DESIGN, DEVELOPMENT AND EVALUATION OF ORAL CONTROLLED DRUG DELIVERY SYSTEM (FOR ACECLOFENAC, AN ANTI-INFLAMMATORY DRUG)

Thesis Submitted To

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY, GUINDY, CHENNAI-600-032

In partial fulfillment of the requirement for the award of the degree of

DOCTOR OF PHILOSOPHY

FACULTY OF PHARMACY

Submitted by

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MARCH 2012

DECLARATION

This is to certify that the thesis, entitled **"Design, development and evaluation of oral controlled drug delivery system (for Aceclofenac, an Anti-inflammatory drug)"** is submitted to The Tamilnadu Dr. M.G.R. Medical University, Chennai in partial fulfillment of the requirements for the award of degree of Doctor of Philosophy is the record of original research work done by me under the guidance and supervision of **Prof. Dr. V. Ravichandiran**, Principal, Vels College of Pharmacy, Pallavaram, Chennai-600117 for the academic year 2008 – 2012 and the thesis has not formed the basis for the award of any Degree, Diploma, Associateship, Fellowship or other similar title.

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Date:

Place: **Prof. Dr. V. RAVICHANDIRAN**

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(K.MASILAMANI)

List of Abbreviations Used

bid	-	twice daily
^{0}C	-	degree celsius
Cm	-	Centimeter
DSC	-	Differential Scanning Calorimetry
EC	-	Ethyl cellulose
eg	-	Example
FT-IR	-	Fourier transform infra red
HCl	-	Hydrochloric acid
hr	-	Hours
KBr	-	Potassium bromide
kg	-	Kilogram
L	-	Litre
µg	-	Microgram
µL	-	Microlitre
µg	-	Microgram
MEC	-	Minimum Effective Concentration
mg	-	milligram
MTC	-	Maximum Therapeutic Concentration
min	-	Minutes
mL	-	Millilitre
mV	-	milliVolt
NS	-	Nanosuspension
NaOH	-	Sodium hydroxide
NS	-	Nanosuspension
NP	-	Nanoparticle
OCDDS	-	Oral controlled drug delivery systems
OT	-	Osmotic tablets
PMMA	-	polymethy (methacryalic acid)
rpm	-	revolution per minute
SD	-	Standard deviation
S.E.M	-	Standard error mean
V/V	-	Volume / Volume

W/V	-	Weight / Volume
W/W	-	Weight / Weight
MLBG	-	modified locust bean gum
%	-	Percentage

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1. INTRODUCTION

ORAL CONTROLLED DRUG DELIVERY SYSTEMS

Oral drug delivery is the most common and frequently used system to deliver drugs and gained more attention because of its flexibility in designing the dosage form when compared to other routes. Oral route is one of the most suitable, convenient, safe, economic and effective way to deliver the drug[1].

For maintaining drug concentration in blood within the therapeutic window, the conventional dosage forms needed to be taken several times a day. This results in significant fluctuations of drug level in blood and thus producing poor therapeutic efficacy, toxicity and unwanted side effects[2].

The goal of any newer drug delivery system is to release the desired amount of drug at the target site in the body. Two parameters have to be considered for the drug delivery system, one is the time which the drug is released from the dosage form and the other is the quantity of the drug which is released. Conventional system fails to control these two parameters.

The problems associated with conventional formulations are

i) The dose missed may lead to poor patient compliance
ii) Maintenance of steady state is difficult
iii) Possibility of side effects

In terms of sustained or controlled systems the drug is released in a predetermined rate for specified time duration.

The novel controlled drug delivery systems provide beneficial in patients with chronic illness, while conventional system have little or no control over drug release and leads to patient non-compliance.

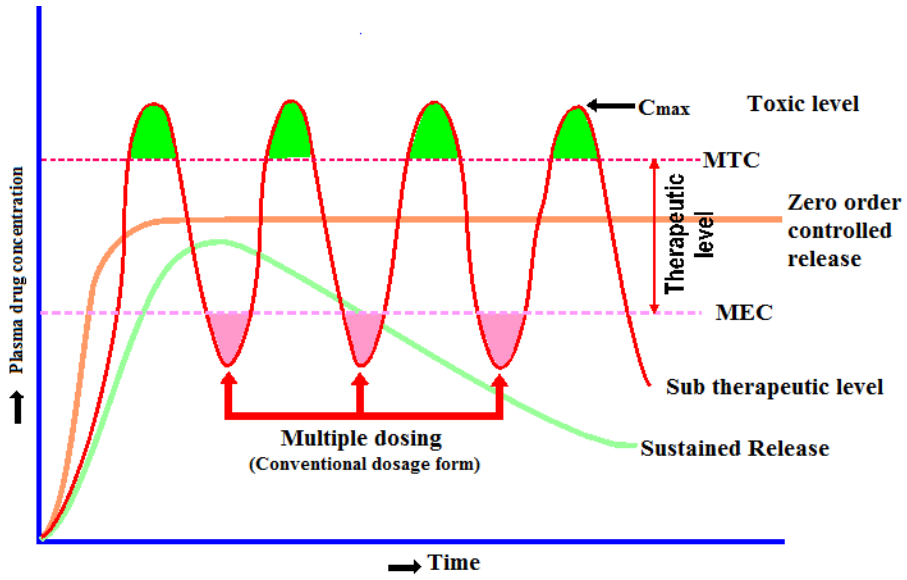


Fig.1.1 Hypothetical Plasma Concentration – Time profile curve of Conventional, SR and CR formulations.

The above figure clearly depicts that,

- Multiple dosing of conventional formulation may not produce steady state concentration; it may cross sub-therapeutic or toxic level easily.
- SR produces long lasting effect, but in certain cases less than therapeutic level.
- CR produces constant level of drug concentration throughout the treatment period

In order to overcome the drawbacks of conventional drug delivery systems, several technical advancements have been led to the development of controlled drug delivery system that could revolutionize the therapeutic regimen and provide a number of therapeutic benefits.

Controlled drug delivery systems are the one which delivers the active medicament at predetermined rate for a specified time period.

Conventional, fast dissolving, sustained or controlled delivery of drugs can also be achieved through oral route. Biological half-life is the major parameter which an R& D person has to look up to design a sustained or controlled drug delivery.

Need for Controlled Oral Drug Delivery Systems:

The development of controlled release systems by oral route will lead to:

- Improved patient convenience due to less frequency of drug administration.
- Minimized fluctuation and maintains steady-state levels of drug concentration in blood.
- More consistent and prolonged therapeutic effect.
- Decreased intensity of adverse effects and toxicity.
- Maximum drug utilization resulting in reduction of total quantity of doses.
- Improved bioavailability of drugs.
- A greater selectivity of pharmacological activity.
- Reduction in health care costs.

Ideal characteristics of drugs to design as controlled drug delivery[3]:

- The drug should possess half-life within the range of 2-8 hrs.
- Drugs possessing wide therapeutic index.
- Drugs which require enhanced bioavailability.
- The drug to be loaded in the dosage form should be less than 1gm.
- Disease conditions which the controlled release is required.
- Reduction in cost due to overall reduction in dose of the drug.
- Drugs possessing side effects in conventional therapy.
- A common dose of the drug must be useful for larger population, rather than individualized dose requirement.

Usually controlled release systems are designed to release the drug for a period of 12hr to 24hr but in some cases it may be designed to release for several days also.

Advantages of oral controlled release systems:

- Therapeutically it reduces the drug plasma level fluctuation and maintains steady plasma level over the specified period of time.
- Reduction in adverse effects.

Usually adverse effects arise when the drug crosses the maximum therapeutic level or toxic level. This usually happens in conventional dosage forms. In controlled delivery, drug is maintained within therapeutic window. Reduction in drug quantity by designing CR also holds responsibility for reduction in side effects.

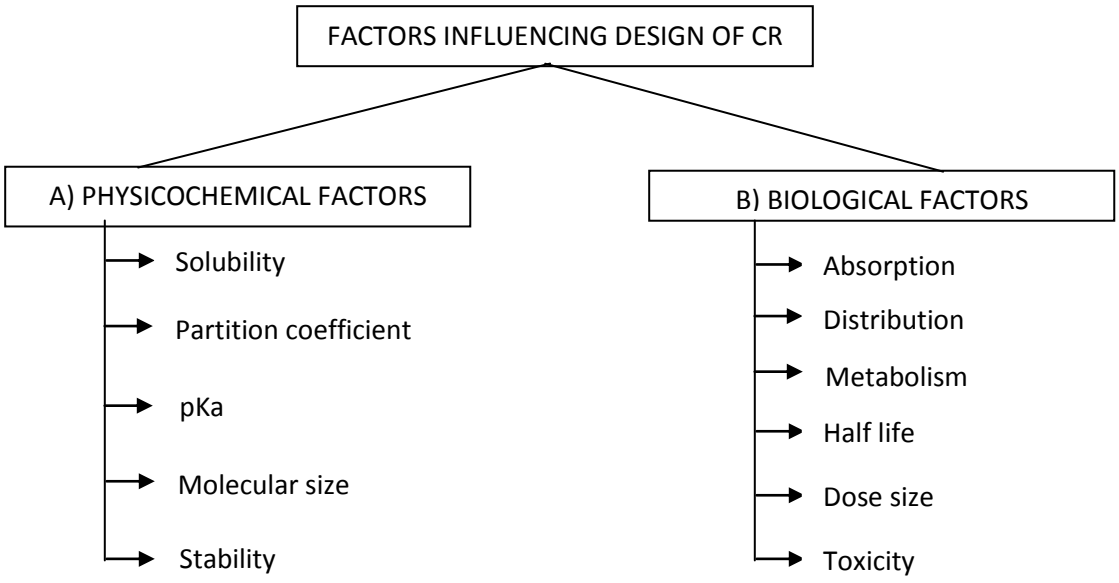
- Reduction in dosing frequency which may solve patient problems
- For better tolerability of some drugs in vivo – E.g. Rivastigmine tartrate[4]
- Cost effective

Disadvantages of Controlled Release Delivery System:

- Difficult to achieve in vitro-in vivo correlations
- In some cases burst release may produce dose dumping
- Patient education is needed to avoid crushing / breaking of CR systems
- High cost due to utilization of advanced technologies for their manufacturing.

Factors influencing design of oral controlled release products:

To make a dosage form successful it is important prerequisite to monitor the following factors carefully.



The physicochemical properties can be studied through in vitro studies and biological properties can be studied through in vivo studies.

MECHANISM OF ORAL CONTROLLED RELEASE:

The mechanisms of oral controlled release are as follows:

i) Diffusion
ii) Dissolution
iii) Diffusion and Dissolution
iv) Osmosis
v) Ion-exchange resin

(i) Diffusion Controlled Systems:

In these systems, the drug release rate is determined by diffusion through a water insoluble polymer. The diffusion devices may be of reservoir or matrix. In reservoir type a core of active medicament is surrounded by a polymeric membrane. Fick's first law of diffusion is applicable for this type[5]. Coating materials like gelatin, celluloses, PMMA, HPC, PVA may be used to design reservoir type[6]. In matrix type the dissolved or dispersed drug is distributed uniformly throughout the polymer. This type follows Higuichi model.

(ii) Dissolution controlled systems:

In these systems dissolution of the therapeutic agent is controlled by coating the drug by encapsulating substances like polymer which shows slow release. The release time can be altered by governing the control of polymeric coating layers[7]. These systems may be encapsulated or matrix products.

(iii) Dissolution & diffusion controlled release system:

Drug is surrounded by a partially soluble membrane. Pores to release the drug are formed by dissolution. The pores, only permits water to entry into it. From the pores the drug is diffused out. Drug release rate is calculated by[8]

$$\text{Release rate} = AD(C_c - C_d)/L$$

Where A= Surface area

D = diffusion coefficient of drug through pore in coating

L = diffusion path length

C_c = concentration of drug in the core

C_d = concentration in the dissolution medium

(iv) Osmosis:

Osmosis is one of the effective mechanisms to achieve oral controlled release. Usually osmotic systems have 3 components namely osmogen, semi-permeable membrane and delivery orifice[9]. The semi-permeable membrane allows water inside the core, but doesn't allow the drug to come out. Osmotic agent creates osmotic pressure which induces the diffusion of drug in controlled manner through the delivery orifice.

(v) Ion-Exchange Resins:

In this system drug is released by exchange of drug ions attached with the resin by the ions of dissolution media. The driving force required for exchange of ions may be derived from electrostatic interactions. Opposite charged resin may suit the attachment for the drug with the resin.

Currently available oral controlled drug delivery systems are osmotic tablets, floating tablets, matrix tablets, colonic release, plastic matrices, ion exchange resin tablets, film coated tablets, enteric coated and delayed release tablets, swellable tablets, mucoadhesive tablets, multiple unit tablets, repeat action tablets, floating capsules, microgranules, spheroids, beads, pellets, microcapsules, microspheres and nanoparticles.

Polymers in the preparation of oral controlled drug delivery systems[10]:

Polymers are macromolecules comprising of small molecular units named monomers. These monomers are linked to form linear polymers, branched polymers or cross linked polymers. The biodegradable polymers can be defined as polymers comprised of monomers linked to one another through functional groups and have unstable linkages. They are biologically degraded or eroded by enzymes.

In the preparation of controlled release dosage forms, the polymer acts as a carrier for drug which aids in achieving controlled and targeted drug delivery. The polymers used should be inert, non-toxic and easy to fabricate.

ORAL OSMOTIC SYSTEMS:

The rate and extent of drug absorption generally depends on various factors such as physicochemical properties of the drug, presence of excipients, etc. Physiological parameters which influence drug concentration include presence/absence of food, pH of GIT, GI motility, pH of stomach etc[11].

Therapeutic agents can be effectively delivered in a controlled pattern over a long period of time by osmotic drug delivery systems.

Interestingly, this system is pH independent and other physiological parameters and hence regulates the release by optimizing the properties of drug and systems.

Merits of Osmotic drug delivery systems[12,13]:

- Provides ease in formulation with simple operational procedures
- Ensures better compliance with reducing dose intervals
- Drug release is independent of gastric pH, food etc.
- Follows zero-order rate in delivery
- Delayed or pulsed delivery possible
- Predictable and programmable delivery rate
- Drugs of varying solubility can be easily incorporated
- Food interaction is minimal

Disadvantages:

- High cost
- Dose dumping

Principle of Osmosis:

It is a phenomenon which a solvent molecule travels from lower concentration part to higher concentration part across a semipermeable membrane.

The effect of osmosis was first reported by Abbe Nollet, in 1748, but Pfeffer reported the osmotic effect by using a selectively permeable membrane to water however impermeable to solute, which showed separation of sugar solution from pure water[14].
Vant'Hoff established the analogy between the Pfeffer results and the ideal gas laws by the expression[15].

$$\Pi = n_2RT$$

Where, n = molar concentration of solute in solution

R= gas constant

T = Absolute temperature

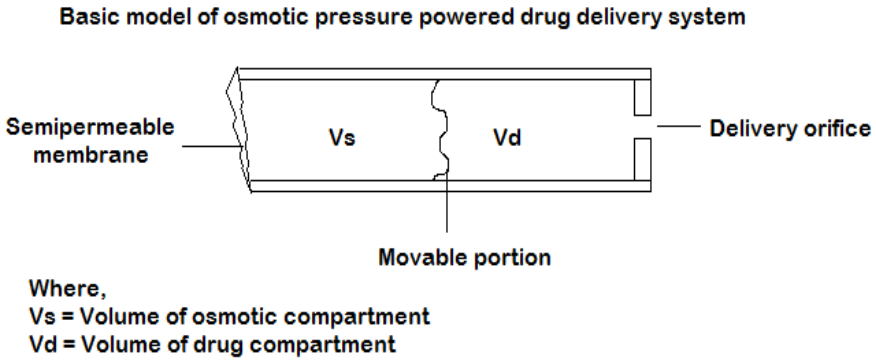


Fig 1.2. Osmotic pressure delivery system

Criteria to be followed to achieve zero order release:

- Amount of osmogen used should be sufficient to maintain saturated solute in osmotic agent compartment
- Osmotic activity of the environment to be either a constant or negligibly small
- Osmotic reflux coefficient should be constant or very close to unity, which means ideally a semipermeable membrane, selectively permeable to water but not to osmotic drug to be used
- Delivery orifice to be made sufficiently large and highly deformable partition should be used

Steps involved in release of drug from an osmotic delivery system:

In general, the release of the drug from an osmotic pump occurs in three stages

- Movement of solvent across a semi-permeable membrane from lower concentration of solute to higher concentration of solute.
- Development of osmotic pressure inside the osmotic system
- Pumping out of drug solution/suspension through the delivery orifice in controlled manner

Factors affecting rate of drug release:

The major factors which affect the rate of drug release from the osmotic drug delivery systems are drug solubility, osmotic pressure, orifice size and semipermeable membrane.

Basic components of osmotic pumps:

The components required for formulation of osmotic pumps are drug, osmogens, semipermeable membrane, polymers, wicking agents, surfactants, coating solvents, plasticizers, flux regulators and pore formers[16].

i) Drug and Osmogen:

Sometimes drug may act as an osmogen and may show good aqueous solubility. Usually drug in salt form is more soluble than the parent drug. If drug does not possess osmogenic property, osmotic salt and other sugar may be added to formula. An ideal osmogen should possess osmotic activity and aqueous solubility. Examples are NaCl, KCl, NaHCO3, methylcellulose etc.

ii) Semipermeable membrane:

The unique property of semipermeable membrane utilized for osmotic pump is that it permits only movement of water into system, and hence effectively isolates the dissolution process from GI environment.The increased permeability would be achieved by ideal membrane material.

Cellulose acetate is most commonly employed as a semipermeable membrane for preparation of osmotic pumps. Other examples include agar acetate, amylasetriacetate, polyacetals, polyglycolic acid, poly lactic acids etc[17].

iii) Hydrophilic/Hydrophobic polymers:

Polymers are mainly incorporated in the formula of osmotic systems containing matrix core. Selection of polymer is based on solubility of drug as well as amount and release rate of drug from pump.

Eg. Ethyl cellulose, HPMC, wax materials, CMC etc[18].

iv) Wicking agents:

It is an agent with capacity to absorb water into porous system of a delivery device. The main function of this agent is to draw water inside the surface of tablet core, thereby creating network of amplified surface area.

Eg. Colloidal silicon dioxide, kaolin, titanium dioxide, alumina, SLS etc[19].

v) Surfactants:

Surfactants are substances which are added to wall forming agents[20]. They act by regulating energy of materials to improve their blending and helps in maintaining integrity in environment during drug release time period.

Eg. Polyoxethylenated glyceryl retinoleate, polyoxyethylenated castor oil, poly ethylene oxide, glyceryl laureates etc.

vi) Coating solvents:

Inert inorganic and organic solvents are used for manufacturing the wall of osmotic device for making polymeric solution.

Eg. Methylene chloride, methanol, acetone, IPA, ethyl acetate, cyclohexane etc.

vii) Plasticizers:

Plasticizers are used to increase permeability of membrane, by increasing water diffusion co-efficient. Film forming properties of polymers can also be enhanced by addition of plasticizers and low molecular weight diluents. Plasticizer converts hard and brittle polymer to soft and pliable material and hence mechanically more strength.

Eg. Dialkyl phthalate, trioctyl Po4, alkyl adipates, triethyl citrate and other citrates[21].

viii) Flux regulators:

These are agents added to wall forming materials, thereby assist to regulate, fluid permeability through membrane.

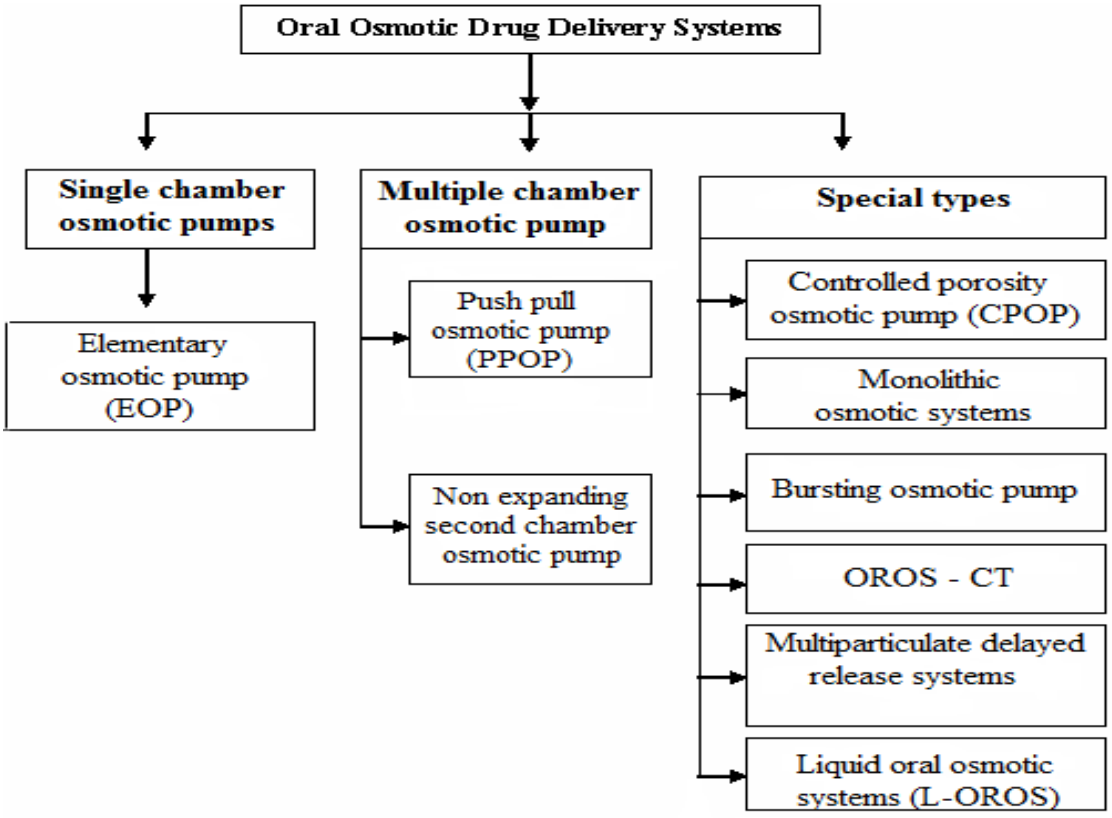
Eg. Polyalkylene glycols, polybutylene, polypropylene, polyamylene etc.

ix) Pore formers:

Pore forming agents are the substances which are added in the coating material to form pores when they come in contact with water. CPOP are designed to release the drug with the help of pores formed by pore forming agents[22].

Eg. PEG 400, diethyphthalate, mannitol

Osmotic pumps classification:



i) Single chamber osmotic pumps:

Elementary osmotic pump (EOP):

EOP is the simplest version of osmotic pump which does not require any special technology or equipment. This consists of moderately water soluble drug with or without an osmotic agent in a single layered core, walled by a semipermeable membrane[23]. When swallowed, water from GIT enters core via membrane, dissolving the drug and the solubilized drug is pumped via the exit orifice. This is a continuous phenomenon; the drug is completely dissolved and pushed out, but at a declined rate. It delivers about 60-80% content at a systematic and constant rate with time lag of 30 – 60 minutes.

ii) Multichambered Types:

a) Push Pull Osmotic Pump (PPOP):

It is modified version of EOP. This system facilitates delivery of poorly as well as highly water soluble drugs at constant rate. This system has two layers one is push layer and another is pull layer. Drug is present in pull layer. The push layer consists of polymeric osmogen with other additives which has the ability to form drug suspension in situ[24]. These layers bonded together by compression and result in single bilayered core. Further the tablet core is coated with semipermeable membrane; followed by a hole which is drilled on the medicament side of the tablet. When placed in water, the osmotic attraction pulls water into compartment to form drug suspension in situ. The same occurs in non-drug layer and results in volumetric expansion causing push of drug suspension out of delivery orifice.

Fig 1.3. PUSH PULL OSMOTIC PUMP (Normal and Expanded)

b) Osmotic pump with non-expanding second chamber:

This contains two compartments, in which the first one contains the drug and the second compartment is intended for the exapandable biologically inert osmogenic agent. Both the compartments are connected with a hole[17]. When water enters, solution of osmotic agent resulted in first chamber passes to the drug chamber via connecting hole and mixes with drug solution leaving device via microporous membrane present in the walls of the chamber. This type is useful to deliver insoluble drugs.

iii) Other special types:

a) Controlled porosity osmotic pumps (CPOP):

In CPOP drug with osmogen and other additives are compressed and coated with semipermeable membrane along with a pore forming material. The specialty of the system

is there is no special drilling required. The drug is released due to the formation of pores by pore forming agents.

The orifice in CPOP is made to form by addition of leachable water soluble component in material used for coating. The rate of flow[16] from CPOP system can be caluculated by the formula.

$$\text{Rate of flow } dV/dt = AK/h\ (D_P - D_R)$$

Where, K = Membrane permeability

A = Area of membrane

D_P = osmotic pressure difference

D_R = hydrostatic pressure difference

The major advantage of the CPOP system is that the drug is released from whole surface of device instead from the single hole hence reduces gastric irritation. Complicated laser drilling is not required and also facilitates very smaller size of tablet with suitable coating material.

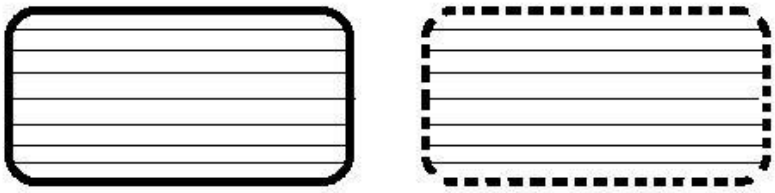


Fig 1.4 CONTROLLED POROSITY PUMP (BEFORE AND AFTER PORE FORMATION)

b) Monolithic osmotic systems:

This system contains polymeric matrix which is water soluble. Hence while in contact with water, imbibition may result in rupture of polymeric matrix, which surrounds the drug capsule. This starts with outer environment initially resulting in a serial fashion to reach the inner matrix.

c) Osmotic bursting pump:

It is identical with EOP and comparatively smaller in size, but with no delivery orifice. Contents are released to the environment by the hydraulic pressure which is exerted on inner wall which rupture resulting because of water imbibition. The thickness of the semipermeable membrane controls the release of drug and hence facilitates pulsated drug release[25].

d) OROS – CT:

It is designed for colon targeting. It is designed as either with single osmotic agent or with numerous (as many as 5-6) push pull osmotic unit compiled in a hard gelatin capsule[13]. While in contact with GIT contents gelatin capsule dissolve and fluids enter through the enteric coating and cause swelling of push compartment and the drug is released from orifice at controlled rate due to formation of flowable gel in drug compartment. Mainly employed for OD or BID dosing of targeted drug delivery of colon.

e) Multiparticulate delayed release systems (MPDRS):

MPDRS comprises mainly pellets of drug with or without osmogen, coated with semipermeable membrane. In this system saturated solution of soluble compartment results due to penetration of water to the core resulting in swelling of membrane and pores are formed. The drug release follows zero order kinetics.

f) Liquid oral osmotic systems (L-OROS):

L-OROS systems were developed to manufacture a drug in dissolved state in liquid and released from a soft gelatin capsule. It is preferred for delivery of liquid form, as being associated with extended release and high bioavailability[26].

EVALUATION:

Drug content, osmotic pressure, swelling properties, orifice diameter, membrane thickness and dissolution test are the evaluation parameters to be checked for osmotic systems.

Table 1.1. APPLICATIONS OF OSMOTIC DRUG DELIVERY SYSTEMS:

S.No	Drug	Category	Osmogen	Type
1	Ibuprofen[27]	Anti-inflammatory	NaCl, PEG 6000	EOP
2	Isradipine &	Anti-hypertensive	PEO	PPOP
	Chlorpheniramine maleate[28]	Anti-histaminic		
3	Isradipine[29]	Anti-hypertensive	PEO	PPOP
4	Diltiazem HCl[30]	Anti-anginal	NaCMC, mannitol	EOP
5	Nifedipine[24]	Anti-anginal	KCl, PEO	PPOP
6	Pramipexole[31]	Anti-parkinsonism	Nacl, NaHCO3, mannitol	PPOP
7	Theophylline[32]	Anti-asthmatic	KCl, tartaric acid	CPOP
8	Pentazocine[33]	Analgesic	NaCl, pectin	PPOP
9	Diclofenac[34]	Anti-inflammatory	NaCl	CPOP
10	Glipizide[19]	Anti-diabetic	Mannitol	CPOP

MICROSPHERES:

Microspheres are the novel drug delivery systems in which the drug can be incorporated into a carrier to provide controlled and targeted release thereby reducing the risk of dose dumping, short gastric residence time and systemic toxicity[35]. Drug release is achieved by diffusion, dissolution, erosion or osmosis.

