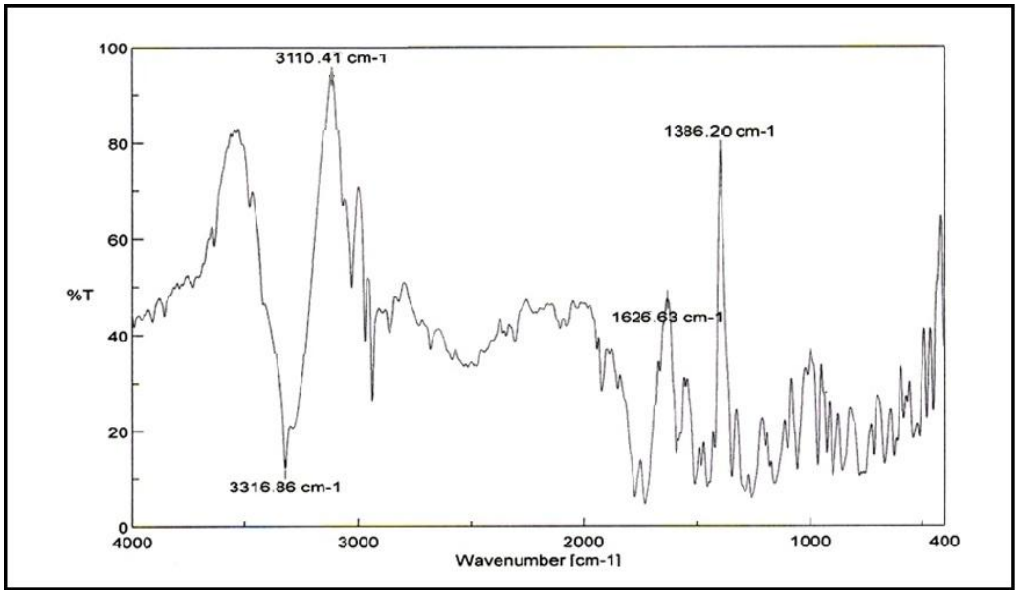


Fig. 9.8: FTIR spectrum of Aceclofenac

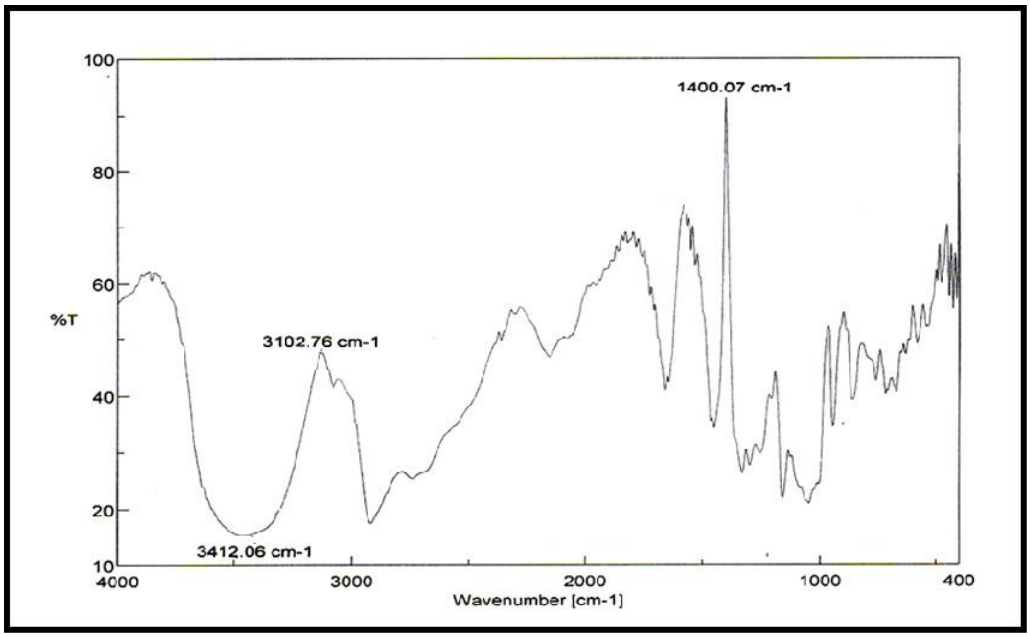


Fig. 9.9: FTIR spectrum of β-cyclodextrin

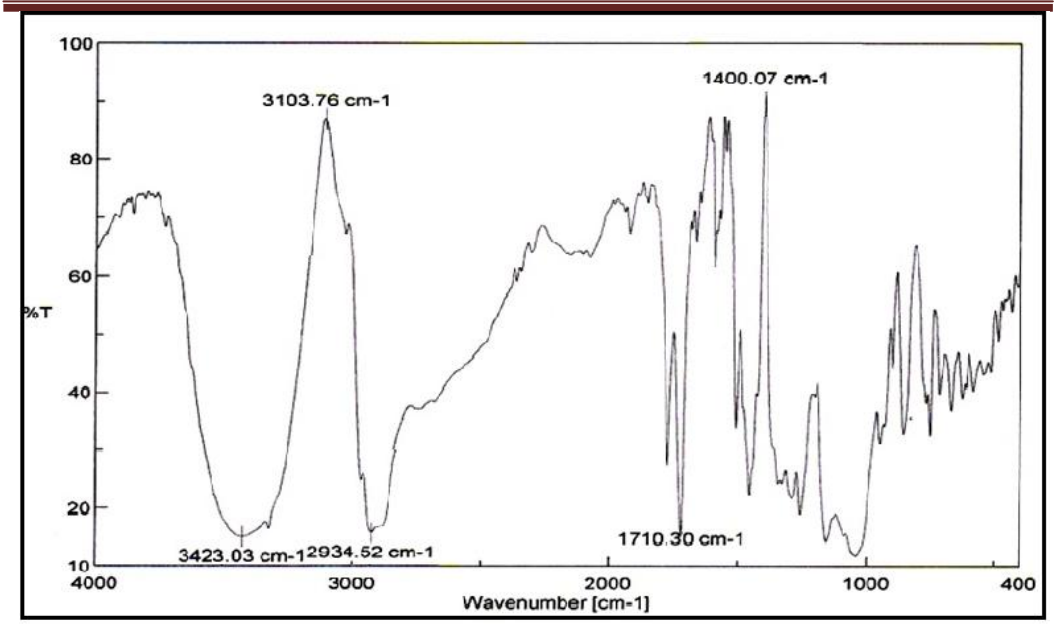


Fig.9.10: FTIR spectrum of formulation F2

Table 9.10: Interpretation of FTIR spectrum of Aceclofenac

S. No.	Wave number (cm^{-1})	Functional group	Peak observed (Yes/No)		
			Range (cm^{-1})	Drug	Drug+ β-CD
1	3316.41	N-H stretching	3600-3300	Yes	Yes
2	3110.41	C-H stretching	Above 3000	Yes	Yes
3	1626.63	C=O stretching	1760-1615	Yes	Yes
4	1386.20	Aromatic C-H stretching	1600-1300	Yes	Yes

From the above Fig. 9.8 to 9.10, it can be seen that, the major functional group peaks like 3316.41, 3110.41, 1626.63 and 1386.20 cm^{-1} showing the functional group N-H stretching, C-H stretching, C=O stretching and aromatic C-H stretching were observed in spectra of drug with β-cyclodextrin complex remains unchanged as compared with spectra of Aceclofenac.

So, from the above IR spectra can be observed that there is no interaction between Aceclofenac and β-cyclodextrin.