Microspheres can be simply defined as mono or multi nuclear materials embedded in spherical coating matrix. These are small solid spherical particles typically with a size range of 1 to 1000μm. They are usually made up of biodegradable polymeric materials which may be synthetic, natural or modified natural type.

Microsphere can solve problems of conventional dosage forms and enhances the therapeutic efficacy of a drug. Microspheres are free flowing powders which make it easy for compressing into tablets or filling in capsules[36,37]. The flow property, elegancy and fine texture are due to its spherical shape.

Microspheres with very small size have colloidal properties. The colloidal property is one of the factors which influence the pharmacological activity of the incorporated drug[38].

Microspheres can be best utilized for targeting to tumor cells, gastro-intestinal (GI) inflammations and diseases related to brain[39]. Microspheres are composed of matrix systems and are spherical in shape whereas microcapsules may either be spherical or non-spherical in shape. Microcapsules are small discrete particles, which contain a core material surrounded by a coating or shell. Microcapsules contain entrapped drug surrounded by capsule wall.

Advantages[40-41]:

The major advantages of the microspheres includes

- Enhanced bioavailability
- Reduced side effects
- Sustained and controlled delivery of the drug can be achieved

- Targeting of drugs to the particular site of action can be achieved
- Manufacturing methods are simple and reproducible
- Free flowing character makes it easy to formulate it into tablets or capsules
- Bitter taste of drugs can be masked
- Wide range of routes of administration

Limitations[41.42]:

- Difficulty in achieving the desired particle size
- Difficulty in large scale manufacturing
- Encapsulation efficiency may be less in most cases
- Polymeric material makes this drug delivery system costly

Factors affecting drug release from microspheres:

In microspheres drug is encapsulated in a polymeric matrix which provides sustained release. The drug release may be affected by many factors such as polymer, drug polymer combined effect, additives used, sphere porosity, crystallinity, size distribution, entrapment efficiency, manufacturing process and pH based target environment.

METHOD OF PREPARATION:-

Different methods are employed for the preparation of microspheres. It includes,

- Solvent evaporation
- Solvent extraction
- Single emulsion method
- Double emulsion method
- Polymerization technique
    1. Normal polymerization
    2. Interfacial polymerization
- Phase separation co-acervation technique
- Spray drying and spray congealing
- Freeze drying
- Precipitation

i) Solvent evaporation:-

It is most frequently used method for the preparation of microspheres. In this method aqueous drug solution is added to an organic polymeric phase. Then, the mixture is stirred continuously until the formation of primary emulsion. Stirring at elevated temperatures may result in evaporation of volatile organic solvent leaving the solid microspheres. Solid microspheres are then washed, centrifuged and freeze dried. The resultant dried microspheres possess good flow property which makes it easy for handling. Polyvinyl alcohol, polyvinyl pyrolidone can be used as emulsifying agent. This method is applicable to wide range of liquid and solid drugs. This technique is suitable for incorporation of both water soluble and insoluble drugs[43].

ii) Solvent extraction:

This is one of the simplest methods used for the preparation of microspheres. In this method the organic phase is removed by addition of a liquid which precipitates the polymer. The liquid added should be miscible in both water and organic solvent. The factors which affect the rate of solvent removal are temperature of aqueous layer and solubility of the polymer[40].

iii) Single emulsion technique:

In this technique, microspheres are usually prepared from natural polymers such as proteins and carbohydrates. The polymeric aqueous phase which contains drug is emulsified with oily phase by using an emulsifying agent. Next step is cross linking which is achieved either by heat or chemical crosslinking agents. Examples of chemical cross linking agents include glutaraldehyde, formaldehyde, terephthaloyl chloride, diacid chloride etc[44]. Exposure of drugs with chemical cross linkers for a prolonged period may cause damage to the drugs. Cross linking by heat is not applicable for heat sensitive materials[45].

iv) Double emulsion technique:

Unlike single emulsion method, both natural and synthetic polymers are used for double emulsion. The steps involved in formation of double emulsion are

i) Formation of primary emulsion (w/o) using low HLB value surfactants

ii) Formation of double emulsion (w/o/w) using high HLB value surfactants

The primary emulsion is formed by emulsifying the aqueous drug solution in polymeric lipophilic dispersed medium. The drug in the aqueous phase is encapsulated by the polymer from the dispersed medium. The primary emulsion so formed is subjected to homogenization and it is re-emulsified with aqueous solution of high HLB value surfactants. This leads to formation of double emulsion. The double emulsion, so formed is then subjected to solvent evaporation/extraction to remove the solvent. This method is suitable for water soluble and macromolecular drugs[46].

v) Polymerization techniques

The polymerization techniques are

1. Normal polymerization
2. Interfacial polymerization

Normal polymerization

Normal polymerization may be carried out by using different techniques as given below:

- Bulk polymerization
- Suspension polymerization
- Emulsion polymerization

Bulk polymerization

Heating monomers with a substance, usually a catalyst is performed to initiate the polymerization reaction[47]. The catalyst accelerates the rate of reaction. The polymer obtained by this reaction can be molded as microspheres. Drug is added during the process of polymerization.

Suspension polymerization

Suspension polymerization is also known as bead, granular or pearl polymerization. In this technique, monomer is heated with drug as droplets dispersion in continuous phase. The droplets may contain an initiator and other additives. Monomer is mechanically dispersed in the continuous phase. The monomer should be insoluble or slightly soluble in

water, so that it precipitates when polymerization reaction takes place which makes it easy for separation. Monomers with high water solubility are not suitable for this technique[48].

Emulsion polymerization

In this technique the monomer is added drop wise with continuous stirring to the phase which contains drug and emulsifying agent. The monomer aggregates to form polymers, subsequently to nuclei which entrap the core material.

The rate of reaction for the conversion of monomers to polymers is rapid. The major disadvantage of suspension and emulsion polymerization is association of polymers with unreacted monomers and/or other additives. In some cases surfactant may degrade the polymer[49].

Interfacial polymerization:

This technique involves reaction of different monomers at the interface between two immiscible phases which leads to the formation of polymers. The monomers from the different phase diffuse rapidly and polymerization takes place in the interface.

vi) Phase separation coacervation:

Coacervation process can be applied for both solids and liquid core materials[6]. This technique involves formation of immiscible phases of core material, solvent and a coating material. These immiscible phases can be achieved by change in temperature, addition of salts or by reaction between two polymers. The next step is deposition of coating material on the core material, followed by rigidization of the coating. If one polymer is used it is called as simple coacervation and when two or more polymers are present it is known as complex coacervation. Simple coacervation may be achieved by the addition of a non-solvent, micro ions, or a temperature change. Complex coacervation is achieved by reaction of two polymers[50].

vii) Spray drying and spray congealing

Spray drying and spray congealing methods involve formation of fine droplets and subsequent drying which leads to the formation of microspheres. Polymeric solution is

prepared by dissolving polymer in suitable volatile solvents such as dichloromethane, acetone etc[51]. The drug is then dispersed in the above polymer solution under high speed mixing. The dispersion formed is sprayed in hot air for drying which leads to formation of fine microspheres. Cyclone separator is used to separate the microparticles. Aseptic conditions can be maintained for spray drying which makes it suitable for parenteral preparation. Plasticizers inclusion may impart spherical and smooth surface to the microspheres[52].

viii) Freeze Drying:

This method involves freezing of prepared emulsion. The mechanism involved in freeze drying is sublimation. Usually the continuous phase is organic, which is removed by sublimation at specified low temperature and pressure. This results in formation of solid polymeric microspheres of the dispersed phase. This method can be used successfully for thermo-sensitive materials, but due to expensive its usage is limited.

ix) Precipitation:

This technique is variation of solvent evaporation. Solvents may be removed by addition of a cosolvent. This results in precipitation of microspheres.

EVALUATION OF MICROSPHERES:

The microspheres are subjected to the following tests.

i) Particle size analysis
ii) Surface Morphology
iii) Drug-Excipient Compatibility studies
iv) Flow Properties
v) Drug content
vi) Percentage yield
vii) Encapsulation efficiency
viii) Drug Release studies
ix) Zeta potential

i) Particle Size Analysis:

The particle size and particle size distribution plays an important role in entrapment of drug and drug release from the microspheres. Particle size can be determined by using a laser diffraction particle size analyzer or by optical microscopy[53]. In the former method, microspheres are suspended in distilled water, sonicated and the particle size is measured by using the designed software. In the later method, the particles are measured by observing it under optical microscope. The particle size is expressed as volume mean diameter in micrometer.

Particle size distribution can be quantified by sieve analysis, optical microscopy, laser diffraction or by coulter counter analysis[54].

ii) Surface Morphology:

Scanning electron microscopy gives information about the surface morphology of microspheres. The microspheres are mounted on a copper cylinder using double sided adhesive tape. Then it is coated by ions using ion sputtering device. The coated sample is examined under microscope and photographed. Atomic force microscopy (AFM) can also be used to study the surface morphology of microspheres[55]. In this technique the samples are placed on mounted slab using double sided adhesive tapes and observed under microscope. Transmission electron microscopy (TEM) can also be used for identifying the surface morphology[56].

iii) Drug –Excipient Compatibility studies:

Drug –excipient compatibility studies can be performed by FTIR, TLC and DSC techniques[57].

iv) Powder flow properties:

Flow property plays an important role in solid substances. Microspheres are subjected to flow properties such as density, angle of repose, porosity and Hausners ratio[58]. The density of microspheres can be determined by pycnometer. Angle of repose is measured by funnel method. Porosity is calculated from the bulk and true density. Hausner's ratio also gives information about flow property.

v) Drug content:

Drug content can be estimated by weighing specified quantity of microspheres, dissolving it in suitable solvent, followed by filtration and analyzing by suitable method.

vi) Percentage yield:

Percentage yield of microspheres is calculated by using the following equation[59].

Percentage yield = [Practical yield / Theoretical yield] x 100

vii) Encapsulation efficiency:

Encapsulation efficiency is the amount of drug that is encapsulated in the formulation of microspheres. A weighed quantity of microspheres are lysed to release the drug and added to suitable buffers and then stirred for specified period. The solution is filtered and diluted appropriately and analyzed by suitable method. The entrapment efficiency is calculated by the following formula[60]

Encapsulation efficiency = [Actual drug content / Theoretical drug content] × 100

viii) Drug release studies:

In vitro studies can be performed by beaker or dissolution apparatus. In the beaker method, microspheres are made to adhere at the bottom of the beaker containing the medium and stirred uniformly by using overhead stirrer. Volume of medium used may vary from 50- 500 ml and the stirring speed from 60-300 rpm[61]. In the dissolution apparatus method, microspheres are placed in dissolution medium in the apparatus, rotated for specified time, and then samples are withdrawn at regular intervals and analyzed by suitable method. Sink conditions should be maintained.

ix) Zeta Potential:

Zeta potential is a property which is exhibited by a particle in suspension. Isoelectric point is defined as the pH at which the zeta potential is zero. Zeta potential can be measured by using zetasizer and the values help to judge the stability of the microspheres[62].

1.2. APPLICATIONS OF MICROSPHERES IN DRUG DELIVERY:

S. No	Drug	Category/Use	Carrier used	Method of Preparation
1	Acetazolamide[63]	Diuretic	Eudragit RL Eudragit RS	Solvent evaporation
2	Atenolol[64]	Anti-hypertensive	Ethyl cellulose	Solvent evaporation
3	Aspirin[65]	Anti-inflammatory	Eudragit RS100	Solvent partition
4	Aceclofenac [66,67]	Anti-inflammatory	Rosin	Solvent evaporation
			Eudragit RS100	Solvent evaporation
5	Piroxicam[68]	Anti-inflammatory	Hyaluronate	Spray drying
6	Diltiazem HCl[69]	Anti-hypertensive	Eudragit, Ethyl cellulose	Solvent evaporation
7	Diclofenac[70]	Anti-inflammatory	Ethyl cellulose	Solvent diffusion
8	Flurbiprofen[71,72]	Anti-inflammatory	Pectin:HPMC	Solvent diffusion
			Eudragit L100, Eudragit S100	Solvent evaporation
9	Ibuprofen[73]	Anti-inflammatory	Ethyl cellulose	Solvent evaporation
10	Indomethacin[74]	Anti-inflammatory	Ethylcellulose	Multiple emulsification
11	Ketorolac[75]	Anti-inflammatory	Ethylcellulose, Eudragit R100, Eudragit S100	Emulsion diffusion
12	Metformin[76]	Antidiabetic	Ethyl cellulose	Solvent evaporation
13	Mephenamic acid[77]	Anti-inflammatory	Chitosan	Emulsion congealing
14	Nifedipine[78,79]	Anti-anginal	Ethyl cellulose	Solvent evaporation
			Methacrylate	Phase separation

NANO DRUG DELIVERY SYSTEMS:

Nano as the name itself implies that these systems are in nano size. Nanotechnology is a broad field which involves variety of applications including drug delivery, medical diagnostics etc. These systems have some peculiar properties such as increased surface area, specific targeting, optical properties and less toxicity when compared to other systems[80].

Norio Taniguchi, in 1974 used the term nanotechnology at first time[81]. These systems provide efficient way of drug delivery particularly for chronic therapy management[82].

Various drug delivery systems available in nanotechnology are nanosuspensions, nanoparticles, nanocapsules, nanoemulsion, SLN, nanocrystals, magnetic nanoparticles, nanotubes, nanoshells etc.

NANOSUSPENSION:

Nanosuspensions are colloidal systems stabilized by surfactants. These are dispersed systems in which the drug acts as dispersed phase[83].

Unique features of nanosuspension[84, 85]:

- Stable for longer period, as it avoids Ostwald ripening
- Drug dissolution/saturation velocity increased
- Improved bioavailability
- Manufacturing methods are simple
- Flexibility in designing dosage forms and route of administration
- Suitable for both lyophilic/lyophobic drug

Problems in nanosuspensions[86]:

- High cost
- Toxicity produced by solvents or stabilizers
- Chances of agglomeration

ADDITIVES IN NANOSUSPENSIONS:

The additives used in nanosuspension are briefly given below:

i) Stabilizer:

Stabilizers are one of the important additives used in the formulation of nanosuspension. These agents help to wet the drug materials fully and also avoid Ostwald ripening, thereby producing stable nanosuspension. Drug and stabilizer at particular ratio yield stable preparation. Usually stabilizers used in nanosuspension are surfactants.

Eg: Poloxamers, lecithins, polysorbates[87]

ii) Co-surfactants:

Co-surfactants have great impact in the preparation of microemulsion template for nanosuspension.

Eg. Trancutol, ethanol, methanol, isopropanol[88]

iii) Organic solvent:

Organic solvents are used in the preparation of nanosuspension to dissolve the solid substances. The solvents used should be water miscible and non-hazardous. The process of removal of solvents from the product should be easy and cost effective.

Eg. Ethanol, methanol, isopropanol, chloroform, dichloromethane[89]

iv) Miscellaneous additives:

Additives such as buffers, osmogens and cryoprotectant are used in the formulation whenever necessary[90].

Eg. Buffer – Phosphate buffer

Osmogen – mannitol

Cryoprotectant – PEG, mannitol

MANUFACTURING METHODS OF NANOSUSPENSION:

The methods followed for the preparation of nanosuspension

- Bottom up approach
- Top down approach
- Other techniques

BOTTOM UP APPROACH:

Bottom up approach methods include precipitation. This method involves assembling of molecules to form nanosize materials. In this technique the drug is dissolved in solvent then added to non-solvent to precipitate the particles. This is simple and user friendly. Precipitation can be combined with high stirring.

The problem in this technique involved is growth of drug particles, but can be rectified by surfactant. In this technique the materials are constructed to form nanoparticles. This method is suitable for the drugs which are soluble in aqueous or non-aqueous solvent. Commonly used methods are microemulsion, microprecipitation, melt precipitation[91].

TOP DOWN APPROACH:

In this top down approach the large particles or microparticles are disintegrated to form nanoparticles.

The technologies include

Milling

- Media milling (nanocrystals)
- Dry co-grinding

Homogenization

- Homogenization with high pressure in aqueous solvents
- Homogenization with high pressure in non-aqueous solvents

Milling:

Media milling:

This method was introduced by Liversidge *et al*[92]. This is wet milling technique. In this method, nanoparticles are prepared by using high shearing force which is produced by impaction which causes the drug to disintegration. Drug, stabilizer and required solvents are kept in the milling chamber and rotated at high shear rate at controlled temperature. These mills contain balls or pearls. These produce impact mechanism, which may produce nanoparticles. This method is well suitable for drugs which are poorly soluble in both aqueous and organic solvents. These are suitable for pilot scale up. Residues in the final product are the problematic issue in this method. Time consuming and scale up may create problem in some cases

Dry co-grinding:

This method involves preparation of nanosuspension by dry milling[93]. Glibenclamide, griseofulvin nanosuspension can be prepared by dry co-grinding. HPMC, PEG, PVP and some souble polymers are used in this method[94]. This method is easy to operate and quietly cost effective as it can be preceded without organic solvents.

Homogenization:

This method involved three steps

- Dispersion of drug in solvent which contains stabilizer
- Homogenization of presuspension formed in step1 with low pressure
- Homogenization with high pressure in aqueous or non-aqueous solvents.

Homogenization with high pressure in aqueous media:

Dissocubes method was introduced by Muller[95]. Dissocubes are prepared by using piston type homogenizers with high pressure. The principle involved in the size reduction is cavitation and further by particles collision. Due to the formation of cavitation, the implosion forces are created which are sufficient to size reduce the micro particles to nanoparticles. The micronized drug is used to produce nanosuspensions. Before homogenization process, the micronized drug is prepared as suspension by stirrers. The suspension is given pressure in the in the homogenization gap to achieve nano size. There is no erosion of the materials. Both dilute and concentrated solutions can be prepared. This method is expensive. The equipment can be operated under varying pressure from minimum of 100 to maximum of 2000 bars. The capacity of the homogenizers may be from 40ml to few thousand liters. The size of particle obtained is indirectly proportional to the pressure applied. Particle size is also indirectly proportional to the number of homogenization cycles.

Homogenization with high pressure in non-aqueous solvents

In this type of homogenization, non-aqueous solvents are used. The drugs which are labile thermally and chemically can be processed by using nonaqueous solvents[96]. The solvents used may be PEG. The nanosuspension can be homogenized at 0ºC and sometimes even at -20ºC which is called as deep freeze homogenization.

OTHER TECHNIQUES:

Emulsion/Microemulsion as templates

Supercritical fluid method

Emulsion/Microemulsion as template[97]:

Emulsion/microemulsion can be used to formulate nanosuspension. The drugs those are soluble either in organic solvent or aqueous solvent. Microemulsion requires less energy for the production of nanosuspension. Nanosuspension may be prepared by loading the drug in organic solvent and dispersing the solution in aqueous phase with the aid of suitable emulgents. The organic solvent is evaporated, so that drug precipitates and forms nanosuspension. The particle size is based on the emulsion droplet size. Nanosuspension may also be prepared from emulsion and diluting it with aqueous phase, which may lead to diffusion of dispersed phase into the dispersed medium, which results in formation of nanosuspension.

Supercritical fluid method[98]:

Solvents normally used in the preparation of nanoparticles may produce hazard to environment. So it is better to use supercritical fluid which may be safe.

By using SC fluids, supercritical anti-solvent (SAS) or rapid expansion of supercritical solution (RESS) method can be employed.

In SAS method, a solvent in liquid state is selected which is miscible with SC fluid. The solute is miscible with solvent but not in SC fluid. This controversy produces precipitation of solute, which results in nanoparticles.

In RESS method, the solute is dissolved in SC fluid and then expanded by nozzle into low pressure area, which leads to formation of precipitate. The technique involves high concentrations of stabilizers. Due to super saturation there may be chance for overgrowth of particles which may lead to form amorphous or polymorph.

NANOSUSPENSION EVALUATION:

Following tests are to be performed for the nanosuspension formulation.

- Particle size
- Zeta potential
- Morphology
- Saturation solubility and dissolution velocity
- Entrapment efficiency

Particle size:

The average particle size is one of the parameter to be considered for the nanosuspension. Particle size affects the properties such as dissolution, saturation solubility, stability etc. This parameter can be calculated by photo correlation spectroscopy, coulter counter, laser diffraction[99]. Particle shape can be visualized by AFM[100]. Polydispersity monitors the physical stability of the nanosuspension. It should be less for prolonged stability.

Zeta potential:

Zeta potential is an important parameter to be considered as it is directly relates the stability of the product. This can be measured by zeta sizer. Drug and stabilizer do have property of affecting the zeta potential. Nanosuspension stabilized by electrostatic method may have zeta potential of $\geq \pm 30$mV and stabilized by combination of steric and electrostatic may have zeta potential of $\geq \pm 20$mV[101].

When zeta potential increases, the particle surface charge increases, which produces increase in electrostatic forces, which ultimately leads to stable preparation.

Morphology:

X-ray diffraction, SEM and TEM analysis are used to identify the morphological characters of the nanosuspension. Due to high pressure, nanosuspension may change in their structure which may be amorphous or other polymorphic forms. The changes occurred in the preparation of nanosuspension can be well identified by these techniques.

Saturation solubility and dissolution velocity:

Nano concept emerged to solve the poor solubility issues. Solubility increases by reducing the particle size. These parameters give information about the dissolution and drug release.

These can be determined in different physiological buffers. These parameters are to be considered carefully particularly in SR/CR dosage forms.

Entrapment efficiency:

This is a parameter which gives information about the entrapment of drug in polymer. From this we can determine what type of carrier is suitable for particular drug. The amount of carrier required can also be determined by this parameter.

Table 1.3. APPLICATIONS OF NANOSUSPENSIONS:

S. No	Drug	Category/Use	Stabilizer	Method of Preparation
1.	Aceclofenac[104]	Anti-inflammatory	Pluronic F-68	Nanoprecipitation
2.	Revaprazan HCl[105]	Proton pump inhibitor	Polaxamer 188	Homogenization (high pressure)
3	Glibenclamide[106]	Anti-diabetic	SLS, Lutrol F68, Tween 80	Media milling
4	Fenofibrate[107]	Antihyperlipidemic	Vitamin E TPGS	Homogenization (high pressure)
5	Itraconazole[108]	Anti-fungal	Poloxamer 407	Milling
6	Atorvastatin[109]	Anti-hyperlipidemic	Polaxamer 188	Homogenization (high pressure)
7	Phenytoin[110]	Anti-convulsant	SLS, Tween 80	Millling
8	Celecoxib[111]	Anti-inflammatory	Tween 80, PVP, SDS	Emulsion diffusion
9	Buparvaquone[112]	Antibiotic	Poloxamer 188,	Homogenization (high pressure)
10	Miconazole[113]	Anti-infective	SDS	milling
11	Piroxicam[114]	Anti-inflammatory	Poloxamer 188,	Homogenization (high pressure)
12	Rutin[115]	Anti-allergic, anti-inflammatory	SDS	Homogenization (high pressure)
13	Griseofulvin[116]	Antifungal	Lecithin	Precipitation Emulsion template Dry co-grinding
14	Amphotericin B[117]	Antifungal	Tween 80 pluronic F68, Sodium cholate	Homogenization (high pressure)

NANOSUSPENSION IN ORAL DELIVERY:

Although nanosuspension can be delivered through different routes such as oral, pulmonary, parenteral and ocular, the oral route of delivery is prominent and safe. The nanosuspension can be freeze dried and filled in capsules or compressed into tablets[102]. Even it can be given as oral liquids. The nanosuspension by oral route makes patient convenience as well as increase in absorption and bioavailability. Bupravaquone, an antibiotic face bioavailability problem in normal delivery, but there is increase in absorption in nanosuspension. Likewise amphotericin B, danazol bioavailability characters are also increased by preparing it in nanosuspension. For GI inflammations, targeting can also be achieved by using polymeric carriers in the formulation of nanosuspensions. Fenofibrate nanosuspensions have higher bioavailability than the ordinary micronized drugs. The first USFDA approved nanocrystal tablet was Rapamune which holds increased bioavailability than the conventional one[103].

INFLAMMATION AND ANTI-INFLAMMATORY DRUGS:

Inflammation is a term which is derived from the Latin word – Inflammare, means burn. Injury to the human body will produce a series of chemical changes in the injured area. Inflammation is the physiological response of vascular tissues to harmful stimuli, such as damaged cells, pathogens, producing edema due to the extravasations of fluid, proteins and accumulation of leucocytes at the inflammatory site[118]. Inflammatory diseases cover a broad spectrum of conditions such as rheumatoid arthritis, osteoarthritis, allergic rhinitis, multiple sclerosis, ankylosing spondylitis, asthma, inflammatory bowel disease, and cardiovascular diseases. The features of inflammation are increased blood flow, increased capillary permeability, increased migration of leucocytes into the affected area, clotting of fluid due to excessive leaking of fibrinogen and other proteins into the interstitial space from the capillaries and swelling of the tissue cells.

Causes of inflammation

- ✓ Physical: Environmental like severe cold and heat, mechanical injury (accident) etc.
- ✓ Chemical: Acid burns, drugs, venom

- ✓ Infection: Bacteria, viruses, fungi and other parasites
- ✓ Immune: Allergies and autoimmune conditions
- ✓ Ischemia: Tissue death due to lack of restricted blood supply known as infarct.

Signs and symptoms of inflammation:

The signs and symptoms of inflammation include redness, pain, swelling and heat.

ACUTE INFLAMMATION[119]

Acute inflammation develops within minutes or hours and generally lasts for hours to days based on the type and severity of the tissue damage. Acute inflammation is a rapid and initial response to foreign substance such as microbes and tissue injury. During this inflammation, leukocytes and plasma proteins are released at the site of injury producing vascular changes and cellular events.

- ➢ **Vascular changes:**

After the tissue damage, within seconds there may be vasoconstriction followed by vasodilatation leading to increased blood flow causing erythema (redness) to the area. Due to increased permeability of blood vessels, protein rich fluids extravasate into the extravascular tissues, causing the RBCs to become more concentrated, thereby increasing blood viscosity and slowing the circulation. The leukocytes adhering the endothelium got activated and proteolytic enzymes are released causing endothelial injury.

- ➢ **Cellular events:**

The leukocytes released from the vascular lumen to the extravascular space. This consists of following events

- **Margination and Rolling along the walls of blood vessels**

  As blood flows from capillaries to venules, circulating cells are removed by laminar flow. Further the small red cells move faster than large white cells, thereby pushing leukocytes out the central column and interact with the endothelial cells. This process is called as margination. Subsequently the leukocytes tumble on endothelial cells, mediated by selectins. This process is called as rolling.

- **Adhesion and Transmigration**

  Transient bond is formed between the leukocytes and endothelial cell surfaces mediated by integrins. This is known as adhesion.

- **Migration to chemotactic stimulus**

  The sticked leukocytes migrate through the vessel wall by squeezing at intercellular junction and escapes out to the extravascular space known as migration. Lecukocytes after crossing endothelium and other parts, finally it reaches the site of injury by a process known as chemotaxis. The chemotactic substance includes leukotrienes B4, cytokines etc.

CHRONIC INFLAMMATION:

In chronic inflammation, active inflammation, injury and tissues healing proceeds simultaneously for long duration. It differs from acute inflammation by infiltration of plasma cells, which are distinguished by vascular changes, edema and neutrophilic infiltrate. It happens due to chronic infections by microbes that are hard to eradicate, immune mediated inflammatory diseases and prolonged exposure to toxic agents like inhaled particulate silica[120].

Cell mediators:

a) Macrophages:

They are diffusely scattered in almost all connective tissues, liver, lymph nodes, spleen, CNS, lungs and migrate to the site of injury within 1 to 2 days from the onset of acute inflammation. It reaches the extravascular tissue and transformed into larger and possess greater ability to kill ingested organisms. After activation, it secrets biological active products like proteases, AA metabolites, cytokines IL-1 and TNF resulting in tissue injury and fibrosis.

a) Lymphocytes:

Both T and B lymphocytes migrate into inflammatory sites. T cells produce cytokines-INF-γ, an activator of macrophages and promote cytokine secretion.

b) Plasma cells:

Plasma cells producing antibodies are developed from activated B lymphocytes.

c) Eosinophil:

Eosinophils are found in inflammatory sites around infections and typically associated with allergies.

d) Mast cells:

These are distributed in connective tissues throughout the body, with IgE antibody mast cells are triggered to release histamines and AA metabolites[121].