9.5. Differential Scanning Calorimetry (DSC) Analysis:

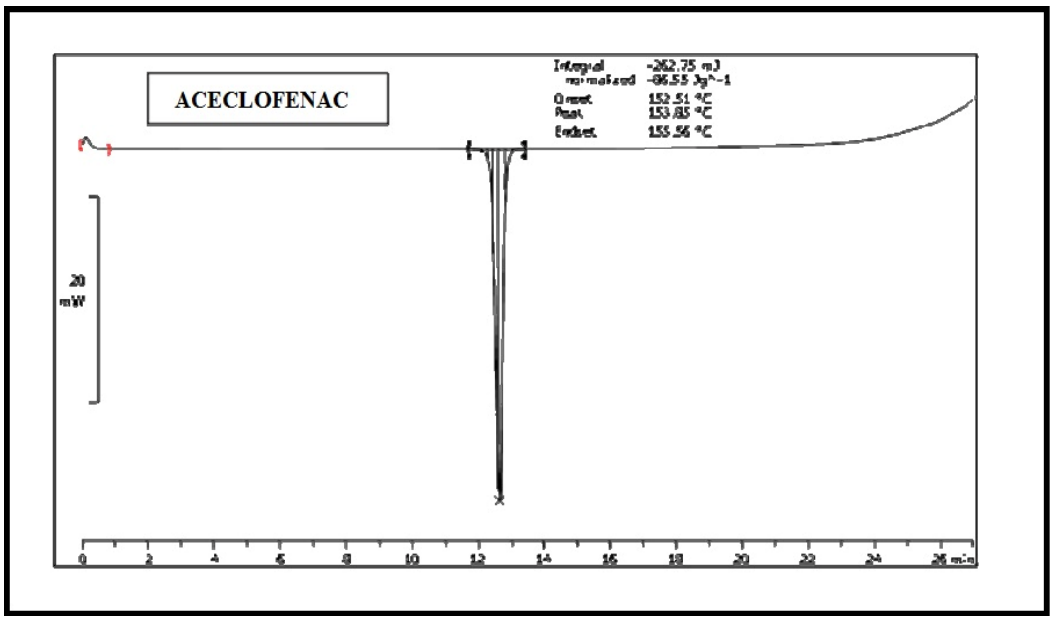


Fig. 9.11: DSC thermogram of Aceclofenac

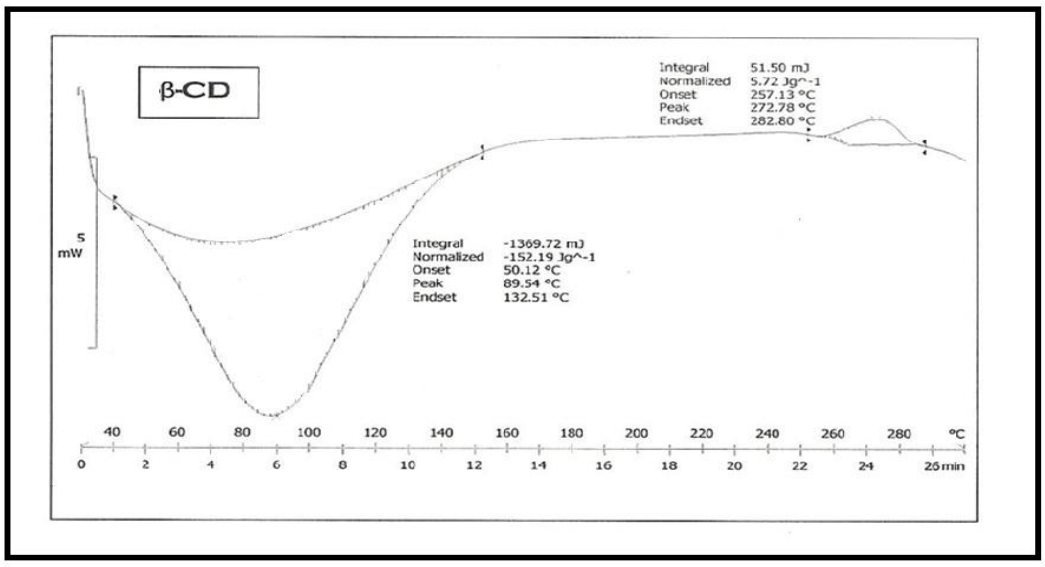


Fig. 9.12: DSC thermogram of β-cyclodextrin

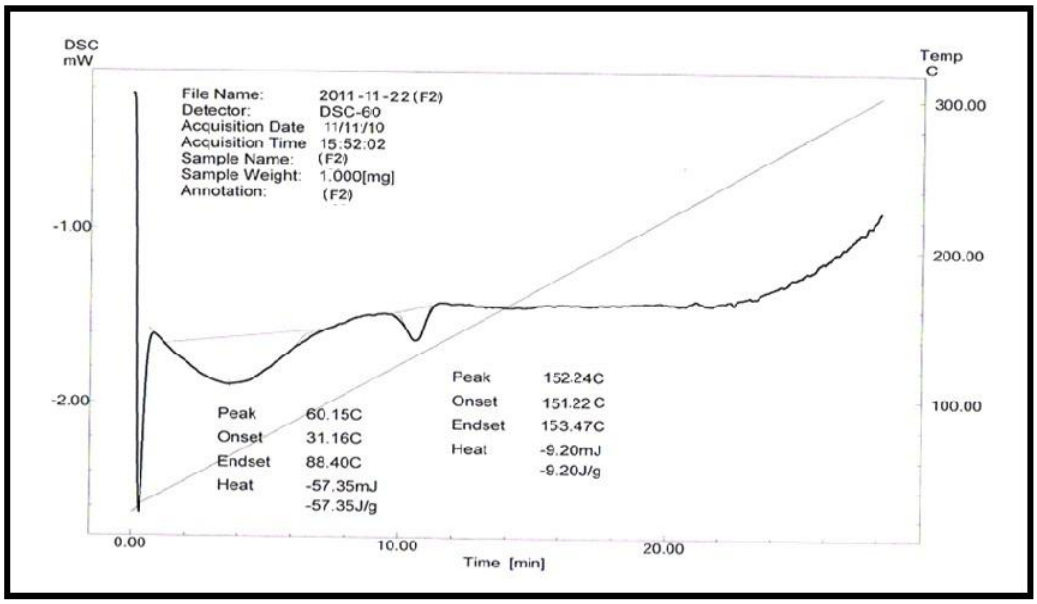


Fig. 9.13 DSC thermogram of formulation F2

Table 9.11: Data for DSC thermogram parameters

S.No.	DSC thermogram sample	Onset temperature (° C)	Peak temperature (° C)	Endset Temperature (° C)
1	Aceclofenac	152.51	153.85	155.36
2	Aceclofenac + β-CD	151.22	152.24	153.47

The thermal behavior of Cyclodextrin inclusion complex was studied using DSC in order to confirm the formation of inclusion complexes. When the molecules are incorporated in cyclodextrin cavity or in the crystal lattice, the boiling point, melting point and sublimation point are usually shifted to different temperature otherwise disappear within the temperature range where the cyclodextrin lattice is decomposed.

The DSC of pure Aceclofenac, β-Cyclodextrin and formulation F2 complexes are shown in Fig.9.11 – 9.13. The results of the study indicate that there are no major changes between the drug and drug carrier with reference to the melting point of Aceclofenac and β-Cyclodextrin.

9.6. X-ray Diffraction Studies:

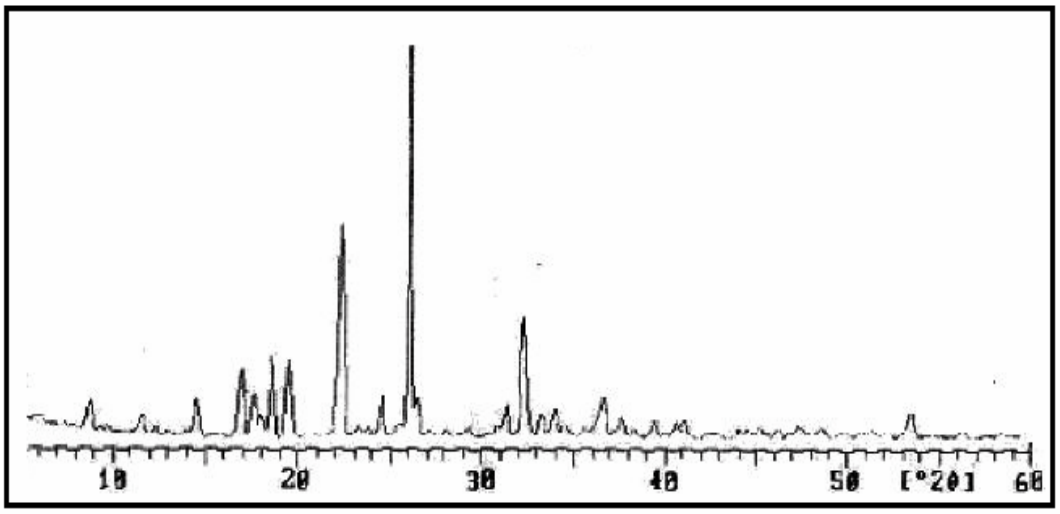


Fig. 9.14: X-ray diffraction of pure Aceclofenac

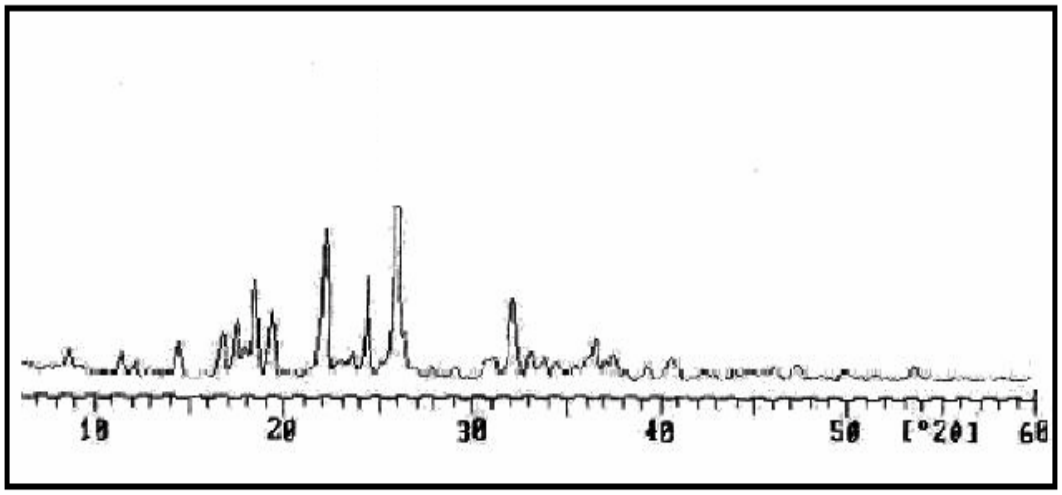


Fig. 9.15: X-ray diffraction of pure β-cyclodextrin

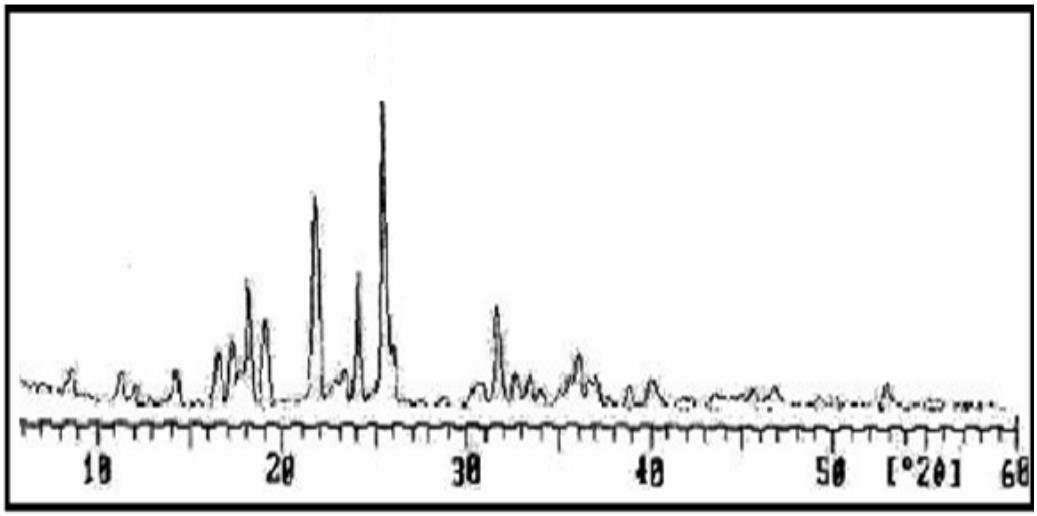


Fig. 9.16: X-ray Diffraction of formulation F2

Powder X-ray diffraction study may be used to detect inclusion complexation in the solid state. The diffraction pattern of newly formed substances clearly differs from that of uncomplexed cyclodextrin. This difference of diffraction pattern indicates the complex formation.

The X-ray diffraction pattern of pure drug shows the peaks that are sharp intense signifying its crystalline nature. Formulation F2 consists of drug and β-cyclodextrin in the ratio 1:2 prepared by kneading technique there is absence of intense peaks of Aceclofenac, signifying amorphous nature of complex. The results of the XRD pattern are shown in the Fig. 9.14-9.16.

9.7. Drug Content Estimation:

Table 9.12: Drug content estimation of Aceclofenac inclusion complexes

Formulation Code	Percent drug content*±SD
F1	98.02±0.13
F2	99.51±0.61
F3	97.23±0.21
F4	96.21±0.58
F5	97.28±0.28
F6	95.38±0.31
F7	98.52±0.84
F8	99.01±0.21
F9	97.23±0.21

*All the values are expressed as mean ± SD, n=3.

Drug content was found to be uniform among all formulations and ranged from 97.23 to 99.51 %. These results showed that all formulations having uniform percentage drug content as per limits given in Indian Pharmacopoeia.

9.8. *In vitro* Dissolution Studies:

Table.9.13: Dissolution profile of formulation F1

S. No.	Time (min)	DR* (%)	Amount (mg)	% DE	MDT
1	0	0.00	0.00	0.00	0.00
2	5	8.25±0.18	8.25	4.79	0.50
3	10	20.48±1.22	20.48	9.49	0.99
4	15	33.86±1.61	33.86	13.88	1.43
5	30	57.26±0.33	57.26	18.02	1.90
6	45	75.09±1.63	75.09	21.86	2.28
7	60	83.37±1.81	83.37	25.72	2.91

*All the values are expressed as mean ± SD, n=3.

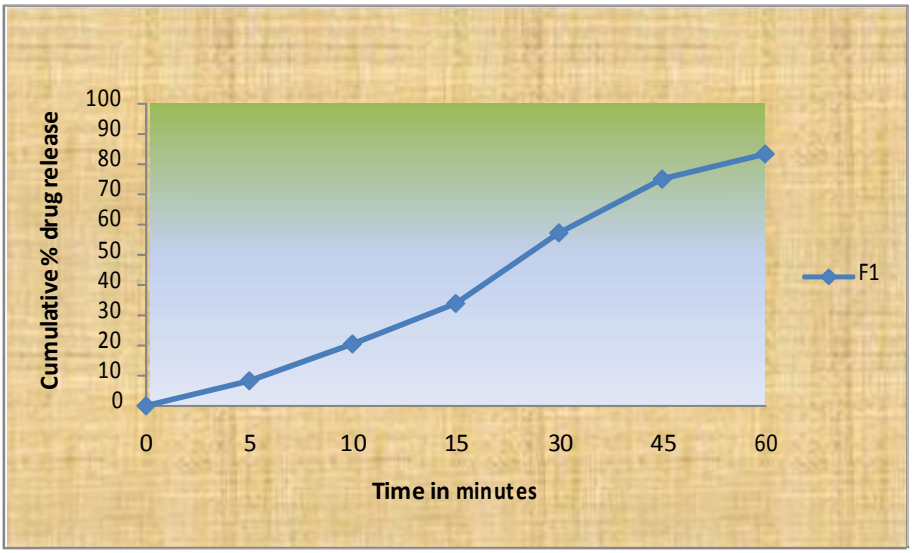


Fig.9.17: Dissolution profile of formulation F1

Table 9.14: Dissolution profile of formulation F2

S. No.	Time (min)	DR* (%)	Amount (mg)	% DE	MDT
1	0	0.00	0.00	0.00	0.00
2	5	26.55±0.80	26.55	12.07	2.50
3	10	43.33±1.05	43.33	23.06	4.75
4	15	55.55±1.92	55.55	32.14	6.49
5	30	85.18±1.20	85.18	51.82	12.00
6	45	93.99±0.29	93.99	64.75	14.28
7	60	97.54±0.89	98.54	73.15	16.93

*All the values are expressed as mean ± SD, n=3.

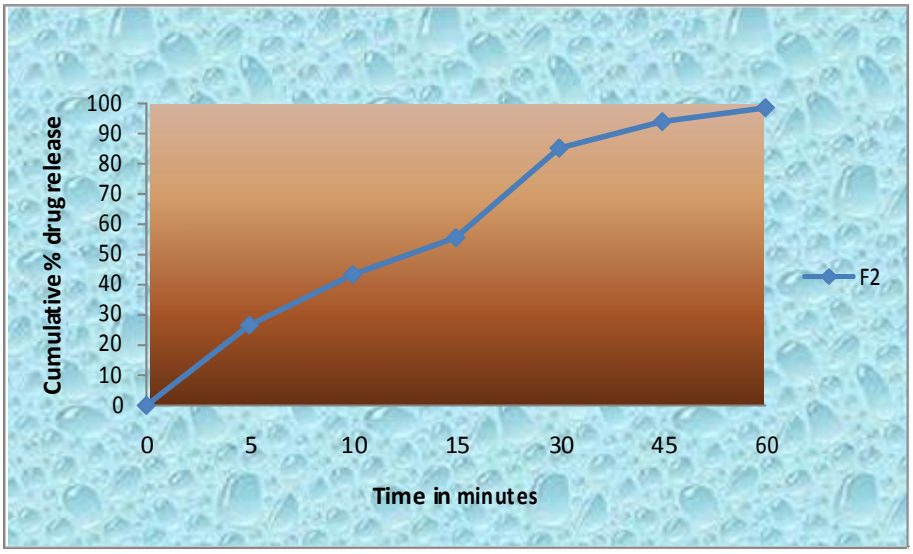


Fig. 9.18: Dissolution profile of formulation F2

Table 9.15: Dissolution profile of formulation F3

S. No.	Time (min)	DR* (%)	Amount (mg)	% DE	MDT
1	0	0.00	0.00	0.00	0.00
2	5	8.27±1.21	8.27	3.59	2.50
3	10	25.46±0.67	25.46	8.92	5.82
4	15	32.83±0.30	32.83	15.17	8.31
5	30	52.28±0.22	52.28	29.56	13.53
6	45	65.89±0.89	65.89	39.77	18.11
7	60	75.09±1.49	75.09	47.71	22.56

*All the values are expressed as mean ± SD, n=3.