INFLAMMATION MEDIATORS

These are the substances producing inflammation through a specific receptor. The mediators for acute and chronic inflammation are given explained below.

Acute inflammation:

- **Plasma proteins:** Complement, coagulation, fibrinolytic and kinin systems.
- **Lipids:** Eicosanoids, Platelet activating factor
- **Others:** Histamine, serotonin, endotoxin, nitric oxide

Chronic inflammation:

Cytokine proteins Interleukin-1, Interleukin-6 and TNF-α

Complement system:

Antigen-antibody complexes formed during infection, the endotoxins of gram negative bacteria, kinin products, fibrinolytic and coagulation systems activates the complement system[122].

Coagulation system:

Coagulation system converts soluble fibrinogen into insoluble fibrin, a major component in the exudate of acute inflammation[123].

Fibrinolytic system:

The lysis of fibrin into degradation products may effect locally on vascular permeability[124.]

Kinin system:

Kinins are activated by coagulation factor XII[125]. Bradykinin a chemical mediator of pain causes pain vasodilation, edema etc[126].

Eicosanoids:

These inflammatory mediators are generated from phospholipids and controls many physiological process[127].

Platelet activating factor:

PAF is an inflammatory mediator released by pro-inflammatory cells, vascular endothelial cells and platelets[128]. Asthma patients have high level of PAF.

Histamine:

It is a chemical inflammatory mediator present in leukocytes, platelets, basophils and mast cells. It causes increased vascular permeability and vascular dilatation immediately[129]. In rheumatoid arthritis and asthma, increased mast cells are present due to increased histamine level.

Serotonin:

Serotonin acts as an inflammatory mediator and presents in high level in platelets and mast cells[130].

Endotoxin:

Endotoxin triggers complement activation causing vasodilation and increased vascular permeability which may be due to formation of anaphylatoxins C3a and C5a[131].

Nitric oxide:

Nitric oxide syntheses enzyme present in endothelium and macrophage produces nitric oxide from arginine may cause cytotoxic, vasodilator and anti-platelet aggregation[132].

Cytokines:

Cytokines are the signaling chemicals released from various immune cells. The duration of inflammatory response is controlled by it. Cytokines may be pro-inflammatory or anti-inflammatory. Microglia produces pro-inflammatory cytokines which increases the inflammatory reactions. The inflammatory reactions are reduced by anti-inflammatory cytokines[133].

TREATMENT OF INFLAMMATION:

Inflammation should be treated if it continues to persist. It is usually persistent in chronic conditions with occasional acute aggravations. Inflammation may be treated by the following drug therapies.

Non-steroidal Anti-inflammatory drugs:

NSAIDs inhibit the release of prostaglandins, a prime chemical mediator of inflammation.

Corticosteroids:

Steroids inhibit the release of prostaglandins by the cells which in turn inhibit the function of WBC,

Anti-histamines:

Anti-histamines inhibit histamine release by blocking basophils and mast cells.

CLASSIFICATION OF NSAIDs:

Currently lots of drugs are available in the market to treat inflammatory disorders but only very few are free from toxicity. Gastrointestinal problems associated with the use of anti-inflammatory drugs are still under research.

NSAIDs can be classified as follows:

Table 1.4: Classification of NSAIDs

NSAIDs	DRUGS
Salicylates	Aspirin, Salsalate, Diflunisal
Propionic acid derivatives	Naproxen, Ibuprofen, Ketoprofen, Loxoprofen, Fenoprofen, Flurbiprofen, Oxaprazin
Acetic acid derivatives	Aceclofenac, Diclofenac, Ketorolac, Sulindac, Indomethacin, Nabumetone, Etodolac
Enolic acid derivatives	Tenoxicam, Piroxicam, Droxicam, Lornoxicam, Meloxicam
Fenamic acid derivatives	Flufenamic Acid, Mefenamic Acid, Tolfenamic Acid
COX-2 inhibitors	Rofecoxib, Celecoxib, Valdecoxib, Parecoxib

2. AIM AND OBJECTIVES

Aceclofenac is a drug commonly used in the management of pain and inflammation in various conditions such as

- ✓ post-traumatic, cervical and low back pain
- ✓ ankylosing spondylitis
- ✓ rheumatoid arthritis and osteoarthritis

This drug, on long term usage, specifically by oral route is reported to cause adverse effects including gastritis. So it is a need to develop safe and effective oral drug delivery system for aceclofenac. The following are the specific reasons which demand the development of oral controlled drug delivery system for aceclofenac:

- ✓ Due to its short half-life of 4- 4.3hrs, frequency of administration is increased. A well designed oral controlled delivery can reduce the frequency of dosing
- ✓ Due to its severe gastrointestinal (GI) irritation and other side effects, controlled delivery of aceclofenac at optimal concentration may considerably reduce the adverse effects
- ✓ Comparing to other routes, oral route is preferable with respect to safety, comfort and reliability. Hence controlled delivery of aceclofenac by oral route is ideal.
- ✓ Controlled release of aceclofenac will reduce the frequency of dosing and may increase patient convenience
- ✓ For many inflammatory disorders including arthritis, administration of NSAID's for long period of time is unavoidable and hence the development of controlled release of aceclofenac, a popular NSAID is most demanding.
- ✓ Wide market opportunities are available

The overall aim and objective of the present work is to:

- ✓ Improve the overall therapeutic efficacy of aceclofenac by controlled release
- ✓ Minimize the adverse effects
- ✓ Reduce the overall dose and dosing frequency of aceclofenac
- ✓ Achieve improved patient compliance

To achieve the above said objectives the plan is executed

- ✓ To formulate oral controlled drug delivery systems like Osmotic tablets, Microspheres and Nanosuspensions of Aceclofenac.
- ✓ To evaluate the formulated products of Osmotic tablets, Microspheres and Nanosuspensions of Aceclofenac
- ✓ To optimize process and formulation variables
- ✓ To perform anti-inflammatory activity and GI tolerability studies
- ✓ To perform stability studies of optimized formulation of Osmotic tablets, Microspheres and Nanosuspension of Aceclofenac

- Bodmeier R and Chen H. (1989) encapsulated indomethacin, ibuprofen, and hetoprofen by solvent evaporation using polymers ethylcellulose, poly(e-caprolactone), PMMA, polystyrene, and Eudragit RS and RL[134]. Indomethacin-poly(e-caprolactone) microspheres were prepared by a melt-dispersion without using organic solvents. The release of indomethacin from EC microspheres was too slow. The release of ibuprofen & ketoprofen was increased by using more permeable polymers. The rate of precipitation of polymer and drug during microsphere formation were dependent upon the solvent selected.

- Malamataris S and Avgerinos A (1990) produced indomethacin CR microspheres by using eudragit RS and RL in different drug polymer ratios using emulsion solvent-diffusion method[135]. The size varied from 7μm to 380 μm. Porosity values ranged from 62.4 – 89.4. Drug loading and release depended on drug polymer ratios, polymer combinations and solvent diffusion method. Increase in polymer content produced increase in size and decrease in porosity. Drug release followed first order kinetics.

- Kim CK *et al*., (1994) prepared terbutaline sulfate microspheres by an emulsion-solvent evaporation method from Eudragit RS[136]. The micropellets formed using aluminium tristearate as a smoothing agent were very spherical and narrow range size distribution. The average size of microspheres decreased as the polymer:drug ratio increased. The 12 h sustained release pattern was observed with formulated microspheres at a polymer/drug ratio of 9:1 which showed equivalent release specifications when compared with commercial TBS.

- Okada H *et al.,* (1994) developedPLGA and PLA microsphere system loaded with leuprorelin acetate by microencapsulation technique[137]. Microspheres of high entrapment and little initial burst release were obtained. The microsphere system using PLGA and PLA is very useful in designing targeted/controlled delivery to attain potent

therapy by reducing the dose than the daily injection and hence improves patient compliance.

- Cuzzolin L *et al.,* (1994) evaluated the anti-inflammatory efficacy of diclofenac and a new original diclofenac-derivate, nitrofenac at doses of 0.3 and 3 mg kg-' *per OS* to adjuvant arthritic rats at the 14th, 21st and 28th days after arthritis induction and also the gastrointestinal tract was examined macroscopically for any presence of lesions[138]. The results showed that the daily oral administration of diclofenac and nitrofenac a.t 3 mg/kg significantly inhibited arthritis development, total disappearance of *Escherichia coli* on 1st and also 7 days after the last drug administration and no ulcers were observed macroscopically with either drug, even if some alterations in the mucosa and haemorrhagic effusions were more evident in rats treated with diclofenac at 3 mg/kg. In conclusion, the better gastrointestinal tolerability observed in nitrofenac-treated rats could be attributed to the release of nitric oxide

- Sriwonanya M and Bodmeier R (1997) entrapped the water soluble cationic drugs (chlorpheniramine maleate, pseudoephedrine HCI and propranolol HC1) with amberlite IRP69 by solvent evaporation[139]. The formed resin particles were entrapped with EC, PMMA and eudragit RS100. Entire resin particles were entrapped with eudragit RS100 and negligible drug release was reported when compared with other polymers which may be due to interaction of polymer with unlike charged resin. All resin particles were encapsulated with Eudragit RS 100, a cationic polymer with quaternary ammonium, and showed negligible drug release was when compared to the other polymers due to the interactions of the polymer with the oppositely charged resin particles, which prevented hydration and swelling of the resin.

- Ganza-Gonzalez A *et al*., (1999) investigated the usefulness of chitosan and chondroitin sulphate microspheres by spray drying of aqueous polymer dispersions for controlled release of metoclopramide hydrochloride in oral administration[140]. The study reveals that the chitosan microspheres showed good control release (more than 8 h) when prepared with more than 15% formaldehyde (w/w with respect to polymer),

and release rates were little affected by medium pH. Release from chitosan microspheres prepared with 20% formaldehyde was independent of pH, suggesting that this may be the most appropriate formulation. The size distribution of the chitosan microparticles was almost-spherical, clearly bimodal, and the release rate is largely governed by rate of diffusion through the matrix.

- Liu L *et al*., (2000) prepared nifedipine monolithic osmotic tablet coated with cellulose acetate and drilled with orifices on both sides[141]. Dissolution profiles of optimized formula have been compared with commercial capsule and PPOT. From the results it was concluded that PEO of molecular weight 300, 000 g/mol was appropriate thickening agent and 0.25–1.41 mm orifice size was optimum orifice size. Plasticizer tiracetin decreased the nifedipine release and PEG increased the release. This system was capable of delivering drug at zero-order upto 24h.

- Tuncay M *et al.,* (2000) formulated diclofenac CR for intra-articular route by solvent evaporation method[142]. The particle size of microspheres were determined approximately 5-10µm. Based on the molecular weight of polymer, the surface morphology varied. The drug release from microspheres depended on molecular weight, loading, particle size and porosity. 99m TC-HIG was utilized as radiopharmaceutical to study the arthritic lesions. There was no remarkable difference between the group treated with PLGA diclofenac sodium microspheres and control group.

- Adibkia K *et al.,* (2001) investigated piroxicam loaded eudragit RS100 nanoparticles by solvent extraction technique[143]. The formulated nanoparticles were smooth spherical shape and followed weibull model for diffusion. Animal studies indicated that the inflammation was inhibited predominantly in nanosuspension than microsuspension.

- Hickey T *et al*., (2002) developed dexamethasone loaded PLGA microspheres for continuous delivery for over a period of 1 month, using an oil-in-water emulsion technique[144]. The standard microsphere systems did not provide the desired release

profile since, following an initial burst release, a delay of 2 weeks occurred prior to continuous drug release. Predegraded microspheres started to release dexamethasone immediately but the rate of release decreased after only 2 weeks. A combination of standard and predegraded system was utilized to avoid the delay and to program a continuous delivery of dexamethasone for one month.

- Esposito E *et al*., (2002) produced ascorbic acid loaded eudragit L, RL, RS microspheres by spray-drying method[145]. The formulation showed a good morphology and size distribution. Encapsulation efficiencies were found to be in the range of 98-100%. 60% of drug release from microspheres of eudragit L, eudragit RL, eudragit RL/L and eudragit RS/L were achieved in 100 min, 82 min, 75-77min, and 70 min respectively. The drug release didn't affected significantly by nature of polymer. The size and morphology made it suitable for oral delivery in treating colorectal cancer.

- Bravo SA *et al* (2002), developed diclofenac sodium uncoated HPMC matrix tablets by wet granulation technique[146]. The weight variation, thickness, hardness and friability were found to be optimal. The effect of MCC, lactose and starch on drug release of tablets were studied. Starch 4% as glidant produced good flowing of powders. Drug release was controlled by starch and lactose (8.5% both) at 1:1 ratio with MCC (5% or 7.5%). HPMC matrix tablets prolonged the release when compared to conventional one. The diclofenac matrix tablets were stable during the storage of 3 months. This matrix tablets avoided the GI side effects.

- Lamprecht A *et al*., (2003) prepared 5-fluorouracil loaded eudragit P4135 microsphere by o/w emulsion process[147]. Process parameters were optimized for drug release and loading. The drug release from eudragit P4135F microsphere was less than 35% up to 6hr at pH 6.8 and immediate release within 30 min at pH 7.4. Addition of RS100 produced only minimal changes in drug release. The formulation proved as promising delivery of 5FU for colon targeting in cancer stages.

- Parikh RH *et al*, (2003) prepared PLGA microspheres of 5-FU by emulsion solvent evaporation method and optimized the process parameters using 3^2 factorial design[148]. Effects on dispersed phase volume of the primary emulsion and continuous phase volume of the secondary emulsion on % yield, particle size and encapsulation efficiency were studied. Increase in dispersed phase volume of primary emulsion was directly proportional to particle size and indirectly proportional to % yield and encapsulation efficiency. Increase in continuous phase volume of the secondary emulsion was indirectly proportional to % yield, particle size and encapsulation efficiency. Reproducibility of results achieved for batch to batch. SEM studies depicted that particles existed as aggregates.

- Lu EX *et al.*, (2003) studied a naproxen MOTS, using gum arabic as osmogen, suspending and swelling agent, cellulose acetate as membrane and PEG 400 as plasticizer[149]. The effects of membrane thickness, orifice diameter, gum arabic and PEG 400 on dissolution were studied. From the results it was concluded that MOTS can be successfully used for water insoluble drugs as oral controlled delivery.

- Kayser O *et al*., (2003) formulated amphotericin B nanosuspension by high pressure homogenization[117]. The diameter of the particles was found to be 528 nm. GI stability was determined in artificial GI fluids at various pH. From the i*n-vivo* study reports, micronised amphotericin B (5 mg/kg) did not show any curative effect whereas, amphotericin B nanosuspension, showed decreased liver parasite by 28.6% compared to untreated controls revealing the therapeutic efficacy of amphotericin B as nanosuspensions for oral administration.

- Lamprecht A *et al*., (2004) designed calcitonin microspheres for colonic delivery by using eudragit P4135F by double emulsion. In vitro release was less than 20% in 4 hr at pH 6.8 buffer and fast release was observed in pH 7.4 buffer[150]. Cmax of carboxyfluorescein was around 60 min, but in microsphere it was identified after 4 hr. Microspheres produced 4 fold increase in the area above the curve of blood level calcium compared to levels reached after calcitonin solution. Chitosan in the

microsphere as absorption enhancer didn't produce any significant effect. This system proved as a promising device for colonic calcitonin delivery.

- Wang Y *et al*., (2004) described coating of silica nanoparticles by SAS method using supercritical CO_2 as anti-solvent and eudragit as coating material[151]. The process involved formation of nucleation with polymer followed by subsequent growth on the surface of the nanoparticles achieved by mass transfer and phase transition forming a matrix structure by agglomeration of the coated nanoparticles. FTIR, TEM, SEM EELS were used to analyze coated silica nanoparticles.

- Okimoto K *et al*., (2004) investigated the use of a controlled-porosity osmotic tablet using $(SBE)_{7m}$-β-CD as solubilizer and also as an osmogen for selected poorly and highly soluble drugs[152]. Dissolution was followed according to Japanese Pharmacopoeia. The osmotic tablets were placed in the dissolution medium for two hours and assayed by HPLC and gravimetry. The appropriate composition ratio correlates to the drug concentration in the core tablet. The present results proved that $(SBE)_{7m}$- β-CD served as a solubilizer and also as an osmogen.

- Patel JK *et al*., (2005) formulated chitosan mucoadhesive microspheres loaded with glipizide by emulsification technique using chitosan as polymer and glutaraldehyde as crosslinker[153]. The microspheres characters were affected by drug polymer ratio, stirring speed and amount of cross linker. Microspheres were spherical, free flowing, exhibited good mucoadhesion property with high entrapment efficiency. A 3^2 factorial design was used to optimize drug polymer ratio and stirring speed. The optimized batch exhibited EE of 75%, swelling index of 1.42, mucoadhesion of 78% after 1 hour and release of more than 12 hours. In vivo studies also supported the presence hypoglycemic effect in wistar rats.

- Hori M *et al*., (2005) prepared chitosan microparticles loaded with ovalbumin and chitosan microparticles of ovalbumin coated with eudragit L100 by ionic gelation and solvent evaporation method[154]. The microparticles contained particle size of 2.3μm and

ovalbumin content of 34.4%. Eudragit L100 coated microparticles contained particle size of 47.9-161.1μm and ovalbumin content of 3.6%-20.5%. The chitosan microparticles were dissolved spontaneously in JP 14 first fluid, but not in second fluid. So release was retarded in second fluid. Eudragit L100 coated microparticles release was retarded in both first and second JP 14 fluid. From the in vivo results it was concluded that eudragit L100 coated microparticles were useful in inducing intestinal immune response.

- Duarte AR *et al*., (2006) prepared naproxen microspheres by precipitation techniques using EC/MC blends[155]. For EC/MC microspheres and encapsulation of naproxen solvent evaporation and SAS method were used. Microspheres formulated by SAS method have high loading with controlled release. It followed Fick's law of diffusion.

- Kocbek P *et al.,* (2006) formulated ibuprofen nanosuspension with particle size of less than 100nm by melt emulsification and compared it with solvent diffusion method[156]. Melt emulsification gained more importance because of its avoidance of organic solvents but particle size is greater than the nanosuspension prepared by solvent diffusion. Tween80 and PVP K25 in combination in melt emulsification produced smallest particle size. Further dissolution rate was increased to 65% within 10 min which produced increased bioavailability with decreased gastric irritation.

- Dashora K. *et al*, (2006) prepared microparticulate system of aceclofenac, an anti-inflammatory drug by modified solvent evaporation using different variables like cellulose acetate and aceclofenac ratios (1:9, 1:6, 1:3 & 1:1), stirring time (5-15 min) and agitation speed (500-1,500 rpm)[157]. The particle size increased from 80.2±1.4 to 97.3 ± 2.06 μm when the cellulose acetate concentration increases and it reduced in increased agitation and stirring time conditions. At higher speed irregular shape produced. A 1:6 (cellulose acetate: aceclofenac) ratio with 10 min stirring time at 1000 rpm created microspheres of high entrapment efficiency and uniform size with good flow property and. Dissolution studies were performed for conventional, SR and

microspheres and the results obtained were 3h, 6h and more than 12h respectively. All preparations followed first order and diffusion mechanism.

- Liu L and Che B[158] (2006) formulated atenolol osmotic tablet by coating the core tablet by compression with punch with needle. PEO was used as suspending agent, sodium chloride as osmogen, ethyl cellulose as polymeric membrane and PEG 400 as plasticizer. The drug release was affected by the size of core tablet in the range of (1.00–1.14) mm. Independent of dissolution media and agitation rate, the osmotic tablet was found to deliver atenolol at a constant rate up to 24 h. The method simplified the preparation of osmotic tablet by elimination of laser drilling.

- Ramesh Babu V *et al.,* (2006) developed carbohydrate polymeric microspheres, containing sodium alginate and methylcellulose loaded with nifedipine by w/o emulsion method[159]. Cross linking was achieved by glutaraldehyde. Prepared microspheres were characterized by DSC, SEM, particle size analyzer and invitro release. The results suggested the distribution of nifedipine through polymer and formation of spherical particles. The drug was released in a controlled fashion up to 12h.

- Ruckmani K *et al.,* (2007) formulated cytarabine HCl loaded eudragit L100 nanoparticles by emulsion polymerization, targeting to the tumor site[160]. The formulation exhibited sustained release up to 30 h, particle size ranged from 400–800 nm, spherical and uniform. The *in-vitro* release studies revealed that the formulation had improved stability in biological fluids. In tumor bearing mice the altered hematological parameters were restored by administering the cytarabine HCl nanoparticles for 9 days and it increased the life span. From these studies it was concluded that increased therapeutic quality of drug can be achieved.

- Shakeel F *et al.,* (2007) formulated aceclofenac nanoemulsion by spontaneous emulsification method[161]. The drug release of optimized formulation by diffusion cell exhibited significant increase in steady state, permeability coefficient and enhancement

ratio. The F1 trial consisted of drug, triacetin, labrafil, tween 80, transcutol and water. In vivo studies also strongly suggested that nanoemulsion are potential than the conventional gel.

- Hanafy A *et al*., (2007) studied the bioavailability of 4 types of fenofibrate formulations including dissocube, SLN and two microsuspensions (reference)[107]. Both the colloidal systems showed two fold increase in bioavailability when compared to reference. Between the two colloidal systems, no significant differences were found in AUC, Cmax and tmax, which revealed that nanosuspensions were suitable delivery improving the bioavailability of poorly soluble drugs.

- Rastogi A *et al*., (2007) produced and evaluated spherical microspheres by using sodium alginate as carrier to prolong the release of INH by modified emulsification method[162]. The results revealed that the microspheres had smooth surface, discrete and spherical in shape with average particle size of 3.719 m. The particle size of the microspheres increased with increase in concentration of polymer, cross-linker and crosslinking time. The EE range was found to be 40–91%. EE increased upto 7.5% increase in concentration of Concentration of the cross-linking agent up to 7.5% caused increase in the EE. Optimized formulation was found to possess good bioadhesion of 72.25±1.015% resulting in sustained retention in the small intestine. Drug loading of 91% was recorded for the optimized formulation. In pH 1.2, nearly 26% of INH was released in 6 h and in pH 7.4, 71.25% was released in 30 h.
- Devarajan PV and Sonavane GS (2007) formulated eudragit L100 and eudragit RSPO nanoparticles loaded with gliclazide by controlled precipitation and solvent evaporation methods respectively[163]. Both the polymeric nanoparticles showed high entrapment efficiency and further addition of surfactants also produced increase in EE. Particle size was altered by variations in drug polymer ratio. The formulation showed sustained release of gliclazide from Eudragit L100 and Eudragit RSPO NP. The prepared nanoparticles showed stability up to 6 months.

- Farhana Yesmin *et al*., (2008) prepared aceclofenac agarose beads by ionotropic gelation method. Entrapment efficiency and swelling index were found to be maximum of 100±5 % and 18.22% respectively for beads containing 1:2 ratio of aceclofenac and agar[164]. Dissolution values for half of the formulations were higuichi and remaining first order. With increased agar concentration, swelling index was increased and the release of aceclofenac was reduced and with increased electrolyte concentration, it was reverse.

- Gonzalez M *et al*., (2008) prepared aspirin nanoencapsules by modified double emulsion, using Eudragit L100 and L30 D-55[165]. The average particle size was around 300 nm, EE found higher than 90%.The release profiles depicted initial small burst release which was suitable to release in intestine rather than gastric medium.

- Mutalik S *et al*., (2008) developed aceclofenac SR double-layer tablet using chitosan, HPMC and CAP[166]. IR and DSC studies showed the absence of drug-additive reactions. Stability and pharmacokinetic studies were performed. M7 trial showed no remarkable difference in in vivo test when compared to commercial product.

- Liu L and Xu X (2008) prepared nifedipine bilayer osmotic tablet consisting push and pull layer coated by ethyl cellulose containing polyethylene glycol 400 using pan coating[167]. PVP as suspending agent, NaCl as osmogen, and CCS as expanding agent were utilized in the formulation. A part of drug layer left uncoated to obtain an aperture for drug release. The optimized formulation produced 24hr release and followed zero order.

- Radhika PR *et al*., (2008) formulated aceclofenac delayed release microspheres by solvent evaporation using CAP as enteric polymer[168]. The effect of HPMCP, eudragit L100 and eudragit S100 on aceclofenac release had been evaluated. HPMCP exhibited positive influence and eudragit L100 and S100 exhibited negative influence. The encapsulation efficiency was found to be 75.65-96.52%. Dissolution release followed

first order kinetics. The ratio 1:8:2 of aceclofenac:CAP:HPMCP produced delayed release and considered as best formulation

.

- Chirag N *et al.*, (2008) studied the formulation of aceclofenac bioadhesive microspheres by double emulsion solvent evaporation technique using polycarbophil as polymer[169]. The microspheres were spherical shape with good flow property. The drug polymer ratio of 1:5 showed drug release of 89% within 10hr, mucoadhesion of 79% and entrapment of 38%. Further IR and DSC of microspheres didn't produce any drug polymer incompatibilities. The in vitro release followed Higuchi model.

- Attama AA *et al.*, (2008) prepared combination of homolipid and phospholipid SLN for diclofenac sodium for human cornea produced from HENC and epithelial cells[170]. The results revealed that the preparation had high entrapment efficiency with expected permeation characters.

- Trivedi P *et al*., (2008) microencapsulated aceclofenac using eudragit S100, RL 100, and RS 100 by emulsion-solvent evaporation[171]. The microspheres were freely flowable, white and spherical in shape. The entrapment efficiency, angle of repose, bulk & tapped density, Carr's index and Hausner's ratio were found to be 60-82%, 16.1 ± 0.62-24 ± 0.59, 0.311 ± 0.006-0.562 ± 0.012, 0.373 ± 0.01-0.735 ± 0.02, 14.04 ± 0.026 to 27.25 ± 1.405 and 1.14 ± 0.026-1.37 ± 0.03 respectively. The particle size was found to be 79.7016-144.840 µm. The drug-polymer concentration of dispersed phase influenced the particle size and drug release properties and all the formulations at higher pH followed the Matrix-Higuchi model.

- Tamizhrasi S *et al*., (2009) prepared lamivudine loaded eudragit nanoparticles by nanoprecipitation method using different ratios of lamivudine to eudragit and evaluated[172]. The formulated nanoparticles had spherical particles; size increased from 121 ± 8 to 403 ± 4 nm with increase in eudragit concentration. The drug release was achieved up to 24hr and followed zero order. The product remained stable for 2 months.

- Lakshmana Prabu S *et al.,* (2009) microencapsulated aceclofenac by o/w emulsion solvent evaporation method using rosin. The effect of formulation variables drug:polymer ratio, PVA concentration and dichloro methane volume were examined[66]. The prepared batches were characterized for particle size distribution, EE and *in vitro* release behavior. The study reveals that drug: polymer ratio had a considerable effect on the entrapment efficiency; however particle size distribution of microspheres was more dependent on the volume of dichloromethane and polyvinyl alcohol concentration rather than on the drug: polymer ratio. Drug, polymer concentrations were varied to obtain optimum release profile for sustaining the action of the drug.

- Ho LTM *et al.,* (2009) prepared ketoprofen loaded polymeric drug nanoparticles with Eudragit E 100 by solvent evaporation[173]. SEM, FTIR and size distribution were performed. The average size of nanoparticles was found to be 150 nm. and the morphology structure was investigated by scanning electron microscopy (SEM).The EE depends on the integration of drug and polymer and glass transition temperature of polymer.

- Gattani YS *et al.,* (2009) formulated aceclofenac gastroretentive microspheres by the emulsification solvent-evaporation technique by using eudragit RS100[67]. Effects of eudragit concentration, temperature and stirring rate on size and drug release were evaluated. The microspheres remained buoyant and released the aceclofenac for more than 12hr. Increase in polymer concentration produced increase in size and decrease in drug release. The prepared microspheres exhibited prolonged drug release and remained buoyant for more than 12 h. No significant effect of the stirring rate during preparation on drug release was observed.

- Prakash Rao B *et al.,* (2009) studied on swellable CPOP tablet containing theophylline by direct compression and wet granulation methods based on Taguchi Orthogonal Array design for core and Fraction Factorial design for coating[32]. Ethyl cellulose

solutions with varying amount of PEG 400 and plasdone were used for spray coating. USP paddle type apparatus was used for dissolution and the release was found to be 98.2%. SEM analysis conformed the formation of pores. Drug release was studied using USP Type I paddle type apparatus and the membrane morphology of the delivery system was determined by scanning electron microscopy (SEM).