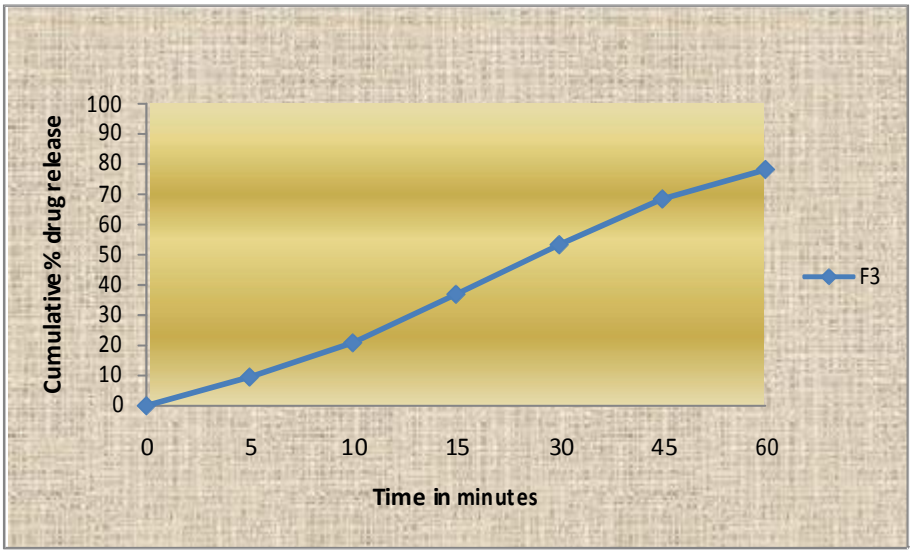


Fig. 9.19: Dissolution profile of formulation F3

Table 9.16: Dissolution profile of formulation F4

S. No.	Time (min)	DR* (%)	Amount (mg)	% DE	MDT
1	0	0.00	0.00	0.00	0.00
2	5	11.89±1.33	11.89	5.71	2.50
3	10	24.68±0.89	24.68	11.04	4.82
4	15	32.37±1.25	32.37	16.59	7.69
5	30	51.09±1.53	51.09	29.56	12.62
6	45	61.28±1.40	61.28	38.83	17.58
7	60	72.85±1.97	72.85	45.76	20.43

*All the values are expressed as mean ± SD, n=3.

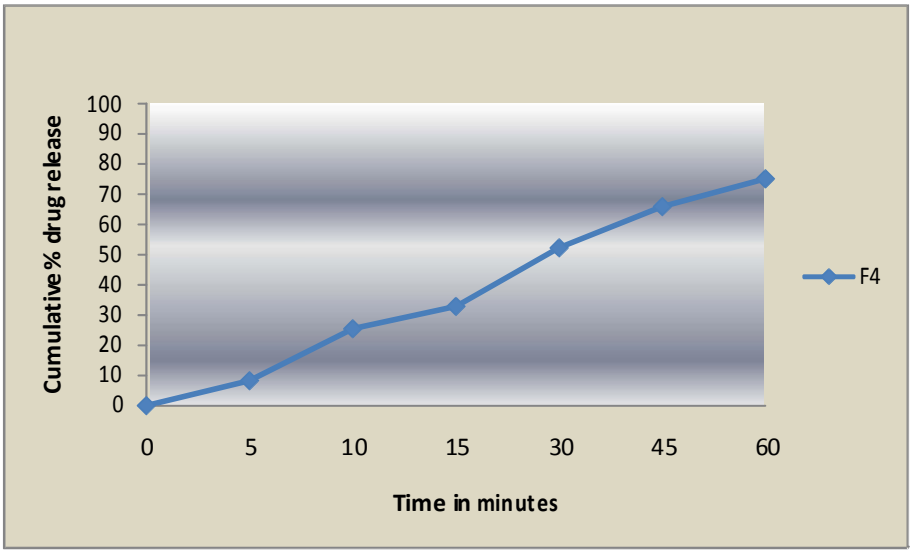


Fig.9.20: Dissolution profile of formulation F4

Table 9.17: Dissolution profile of formulation F5

S. No.	Time (min)	DR* (%)	Amount (mg)	% DE	MDT
1	0	0.00	0.00	0.00	0.00
2	5	10.45±1.17	10.45	5.00	2.50
3	10	20.74±1.49	20.74	9.63	4.79
4	15	37.74±1.65	37.74	15.65	8.64
5	30	53.90±0.96	53.90	30.15	12.75
6	45	67.73±0.77	67.73	39.46	17.14
7	60	80.82±0.96	80.82	47.65	24.57

*All the values are expressed as mean ± SD, n=3.

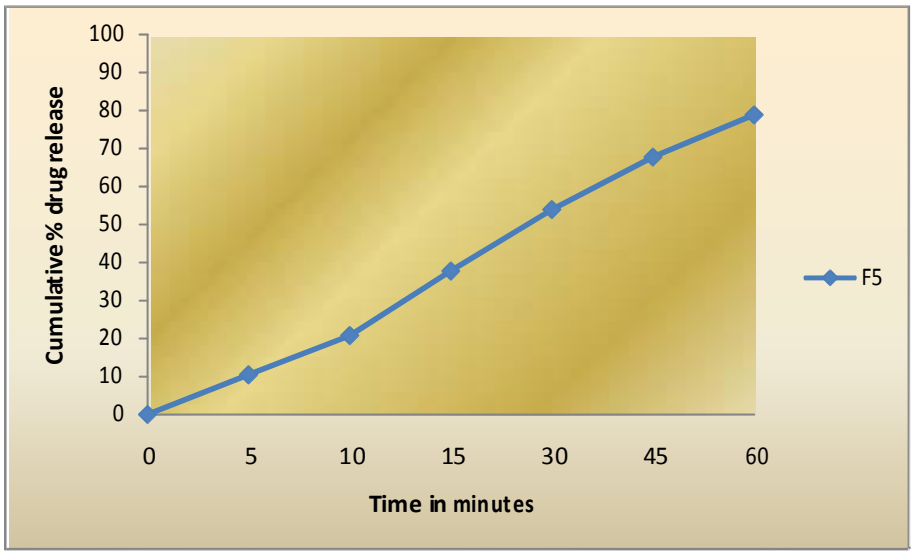


Fig.9.21: Dissolution profile of formulation F5

Table 9.18: Dissolution profile of formulation F6

S. No.	Time (min)	DR* (%)	Amount (mg)	% DE	MDT
1	0	0.00	0.00	0.00	0.00
2	5	9.48±1.22	9.48	5.71	2.50
3	10	20.83±1.89	20.83	11.04	4.82
4	15	36.84±1.55	36.84	16.59	7.69
5	30	53.31±1.72	53.31	29.56	12.62
6	45	61.44±0.91	61.44	38.83	17.58
7	60	68.16±2.17	68.16	45.76	20.43

*All the values are expressed as mean ± SD, n=3.

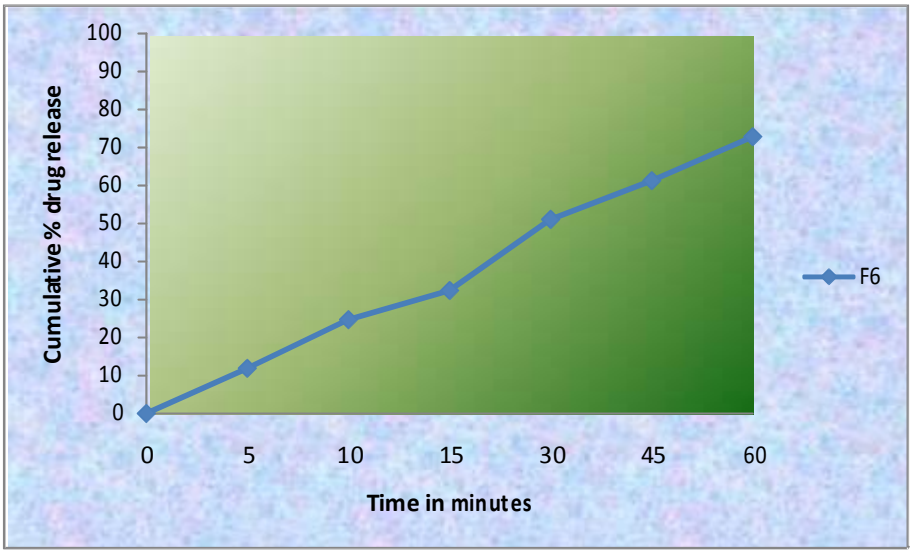


Fig.9.22: Dissolution profile of formulation F6

Table 9.19: Dissolution profile of formulation F7

S. No.	Time (min)	DR* (%)	Amount (mg)	% DE	MDT
1	0	0.00	0.00	0.00	0.00
2	5	10.38±0.44	10.38	5.00	2.50
3	10	26.59±0.89	26.59	10.69	5.30
4	15	36.85±1.41	36.85	16.82	7.88
5	30	56.59±1.57	56.59	31.44	13.35
6	45	68.38±1.26	68.38	41.97	17.78
7	60	77.47±0.80	77.47	49.89	21.56

*All the values are expressed as mean ± SD, n=3.

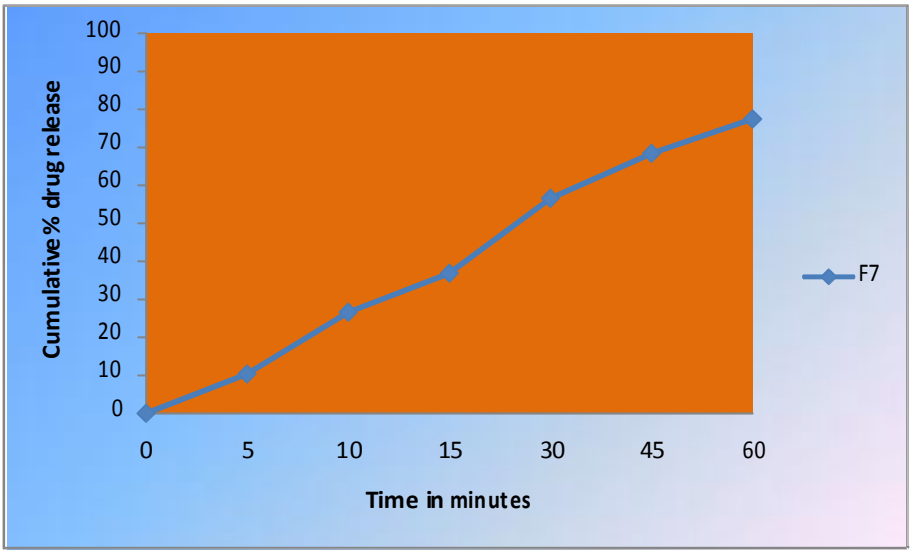


Fig.9.23: Dissolution profile of formulation F7

Table 9.20: Dissolution profile of formulation F8

S. No.	Time (min)	DR* (%)	Amount (mg)	% DE	MDT
1	0	0.00	0.00	0.00	0.00
2	5	11.63±0.96	11.63	5.71	2.50
3	10	24.79±0.89	24.79	11.39	4.99
4	15	37.49±1.19	37.49	17.53	7.87
5	30	55.27±0.89	55.27	32.50	13.21
6	45	70.71±1.06	70.71	42.68	16.75
7	60	83.68±0.86	83.68	50.95	23.40

*All the values are expressed as mean ± SD, n=3.

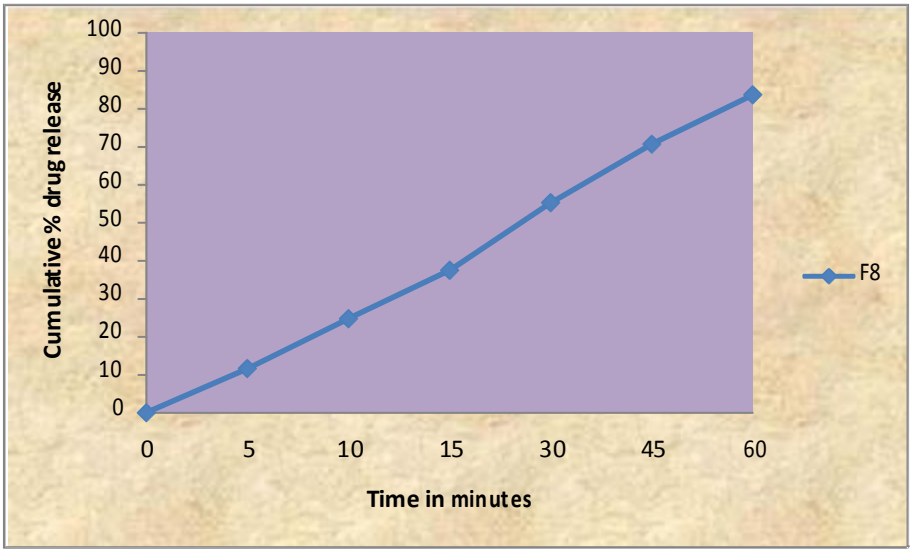


Fig.9.24: Dissolution profile of formulation F8

Table 9.21: Dissolution profile of formulation F9

S. No.	Time (min)	DR* (%)	Amount (mg)	% DE	MDT
1	0	0.00	0.00	0.00	0.00
2	5	7.74±1.33	7.74	2.88	2.50
3	10	19.82±1.20	19.82	7.15	5.81
4	15	31.18±0.96	31.18	12.82	8.84
5	30	48.63±1.17	48.63	26.26	13.65
6	45	63.52±1.65	63.52	36.39	19.86
7	60	73.31±1.79	73.31	44.82	24.17

*All the values are expressed as mean ± SD, n=3.

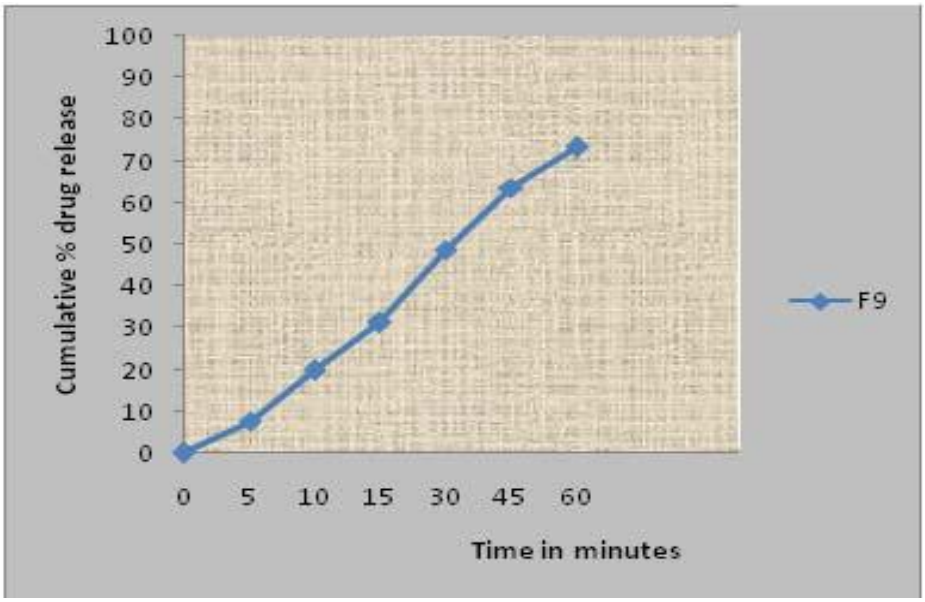


Fig.9.25: Dissolution profile of formulation F9

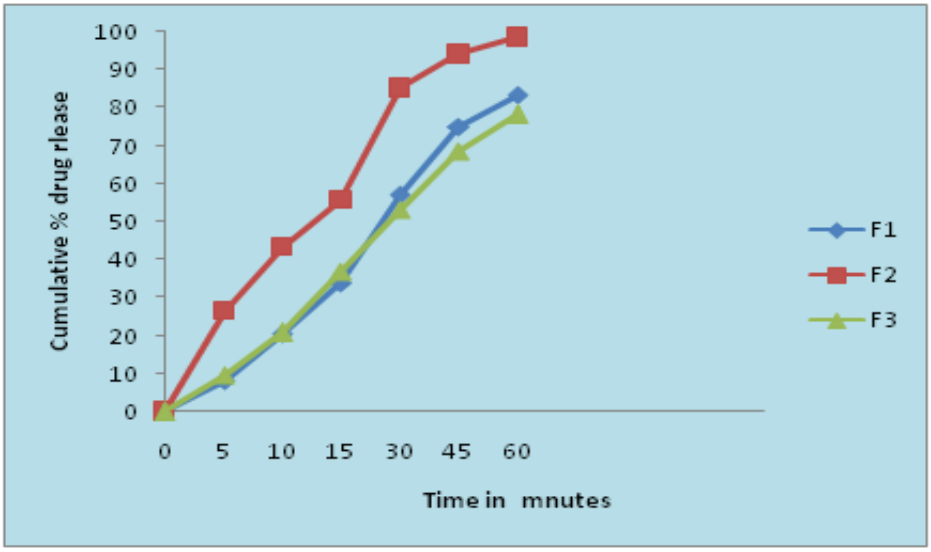


Fig.9.26: Dissolution profile of inclusion complex (kneading method) containing various ratio i.e. 1:1, 1:2, 1:3