- Manjanna KM *et al*., (2009) formulated sodium alginate microbeads of aceclofenac by ionotropic gelation method[174]. The microbeads exhibited no significant drug polymer interactions, particle size of 596.45 ± 1.04µm to 880.10 ± 0.13µm and % EE of 63.24 - 98.90%. Sphericity, particle size, size distribution, swelling ratio and EE were directly proportional to the concentration of sodium alginate, $CaCl_2$ and cross-linking time. The release of aceclofenac sodium was examined in pH 1.2 for initial 2 h, in pH 6.8 up to 6h and in pH 7.2 up to 24h. Drug release was pH dependent and showed negligible in pH 1.2. At neutral aceclofenac was released by swelling and erosion.

- Yadav AV *et al*., (2009) formulated aceclofenac enteric microcapsules by emulsion solvent evaporation using retardant polymer ethyl cellulose[175]. Microcapsules prepared were discrete, free flowing and spherical in shape. Particle size, %Entrapment efficiency and % yield were 1350 µm to 532.5µm, 24.56% to 41.45% and 26.69% to 40.34% respectively. The drug release continued over a period of 12hrs and followed higuchi model.

- Selvakumar K and Yadav AV (2009) prepared Carvedilol loaded eudragit E100 nanoparticles by the nanoprecipitation method using poloxamer 407 as stabilizer[176]. The particle size varied from 190-270nm and encapsulation efficiency of 85-91%. The drug released within 5 minutes. By the results it was concluded that eudragit E100 nanoparticles loaded with carvedilol can be successfully used for the management of hypertension.

- Patel DJ *et al*., (2009) formulated famotidine nanosuspension by nanoprecipitation technique[177]. The combination of less amount of stabilizer with low speed produced

transparentbluish-whitenanosuspensions withmean particle size of 566 nm. The resultant nanosuspension had enhanced release rate. The nano sized drug batch,F1 released 42% within 10 min whereas micronized drug batch, F7 released 2.5% only.

- Shakeel F *et al*., (2009) compared pharmacokinetic profile of nanoemulsion gel, nanoemulsion, and marketed tablet of aceclofenac by transdermal and oral application on Wistar male rats[178]. The absorption of aceclofenac when compared to oral tablet formulation, the transdermally applied nanoemulsion gel and nanoemulsion resulted in 2.60 and 2.95 fold increase in bioavailability indicating that the nanoemulsions can be fruitfully used as effective vehicle for enhancement of bioavailability of aceclofenac.

- Jawahar N *et al*., (2009) developed carvedilolnanoparticles by nanoprecipitation method using PLGA and Pluronic F-68. Particle size of 132-234nm were achieved[179]. EE and drug release was found to be 77.6% and 72% at 24hr respectively. Biodistribution studies suggested that at higher concentration carvedilol may be delivered to the target sites.

- Agnihotri SM and Vavia PR. (2009) Prepared PLGA and poly (lactide-co-glycolide-leucine) nanosuspensions of diclofenac sodium by solvent evaporation[180]. This system possessed improved bioavailabilty. In vitro studies produced extended release of diclofenac. The stability of this formulation was good throughout the period of six months. The drug release data evidenced that the product can deliver drug for 24hrs.

- Khushwant S *et al*., (2009) developed nine batches of Cytarabine-loaded PLGA nanoparticles by modified nanoprecipitation[181]. A 3^2 factorial design was used to optimize the volume of cosolvent (0.22 to 0.37 ml) and non-solvent (1.7 to 3.0 ml). Again 3^2 optimization factorial design was used for drug:polymer ratio considering the mean particle size (125±2.5 nm), and % entrapment efficiency (21.8±2.0%). Optimized formulation showed minimum increase in their particle size, and zeta potential of −29.7 mV. Nanoparticles released the drug up to 24 h. The formulations were stored in freeze

dried condition at 2-8°C and the stability studies indicated that the formulation was stable in particle size and drug content for a period 2 months.

- Raghavendra Rao NG *et al.*, (2010) developed chitosan microparticles loaded with aceclofenac by ionotropic gelation technique[182]. Increase in TPP concentration; pH and cross-linking time decreased the drug release. With increase in cross-linking time, the particle size reduced and found to be between 1194.1 to 1568.9 μm. In pH 7.4 phosphate buffer showed slight burst release in first hour followed by prolonged release for 8 hrs. The values of regression coefficient r^2 were found to be greater ($\leq$ 0.9541) for first order than for zero order ($\leq$ 0.8740) and the r^2 value for Higuchi was $\leq$ 0.9805 suggesting diffusion controlled process.

- Xia D *et al.*, (2010) prepared and evaluated nitrendipine nanosuspensions by the precipitation–ultrasonication[183]. The particle size was 209±9 nm and zeta potential of was −13.9±1.9 mV. XRPD and DSC results showed that the shape of nanocrystals was flaky and no consdierable crystalline changes were observed. By reducing the particle size, the dissolution rate markedly increased. In rats Cmax and $AUC_{0\rightarrow12}$ values of nanosuspension was approximately 6.1 and 5.0 fold increase respectively than that of marketed tablets.

- Mothilal M *et al.*, (2010) formulated osmotically controlled oral drug delivery system by wet granulation for metoprolol succinate using varying concentrations of mannitol[184]. The tablets were subjected to dip coating with cellulose acetate. All evaluation tests were performed. Orifice size was analyzed by SEM. The amount of osmogen used and pore size were directly proportional to rate of drug release upto the optimum concentration.

- Verma A *et al.*, (2010) encapsulated selected drugs within PMMA copolymer (Eudragit RS100 and Eudragit RL100), by solvent evaporation method[185]. Magnesium stearate was used as stabilizer in concentration of 0.3% (v/v). *In vitro* dissolution tests for all the selected formulations exhibited a prolonged release for almost 24 hr. The

mean particle size of microspheres ranged from 75 to 225 μm, a spherical and uniform appearance with rough surface and encapsulation efficiency ranged from 72.72 to 95.88% (w/w). Mechanism of release was found to be Higuchi type. *In vivo* study of microspheres showed significant analgesic and anti-inflammatory activities of microspheres for longer period of time in albino wistar rats when compared to the parent drug. This study indicated that eudragit microspheres sustain the release of drug but also minimize the side effects.

- Kumar KK *et al*., (2010) prepared controlled release aceclofenac microcapsules by solvent evaporation[186]. The granules are encapsulated into the capsules and subjected to particle size, dissolution, encapsulation efficiency and compared with commercial marketed product. Ethylcelllulose, liquid paraffin, cyclohexane and emulsifying agent were added in the formulation. Results showed that 1:6 ratio had 99.08% EE, and 98% drug release.
- Sheaikh S *et al*., (2010) designed OCDDS containing aceclofenac[187]. This system utilized the osmotic principle for drug delivery, so that the release rate was independent of pH, food and physiological factors etc. Formulation 2a released maximum of 96% in controlled passion.

- Cetin M *et al*., (2010) formulated and evaluated Eudragit L100 & Eudragit L100/PLGA nanoparticles loaded with diclofenac sodium[188]. Prepared Nanoparticles were spherical in shape with particle size of 241-274nm. Entrapment efficiency was found to be 25.8-62%. Drug release studies showed that initial burst release of 38-47% occurred during the first 4 hr. Maximum drug release for Eudragit L100 nanoparticles was 92% at 12hr. Eudragit L 100/PLGA (20:80), Eudragit L100/PLGA (30:70), Eudragit L100/PLGA (50:70) nanoparticles produced drug release of 56%, 69% and 81% at 72h respectively.

- Deveswaran R *et al*., (2010) formulated aceclofenac SR microspheres using egg albumin[189]. The particle size, entrapment efficiency and percentage yield was found to be 99.6 μm, 65.2% and 96.99% respectively. The drug released within 10hrs for 1:1

drug polymer ratio and sustained for 12 hrs for 1:2 drug polymer ratio. Ths study reduced the dosing frequency and improved the patient compliance.

- Chakraborty S *et al.*, (2010) prepared microspheres of aceclofenac by gelation technique[190]. Combination of sodium alginate and carbopol934 were used as retardant. All formulations showed good flow property, particle size and % swelling. Microspheres are spherical in shape. Mucoadhesive property increased with increase in polymer concentration. In vitro results showed that increase in polymer concentration caused decrease in drug release. F7 trial sustained release for 12 hr.

- Manjanna KM *et al*., (2010) developed oral SR microbeads of aceclofenac by ionic gelation using sodium alginate and calcium chloride[191]. The process was optimized for parameters drug:polymer ratio, rotational speed and cross-linking time. Increase in polymer and crosslinker concentration, resulted in increased size, flow properties and entrapment efficiency. The average particle size and EE were found to be 596.45 ± 1.04 to 880.10 ± 0.13 μm and 63-98%. The drug was released at negligible rate for first 2 hr and controlled rate for upto 24hrs.

- Patel HR and Patel MM. (2010) prepared and evaluated osmotic controlled mucoadhesive cup-core containing aceclofenac[192]. Formulations containing HPMC had higher swelling index. The drug release studies showed a release up to 12 h following zero order kinetics with diffusion mechanism. Further the formulation showed significant anti-inflammatory activity ($P<0.001$) without hypersensitive reaction. The results revealed that OCMC overcomes the problems associated with bucoadhesive tablet.

- Chakraborty S *et al*., (2010) prepared and evaluated algino-pectinate bioadhesive microspheres of aceclofenac[193]. Drug release was fast in alginate and prolonged in algini-pectinate microspheres. The microspheres exhibited good biodhesion character with high EE. Based on the in vivo test results the algino-pectinate microspheres exhibited sustained release.

- Khandai M *et al.,* (2010) prepared aceclofenac loaded sericin- alginate microspheres by gelation technique using a blend of sodium alginate and sericin as release retardant and evaluated for particle size, flow behavior, swelling properties, surface morphology study by SEM and in vitro drug release etc[194]. All the formulations showed good flow property as compared to the pure drug. The result showed that the release of all the formulations was gradually decreased on increasing the polymer concentration following zero order kinetics followed by case-II diffusion. Ex- vivo mucoadhesion study depicts that when the polymer concentration was increased the mucoadhesion nature was also increased. FTIR study showed that the major peaks of pure drug were almost intact in the formulation.

- Ahad HA *et al*., (2010) developed matrix tablets of Aceclofenac with *Prosopis cumanensis* gum and evaluated once-daily sustained release of the tablets[195]. The formulated tablets found to have better uniformity of weight and drug content with low SD values. The dissolution study proved that the dried *Prosopis cumanensis* gum can be used as a matrix forming material for making once daily sustained release matrix tablets.

- Chella N *et al*., (2010) formulated microspheres of diclofenac sodium using EC as retardant material by w/o emulsion method using methylenechloride/ethanol solvent system; span 80 and n-hexane were added in the formulation of microspheres[196]. Prepared microspheres were sphere shape, excellent flowing with EE of 51.2% and release upto 10hr. The system follows diffusion controlled mechanism.

- Kakran M *et al*., (2010) fabricated artemisinin nanoparticles by evaporative precipitation and investigated by full factorial design[197]. The result revealed that the prepared nanoparticles showed decreased crystallinity with increased drug concentration. Particle size was found to be 100–360 nm, the optimized formulation holds dissolution of 75.9%.

- Kietzmann D *et al*., (2010) formulated carboxyfluorescein microspheres with eudragit 100, for colon targeting[198]. Kinetic parameters of microspheres were compared to oral and rectal. Bioavailability of oral route was lowered in colitis than the healthy group (colitis group 3.0 ± 0.9, healthy group 8.4 ± 1.5) and comparable results were obtained in rectal route (colitis group 1.8 ± 0.8, healthy group 5.6 ± 2.1). But the microspheres showed minimal difference between colitis and healthy groups (colitis group 2.3 ± 0.4, healthy group 1.9 ± 0.8).

- Arias JL *et al*., (2010) investigated polycaprolactone loaded with ftorafur and diclofenac sodium aimed to achieve better therapeutic activity[199]. Drug was loaded by surface adsorption and interfacial polymer disposition methods. High loading with small burst release achieved by these techniques.

- Pandey S. (2010) formulated itraconazole nanosuspension by pearl milling method using ZrO_2 beads as a milling media, poloxamer 407 as stabilizer and glycerol as wetting agent[200]. The nanosuspension was optimized by considering parameters like stirring period and ratio of milling media keeping. The optimized formulation showed a mean particle size of 294nm, high assay value, higher release in 0.1N HCl, with no significant change in crystalline nature.

- Wakode R *et al*., (2010) developed pramipexole PPOP tablets for parkinsonism[31]. Pramipexole PPOP consists of polymeric push layer and drug pull layer coated with cellulose acetate and PEG. *In vitro* and *in vivo* studies conformed that the developed formulation persists its pramipexole plasma level for 24 hr period.

- Gowda DV *et al.,* (2010) formulated a drug loaded transdermal film by casting method using sodium alginate and MLBG[201]. The effects of drug polymer ratio, plasticizer concentration on drug release were studied. The films showed 1.90 to1.95 mg/sq.cm of drug content, 0.29 to 0.38 mm thickness and found to be compatible and stable. Increase in MLGB and decrease in sodium alginate caused decreased folding endurance. *In-vitro* tests produce controlled release for 24hr. Absence of erythema,

edema or ulceration suggested that prepared transdermal films were effective with good skin tolerability.

- Gaikwad A *et al*., (2010) prepared furosemide loaded eudragit RS100 nanoparticles with various drug carrier ratios by using nanoprecipitation method for oral delivery[202]. The results indicated the sphericity with particle size of 163nm to 378 nm, entrapment efficiency of 14.95 ± 0.06 to 69.73±0.03%, and drug release up to 24 hrs. It followed higuchi kinetics. Zeta potential supported the stability, and further stability study for 60 days produced the good stability of the product.

- Guerrero S *et al.*, (2010) prepared ketotifen loaded chitosan microspheres by spray drying followed by addition of glutaraldehyde in methanol as cross-linker for controlled release[200]. Results indicated that high load of drug (92 ± 6 μg KT/mg) and spherical microspheres of size 1.0–1.3 μm were achieved. Further results showed that loading of KT decreased with cross-linking (52 ± 2– 46 ± 7 μg KT/mg). KT release was detected in blood at 24 h.

- Yadav IK *et al*., (2010) developed aceclofenac matrix tablets consisting of hydrophilic & hydrophobic polymers by direct compression using optimizing parameter as drug polymer ratio[204]. In vitro release of F1, F4 and F7 batches extended up to 12 hrs and followed zero order. Drug release was reduced by increasing the polymer concentration. Stability studies for 3 months indicated that the product was stable and the polymers used were suitable for delivery of aceclofenac.

- Ahad HA *et al.*, (2010) investigated *Prosophis juliflora* as matrix material for once a daily aceclofenac tablets by using drug and matrix material ratios of 1:0.25, 1:0.375, 1:0.5, 1:0.675 & 1:0.750[205]. The flow properties of the gum were recorded. The tablets were evaluated for thickness, hardness, friability, uniformity of weight, swelling and in vitro release. All the recorded values were within the limit. From the dissolution values it can be concluded that Prosophis juliflora can be used as polymeric matrix carrier for SR tablets.

- Xu WJ *et al.,* (2011) prepared and optimized controlled porosity osmotic tablets for salvianolic acid (SA) using experimental design methods including an artificial neutral network method[206]. Three causal factors, i.e., drug, osmotic pressure promoting agent rate, PEG400 content in coating solution and coating weight, were evaluated based on their effects on drug release rate.The linear correlation coefficient of the accumulative amount of drug release and the time of 12h, r(Y1), and the sum of the absolute value between measured and projected values, Y2, were used as outputs to optimize the formulation. The weight expression Y¼(1_Y1)2þY2 2 was used in the calculation. Furthermore, the ANN and uniform design gave similar optimization results, but ANN projected the outputs better than the uniform design. This paper showed that the release rateof salvianolic acid B and that of the total salvianolic acid was consistent in the optimized formulation.

- Reza KhZ *et al.,* (2011) formulated aceclofenac monolithic osmotic tablet and investigated the formulation variables including amount of explotab and sodium chloride[207]. Both explotab and sodium chloride produced positive effects. The optimal orifice size was 800 μm. Incorporation of polyethylene glycol (PEG) in CA membrane improved drug release. This system followed zero order for 24 hrs.

- Li W *et al*., (2011) prepared 3 suspensions containing revaprazan hydrochloride by high pressure homogenization and evaluated the same[105]. The reports revealed that both microsuspension and nanosuspension exhibited and remained as crystalline in nature. In vivo tests for coarse, micro and nano suspension were determined. Nanosuspension exhibited notable increase in AUC, Cmax, Tmax and MRT when compared with coarse, but microsuspension didn't produce any difference in pharmacokinetic parameters. The work made an impact on enhanced oral bioavailability of revaprazan HCl by reducing the particle size reduction.

- Gao L *et al*., (2011) prepared a stable quercetin nanosuspension by using evaporative precipitation and high homogenization process[208]. The particle size and zeta potential

of the nanosuspension prepared by first method was similar to that of second one. In first method the dried powder showed crystalline to amorphous state and in second crystallinity was maintained. First method produced higher dissolution than the second. Both the methods were feasible and superior over the quercetin solution.

- Zhang ZH *et al*., (2011) developed an expert system for poorly water soluble drugs as PPOP[209]. Hundreds of PPOP were considered to poorly soluble drugs with inert additives. By utilizing knowledge database, rule base and from researchers an expert system was builded based on the reports of PPOP formulations. Famotidine was selected as model drug to validate the expert system.

4. PLAN OF WORK

The plan of work was given below

I. PREFORMULATION STUDIES

i) Analysis of Aceclofenac

- UV Spectroscopy
- IR Spectroscopy
- Melting point
- Loss on drying
- Angle of repose
- Bulk density and tapped density
- Compressibility index
- Hausner's ratio

ii) Drug-Excipient compatibility studies

- IR Spectroscopy
- DSC technique

iii) Preparation of standard graph of aceclofenac

II. FORMULATION AND EVALUATION STUDIES:

i) Formulation and evaluation of Aceclofenac osmotic tablets

Formulation of aceclofenac oral osmotic tablets (OT1-OT13)

Evaluation of aceclofenac oral osmotic tablets (OT1-OT13)

- Content uniformity
- Weight variation test
- Thickness
- Hardness test
- Friability test
- Osmotic pressure
- In vitro dissolution

ii) Formulation and evaluation of Aceclofenac microspheres

Formulation of aceclofenac microspheres (M1 – M15)

Evaluation of aceclofenac microspheres (M1 - M15)

- Flow properties
- Particle size
- Morphology
- Drug content
- Entrapment efficiency
- In vitro release

iii) Formulation and evaluation of Aceclofenac nanosuspension

Formulation of aceclofenac nanosuspension (F1 – F27)

Evaluation of aceclofenac nanosuspension (F1 – F27)

- Particle size
- Morphology
- Zeta potential
- Drug content
- Entrapment efficiency
- In vitro release

III. PHARMACOLOGICAL STUDIES:

- Anti-inflammatory studies
- GI tolerability studies

IV. STABILITY STUDIES:

- Stability studies of best formulation (Osmotic tablets - **OT10)**
- Stability studies of best formulation (Microspheres - **M4)**
- Stability studies of best formulation (Nanosuspension - **F-14BYM)**

5. DRUG PROFILE AND ADDITIVES PROFILE

ACECLOFENAC

Synonyms:

Airtal, Falcol, PR-82/3, Gerbin, Preservex

Chemical Name:

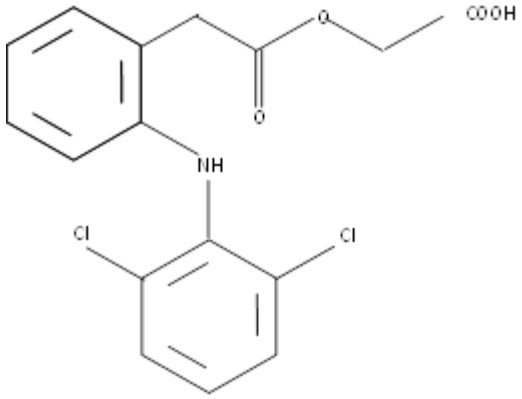
- 2,[(2,6-dichlorophenyl)amino]benzene acetic acid carboxy methyl ester
- 2-[(2,6-dichlorophenyl)amino]phenyl acetoxy acetic acid
- Glycolic acid [O-(2,6 – dichloroanilino)phenyl] acetate ester

Molecular Formula:

$C_{16}H13C_{l2}NO_4$

Molecular structure:

Fig.5.1 ACECLOFENAC



Molecular Weight:

354.2

Where C → 54.26%, H → 3.7%, Cl →20.02%, O→ 18.07%, N → 3.95%

Description:

Aceclofenac is a new Non-steroidal anti-inflammatory drug (NSAID) which acts on inflammatory sites.

Physical Appearance:

Crystalline white powder

Percentage purity:

99.0 – 101.0%

Melting point:

149 - 153ºC

Loss on drying:

Maximum of 0.5% calculated on basis of drying 1gm at 100 - 105ºC

Solubility:

Insoluble in water, however spontaneously soluble in acetone, dimethyl formamide, ethanol and in methanol.

Identification[210]:

i) 50mg of drug is dissolved in methanol and diluted to 100ml. From the above solution 2ml is diluted to 50ml with the same solvent. The diluted solution is examined at nanometer of 220 to 370. The absorption maximum shows at 275nm.

ii) It can be identified by IR spectrum. Sample spectrum is compared with aceclofenac standard.

iii) 10mg of drug is dissolved in 10ml of alcohol. From this 1ml of solution is taken, to this, 0.2ml of mixture (6g/l Potassium ferricyanide solution and 9g/l ferric chloride solution) is added. This solution is allowed to stand for 5 minutes in light protected place. Then 3ml of 10g/l HCl solution is added. Again this solution is allowed for 15 minutes. A blue color is developed and precipitate formed.

PHARMACOLOGY:

Mechanism of Action[211]:

The multifactor mechanism of aceclofenac may be given as follow

i) Inhibition of interleukins-beta and tumor necrosis factor in the inflammatory cells (Intracellular action):

Interleukins-beta, tumor necrosis factor are released by inflammatory cells which are responsible for the production of prostaglandin E2. Aceclofenac inhibits COX (cyclooxygenase) activity; thereby suppress the prostaglandin E2 synthesis. Aceclofenac penetrates into the affected cells and hydrolyzed to active drug. This active drug inhibits interleukin and tumor necrosis factor, which may result in suppression of synthesis of prostaglandins.

ii) Stimulation of synthesis of extracellular matrix in human articular cartilages:

Aceclofenac stimulates extracellular matrix synthesis by inhibiting the action of cytokines. This leads to inhibition of development of inflammatory cells, inhibition of increased synthesis of MMP and proteoglycan production.

iii) Inhibition of neutrophil adhesion and accumulation:

Aceclofenac contains diphenylamine core which is responsible for inhibition of transmigration of neutrophils to inflammatory cells and tissues.

PHARMACOKINETICS[212]:

Absorption:

After oral administration, aceclofenac immediately and completely absorbed. Tmax reached between 1.25 - 3 hours of administration. Food may alter the absorption rate. Bioavailability is 60-70%.

Distribution:

Aceclofenac is having high affinity to protein (99.7%). It penetrates to the synovial fluid. The Volume of distribution (Vd) is around 30 Litres.

Metabolism:

Aceclofenac is metabolized in human microsomes and hepatocytes as hydroxyl metabolites. The chief metabolite is 4'-hydroxyaceclofenac. Some other metabolites include 5-hydroxyaceclofenac, diclofenac and 5-hydroxydiclofenac.

Elimination:

Approximately 70 -80% of dose administered is eliminated through renal excretion. Around 20% is eliminated through faeces. Elimination half-life is 4-4.3 hrs. Eliminated as glucuronides of aceclofenac.

THERAPEUTIC USES[213]:

Aceclofenac is catergorized as anti-inflammatory and analgesic.

It is used in conditions of pain and inflammations in

- Rheumatoid arthritis
- Osteoarthritis
- Ankylosing spondylitis
- Post-traumatic pain

- Cervical pain
- Low back pain

DOSING PARAMETERS:

- Normal adults – Maximum 200mg/day
- Elderly patients- Same as adult as there is no change in ADME
- Children – No clinical data
- Hepatic insufficiency
- Severe – not recommended
- Mild/moderate – 100mkg/day

DRUG INTERACTIONS[214]:

Aceclofenac produces interactions with some drugs which are listed below:

- Lithium, methotrexate and digoxin. Acelclofenac increases the blood level of these drugs which may cause toxicity.
- Risk of bleeding in GIT with anti-clotting drugs like ticlopidine, warfarin, heparin
- Aceclofenac and ketoralac cause haemorrhage
- Aceclofenac and quinoline antibiotics produce convulsion
- Aceclofenac with frusemide, reduces the effect of the later/latter
- Aceclofenac with cyclosporine may induce risk of nephrotoxicity
- Aceclofenac with corticosteroids may produce stomach irritation

ADVERSE DRUG REACTIONS[215, 216]:

Aceclofenac produces the adverse reactions give below

- Dyspepsia, vomiting diarrhoea, abdominal pain
- Flatulence, constipation, gastritis, constipation, ulcerative stomatitis
- Dizziness, headache, allergic reactions, increase in weight
- Rash, pruritis
- Increased serum creatinine and urea
- Blood disorders such as anaemia, thrombocytopenia, granulocytopenia, neutropenia
- Cardiovascular disorders such as palpitation, edema, flushing
- Nephrotic syndrome

- Psychiatric disorders such as depression, somnolence, insomnia

PRECAUTIONS/WARNINGS[215, 216]:

In some disease state aceclofenac should be used with caution. Such states include

- Hepatic Porphyria (Blood disorder caused by liver defect)
- Bleeding conditions
- Crohn's disease
- Reduced heart function
- Elderly patients those easily affected by adverse reactions
- Peptic ulcers
- GI inflammation
- Reduced kidney function
- Patients undergone recently major surgery
- Stomach and intestinal disorder

CONTRAINDICATIONS[217]:

In some disease conditions aceclofenac should be avoided. Such states include

- Peptic or duodenal ulcer
- Bleeding in Stomach or intestine
- Kidney failure
- Asthma, acute rhinitis or urticarial
- 7th to 10th months of pregnancy which may cause delayed delivery, neonatal respiratory hypertension, renal function
- During lactation. If it is essential can be given

STORAGE:

It should be stored in air tight container. It should be protected away from light.

PREPARATIONS:

Aceclofenac is available as conventional tablets, SR tablets, capsules and gel.

SODIUM BICARBONATE[218,219]:

Synonyms:	Baking soda, sodium acid carbonate, monosodium carbonate
Chemical Name:	Carbonic acid monosodium salt
Molecular formula:	NaHCO3
Molecular weight:	84.01
Description:	White, odourless crystalline inorganic salt.
Solubility:	Soluble in water (96g/l at 20ºC), Insoluble in ether & ethanol.
Melting point:	270ºC
Particle size:	Based on the grades particle size may range from 15 to 300 μm.
Density:	2.15 at 20ºC
Bulk Density:	0.8690 g/cubic cm
Tapped density:	1.3690 g/cubic cm
LOD:	40%
Storage:	Should be stored in well closed container.
Incompatibilities:	It may produce CO2 with acidic and alkaloidal salts.
Applications:	Antacid and osmotic agent. In effervescent tablets it is used to produce CO2. It can be used as buffering agent or as stabilizer in tooth paste.

SODIUM CHLORIDE[219, 220]:

Synonym:	Hopper salt, rock salt, table salt
Molecular formula:	NaCl
Molecular weight:	58
Description:	White, isometric orthorhombic cubic crystal form.
Solubility:	Soluble in water, slightly soluble in ethanol
Melting point:	800.8 ºC
Boiling point:	1,465 ºC
Refractive index:	1.544
Density:	2.16 g/cc

Bulk density: 1.154 g/cc

Angle of repose: 32º

LOD: < 0.5%

Hardness: 2.5 (Moh scale)

Storage: Stored in well closed container

Applications: Solid dosage form diluent, osmogen

POTASSIUM CHLORIDE[219,221]:

Synonyms: K-lyte, k-lor

Molecular formula: KCl

Molecular weight: 74.55

Description: Crystalline, white to colourless, KCl naturally occurs as sylvite.

Solubility: Soluble in water and ethanol, insoluble in ether.

Melting point: 773ºC

Refractive index: 1.456

Specific gravity: 1.988

Bulk density: 0.96 g/cc

Angle of repose: 28-30º

LOD: Maximum 1% (105 ºC for 2 hrs)

Percentage purity: 99-100.5%

Toxicity: Cardiac, GI ulcer, diarrhea, vomiting and hyperkalemia

Storage: Store in air tight container

Incompatibility: incompatible with amphotericin, phenytoin, mannitol

Applications: Pharmaceutically used as an osmogen and electrolyte replenisher. Widely used in food materials like cheeses, soup, sauces and drinks

HYDROXYPROPYL METHYL CELLULOSE[219, 222]:

Synonyms: Methocel, Methylcellulose, cellulose, hydroxypropylmethyl ether, hypromellose

Molecular weight: Ranges between 10000 to 1500000

Description: Odourless, tasteless, creamy white powder

Solubility: Forms viscous solution in water. Insoluble in ethanol, ether and

chloroform.

Melting point: Browning at 190 - 200ºC and charring at 225 - 230 ºC

Viscosity: 4000 mPa s (2% aqueous solution of HPMC K4M)

Specific gravity: 1.26

True density: 1.326 g/cm3

Bulk Density: 0.341g/cm3

Tapped density: 0.557g/cm3

Loss on drying: Maximum of 5%

Residue on ignition: 1.5%

Storage: Well closed container.

Incompatibility: It produces molecular adducts with sodium salicylate, sulfathiazole and tannins.

Applications: Used in tablet manufacturing as binder, sustained release and film material. It can also be used as thickening and suspending agent in ophthalmic preparations.

MICROCRYSTALLINE CELLULOSE[219, 223]:

Synonyms: Avicel, cellulose crystalline, tabulose, fibrocel

Molecular formula: $(C_6H_{10}O_5)_n$ Where n ≈ 220

Molecular weight: ≈ 36000

Description: White, odourless, crystalline powder comprising porous particles

Solubility: Insoluble in water, organic solvents, slightly soluble in 5% NaOH

Melting point: 260 - 270ºC

True Density: 1.512 to 1.668 g/ml

Bulk Density: 0.32 g/cc

Tap Density: 0.45 g/cc

Particle size: Ranges from 20 to 200µm

Moisture level: < 5%

Storage: Stored in well closed container free from moisture

Incompatibility: It may produce incompatibility with strong oxidants

Applications: MCC at different concentrations it can be used for different purposes. 20 to 90% is used as adsorbent, 5 to 20%, and 5 to 15% used as anti-adherent and disintegrants respectively.

PVP K30[224]:

Synonym: Povidone

Chemical Name: 1-Ethenyl-2-pyrolidinone homopolymer

Molecular weight: 50,000 (approx.)

Molecular formula: $(C_6H_9NO)n$

Description: White creamy odourless powder, hygroscopic nature.

Solubility: Soluble in chloroform, methanol, ethanol and water .
Insoluble in ether.

Preparation: By spray drying

Storage: Air tight containers

Applications: Used in pharmaceutical formulations as granulating agent, suspending agent, coating material or viscosity builders.

MAGNESIUM STEARATE[219, 224]:

Synonyms: Magnesium salt, octadecanoic acid, stearic acid

Chemical Name: Octadecanoic acid magnesium

Molecular Formula: $[CH_3(CH_2)_{16}COO]_2Mg$

Molecular weight: 591.34

Description: Fine, white, precipitated powder having stearic acid faint odour and taste. It adheres to the skin.

Solubility: Slightly soluble in benzene and ethanol (warm conditions).
Insoluble in water, ethanol and ether.

Melting point: 126-130ºC

True density: 1.09 g/ml

Bulk density: 0.15 g/ml

Tapped density: 0.28 g/ml

Surface area: 1.6-14.8m^2/g

Storage: Well closed container, cool and dry place

Incompatibilities: With oxidizing substances, strong acids, alkalis, and some vitamins.

Applications: Used as lubricant in tablet/capsule preparation.

Commonly used in food, cosmetic and pharmaceutical industries.

TALC[224]:

Synonyms: Purtalc, Magsil star, soap stone

Formula: $Mg_6(Si_2O_5)_4(OH)_4$

Description: White to dim colour, odourless, crystalline in nature. Sticks on skin.

Solubility: Insoluble in organic solvents and water.

pH: 6.5 – 10 (20% aqueous soln.)

Specific gravity: 2.7 to 2.8

Bulk density: 0.21 g/cc

Hardness: 1-1.5 (Moh scale)

LOD: Less than 1%

Storage: Stored in well closed container.

Incompatibilities: With quarternary ammonium type of compounds

Applications: Lubricant in tablet and capsule formulations.

It is also used in the CR formulation as dissolution retardant.

It is also used in clarification of liquids.

CELLULOSE ACETATE[225]:

Synonyms: Acetylcellulose

Chemical name: Cellulose diacetate and cellulose triacetate

Description: Powder or pellets with no taste and odour.

Solubility: Soluble in acetone-water mixture.

Melting point: 230 - 300 ºC

Glass transition (Tg): 170-190ºC

Density: 0.4g/ml

Loss on drying: less than or equal to 5%

Storage: Stored in cool and dry place.

Incompatibility: With acidic and alkaline materials.

Applications: As a coating material for SR systems.

POLYETHYLENE GLYCOL 400[225]:

Synonyms: Polyoxyethylene glycol, carbowax, Macrogol

Chemical name: α hydro-ω-hydroxypoly(oxy-1,2-ethanediol)

Molecular formula: $HO\text{-}CH_2\text{-}(CH_2\text{-}O\text{-}CH_2)_{8.7}\text{-}CH_2\text{-}OH$

Avg mol weight: 380-420

Description: Clear, colourless viscous liquid with characteristic odour and bitter taste

Solubility: Miscible in ethanol, chloroform, fixed oils and in organic solvents. Immiscible in water, methanol, ethyl acetate.

Density: 1.12 g/cm3 at RT

Viscosity: 105-130 cP

Refractive index: 1.46

Storage: Store in well closed container.

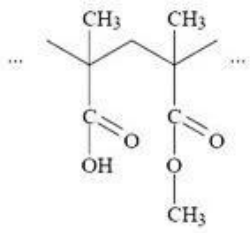
Applications: Plasticizer, ointment & suppository base, poreformer in osmotic systems.

EUDRAGIT L100[226]:

Synonyms: Polymethymethacrylate, Kollicoat

Chemical name: Poly(methacrylic acid methylmethacrylate)

Structure:



Avg. mol. wt.: 1, 23,000 (approx.)

Description: White powder, with faint odour

Solubility: Soluble in methanol & acetone, insoluble in water and ethylacetate.

Glass ignition temp: >150ºC

RI: 1.39

Acid value: 315 mg Potassium chloride/gram Eudragit L100

True density: 0.83-0.85 g/ml

Bulk density: 0.39 g/ml

Tapped density: 0.42 g/ml

LOD: 5% dried at 110 ºC for 6 hrs

Storage: Protected from heat and moisture

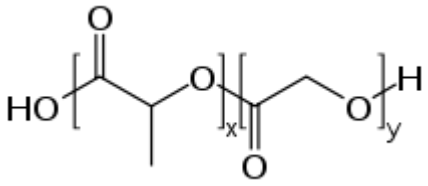
Applications: Enteric coating polymer, binder, film former.

PLGA[227, 228. 229]:

Synonyms: Resomer

Molecular formula: $[(C_6H_8O_4)x(C_4H_4O_4)y]n$

Molecular structure:



x = no. of lactic acid units, y = no. of glycolic acid units

Wt. Avg. mol. wt: Varies from 5000 to 20000.

Description: It is a biocompatible/biodegradable polymer synthesized from lactic acid and glycolic acid. Amorphous in nature.

Solubility: Soluble in acetone, ethylacetate, but insoluble in water, methanol and ethanol.

Tg: 40-60ºC

Degradation: Degraded into glycolic and lactic acids in body by hydrolysis.

Storage: Stored in a temperature of -20ºC.

Applications: It can be successfully used for sustained release formulations. It can be used for preparation of microspheres, nanoparticles for effective and targeted delivery of drugs.

POLYVINYL ALCOHOL[219]:

Synonyms: Gelvatol, mowiol, PVA.

Solubility: Soluble in water.

Formula: $(C_2H_4O)_x$

Molecular weight: 20,000 to 2,00,000

Melting point: 228ºC

Specific gravity: 1.19-1.31 at RT

Density: 1.19-1.31g/cm^3

Properties: Odourless, high tensile strength. Water reduces the tensile strength. It is completely degradable nontoxic material.

Uses: Film forming agent, stabilizer in emulsions, suspensions etc.

ETHANOL[219]:

Synonym: Alcohol, ethyl alcohol, synasol

Molecular Formula: C_2H_5OH

Molecular weight: 46.07

Boiling point: 78.5ºC

Density: 0.789 g/cc

Properties: Polar because of –OH group. It can form hydrogen bond easily.

Miscibility: Miscible with water at all concentrations.

Applications: Wide range of applications in pharmaceutical field and in other fields. It is used as a solvent for extraction of many drugs. The major advantage of ethanol is its capability of free from microorganisms.

METHYLENE CHLORIDE[219]:

Synonym: Dichloromethane

Molecular formula: CH_2Cl_2

Description: Colourless liquid and volatile in nature.

Applications: Used as solvent in preparation of microspheres and nanoparticles.

METHANOL[219]:

Synonyms: Methyl alcohol, carbinol

Molecular Formula: CH_3OH

Molecular Weight: 32.04 g/mol

Description: White colourless liquid with alcoholic odour, miscible in water.

Boiling point: 64.7ºC

Melting point: -97 ºC

Flash point: 12ºC

Applications: Used as solvent in preparation of bulk drugs, micro & nanoparticles.

ACETONE[219]:

Synonyms: dimethyl ketone, dimethyl formaldehyde

Chemical name: 2-Propanone

Molecular formula: C_3H_6O

Molecular weight: 58.08

Description: Colourless volatile liquid.

Solubility: Miscible in water and ethanol.

Odour: Fruity

Boiling point: 56.2ºC

Melting point: 94.35ºC

Density: 0.7899 g/ml

Applications: Used as solvent in formulation of microspheres and nanoparticles. Widely used as a solvent in production of various compounds like methacrylic acid, bisphenol etc.

TWEEN 80[219]:

Synonym: Polysorbate 80, Alkest, Crillet 4

Formula: $C_{64}H_{124}O_{26}$

Molecular weight: 1310 daltons

Description: Non-ionic surfactant obtained from polyethoxylated sorbitan and oleic acid. Amber colour viscous liquid.

Solubility: Soluble in water, methanol and ethanol. Insoluble in mineral oil.

Relative density: 1.07 (RT)

Viscosity: 400 to 620 cps

Melting point: -20.5ºC

HLB Value: 15

Surface tension: 42.5 dynes/cm

CMC: 13 to 15 mg/litre

Applications: Emulsifier, stabilizer.

6. MATERIALS AND EQUIPMENTS

The materials and equipments used for the preparation of osmotic tablets, microspheres and nanosuspension were given below:

Table 6.1. MATERIALS USED

S.No	Material	Source
1.	Aceclofenac	Tablets India Ltd., Chennai
2.	Sodium bicarbonate	Sisco research laboratories pvt ltd, Mumbai
3.	Sodium chloride	Sisco research laboratories pvt ltd, Mumbai
4.	Potassium chloride	Sisco research laboratories pvt ltd, Mumbai
5.	Microcrystalline cellulose	Indian research products,Chennnai
6.	PVP K30	Sisco research laboratories pvt ltd, Mumbai
7.	HPMC K4M	β-pura laboratories pvt., ltd,chennai
8.	Talc	Otto Chemika-Biochemika reagents, Mumbai
9.	Magnesium stearate	Loba chemie pvt, ltd,mumbai
10.	PEG 400	β-pura laboratories pvt ltd,chennai
11.	Cellulose acetate	Otto Chemika-Biochemika reagents, Mumbai
12.	Eudragit L100	Loba chemie pvt, ltd,mumbai
13.	PLGA	Sigma Aldrich, St.Louis
14.	Acetone	Sisco research laboratories pvt ltd, Mumbai
15.	PVA	Indian research products,Chennai
16.	Tween 80	Otto Chemika-Biochemika reagents, Mumbai

17.	Ethanol	Loba chemie pvt ,ltd,mumbai
18.	Methylene chloride	RFCL Limited, Rankem, Newdelhi
19.	Methanol	Loba chemie pvt ,ltd,Mumbai
20.	Potassium Bromide (IR Grade)	Merck, Goa
21.	HCl	RFCL Limited, Rankem, Newdelhi
22.	Potassium dihydrgen sulphate	Central drug house (p) ltd,Mumbai(andheri)
23.	Sodium hydroxide pellets	Merck Ltd., Mumbai

Table 6.2. EQUIPMENTS USED

S.No.	Equipments	Model / Source/Supplier
1.	Compression machine	Rimek, minipress, UK
2.	Mechanical sieve shaker	Hicon, grover enterprises New Delhi.
3.	Hot air oven for drying	Hicon, grover enterprises New Delhi.
4.	Coating pan	Hicon grover enterprises, New Delhi
5.	Bulk density apparatus	Veego, Mumbai
6.	Vernier caliper	Mitutoyo cd - 6 cs, Japan
7.	Hardness tester	Tablet hardness tester,Shankar
8.	Friabilator	EF - 2 Electrolab, Mumbai
9.	Weighing balance	Sartorious, Germany
10.	Ultraprobe sonicator	Electrosonic industries, EI 250 UP, Mumbai
11.	Magnetic stirrer	Remi instruments, Mumbai
12.	Ultra centrifuge	Remi instruments, Mumbai

13.	FTIR	Alphar T0, Bruker, New Delhi
14.	Ultraviolet spectrophotometer	UV-1700, Shimadzu, Mumbai
15.	Differential Scanning calorimeter	DSC 60 Shimadzu, Mumbai
16.	Melting point apparatus	Campbell Electronics, Mumbai
17.	Scanning Electron Microscope	FEI-Quanta 200F, USA
18.	Zetasizer	Malvern zetasizer ,ZS90, UK
19.	Franz diffusion cell	Aar Gee Automation & Control, Mohali, India
20.	Dissolution tester	Electrolab TDT - 08 L, Mumbai
21.	pH meter	DI-707Digisun Electronics. Hyderabad
22.	Stability chamber	Thermolab Scientific equipments Pvt. Ltd., Mumbai

7. PREFORMULATION

Preformulation is defined as application of biopharmaceutical principles to the physicochemical properties of the drug. It is a phase of R & D process developed to achieve a safe and effective dosage form.

PREFORMULATION STUDIES:

ANALYSIS OF ACECLOFENAC:

i) UV Spectroscopy[231]:

50mg of sample was dissolved in methanol and then diluted to 100ml with the same. 2ml of the above solution was diluted to 50ml with methanol. This solution was scanned between 220nm to 370nm. The UV curve obtained was given in results and discussion section as Fig 7.1.

ii) IR Spectroscopy[231]:

IR spectra of aceclofenac, was obtained by a Perkin-Elmer Fourier transform infrared spectrophotometer using KBr pellets. KBr pellets were prepared by gently mixing the aceclofenac with KBr (1:100). The scanning range used was 4000 to 400cm-1. The obtained graph was compared with the reference standard. The IR graph was given in results and discussion part Fig 7.2.

iii) Melting point:

Aceclofenac MP was measured by capillary tube method[232]. The readings were given in table 7.1

iv) Loss on drying:

One gram of Aceclofenac was heated to a temperature of 105ºC in hot air oven until it attained constant weight. The formula to calculate LOD was[233]

$$\text{Percentage LOD} = \frac{\text{Initial weight - Final weight}}{\text{Initial weight}} \text{ X } 100$$

The results were recorded and given in the table 7.1.

v) Angle of repose:

It was measured by fixed funnel technique[234]. In this technique a funnel containing aceclofenac was kept at a fixed height, and it was allowed to flow to the ground surface which contains graph paper. The height (h) and radius (r) of the heap formed was measured and from this value angle of repose (θ) was determined by the formula

$$\theta = \tan^{-1}(h/r)$$

The results were recorded and given in table 7.1.

vi) Bulk density & Tapped density:

Weighed amount of aceclofenac was placed in a measurable cylinder, the volume (untapped) was noted and then the measurable cylinder was tapped until the volume remains constant. Bulk and Tapped densities were calculated by the following formulas[234]

$$\text{Bulk Density} = \frac{\text{Mass of Powder}}{\text{Volume of Powder (Untapped)}}$$

$$\text{Tapped Density} = \frac{\text{Mass of Powder}}{\text{Volume of Powder (Tapped)}}$$

The results were recorded and presented in results and discussion section, table 7.1.

vii) Compressibility Index:

CI of the powder was determined from the bulk and tap density as follows[4]

$$\text{Percentage Compressibility Index} = 100 \text{ X } \frac{(\text{Tapped Density - Bulk Density})}{\text{Tapped Density}}$$

The results were recorded and presented in results and discussion section, table 7.1.

viii) Hausner's ratio:

It was calculated as

$$\text{Hausner ratio} = \frac{\text{Tapped Density}}{\text{Bulk Density}}$$

The results were recorded and presented in results and discussion section, table 7.1.

DRUG-EXCIPIENT COMPATIBILITY STUDIES

- **IR Spectroscopy**

  IR spectra of pure aceclofenac, additives and combination of aceclofenac with additives were obtained by using Perkin-Elmer Fourier transform infrared spectrophotometer using KBr pellets[235]. KBr pellets were prepared by gently mixing the sample with KBr. The scanning range used was 4000 to 400cm^{-1}. IR spectra for the samples were given in results and discussion section from Fig. 7.2 to Fig. 7.30.

- **DSC technique**

  For DSC studies aceclofenac, additives and combination of aceclofenac with additives were sealed in aluminum pans and the DSC thermograms were recorded at a heating rate of 10°/min[235]. DSC thermograms were given in results and discussion section from Fig. 7.31 to Fig. 7.58.

PREPARATION OF STANDARD GRAPH OF ACECLOFENAC:

Standard graphs of the drug were prepared using standard aceclofenac solution in acid buffer pH 1.2, phosphate buffer pH 6.8 & pH 7.4 containing 5 to 50µg. The absorbance was measured at 275nm. Linear relationship was observed with absorption to concentration of drug. The values of absorbance related to concentration were given in table 7.2 and graphs were given in fig 7.59, 7.60, 7.61.

RESULTS AND DISCUSSION:

The UV, IR and melting point studies helped to identify the aceclofenac. The obtained UV and IR spectra of the sample were similar to that of standard. The spectra were represented in Fig 7.1 & 7.2.

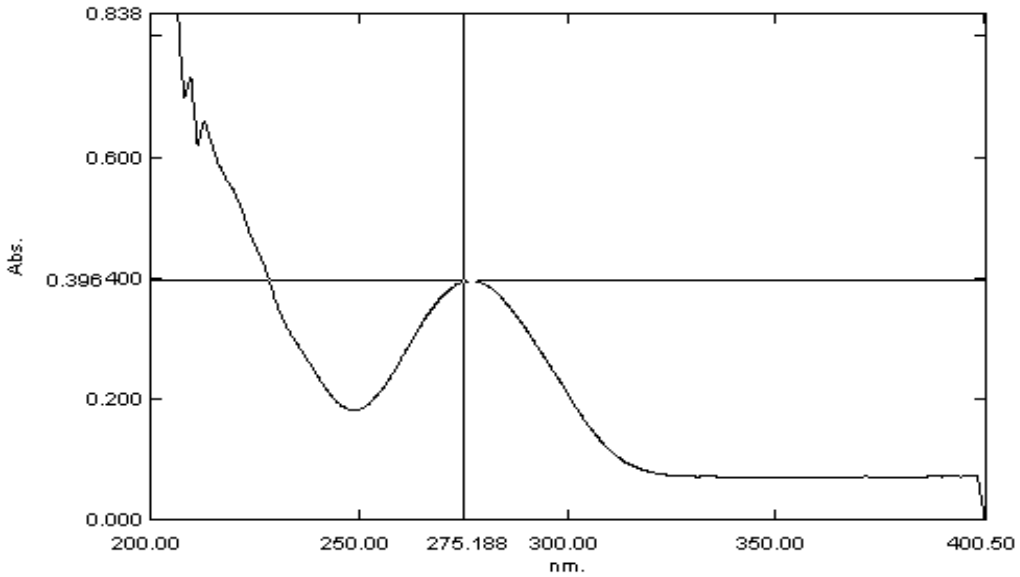


Fig. 7.1. UV Spectra of Aceclofenac

Physical Characteristics:

Physical characteristics indicated that aceclofenac possess poor flow. The results were given below:

Table 7.1. Physical characteristics of Aceclofenac

S.No	Particulars	Results
1.	Melting point	150ºC
2.	LOD	0.2%
3	Angle of repose	40.32 ± 0.16
4	Bulk density	0.560 ±0.015
5	Tapped density	0.720 ± 0.025
6	Compressibility Index	22.21 ± 0.7
7	Hausner's ratio	1.285 ± 0.013

DRUG-EXCIPIENT COMPATIBILITY STUDIES

IR and DSC analysis:

Aceclofenac contains one carbonyl, one ester and one secondary amine group which have characteristic band values around 3276, 1770 and 3317cm^{-1}. These characteristic bands were observed in all of the recorded IR spectra. The DSC thermogram revealed that the additives showed superimposition on thermogram, however mild preshift was observed. The FTIR and DSC results revealed that there was no interaction between the drug and additives used in the formulation. The IR and DSC were shown from Fig. 7.2 to Fig. 7.58.

STANDARD GRAPH OF ACECLOFENAC:

Table 7.2. Concentration and absorbance of aceclofenac in pH 1.2, pH 6.8 and pH 7.4

S.No	Conc (µg/ml)	Absorbance		
		pH 1.2	pH 6.8	pH 7.4
1	5	0.042	0.035	0.138
2	10	0.080	0.067	0.260
3	15	0.120	0.095	0.382
4	20	0.152	0.123	0.488
5	25	0.192	0.148	0.612
6	30	0.228	0.178	0.722
7	35	0.265	0.210	0.852
8	40	0.298	0.241	0.973
9	45	0.335	0.267	1.099
10	50	0.375	0.296	1.218

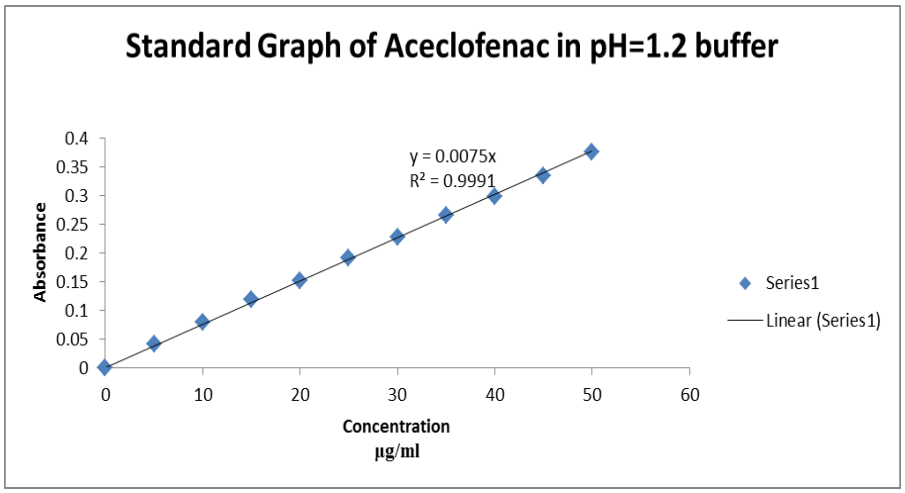


Fig 7.59

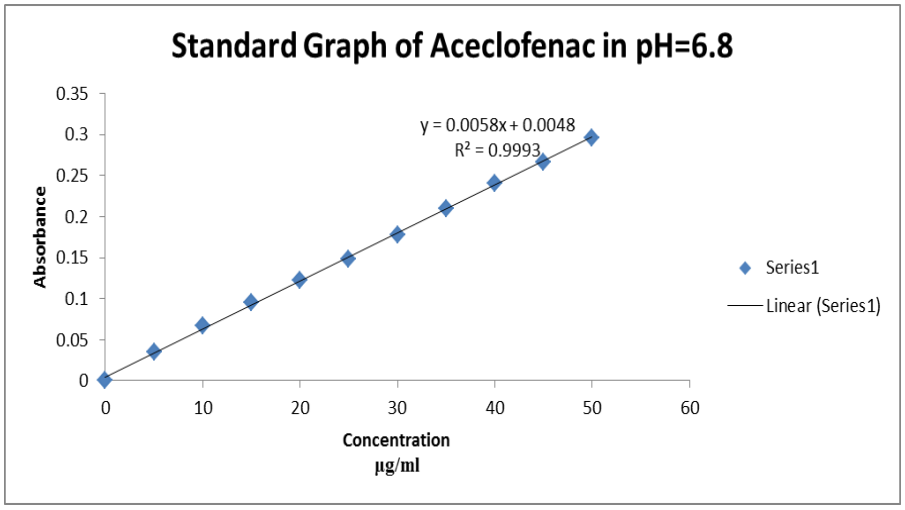


Fig 7.60

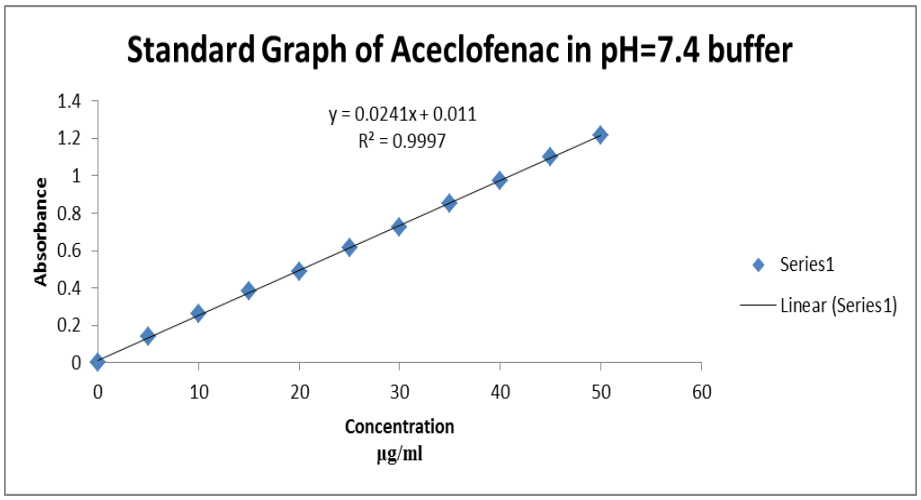


Fig 7.61

8. ACECLOFENAC OSMOTIC TABLETS

METHOD OF PREPARATION:

Aceclofenac osmotic tablets were prepared by wet granulation method[31] using different osmotic agents - sodium bicarbonate sodium chloride and potassium chloride. Osmogen concentrations were optimized by performing 13 trails (OT1-OT13). The prepared osmotic tablets contain two layers: push and pull layers. Aceclofenac, osmotic agents, MCC were blended together using alcoholic solution of PVP K30 as binder. The wet mass was sieved and dried. Then the dried granules were lubricated. Similarly the push compartment was also prepared. Colouring agent was added to the push layer for identification. The prepared push and pull layer granules were compressed as bilayer tablets. The prepared core tablets were coated with cellulose acetate. PEG 400 was used as pore forming agent

Table 8.1. Formulation of Aceclofenac Osmotic Tablets

Pull compartment								
		Trials (in mg)						
S.No	**Ingredients**	**OT1**	**OT 2**	**OT 3**	**OT4**	**OT 5**	**OT 6**	**OT 7**
1.	Aceclofenac	200	200	200	200	200	200	200
2.	sodium bicarbonate	50	-	-	25	25	-	75
3.	Sodium chloride	-	50	-	25	-	25	-
4.	Potassium chloride	-	-	50	-	25	25	-
4.	MCC	50	50	50	50	50	50	25
5.	PVP K30	20	20	20	20	20	20	20
6.	Talc	4	4	4	4	4	4	4
7.	Magnesium stearate	1	1	1	1	1	1	1
Push compartment								
1.	MCC	50	50	50	50	50	50	50
2.	HPMC K4M	50	50	50	50	50	50	50
3.	Sodium bicarbonate	75	75	75	75	75	75	75
4.	PVP K30	20	20	20	20	20	20	20
5.	Magnesium stearate	5	5	5	5	5	5	5

Table 8.2. Formulation of Aceclofenac Osmotic Tablets

Pull compartment							
S.No	Ingredients	Trials (in mg)					
		OT 8	OT 9	OT 10	OT11	OT12	OT13
1.	Aceclofenac	200	200	200	200	200	200
2.	sodium bicarbonate	-	-	50	50	25	25
3.	Sodium chloride	75	-	25		50	
4.	Potassium chloride	-	75	-	25	-	50
4.	MCC	25	25	25	25	25	25
5.	PVP K30	20	20	20	20	20	20
6.	Talc	4	4	4	4	4	4
7.	Magnesium stearate	1	1	1	1	1	1
Push compartment							
1.	MCC	50	50	50	50	50	50
2.	HPMC K4M	50	50	50	50	50	50
3.	Sodium bicarbonate	75	75	75	75	75	75
4.	PVP K30	20	20	20	20	20	20
5.	Magnesium stearate	5	5	5	5	5	5

EVALUATION:

Evaluation of pre compression parameters of granules

The powder blend was evaluated for its physical characteristics bulk density, tapped density, angle of repose, compressibility index, and Hausner's ratio[30]. The results were given in results and discussion in table 8.3.