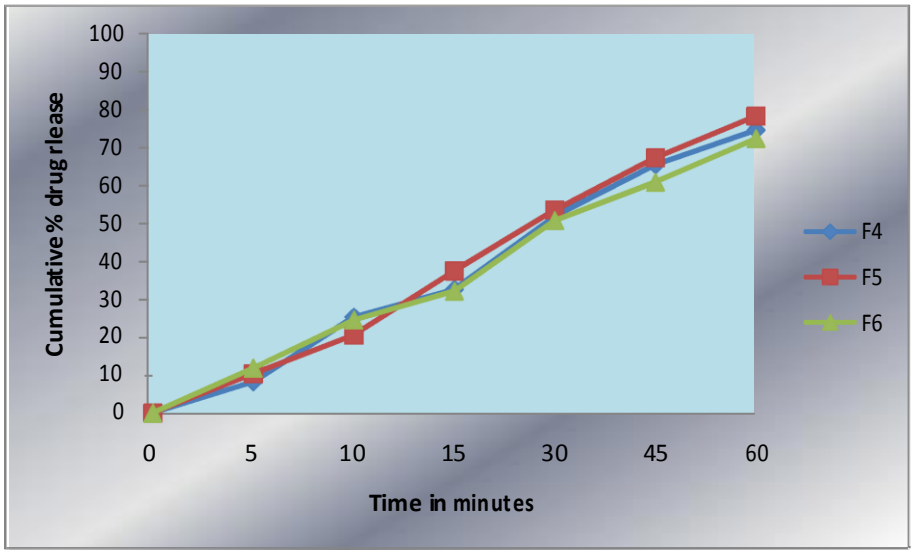


Fig.9.27: Dissolution profile of inclusion complex (common solvent method) containing various ratio i.e. 1:1, 1:2, 1:3

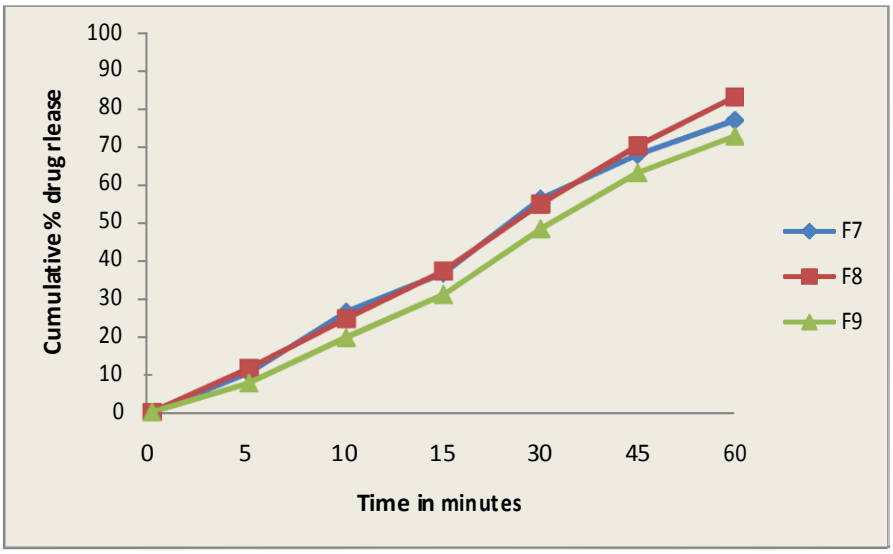


Fig.9.28: Dissolution profile of inclusion complex (physical mixture) containing various ratio i.e. 1:1, 1:2, 1:3

Table 9.22: Comparative dissolution profile of all formulations

Time in (min)	Percentage drug release (%)								
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
0	0	0	0	0	0	0	0	0	0
5	8.25± 0.81	26.55± 0.80	8.27± 1.21	11.89± 1.33	10.45± 1.17	9.48±1. 22	10.38± 0.44	11.63± 0.96	7.74± 1.33
10	20.48± 1.22	43.33± 1.05	25.46± 0.67	24.68± 0.89	20.74± 1.49	20.83± 1.89	26.59± 0.89	24.79± 0.89	19.82 ±1.20
15	33.86± 1.61	55.55± 1.92	32.83± 0.30	32.37± 1.25	37.74± 1.65	36.84± 1.55	36.85± 1.49	37.49± 1.19	31.18 ±0.96
30	57.26± 0.33	85.18± 1.20	52.28± 0.22	51.09± 1.53	53.90± 0.96	53.31± 1.72	56.59± 1.57	55.27± 0.89	48.63 ±1.17
45	75.09± 1.63	93.99± 0.29	65.89± 0.89	61.28± 1.40	67.73± 0.77	61.44± 0.91	68.38± 1.26	70.71± 1.06	63.52 ±1.64
60	83.37± 1.81	97.54± 0.89	75.09± 1.49	72.85± 1.97	80.82± 0.96	68.16± 2.17	77.47± 0.80	83.68± 0.86	73.31 ±1.79

*All the values are expressed as mean ± SD, n=3.

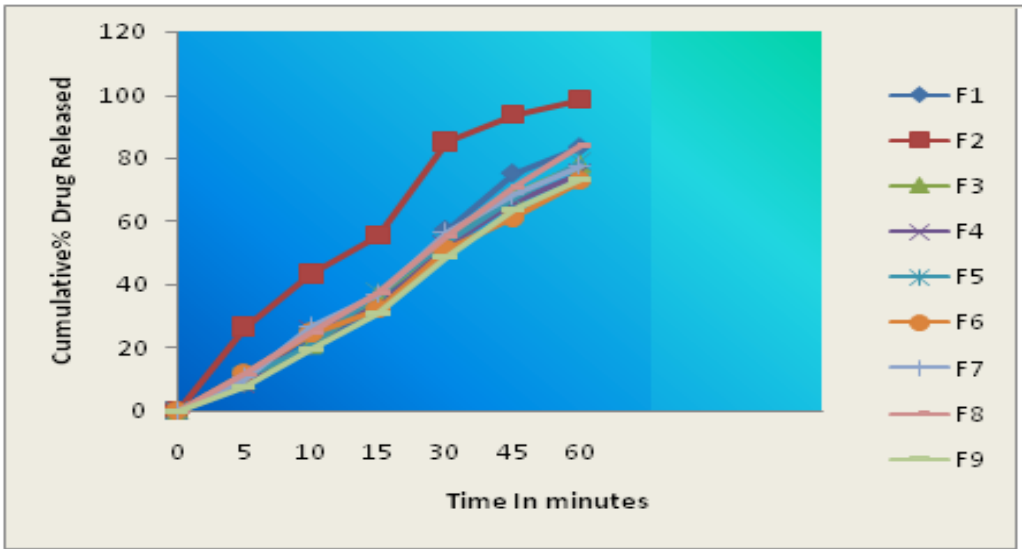


Fig.9.29: Comparative dissolution profile of all formulations

Fig.9.29: Comparative dissolution profile of all formulations

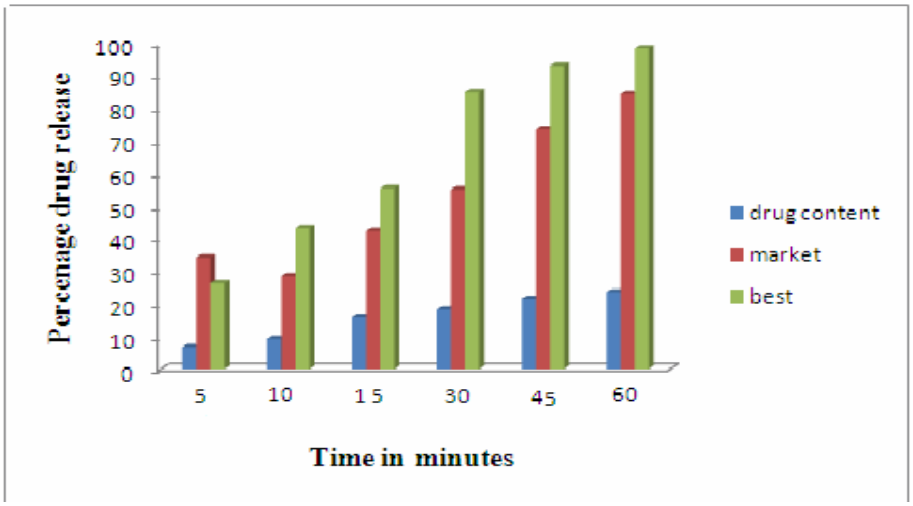


Fig. 9.30: Comparative dissolution profile of drug content, marketed formulation and formulation F2

The dissolution studies revealed that all the formulations showed and increased rate the complex prepared by kneading method was found to yield a complex of higher rate of dissolution over common solvent and physical mixture method. The results of the dissolution study indicates that formulation F2 prepared by kneading method released to the maximum amount of drug and gave maximum percentage of drug release was above 97.54 %±0.89 within hour when compared to all other formulations.