Evaluation of post compression parameters of granules

The prepared tablets were evaluated for thickness, hardness, friability, weight variation, drug content and *in vitro* drug release[25].

Thickness

Thickness of a table is measured by using vernier calipers. The results were given in table 8.4

Hardness Test:

Hardness of a tablet depends on wt. of the material utilized, space between upper and lower punches, quantity of excipients used in formulation. Hardness was carried out by hardness tester. The results were given in table 8.4

Friability Test

Friability test indicated the withstand abrasion during handling and transportation. Friabiliator used for this purpose. Twenty tablets were weighed and subjected to rotating chamber of the apparatus and rotated for 25 rpm for 4 minutes. After that, the tablets were collected and reweighed. The formula to calculate the friability was

$$F = 100\ \frac{[W_0 - W]}{W}$$

Where W_o = Initial weight of 20 tablets.

W = Final weight of 20 tablets.

The results were given in table 8.4

Weight variation:

Twenty tables were weighed and the average weight was calculated. Then the tablets were individually weighed and the percent weight variation was calculated. The results were given in table 8.5

Drug content

Twenty tablets were weighed and powdered. 0.05gm equivalent to aceclofenac was weighed and transferred into 100ml volumetric flask, dissolved and volume made upto 100ml. From this solution 5 ml was to diluted to 50ml with ethanol. The absorbance was measured at 275nm using UV spectrophotometer. The results were given in table 8.5

***In Vitro* Drug Release Study**

The *in vitro* dissolution[34] was performed using pH1.2, 6.8 and 7.4 buffers. Samples were taken at regular intervals upto 24hrs and analyzed spectrophotometrically. The results were given in table 8.6, 8.7 and 8.8.

RESULTS AND DISCUSSION:

Pre compression parameters of granules:

Table 8.3 Pre compression parameters of granules

Trial	Angle of repose (º)	Bulk density (g/ml)	Tapped density (g/ml)	Compressibility index (%)	Hausner's Ratio
OT1	27.05± 0.02	0.62 ± 0.01	0.71 ± 0.01	12.67±0.01	1.14±0.01
OT2	27.27± 0.06	0.64 ± 0.02	0.72 ± 0.01	11.11±0.03	1.12±0.03
OT3	27.49± 0.17	0.65 ± 0.04	0.73 ± 0.04	10.90±0.04	1.12±0.03
OT4	27.98± 0.24	0.66 ± 0.05	0.73 ± 0.04	9.51±0.01	1.10±0.01
OT5	28.44± 0.21	0.63± 0.02	0.74± 0.05	14.86±0.04	1.17±0.01
OT6	28.43± 0.12	0.67± 0.01	0.74 ± 0.03	7.45±0.03	1.10±0.02
OT7	28.51± 0.21	0.67± 0.03	0.75 ± 0.03	10.66±0.01	1.11±0.01
OT8	27.94± 0.22	0.66± 0.05	0.71± 0.08	8.57±0.04	1.07±0.02
OT9	28.13± 0.18	0.62± 0.01	0.73± 0.04	15.06±0.01	1.17±0.02
OT10	28.21± 0.11	0.67 ± 0.01	0.74 ± 0.03	9.45±0.01	1.10±0.04
OT11	28.22± 0.21	0.66± 0.05	0.72± 0.01	8.69±0.03	1.09±0.03
OT12	28.13± 0.18	0.62± 0.01	0.73± 0.03	14.82±0.03	1.19±0.02
OT13	28.26± 0.23	0.65± 0.04	0.71± 0.06	8.45±0.01	1.09±0.01

The precompression parameters of the granules indicated that the powder was freely flowing. As the granules were freely flowable, it was suggested that the granules were suitable for compression. All the batches of granules passed the test.

Table 8.4. Results of thickness, hardness and friability of aceclofenac osmotic tablets

Formulations	Thickness (mm)	Hardness (kg/cm^2)	Friability (%)
OT1	5.49 ± 0.19	7.5 ±0.11	1.20 ± 0.10
OT2	5.44 ± 0.01	8 ± 0.17	1.24 ± 0.15
OT3	5.48 ± 0.11	8 ± 0.17	1.21 ± 0.07
OT4	5.42 ± 0.26	8 ± 0.17	1.13 ±0.05
OT5	5.48 ± 0.10	8 ± 0.17	1.14 ± 0.02
OT6	5.47 ± 0.05	7.5 ±0.11	1.02 ± 0.06
OT7	5.42 ± 0.26	8 ± 0.17	1.09 ± 0.12
OT8	5.27± 0.23	7.5 ±0.11	1.21 ± 0.07
OT9	5.41 ± 0.22	8 ± 0.17	1.13 ±0.05
OT10	5.27 ± 0.23	7.5 ±0.11	1.09 ± 0.12
OT11	5.43 ± 0.32	8 ± 0.17	1.21 ± 0.07
OT12	5.27± 0.23	7.5 ±0.11	1.09 ± 0.12
OT13	5.23 ± 0.12	8 ± 0.17	1.20 ± 0.10

There is no significant change in the thickness, hardness and friability for all the batches (OT1-OT10).

Table 8.5. Results of average weight and drug content

Formulations	Avg. wt (mg)		Assay (%)
	Before coating	After coating	
OT1	524.90 ± 0.45	550.12± 0.43	98.55± 0.97
OT2	525.22 ± 0.14	551.48± 0.38	98.39 ± 0.51
OT3	524.97 ± 0.15	550.34± 0.54	99.22 ± 1.01
OT4	524.92 ± 0.10	551.35± 0.65	98.07 ± 1.26
OT5	522.20 ± 0.21	550.34± 0.39	99.53 ± 1.82
OT6	523.89 ± 0.11	550.47± 0.39	99.22 ± 0.33
OT7	526.04 ± 0.25	551.43± 0.43	97.22 ± 0.58
OT8	524.04 ± 0.16	550.75± 0.28	99.34 ± 0.58
OT9	523.04 ± 0.17	550.29± 0.61	99.55 ± 0.58
OT10	525.04 ± 0.19	551.18 ± 0.47	99.80 ± 0.58
OT11	526.04 ± 0.13	551.32 ± 0.63	99.62 ± 0.58
OT12	524.04 ± 0.27	550.26 ± 0.84	99.28 ± 0.58
OT13	523.04 ± 0.18	550.13 ± 0.35	99.44 ± 0.58

Average weight and assay

- There was no significant changes in average weight and assay for all formulations (OT1-OT13)
- The average weight before coating was around 525mg and after coating was 550mg. The drug content for all the formulations were nearly 100% which indicated that there was no drug loss by manufacturing process or by additives.

Table 8.6. *In vitro* drug release profile of aceclofenac osmotic tablets (OT1- OT4)

S.No	Time (hrs)	Cumulative percentage release of aceclofenac			
		OT1	OT2	OT3	OT4
1	0	0	0	0	0
2	2	17.3± 0.11	18.5± 0.11	12± 0.12	9.05± 0.15
3	4	36.3± 0.12	37.3± 0.24	29± 0.09	16.32± 0.11
4	6	44.3± 0.09	47.12± 0.09	37± 0.08	33.21± 0.13
5	8	56.7± 0.13	58.24± 0.24	48.13± 0.11	42.23± 0.12
6	12	62.01 ± 0.14	65.25 ± 0.31	59.32± 0.12	52.42± 0.26
7	16	70.18± 0.21	76.31± 0.17	68.42± 0.08	62.34± 0.23
8	20	82.24± 0.25	84.56± 0.22	75.3± 0.12	71.27± 0.16
9	24	85.27± 0.19	87.12± 0.24	80.15± 0.22	78.32± 0.31

Table 8.7. *In vitro* drug release profile of aceclofenac osmotic tablets (OT5- OT8)

S.No	Time (hrs)	Cumulative percentage release of aceclofenac			
		OT5	OT6	OT7	OT8
1	0	0	0	0	0
2	2	9.27± 0.25	8.0± 0.14	8.76± 0.12	9.24± 0.15
3	4	19.26± 0.23	16.0± 0.08	19.23± 0.09	20.45± 0.11
4	6	39.24± 0.35	25.3± 0.11	27.41± 0.08	28.46± 0.13
5	8	49.36± 0.19	34.8± 0.09	36.61± 0.11	41.54± 0.12
6	12	66.13± 0.25	49.8± 0.13	52.72± 0.12	53.72± 0.09
7	16	79.23± 0.27	66.8± 0.08	68.28± 0.08	69.41± 0.13
8	20	84.29± 0.24	82.8± 0.14	85.54± 0.39	87.54± 0.23
9	24	89.23± 0.25	88.9± 0.12	90.32± 0.28	91.82± 0.36

Table 8.8. *Invitro* drug release profile of aceclofenac osmotic tablets (OT9- OT13)

S.No	Time (hrs)	Cumulative percentage release of aceclofenac(%)				
		OT9	OT10	OT11	OT12	OT13
1	0	0	0	0	0	0
2	2	10.18± 0.09	12.36± 0.14	12.23± 0.12	11.82± 0.11	12.83± 0.19
3	4	28.24± 0.12	29.56± 0.08	27.56± 0.04	26.23± 0.17	25.54± 0.15
4	6	42.32± 0.14	45.73± 0.11	41.21± 0.09	39.12± 0.12	38.92± 0.28
5	8	68.25± 0.09	68.92± 0.09	62.35± 0.02	60.24± 0.15	59.25± 0.11
6	12	74.13± 0.13	76.57± 0.13	72.24± 0.08	70.25± 0.22	69.73± 0.22
7	16	81.96± 0.12	86.68± 0.08	82.16± 0.14	81.81± 0.19	80.08± 0.14
8	20	84.26 ± 0.36	91.28± 0.14	89.02± 0.11	87.56± 0.34	86.74± 0.25
9	24	90.79± 0.23	94.3± 0.12	92.86± 0.09	91.12± 0.23	90.58± 0.22

IN VITRO RELEASE:

- The effect of same amount of three different osmogens (sodium bicarbonate, sodium chloride and potassium chloride) and the combinations of these osmogens exhibits significant increase in rate and extent of drug release were observed.
- All the prepared osmotic tablets of aceclofenac showed one hour delayed drug release, which may be attributed to time elapsed for imbibition of osmotic core with the release medium. After one hour, almost all the batches exhibited linear and controlled drug release profiles.
- Sodium chloride (25mg) and sodium bicarbonate (50mg) based push pull osmotic tablets of aceclofenac (OT10) exhibited little higher rate and extent of drug release than potassium chloride(25mg) and sodium bicarbonate (50mg) based tablets.
- The results showed that the concentration of osmotic agent is directly proportional to the drug release. Hence there was considerable increase in drug release based on the increase in concentration of osmogen.

- Three types of medium were chosen to carry out the *in vitro* dissolution studies from the result indicated that the rate of release was moderately affected by PH. it reveals that osmotic tablets are unaffected by the ph off the medium.
- The *in vitro* drug release results of formulations (OT1- OT6) were not found to be satisfactory for controlled release due to lesser concentration of osmogens.
- The formulations (OT7-OT13) showed profound increase in the dissolution profile due the combination of increased concentration of osmogens.
- The formulation (OT10) gave desired drug release profile (94.2%) in 24 hrs. Hence formulation (OT10) was selected as an optimized batch and was chosen for stability studies. The concentration of combination of osmogen used in this (OT10) batch was found to be sodium chloride 25mg and sodium bicarbonate 50mg

RELEASE KINETICS:

To know the mechanism of drug release from various preparations the data were treated according to zero order, first order, Higuchi and korse mayer equation. The release rate kinetic data for all the equations were shown in graph. Fig 8.1 to 8.5. The value fitted to zero order plot and its regression value was 0.9912, as its value is close to 1, it was conformed that it followed zero order release. The mechanism of drug release was further cconformed by korsemayer and peppas plot. According to this 0.5 is Fickian diffusion, $0.5 < n < 1$ is anomalous transport or Non-Fickian transpory, 1 is Case II transport, $n > 1$ is Super case II transport. The n value of the formulation OT10 was 1.2664 and hence it suggests supercase II transport.

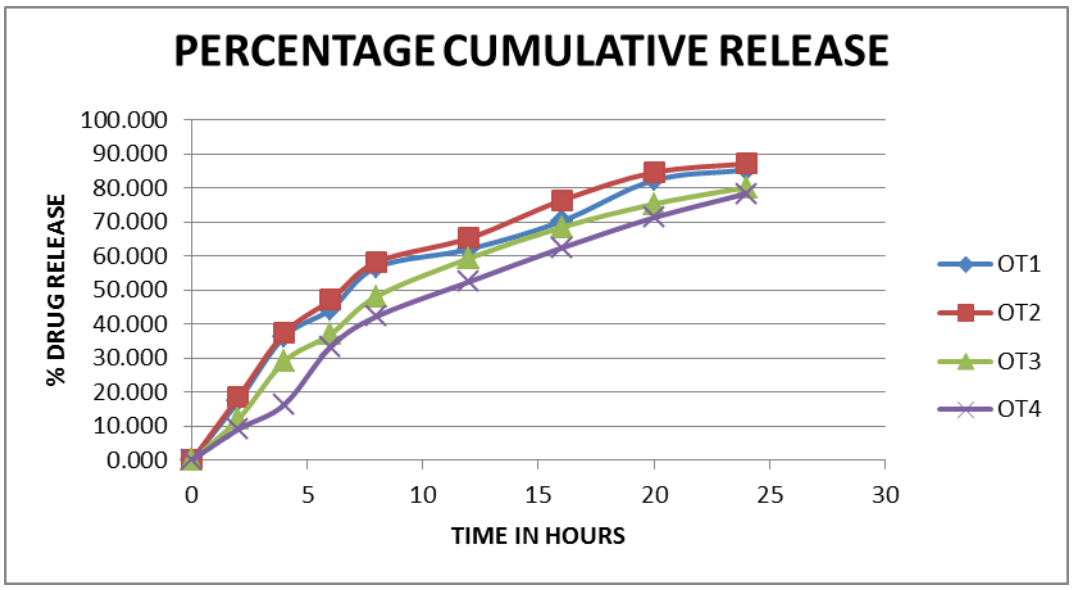


Figure:8.1 Percentage cumulative drug release of osmotic tablets containing aceclofenac(OT1-OT4)

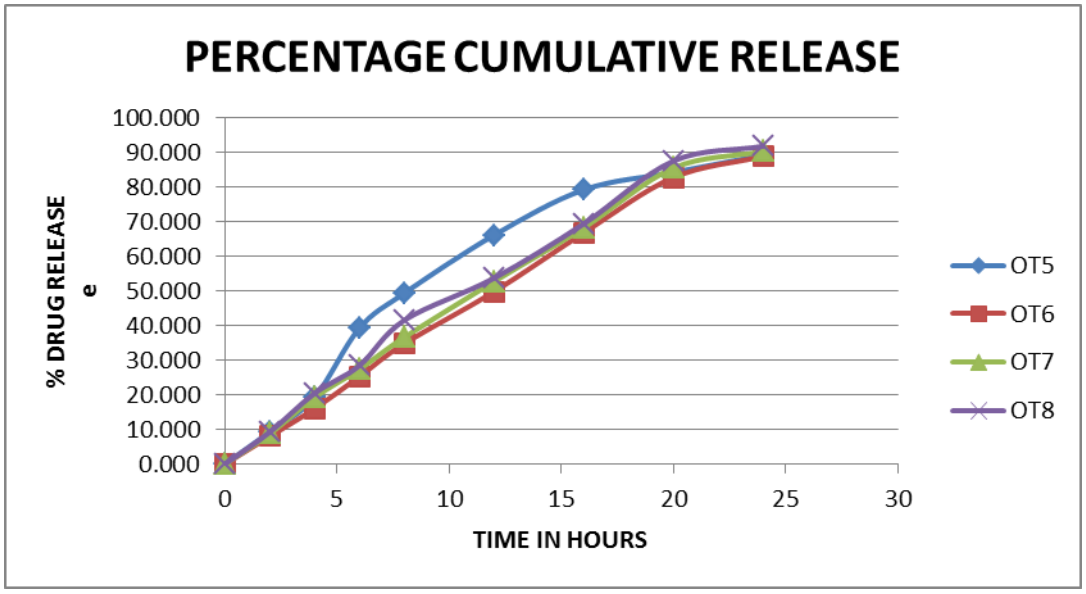


Figure:8.2 Percentage cumulative drug release of osmotic tablets containing aceclofenac(OT5-OT8)

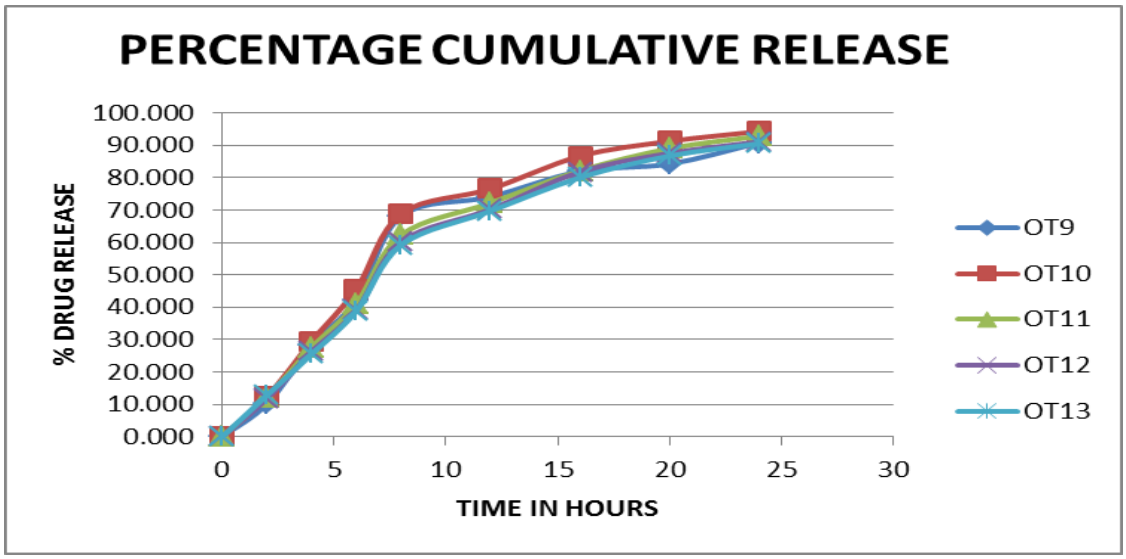


Figure:8.3 Percentage cumulative drug release of osmotic tablets containing aceclofenac(OT9-OT13)

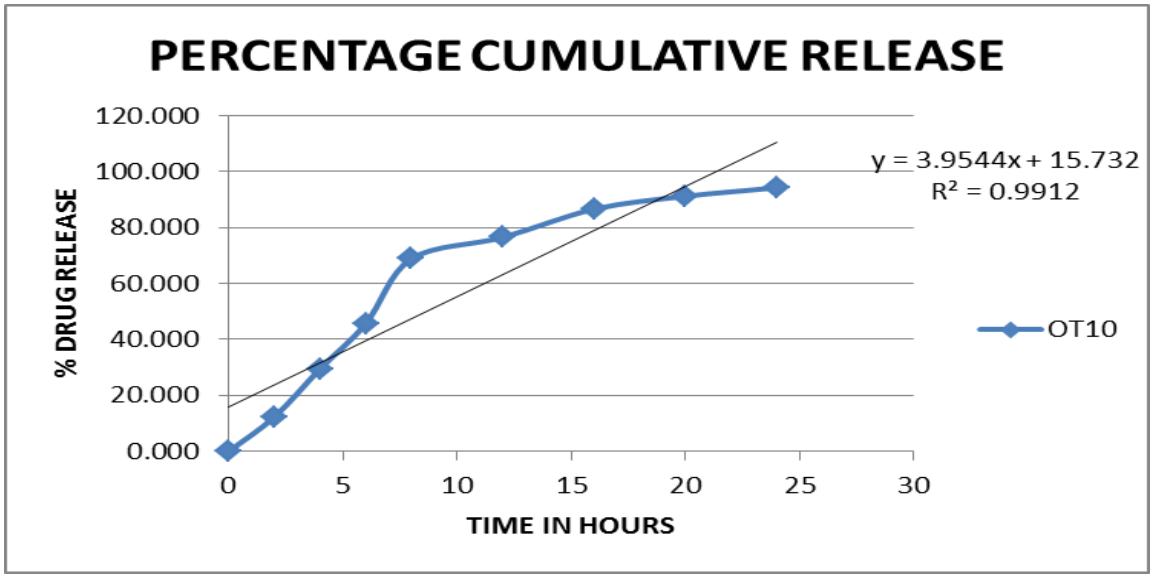


Figure:8.4 Percentage cumulative drug release of osmotic tablets containing aceclofenac(OT10)

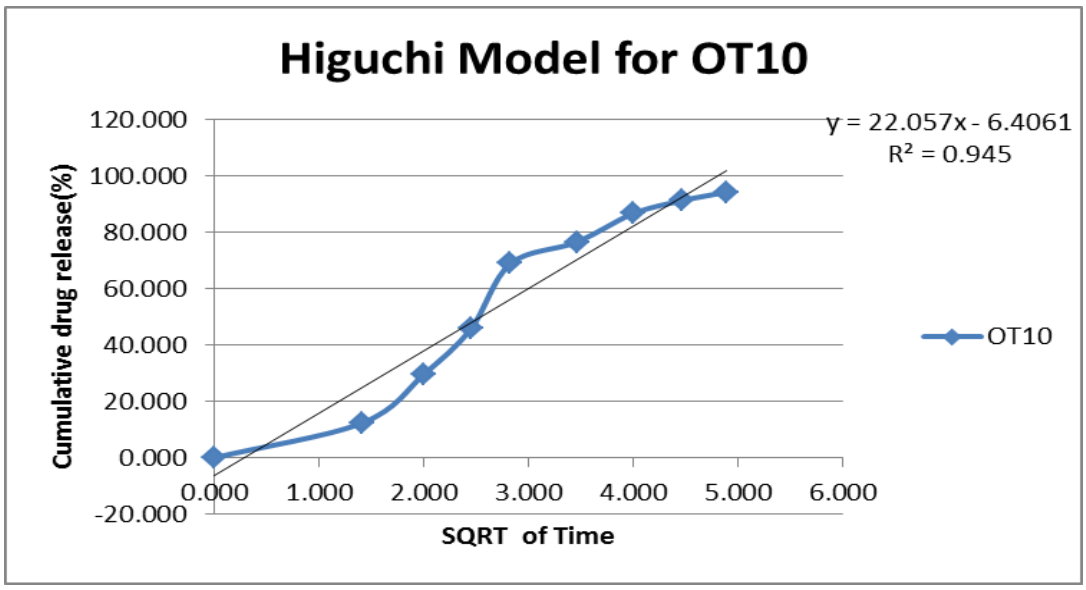


Figure:8.5 Release kinetics of osmotic tablets containing aceclofenac(OT10)-Higuchi Model

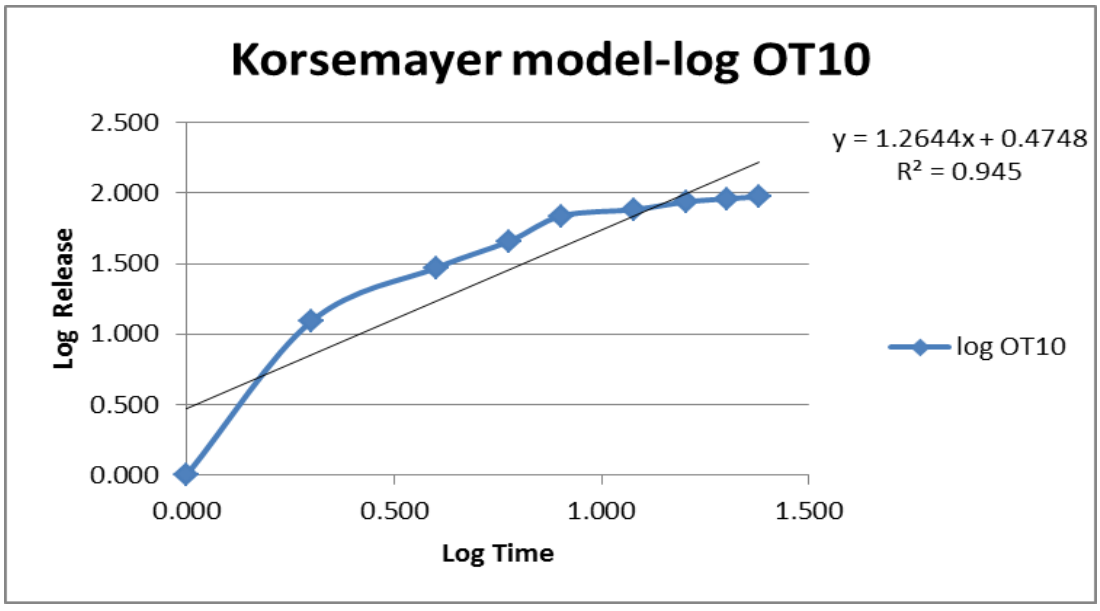


Figure:8.6 Release kinetics of osmotic tablets containing aceclofenac(OT10)- Korsemayer model.

CONCLUSION:

The osmotic tablets containing aceclofenac (OT10) with sodium chloride and sodium bicarbonate had exhibited ideal controlled release. The formulation (OT10) gave desired drug release profile (94.2%) in 24 hrs The formulation fitted to zero order release with super case II transport.

9. ACECLOFENAC MICROSPHERES

METHODS:

Formulation of aceclofenac microspheres:

Preparation of polymer mix:

The microsphere formulations were made with varying proportion of the drug polymer ratio as well as varying concentrations of two polymers (polymer mix) Eudragit L 100 and PLGA. Polymer mix of Eudragit L100: PLGA in the ratios of 1:1, 1:2 and 1:3 were prepared. The internal variation in the polymer mix were designated as a, b and c.

Table 9.1. Polymer mix Eudragit L100: PLGA(ratio)

Polymer mix code	Eudragit L100:PLGA
A	1:1
B	1:2
C	1:3

Preparation of Aceclofenac Microspheres:

Mixture of EudragitL100 and PLGA polymers in different ratios were used for the formulation of aceclofenac loaded Eudragit:PLGA microspheres as given in table 9.1 and table 9.2. PVA (3%w/v) was added for stabilizing the microspheres[188]. Eudragit L100 and drug were dissolved in ethanol and PLGA was dissolved in acetone, respectively. Subsequently the Eudragit solution in ethanol was added slowly to the PLGA solution in acetone with a constant stirring and stirring is continued to remove the solvent. Then the microspheres were washed and resuspended in distilled water and lyophilized.

TABLE 9.2. FORMULATION OF ACECLOFENAC MICROSPHERES

S. No	Ingredients	Batches of Microspheres														
		M1	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**	**M8**	**M9**	**M10**	**M11**	**M12**	**M13**	**M14**	**M15**
1	Aceclofenac **(mg)**	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
2	Eudragit L100 **(mg)**	50	33.3	25	75	50	37.5	100	66.6	50	125	83.3	62.5	150	100	75
3.	PLGA **(mg)**	50	66.7	75	75	100	112.5	100	134.4	150	125	166.7	187.5	150	200	225
4	Aceclofenac: Polymer mix	1:1a	1:1b	1:1c	1:1.5a	1:1.5b	1:1.5c	1:2a	1:2b	1:2c	1:2.5a	1:2.5b	1:2.5c	1:3a	1:3b	1:3c
5.	PVA(%w/v)	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
6	Ethanol(ml)	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70
7	Acetone(ml)	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30

CHARACTERIZATION OF ACECLOFENAC MICROSPHERES

Flow property studies[142]:

Angle of repose, bulk density, tapped density, compressibility index and Hausners ratio were determined and the values recorded were recorded in table 9.3.