The dissolution study of all the formulations was showed in Table 9.17-9.21 and Fig. 9.17-9.25.

9.9. Stability Studies:

After storage the formulation F2 was analyzed for various parameters, results are showed in Table 9.23.

Table 9.23: Stability study of formulation F2 of inclusion complex Aceclofenac at room temperature 25° C ± 2° C / 60 % RH ± 5 % and accelerated temperature 40° C ± 2° C / 75 % RH ± 5 %.

Parameter	Initial	At 25° C ± 2° C / 60 % RH±5 %			At 40° C ± 2° C / 75 % RH ± 5 %.		
		1st Month	2nd Month	3rd Month	1st Month	2nd Month	3rd Month
Drug content (%)	99.69± 1.20	99.31± 1.54	99.20± 0.32	99.06± 0.28	99.54± 0.52	99.17± 0.35	99.02± 0.41
***In vitro* drug release at end of 60 minute.**	97.54± 0.89	97.39± 0.19	97.07± 0.98	96.81± 0.68	96.62± 1.11	96.35± 0.32	96.11± 1.01

*All the values are expressed as mean ± SE, n=3.

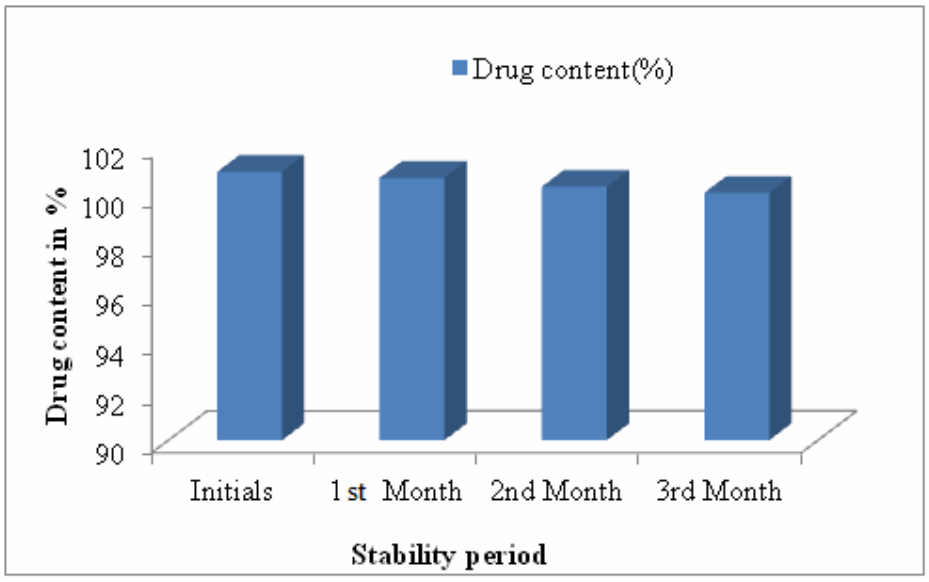


Fig.9.31: Comparisons of drug content before and after stability period at room temperature (25° C ± 2° C / 60 % RH ± 5 %)

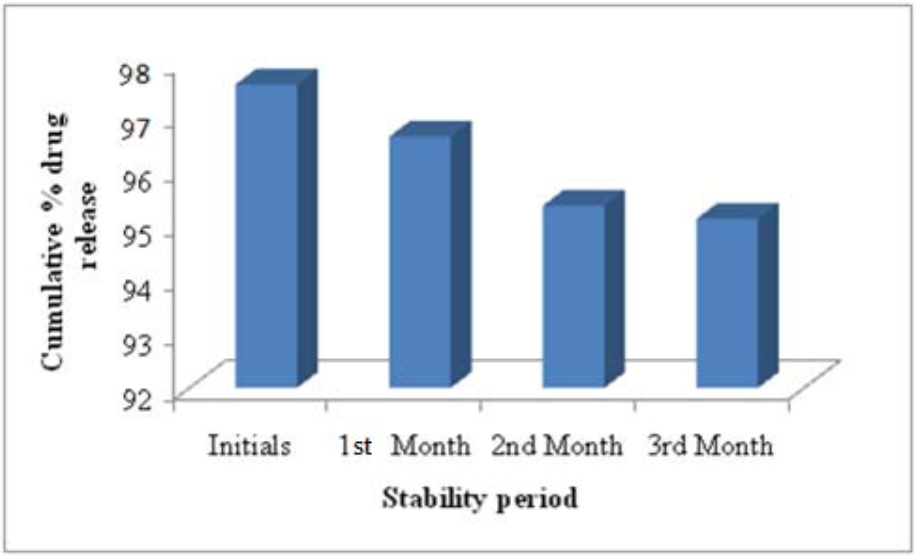


Fig.9.32: Comparisons of *in vitro* cumulative % drug release before and after stability period at room temperature (25° C ± 2° C / 60 % RH ± 5 %)

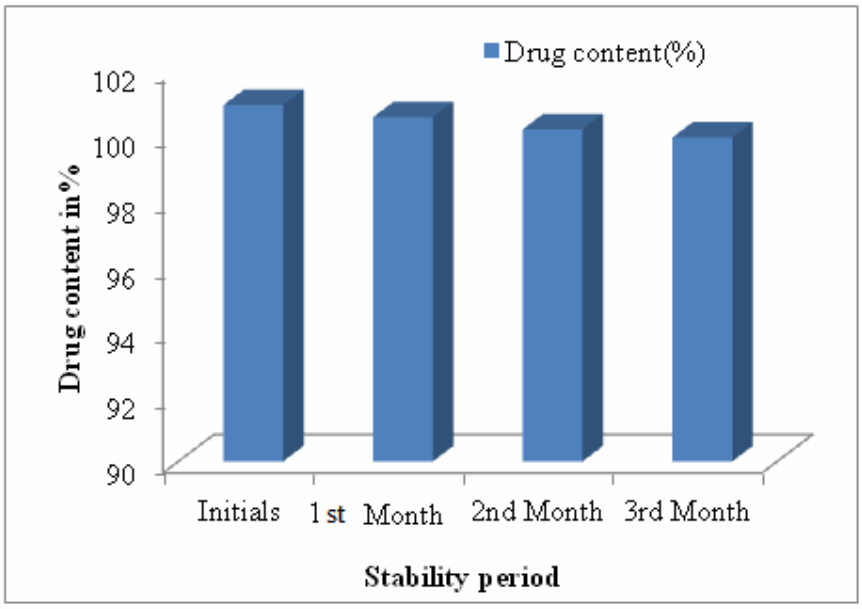


Fig.9.33: Comparisons of drug content before and after stability period at accelerated temperature (40° C ± 2° C / 75 % RH ± 5 %)

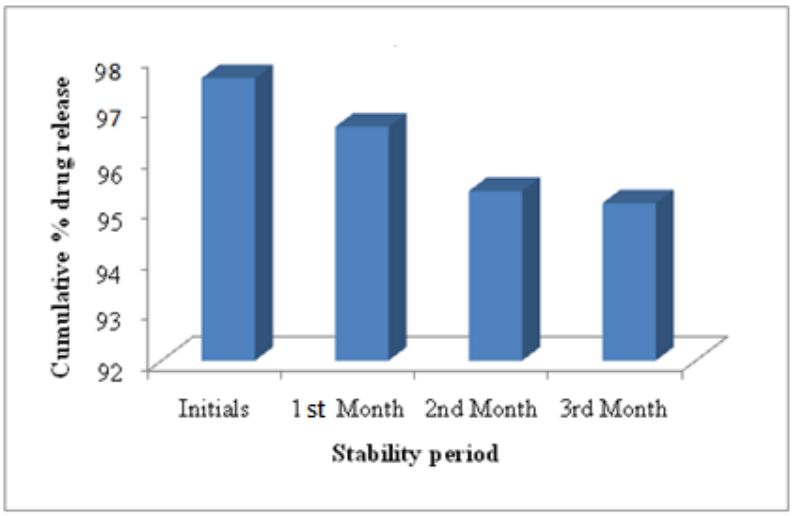


Fig.9.34: Comparisons of *in vitro* cumulative % drug release before and after stability period at accelerated temperature (40° C ± 2° C / 75 % RH ± 5 %)

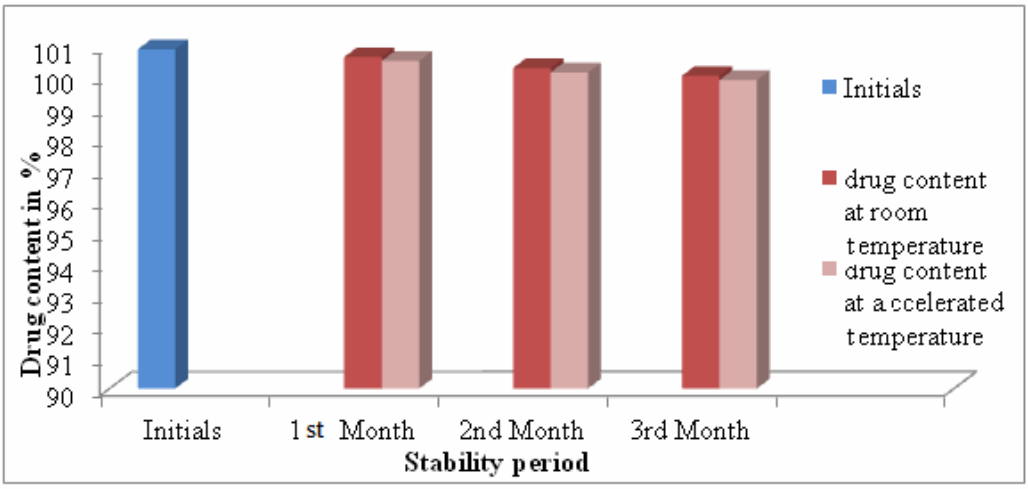


Fig.9.35: Comparisons of drug content before and after stability period at room temperature and accelerated temperature

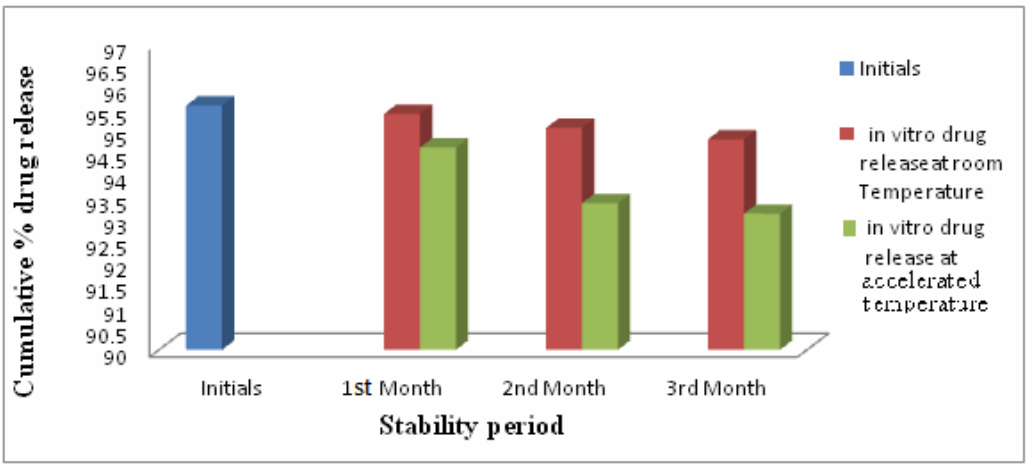


Fig.9.36: Comparisons of *in vitro* cumulative % drug release before and after stability period at room temperature and accelerated temperature

The complex prepared by kneading method in the molar ratio of 1:2 (formulation F2) was found to exhibit a better stability under certain storage condition.

There were no major differences between the evaluated parameters like drug content, *in vitro* dissolutions and all the results were acceptable limits.

SUMMARY AND CONCLUSION

10.SUMMARY AND CONCLUSION

Among the emerging new chemical entities, most are poorly water soluble drugs putting impact on their bioavailability and therapeutic effect. The solubility enhancement techniques also play an important role in getting the excellent dissolution properties of poorly soluble drugs. Successful improvement in aqueous solubility is mainly depends on the method which we choose. Inclusion complex with β-cyclodextrins is the most attractive technique to enhance aqueous solubility of poorly soluble drugs. β-cyclodextrins, act as the useful solubilizer enabling both solid/liquid oral and parenteral dosage forms. Solid binary system of drug and β-cyclodextrins are capable to modify the physicochemical properties of drugs such as solubility, particle size, crystal habit, thermal behavior, and there by forming a highly water soluble amorphous forms. The β-cyclodextrins, due to their extreme high aqueous solubility, they became capable to enhance the dissolution rate and bio-availability of the poorly soluble drugs. The permeation of insoluble drugs through various biological membranes can also be enhanced by preparing drug- β-cyclodextrins inclusion compounds.

The present study was aimed to improving the dissolution of poorly soluble drug Aceclofenac, being a drug acidic in nature shows a poor solubility in biological fluids. Hence, an attempt was made to prepare β-cyclodextrins inclusion complex of Aceclofenac by different techniques.

Literature survey revealed that cyclodextrin has been extensively study to improve solubility, dissolution rate of various drugs. Due to its less price and easy

availability, β-cyclodextrin is widely used. It is a less toxic among all other derivatives of cyclodextrins.

The preliminary work was studied by performing preformulation studies. The identification drugs was determined by conducting the experiment on melting point, solubility, loss on drying, FTIR and assay. The data obtained from these studies indicated that the drugs are confirmed as Aceclofenac.

β-cyclodextrin inclusion complex were prepared by different methods like kneading, common solvent and physical mixture in the ratio of 1:1, 1:2 and 1:3.

The prepared inclusion complex characterised by various analytical techniques like UV spectrophotometer, Fourier Transform Infrared Spectroscopy, Differential Scanning Calorimetry and X-ray diffraction studies.

The phase solubility studies were carried out according to the method Higuchi and Connors. It revealed that the solubility of Aceclofenac increased linearly with the increase in concentration of β-cyclodextrin.

The UV spectra of Aceclofenac and inclusion complex in the Methanol were studied to know maximum absorbance (λ_{max}). There was no shift in the λ_{max} of Aceclofenac in the presence of β-cyclodextrin.

Fourier Transform Infrared Spectroscopy and Differential Scanning Calorimetry indicated the formation of true inclusion complexes of Aceclofenac with β-cyclodextrin in 1:2 ratio prepared by kneading method.

The X-ray diffraction pattern confirmed the formation of complex with β-cyclodextrin and the substance exhibits amorphous form.

The drug content of **formulation F2** was found to be official limit.

From the dissolution profiles of inclusion complex prepared by kneading method concludes that β-cyclodextrin is more meaningful for the enhancement of solubility and dissolution rate of Aceclofenac.

The inclusion complex prepared by kneading method revealed that more stable in the molar ratio of 1:2 at the end of three months.

So, it can be concluded that inclusion complex of Aceclofenac with β-cyclodextrin could be prepared successfully by kneading method in a molar ratio of 1:2.

It can be concluded that an inclusion complex of Aceclofenac with β-cyclodextrin could be prepared successfully by kneading in a molar ratio of 1:2 and this was confirmed by phase solubility studies, UV spectrophotometry, Fourier Transform Infrared Spectroscopy, Differential Scanning Calorimetry, X-ray diffraction, drug content estimation, *in vitro* dissolution study and stability studies.

From the overall studies **formulation F2** considered as best formulation.

FUTURE PROSPECTUS

11.FUTURE PROSPECTUS

In the present work summarized and concluded the β-cyclodextrin inclusion complex of Aceclofenac were prepared successfully by kneading method and it was characterised by various analytical techniques. In the present investigation *in vitro* characterisation was performed. Along with this study there is need of *In vivo* studies to set up the *in vitro – in vivo* correlation for the better efficacy of Aceclofenac in the treatment of rheumatoid arthritis with reducing gastrointestinal side effects.

Long term stability to be performed in future as per the ICH guidelines.

The best inclusion complex of Aceclofenac was chosen in future for manufacturing of tablet formulation and evaluated for *in vitro* and *in vivo* performance.

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