Determination of mean particle size of microspheres[236]

Particle size analysis of drug-loaded microspheres was performed using stereomicroscope which was calibrated using calibrated micrometers. The microscope was equipped with the software Bioplus-55 Video Plan-11UP through a camera. A small amount of dry microspheres were suspended in glycerin. A small drop was of suspension was placed on a clean glass slide. The slide with specimen was observed under the microscope (100X). An image was taken with the help of camera and the particle size was determined using software. The particle size calculation was carried out in triplicate and the values were given in table 9.4.

Scanning electron microscopy[199]:

For the external morphology studies, air dried particles were visualized using scanning electron microscopy. The samples were mounted on a metal slab with double adhesive tape and coated with platinum under vacuum.

Drug content[160]

For drug content analysis the microsphere formulations were centrifuged in a centrifuge and the amount of drug was measured by ultraviolet (UV) spectrophotometer at 275nm. Loading capacity is the maximum amount of drug that can be incorporated in the microspheres.

$$\text{Percentage Drug Content} = \frac{W_{act}}{W_{ms}} \times 100$$

where W_{act} is the actual drug content in a weighed quantity of microspheres and W_{ms} is the weighed quantity of microspheres.

Entrapment efficiency[5]:

The encapsulation efficiency is the amount of added drug (in percent) that is encapsulated in the formulation of microspheres.

$$\text{Percentage Entrapment Efficiency} = \frac{W_{act}}{W_{thy}} \times 100$$

where W_{act} is the actual drug content in a weighed quantity of microspheres, and W_{thy} is the theoretical amount of drug in microspheres calculated from the quantity added in the process

***In Vitro* Drug Release Studies[2]**

In vitro release studies were carried out using USP type I apparatus at 37 ± 0.5□C in 900 ml dissolution medium (pH1.2, 6.8 and 7.4) for 24 h. Microspheres equivalent to 200 mg drug was placed into the baskets (tied using muslin cloth), and rotated at 100 rpm. Samples were taken at regular intervals up to 24hr and analyzed by spectrophotometrically at 275 nm.

Stability Studies[5]

The optimized formulation of microspheres were selected and subjected to stability studies as per ICH guidelines. Samples were withdrawn at predetermined time intervals of 15, 30, 45, 60 and 90 days, and then evaluated.

RESULTS AND DISCUSSION:

Flow property studies:

Angle of repose, bulk density, tapped density, CI and Hausner's ratio were calculated and given below table 9.3. From the values it was indicated that all the prepared microspheres had good flow property.

Table 9.3 Flow property studies of aceclofenac microspheres

Batch	Angle of repose (Mean± SD)	Bulk density(g/ml Mean± SD	Tapped density(g/ml) Mean± SD	CI (%) Mean± SD	Hausner's ratio Mean±SD
M1	18.6 ± 0.20	0.31±0.01	0.37±0.01	16.4±1.1	1.19±0.1
M2	15.1± 0.62	0.42±0.01	0.507±0.13	16.3±1.3	1.20±0.2
M3	17.5 ± 0.19	0.52±0.01	0.594±0.01	13.4±0.9	1.14±0.01
M4	18.3±0.14	0.42±0.02	0.509±0.02	17.3±1.1	1.21±0.1
M5	20.7± 0.58	0.49±0.02	0.582±0.01	15.8±0.8	1.18±0.05
M6	24.0 ± 0.59	0.62±0.03	0.735±0.04	15.6±2.2	1.18±0.02
M7	19.1± 0.56	0.56±0.02	0.652±0.22	14.1±2-3	1.16±0.1
M8	18.5 ± 0.16	0.61±0.10	0.726±0.01	15.9±3.2	1.18±0.1
M9	16.1 ± 0.62	0.51±0.12	0.614±0.01	16.9±1.4	1.01±0.2
M10	17.5 ± 0.16	0.45±0.08	0.517±0.03	12.9±1.2	1.14±0.4
M11	18.1 ± 0.64	0.52±0.09	0.626±0.20	16.9±1.2	1.20±0.1
M12	17.6 ± 0.16	0.55±0.02	0.652±0.01	15.6±1.2	1.18±0.2
M13	19.8 ± 0.54	0.63±0.01	0.723±0.01	14.7±0.6	1.14±0.2
M14	18.6 ± 0.18	0.60±0.01	0.716±0.09	16.2±0.9	1.19±0.3
M15	18.8 ± 0.54	0.59±0.01	0.703±0.09	16.0±1.1	1.19±0.01

Mean Particle Size, Drug content and Entrapment Efficiency:

Mean particle size was determined by optical microscopy and the average particle size was calculated. The mean particle size and entrapment efficiency were determined for all the batches (M1- M15) and results were given in Table 9.4. Particle sizes of all the formulations were in micro particular size range and it was maximum of 630±36µm with M13 and minimum of 20±10µm with M4**.** The increase in the concentration of the dissolved Eudragit L100 might have caused a considerable increase in the viscosity of organic phase and reduced the stirring efficiency resulted in the formation of the larger emulsion droplets, which have yielded microspheres with increased particle size[237]

The molecular weight of the polymers used in the formulation is an important factor which decides the drug entrapment. The molecular weight of Eudragit L100 is 320,000 g/mol and PLGA is above 30,000 and this may considerably increased the entrapment efficiency and maximum entrapment efficiency was reported at a drug: polymer ratio of 1:1.5a, in which the proportion of the polymers Eudragit L100 and PLGA are equal and this is in agreement with earlier reports. In addition, a higher viscosity of the organic phase causes a better distribution of the drug in the matrix. On the contrary, lowering the viscosity of the organic phase allows drugs to come close to the surface during the formation of and to dissolve in the surrounding aqueous medium, resulting in lower drug content. The entrapment efficiency was found to be maximum 86.6±0.6% with M4 and the minimum was indicated by 25±1.1% with M3. Drug content was minimum of 21.1%±0.5 in M8 and maximum of 64.1±0.9 in M4. The results indicated that the entrapment efficiency was directly related to drug content based on the polymer concentration.

Among all the microsphere formulations prepared, M4, M5 and M13 shows desirable entrapment efficiency and hence selected for the further studies where the concentration of aceclofenac:polymer mix were 1:1.5a, 1:1.5b and1:3a respectively.

SCANNING ELECTRON MICROSCOPY

The morphology of the optimized formulation of microspheres was found to be discrete, smooth and spherical. The SEM photos were given in fig 9.1, 9.2 and 9.3.

Table 9.4. Mean Particle Size, Drug content and Entrapment Efficiency (M1-M15)

Parameter	Trials														
	M1	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**	**M8**	**M9**	**M10**	**M11**	**M12**	**M13**	**M14**	**M15**
Particle size(µm)	310 ±24	255 ±42	120 ±58	20 ±10	50 ± 15	35 ± 23	442 ±56	338 ±42	147 ±26	302 ±43	282 ±53	253 ±52	630 ±36	575 ±34	502 ±15
Drug Content (%)	42.5 ±0.9	24.1 ±0.5	27.3 ±0.6	64.1 ±0.9	31.0 ±0.5	35.5 ±0.8	19.9 ±0.3	21.1 ±0.5	21.5 ±0.4	30.4 ±0.7	34.7 ±0.7	41.4 ±1.0	28.3 ± 0.4	40.7 ± 0.6	36.8 ± 0.5
Entrapment efficiency (%)	32.3 ±1.0	29.4 ±0.7	25 ±1.1	86.6 ±0.6	50.3 ±0.5	45.6 ±1.2	60.5 ±0.8	52.7 ±0.6	49 ±0.4	65.3 ±0.7	53.7 ±1.4	50.7 ±1.2	67.1 ±0.5	55.5 ±1.3	53.6 ±0.9

Particle size values expressed as ± S.E.M in µm of triplicate trials

Entrapment efficiency values expressed as ± S.D in µm of triplicate trials

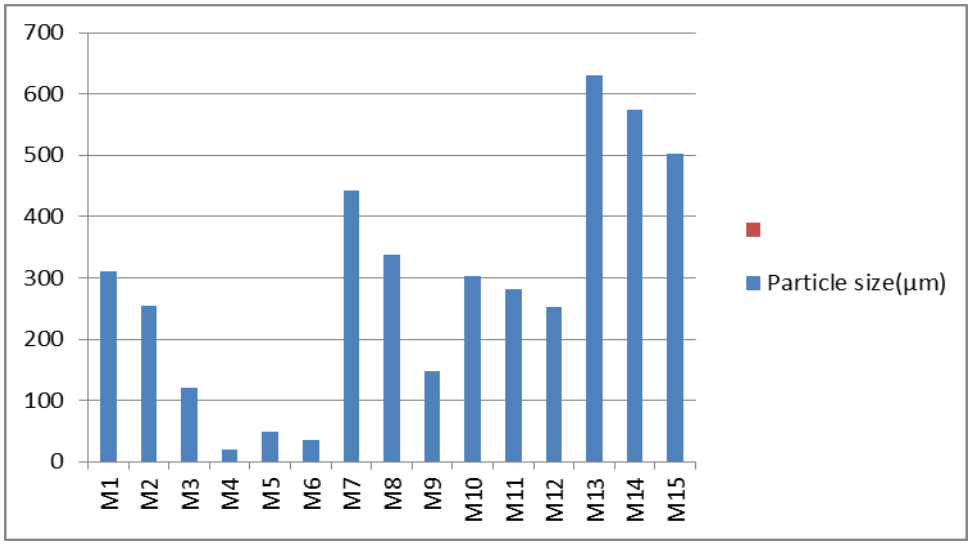


Fig. 9.1. Particle size of Aceclofenac Microspheres formulations

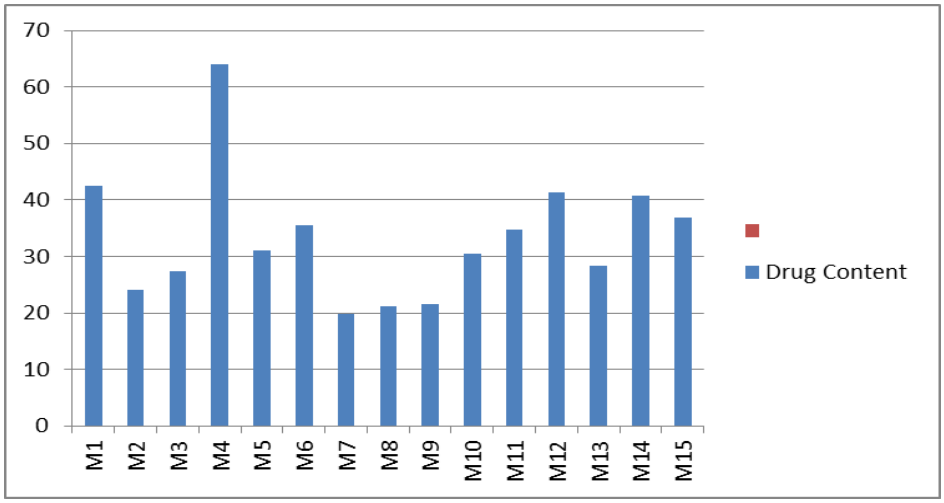


Fig. 9.2. Drug content of Aceclofenac Microspheres formulations

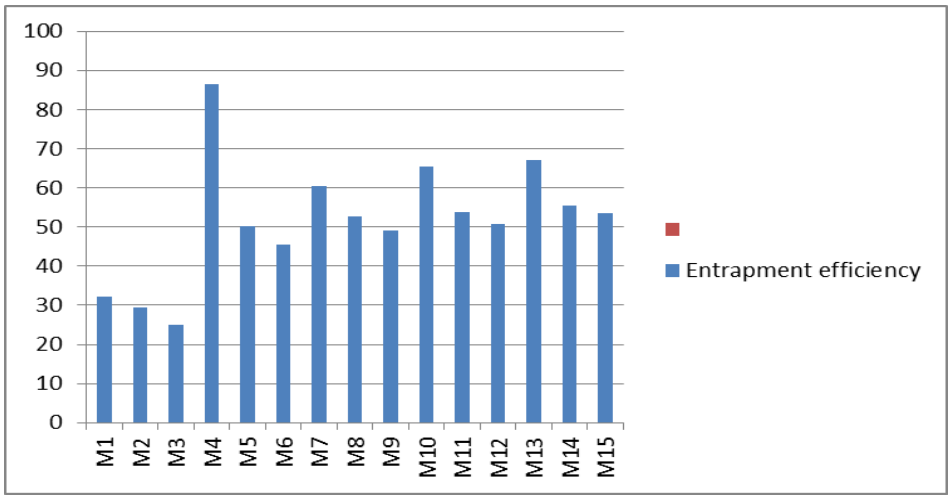


Fig. 9.3. Entrapment efficiencies of Aceclofenac Microspheres formulations

SEM PHOTOS OF FORMULATIONS M4, M5, M13

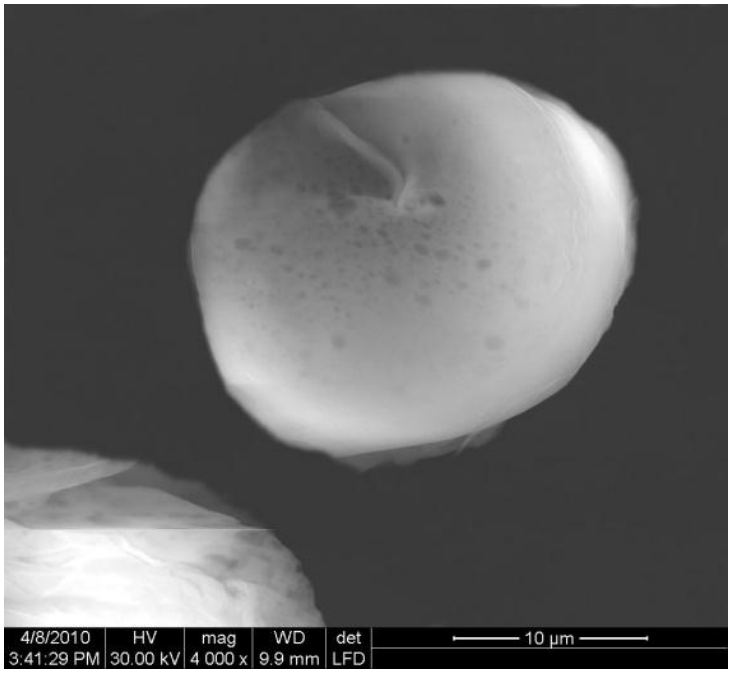


Fig 9.1. SEM picture of M4 formulation

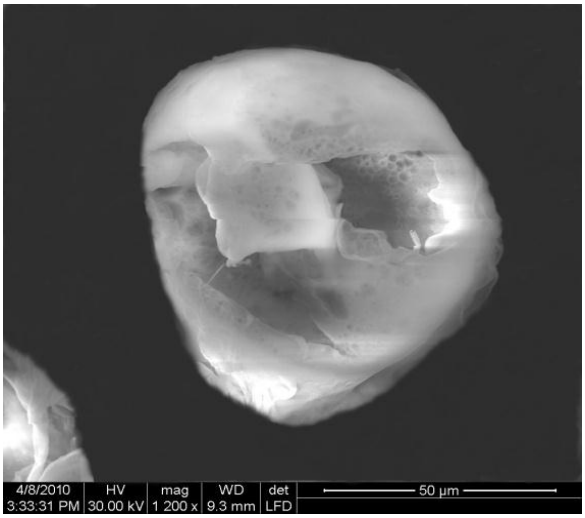


Fig 9.2. SEM picture of M5 formulation Fig 9.3. SEM picture of M13 formulation

IN-VITRO RELEASE STUDIES

Based on the particle size and entrapment efficiency the optimized formulations (M4, M5 and M13) were selected for further studies. The *in vitro* release profile of M4, M5 and M13 indicated controlled release of aceclofenac with maximum of 96.6±1.6%, 88.6±1.8% and 76.2±1.9%, respectively. The analysis of the above data indicates that the formulation M4 possess the ideal particle size of 20±10µm and higher entrapment efficiency of 86.6±0.6%. The *in vitro* release profile of this formulation was found to follow zero order kinetics with a controlled release for 24h. Hence, the formulation M4 was subjected for in vivo anti-inflammatory, anti-arthritic and gastro intestinal tolerability tests.

Table 9.5 % Cumulative Drug Release of M4, M5 and **M13**

% CUMULATIVE DRUG RELEASE			
Hours	**M4**	**M5**	**M13**
0.5	2.2±0.2	2.7± 0.2	2.5±0.3
1	3.4±0.1	4.2±0.4	3.8±0.1
1.5	7.2±0.4	6.9±0.2	4.9±0.2
2	9.5±0.7	7.5±0.8	6.2±0.4
2.5	12.7±0.6	9.1±0.7	8.1±0.3
3	15.4±1.1	11.5±1.5	11.7±0.5
4	19.9±0.9	14.7±1.2	14.5±0.7
5	26.8±0.9	17.9±1.0	17.2±0.6
6	31.7±1.3	24.6±1.2	20.3±1.0
8	36.8±1.5	32.1±1.7	25.9±1.8
10	45.1±1.2	49.7±1.5	33.7±2.6
12	57.6±1.5	58.5±0.9	46.1±1.7
16	71.3±1.3	65.9±1.5	59.0±2.0
20	82.5±1.7	73.0±1.3	67.2±1.9
24	96.6±1.6	88.6±1.8	76.2±1.9

RELEASE KINETICS:

To know the mechanism of drug release from various preparations the data were treated according to zero order, first order, Higuchi and korse mayer equation. The release rate kinetic data for all the equations were shown in graph. Fig 9.4, 9.5 and 9.6. The value fitted to zero order plot and its regression value was 0.982, as its value is close to 1, it was conformed that it followed zero order release. The mechanism of drug release was further cconformed by korsemayer and peppas plot. According to this 0.5 is Fickian diffusion, $0.5 < n < 1$ is anomalous transport or Non-Fickian transpory, 1 is Case II transport, $n > 1$ is Super case II transport. The n value of the formulation M4 was 0.9904 and hence it suggests non fickian diffusion.

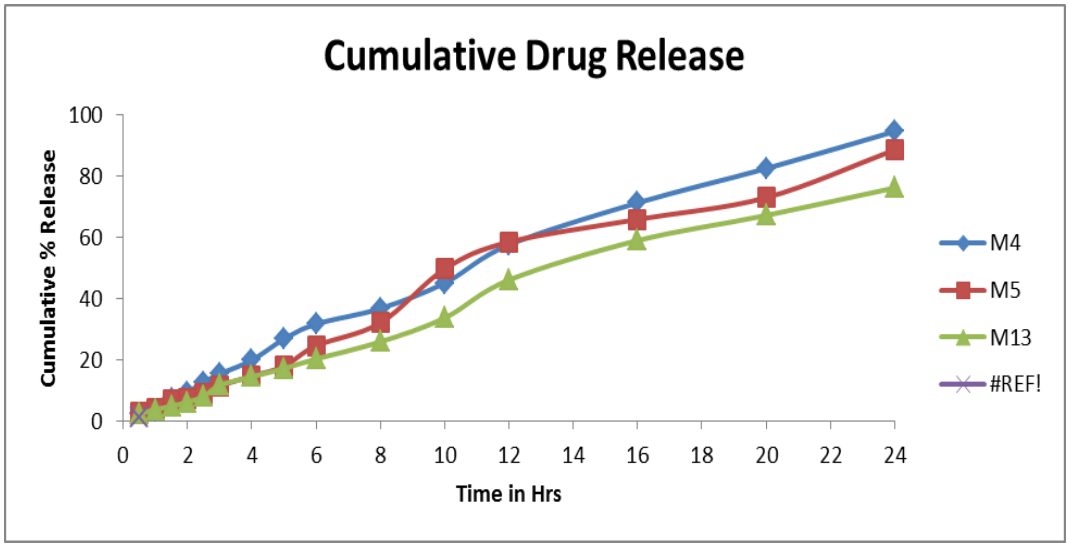


Fig. 9.4 In vitro Release profile of aceclofenac from microspheres

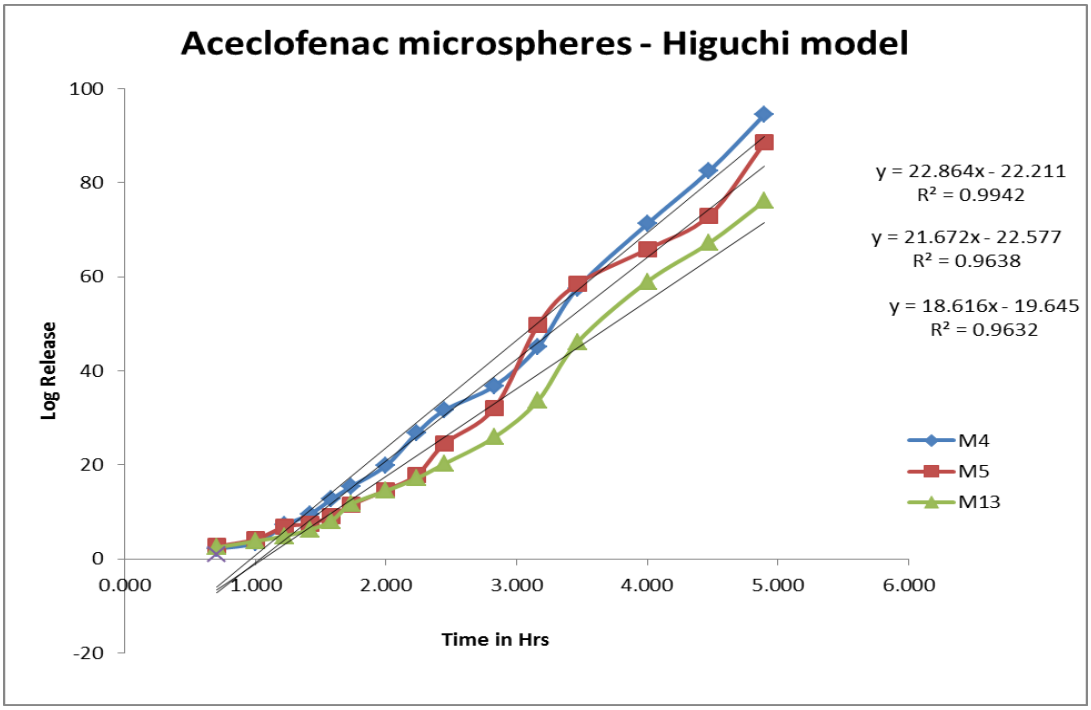


Fig 9.5 HIGUCHI MODEL

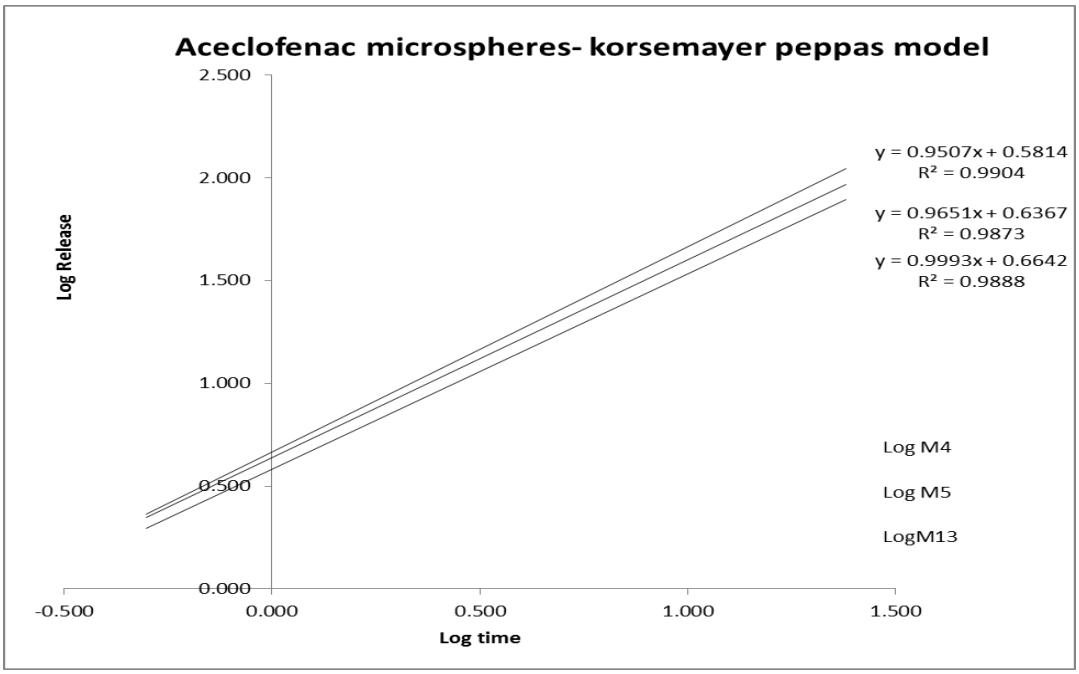


FIG 9.6. KORSE MAYER MODEL

CONCLUSION:

Aceclofenac microspheres prepared with drug polymer mix in the ratio of (1:1.5a) and 3% PVA concentration **(M4)** was observed to possess better entrapment efficiency of **86.6%**, *invitro* release of **96.6±0.11%**.The particle size value of **M4** also found to be

good when compared to other formulations. The least particle size of **20±10nm** was also observed with this formulation. Hence, **M4** can be selected as best formulation among the other formulations.

Microparticulate drug delivery systems are considered and accepted as a reliable tool to deliver the drug to the target site with specificity, to maintain the desired concentration at the site of interest without untoward effects. Microencapsulation is a useful method which prolongs the duration of drug effect significantly and improves patient compliance.

Eventually the total dose and few adverse reactions may be reduced since a steady plasma concentration is maintained. In recent years much research in drug delivery has been focused on degradable polymer microspheres. Administration of medication via such systems is advantageous because microspheres can be ingested or injected, can be tailored for desired release profiles and in some cases it can provide organ-targeted release. The microencapsulation process enables conversion of liquids to solids, altering colloidal and surface properties, providing environmental protection and controlling the release characteristics by using the coating materials.

The success of any microencapsulation method depends on many factors such as the drug solubility, partition co-efficiency, polymer composition, molecular weight etc. Among the various microencapsulation methods, emulsion solvent evaporation technique is often widely used to prepare microcapsules of water insoluble drugs (within the water insoluble polymer). Microspheres are formed by the evaporation of an organic solvent from dispersed oil droplets containing both polymer and drug

The aim of the present work was to encapsulate aceclofenac with Eudragit L100 and PLGA, the effect of different formulation variables such as concentration of polymer and eudragit L 100 and PLGA and the effect of these variables on particle size distribution, encapsulation efficiency and its *in vitro* release behavior. Aceclofenac is an ideal candidate for this controlled release formulation, resulting in more reproducible drug absorption and reducing the risk of local irritations compared to conventional dosage forms.

10. ACECLOFENAC NANOSUSPENSION

Table 10.1. Formulation of Aceclofenac Nanosuspension

S.No.	Ingredients	Batches of nanosuspension containing Aceclofenac								
		F-1AXL	**F-2AXM**	**F-3AXH**	**F-4AYL**	**F-5AYM**	**F-6AYH**	**F-7AZL**	**F-8AZM**	**F-9AZH**
1	Drug:Polymer	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1
2	Tween 80	0.01%	0.01%	0.01%	0.02%	0.02%	0.02%	0.03%	0.03%	0.03%
3	Methylene Chloride	5ml	5ml	5ml	5ml	5ml	5ml	5ml	5ml	5ml
4	Methanol	2ml	2ml	2ml	2ml	2ml	2ml	2ml	2ml	2ml
5	Sonication time	2min	4 min	6 min	2min	4 min	6 min	2min	4 min	6 min
6.	Agitation Speed	2000rpm	4000rpm	6000rpm	2000rpm	4000rpm	6000rpm	2000rpm	4000rpm	6000rpm
7.	Distilled water	45 ml	45 ml	45 ml	45 ml	45 ml	45 ml	45 ml	45 ml	45 ml

Table 10.2. Formulation of Aceclofenac Nanosuspension

S.No.	Ingredients	Batches of nanosuspension containing Aceclofenac								
		F-10BXL	**F-11BXM**	**F-12BXH**	**F-13BYL**	**F-14BYM**	**F-15BYH**	**F-16BZL**	**F-17BZM**	**F-18BZH**
1	Drug:Polymer	1:2	1:2	1:2	1:2	1:2	1:2	1:2	1:2	1:2
2	Tween 80	0.01%	0.01%	0.01%	0.02%	0.02%	0.02%	0.03%	0.03%	0.03%
3	Methylene Chloride	5ml	5ml	5ml	5ml	5ml	5ml	5ml	5ml	5ml
4	Methanol	2ml	2ml	2ml	2ml	2ml	2ml	2ml	2ml	2ml
5	Sonication time	2min	4 min	6 min	2min	4 min	6 min	2min	4 min	6 min
6.	Agitation Speed	2000rpm	4000rpm	6000rpm	2000rpm	4000rpm	6000rpm	2000rpm	4000rpm	6000rpm
7.	Distilled water	45 ml	45 ml	45 ml	45 ml	45 ml	45 ml	45 ml	45 ml	45 ml

Table 10.3. Formulation of Aceclofenac Nanosuspension

S.No.	Ingredients	Batches of nanosuspension containing Aceclofenac								
		F-19CXL	**F-20CXM**	**F-21CXH**	**F-22CYL**	**F-23CYM**	**F-24CYH**	**F-25CZL**	**F-26CZM**	**F-27CZH**
1	Drug:Polymer	1:3	1:3	1:3	1:3	1:3	1:3	1:3	1:3	1:3
2	Tween 80	0.01%	0.01%	0.01%	0.02%	0.02%	0.02%	0.03%	0.03%	0.03%
3	Methylene Chloride	5ml	5ml	5ml	5ml	5ml	5ml	5ml	5ml	5ml
4	Methanol	2ml	2ml	2ml	2ml	2ml	2ml	2ml	2ml	2ml
5	Sonication time	2min	4 min	6 min	2min	4 min	6 min	2min	4 min	6 min
6.	Agitation Speed	2000rpm	4000rpm	6000rpm	2000rpm	4000rpm	6000rpm	2000rpm	4000rpm	6000rpm
7.	Distilled water	45 ml	45 ml	45 ml	45 ml	45 ml	45 ml	45 ml	45 ml	45 ml

METHODS

FORMULATION OF ACECLOFENAC NANOSUSPENSION (NS):

Nanosuspension (NS) containing aceclofenac were prepared by o/w emulsion method using Eudragit L100[180]. The polymer and drug (200mg) were dissolved in methanol and added to 5ml of methylene chloride by stirring. This solution was then slowly added into water containing Tween 80 and was kept at a low temperature using an ice water bath. During addition, the mixture was vigorously mixed using magnetic stirrer. The resulting emulsion obtained was sonicated in a probe sonicator, and further stirring was continued for 60 minutes. Solvent residues were allowed to evaporate under a slow magnetic stirring of the NS at room temperature (20°- 23°C) for 8–12 hours. The following critical parameters were optimized:

1. Drug polymer ratio
2. Concentration of surfactant(Tween 80)
3. Sonication time/Agitation speed

OPTIMIZATION OF FORMULATION

Multifactorial design was applied for determining the effect of drug: polymer ratio(X1), Concentration of the surfactant(X2) and effect of agitation(X3) on two responses, particle size (Y1), entrapment efficiency and drug content (Y2). With different formulation parameters 27 trials of nanosuspension were prepared. The results were categorized as: positive effect (+1), no effect (0) and negative effect (-1). The details of the parameters studied were represented in the table 10.4.

CHARACTERIZATION OF NANOSUSPENSION:

Scanning Electron Microscopy (SEM)

The morphology studies of the prepared formulations were investigated using scanning electron microscopy[199]. The samples were mounted on a metal stab with double adhesive tape and coated with platinum under vacuum. The results were given in results and discussion section.

Determination of Particle Size and Surface Charge

The particle size analysis (using dynamic light scattering as the basic principle of operation) and zeta-potential measurement (using Doppler electrophoresis as the basic principle of operation) were analysed by Zetasizer Nano ZS[238] (Malvern Instruments, UK). For the analysis, the nanoparticle sample of the desired concentration was flushed through a folded capillary cell (DTS1060) and the measurement was carried out on the second filling; a sufficient sample volume was

used to completely cover the electrodes of the cell. To avoid air bubbles in the cell, the sample was injected slowly and analysis was only carried out if there were no visible air bubble inclusions present. After successful inspection, the cell was placed into the Zetasizer and equilibrated at 20ºC (close to the average temperature in the laboratory) for 2 min prior to the particle size measurements, of which there were six replicates. The measurement temperature was set and maintained by the Peltier elements in the sample holder of the instrument. The corresponding zeta-potential measurements (triplicate) were taken immediately after the particle size measurements. The sample and the cell were then discarded. Malvern Instruments Dispersion Technology software (Version 4.0) was used to control and analyze all data from the instrument. The instrument uses dynamic light scattering, which is sometimes referred to as photon correlation spectroscopy (PCS), to measure time dependent fluctuation of scattered light arising from the suspension of the nanoparticles undergoing random Brownian motion.

The diffusion coefficient of the particles is deduced and analyzed to give their mean diameter, which is the size data presented in this study. For the zeta-potential measurement, the instrument uses laser Doppler electrophoresis to measure the net velocity of the nanoparticles in the liquid that results when an electric field is applied.

Determination of Drug Content

For drug content analysis, a specified volume of NS formulations was ultra-centrifuged at 25000xg and the amount of drug in the supernatant was estimated by UV spectrophotometer at 275 nm. The drug content of the prepared NS was determined by the formula[4]:

$$\text{Drug content (\%)} = \frac{\text{Weight of drug in nanoparticles X 100}}{\text{Weight of nanoparticles}}$$

The results were given in results and discussion section.

Determination of Drug Entrapment Efficiency

The drug entrapment efficiency of various NS formulations prepared were calculated and was expressed in percentage. The following formula was used to calculate the percentage drug entrapment[160]:

$$\text{Drug entrapment(\%)} = \frac{\text{mass of drug in nanoparticles x100}}{\text{mass of drug used in formulation}}$$

The results were given in results and discussion section.

***In Vitro* Drug Release Studies[239]**

The in vitro release studies of the prepared NS were carried out using a modified Franz diffusion cell over a period of 24 h at a temperature of 37±1ºC. For each formulation a specified amount of NS was introduced into compartment and the open ends of the apparatus sealed with non-porous membrane to prevent evaporation. Phosphate buffer (pH 7.4) was used and stirred. Samples were withdrawn at regular intervals and analyzed for drug content by spectrophotometer at 275nm. Sink condition were maintained throughout the release period. The results were given in results and discussion section.

Stability Studies[239]:

The optimized NS were investigated for their stability and their ability to withstand temperature conditions during their storage. The NS formulations and aqueous solution of aceclofenac were sealed in Type-I amber colored glass vials and were stored at 2–8°C, 25°C, and 40°C. Samples from these formulations were withdrawn at 15, 30, 45, 60 and 90 days interval and analyzed for mean particle size and drug content. Each study was performed in triplicate. The results were given in results and discussion section.

RESULTS AND DISCUSSION:

Preparation of Nanosuspension:

Aceclofenac loaded Eudragit L100 nanosuspension was prepared by o/w emulsion method. The prepared were found to be turbid and stable. No visible sedimentation was noticed atleast for a period of 7 days, when stored at 4°C. However, stability studies of the selected formulations were carried out separately. The preparation was shown in fig.10.1

SEM analysis:

The SEM analysis was performed and the SEM photos of aceclofenac nanoparticles were recorded. The particles of all the batches are almost spherical with smooth surface, however they shown variation in size. The SEM photos of 4trials were given in fig 10.2.

Optimization of formulation

The multifactorial design applied for determining the effect of drug: polymer ratio(X1), Concentration of the surfactant(X2) and effect of agitation(X3) on two

responses, particle size (Y1) and drug content(Y2) shown evident variations with the three parameters used for the investigation. From the results of all 27 trials the formulations F-14BYM, F-15BYH, F-23CYM and F-24CYH were considered as ideal and selected for further studies. They have particle size of 210±15nm, 212±23nm, 225±30nm and 220±42nm and entrapment efficiency of 89.6±0.9%, 86.2±1.6%, 88.9±1.3% and 88.6±1.5%, respectively. The formulations with F-14BYM, F-15BYH, F-23CYM and F-24CYH were identified as the optimized formulations with respect to particle size, drug content and entrapment efficiency, and these were selected for the other studies. The details were given in table 10.4

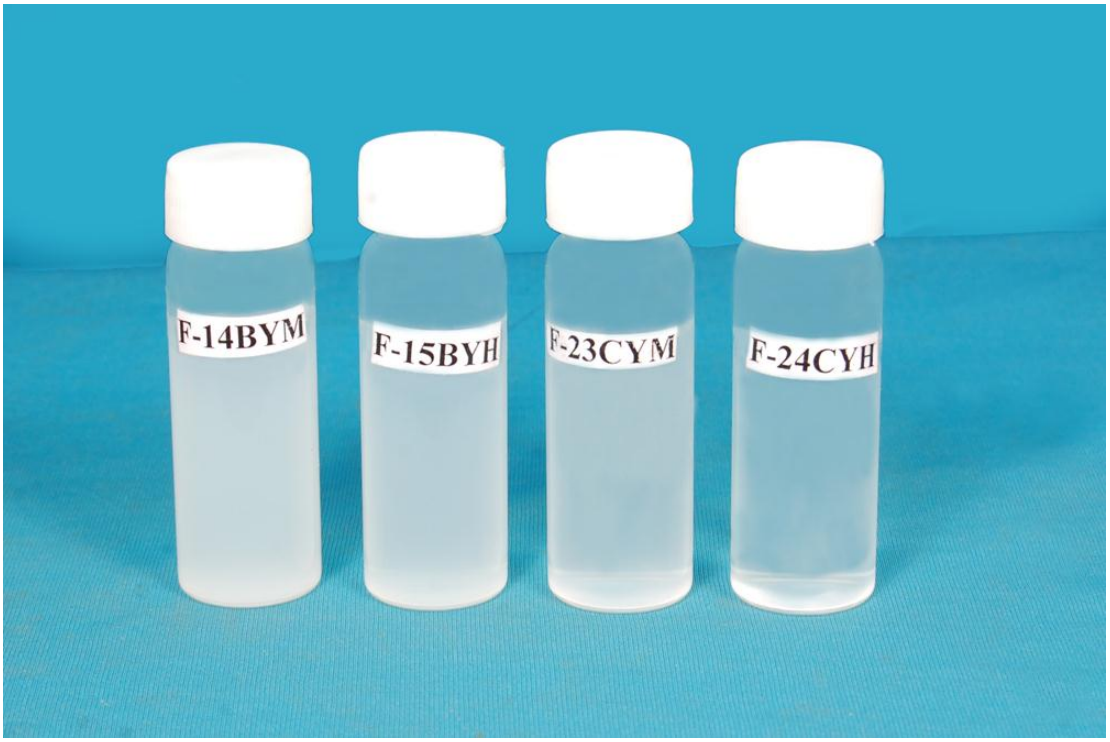


Fig 10.1. Nanosuspensions of aceclofenac
(F-14BYM, F-15BYH, F-23CYM & F-24CYH)

Fig 10.2. SEM PHOTOS OF F-14BYM, F-15BYH, F-23CYM & F-24CYH

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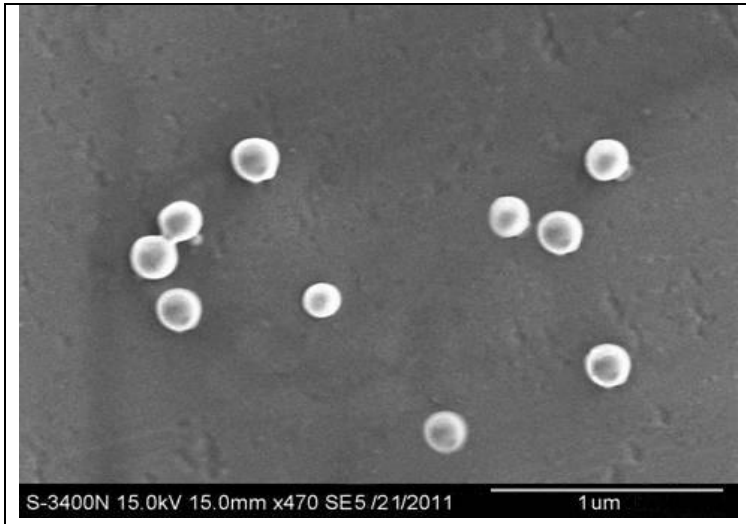
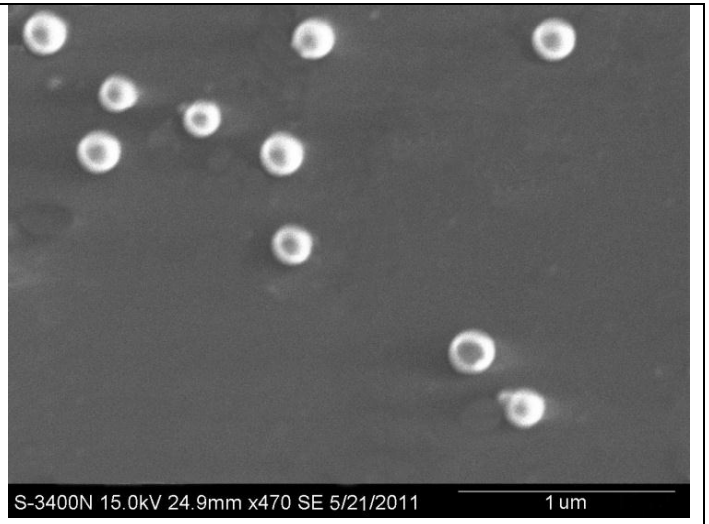
<tr><td> 
</td><td> 
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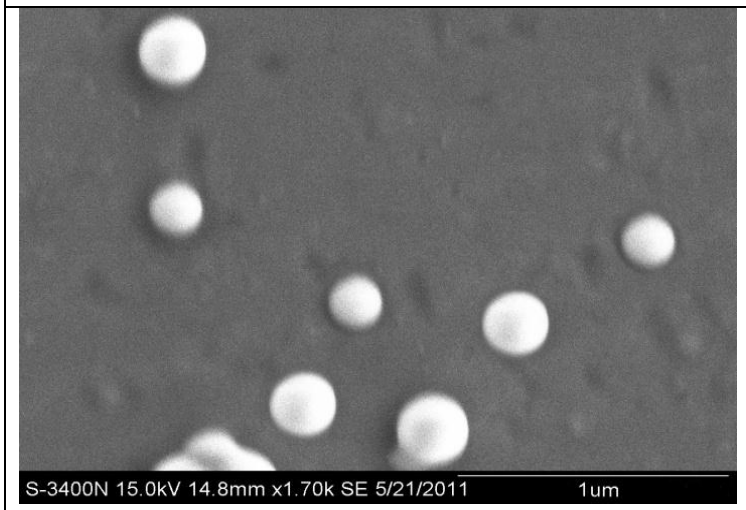
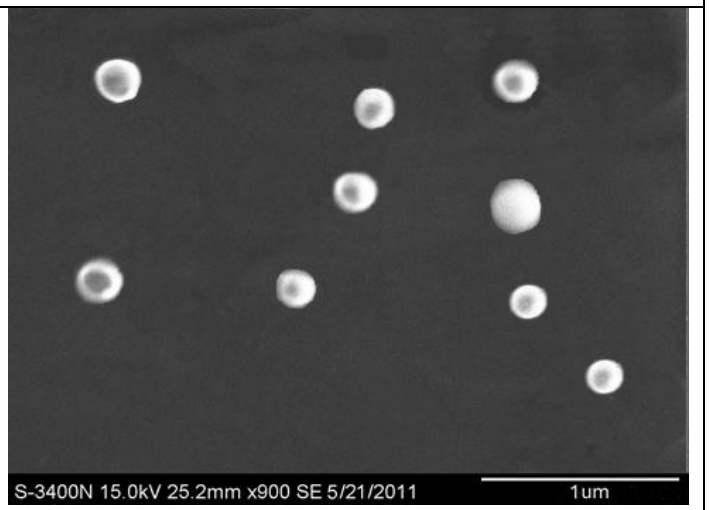
<tr><td> 
</td><td> 
</td></tr>
<tr><td>F-23CYM</td><td>F-24CYH</td></tr>
</table>

Table 10.4. Optimization of Aceclofenac Nanosuspension formulation

Batches	**F-1AXL**	**F-2AXM**	**F-3AXH**	**F-4AYL**	**F-5AYM**	**F-6AYH**	**F-7AZL**	**F-8AZM**	**F-9AZH**
P size	-1	-1	+1	+1	+1	-1	-1	-1	0
Drug content	-1	-1	-1	-1	-1	-1	-1	-1	-1
E.E	-1	-1	-1	-1	-1	-1	-1	-1	-1
Batches	**F-10BXL**	**F-11BXM**	**F-12BXH**	**F-13BYL**	**F-14BYM**	**F-15BYH**	**F-16BZL**	**F-17BZM**	**F-18BZH**
P size	-1	0	+1	+1	**+1**	**+1**	-1	-1	-1
Drug content	-1	+1	+1	+1	**+1**	**+1**	-1	0	0
E.E	0	+1	+1	+1	**+1**	**+1**	-1	0	0
Batches	**F-19CXL**	**F-20CXM**	**F-21CXH**	**F-22CYL**	**F-23CYM**	**F-24CYH**	**F-25CZL**	**F-26CZM**	**F-27CZH**
P size	-1	0	+1	+1	+1	+1	-1	-1	-1
Drug content	+1	+1	+1	+1	+1	+1	+1	+1	+1
E.E	+1	+1	+1	+1	+1	+1	+1	+1	+1

+1= Positive effect, 0= No effect, -1 = Negative effect

Particle Size

Increase in sonication time/agitation speed there is reduction in particle size. Entrapment efficiency increased with the increase in polymer concentration. It is well evident that the concentration of tween 80 played a significant role in achieving the particle size and entrapment efficiency. The increase in tween 80 concentration, from 0.01% to 0.02% there is an increase in entrapment efficiency and decrease in particle size. But the increase in the concentration of tween 80 from 0.02 to 0.03% the particle size increased and entrapment efficiency decreased. This may be due to increase in concentration of the surfactant beyond the critical micellar concentration, reduces the effectiveness in reducing the surface tension with a consequent increase in particle size and reduced entrapment efficiency. But this occurs only when the drug polymer ratio 1:1 and 1:2. For drug:polymer ratio 1:3 at 0.03% of tween 80 there is reduction in particle size and entrapment efficiency. The reduction of entrapment efficiency might be due to the formation of aggregates. The increase in polymer concentration indicated a direct impact on entrapment efficiency and has no impact on particle size.

Increase in sonication time/agitation speed shown a reduction in particle size and increase in entrapment efficiency. But increase in sonication time/agitation time for drug:polymer ratio of 1:2, the particle size is increased and entrapment efficiency is reduced for 0.02% from 4min/4000 to 6 min/6000. This may be due to particle aggregation due to over speed. Similar phenomenon was exhibited in drug:polymer ratio of 1:3, but the particle size and entrapment efficiency is reduced. With higher concentration of polymer(1:3), no increase in particle size in the nanoparticle (F-24CYH) was experienced.

This size ranges of the nanoparticles were reported to possess higher intracellular uptake than microparticles and are considered ideal for the delivery of biopharmaceuticals[240] . The details were given in table 10.5 and fig.10.3.

Table 10.5. Particle size analysis of aceclofenac nanosuspension

Batches	**F-1AXL**	**F-2AXM**	**F-3AXH**	**F-4AYL**	**F-5AYM**	**F-6AYH**	**F-7AZL**	**F-8AZM**	**F-9AZH**
Particle size (nm)	780± 36	650 ±27	510 ±22	405± 40	380 ± 36	215± 16	680± 40	602 ± 31	570± 30
Batches	**F-10BXL**	**F-11BXM**	**F-12BXH**	**F-13BYL**	**F-14BYM**	**F-15BYH**	**F-16BZL**	**F-17BZM**	**F-18BZH**
Particle size (nm)	710 ± 50	590 ± 48	436± 40	405± 30	210± 15	212± 23	809± 60	760± 72	710±75
Batches	**F-19CXL**	**F-20CXM**	**F-21CXH**	**F-22CYL**	**F-23CYM**	**F-24CYH**	**F-25CZL**	**F-26CZM**	**F-27CZH**
Particle size (nm)	940± 60	600± 52	500±60	460± 60	225± 30	220± 42	880± 40	780 ± 65	750 ± 50

Values expressed as ± S.E.M of triplicate trials

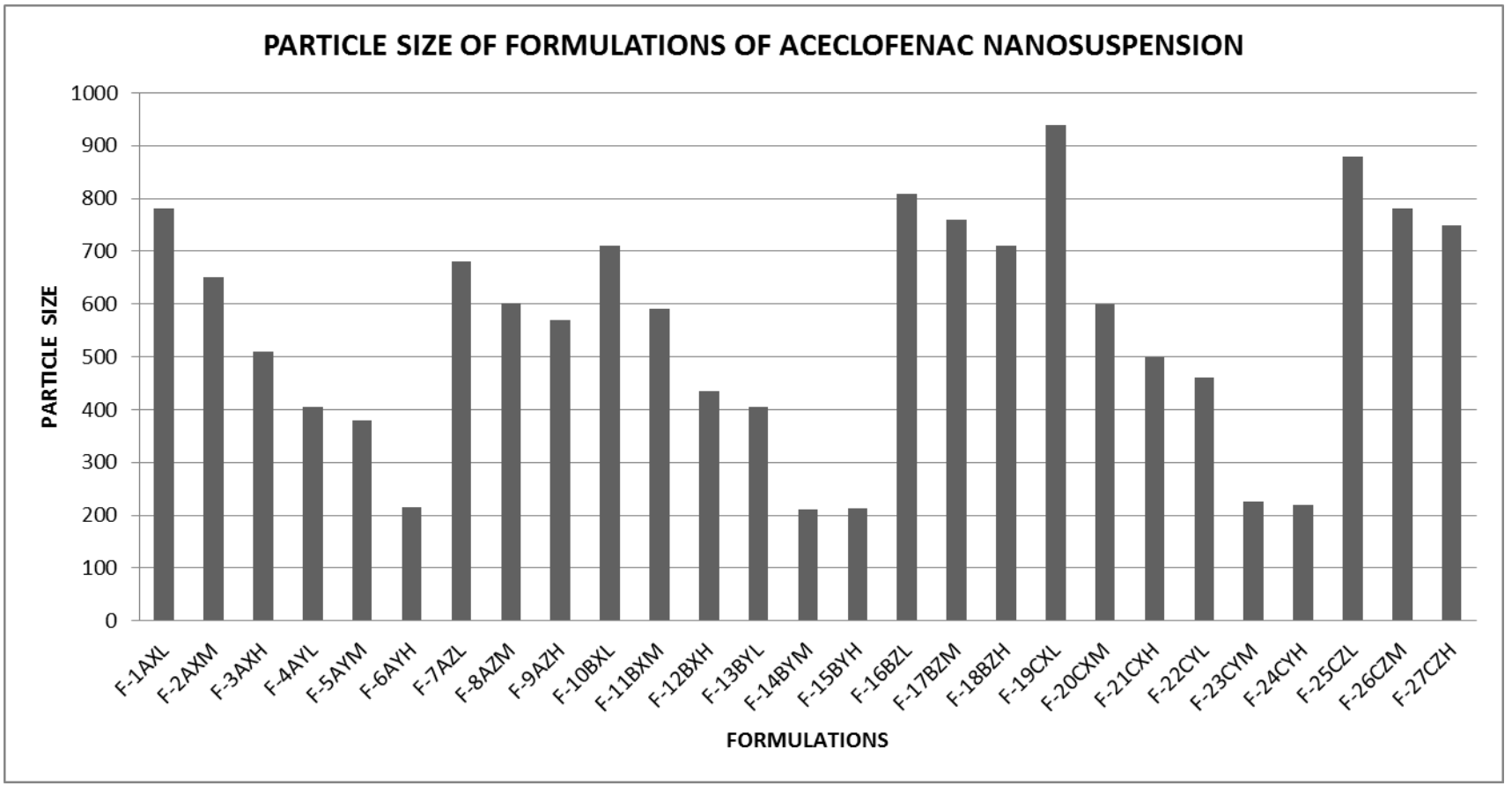


Fig 10. 3. PARTICLE SIZE OF FORMULATIONS OF ACECLOFENAC NANOSUSPENSION

Zeta Potential:

The particle charge is one among the factors that determines the physical stability of emulsions and suspensions. The higher particles are equally charged, the higher is the electrostatic repulsion between the particles and the higher is the physical stability. The particle charge can be quantified as surface charge in terms of zeta potential, which is measured e.g. via the electrophoretic mobility of the particles in an electrical field.

Zeta potential is the electrical charge at the hydrodynamic shear plane and can be determined from the particle mobility under an applied external electric field. The movement of the particles will depend on the effective charge on the surface of the individual particles. The zeta potential values are highly useful in predicting the storage stability of colloidal dispersions. According to the zeta potential theory[241] the particles possess a surface charge, which occurs due to the dissociation of surface functional groups, called as Nernst potential. The degree of dissociation of the functional groups depends of the pH of the suspension, and hence the zeta potential is pH dependent. The evaluation of zeta potential is considered as critical because the nanosuspension is designed for oral administration and after administration they undergo a pH changes, particularly acidic pH in the stomach, increasing to alkaline pH (pH 7) in the gastro intestinal tract (GIT).

In general, greater the zeta potential value of a nanoparticulate system better is the colloidal suspension stability due to repulsion effect between charged nanoparticles. Zeta potential values in the −15 to −30 mV are common for well-stabilized nanoparticles[242]. The zeta potential values were found to be -18.6±0.3mV, -18.3±0.4 mV, -16.3±0.4 mV and -16.4±0.3 mV for the formulations F-14BYM, F-15BYH, F-23CYM and F-24CYH respectively. These results indicate that the four batches of the nanosuspension will be more stable on storage. Eudragit L100 is an anionic polymer and the hence the formulations indicated negative surface charge and the variation in the charge may be attributed by the presence of aceclofenac and tween 80.

Polysorbate 80 (Polyoxyethylen(20)sorbitan monooleate), Tween 80 possess HLB value of 15 and it is a non-ionic surfactant with a relatively low toxicity. It is widely used in the commercial preparations for oral use. Eg. Combantrin tablet and oral suspension (Pfizer Consumer Healthcare 2000) contains this surfactant.

Table 10.6. Zeta potential values of optimized formulations

S.No	Formulations	Zeta Potential (mV)
1	F-14BYM	-18.6±0.3
2	F-15BYH	-18.3±0.4
3	F-23CYM	-16.3±0.4
4	F-24CYH	-16.4±0.3

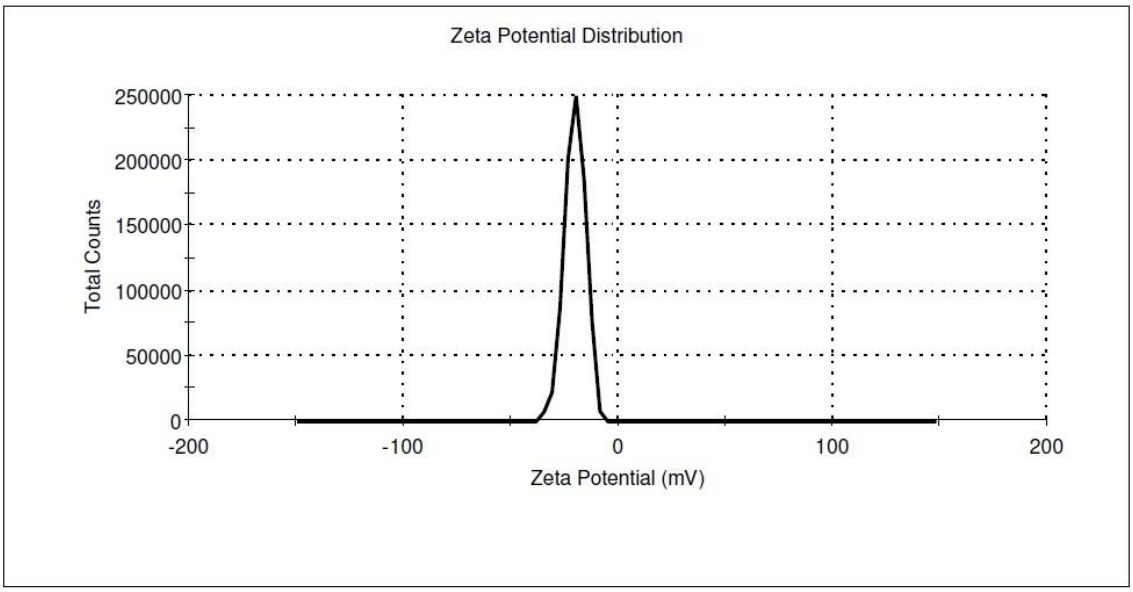


Fig 10.4. Zeta potential of Nanosuspension of Aceclofenac- F-14BYM

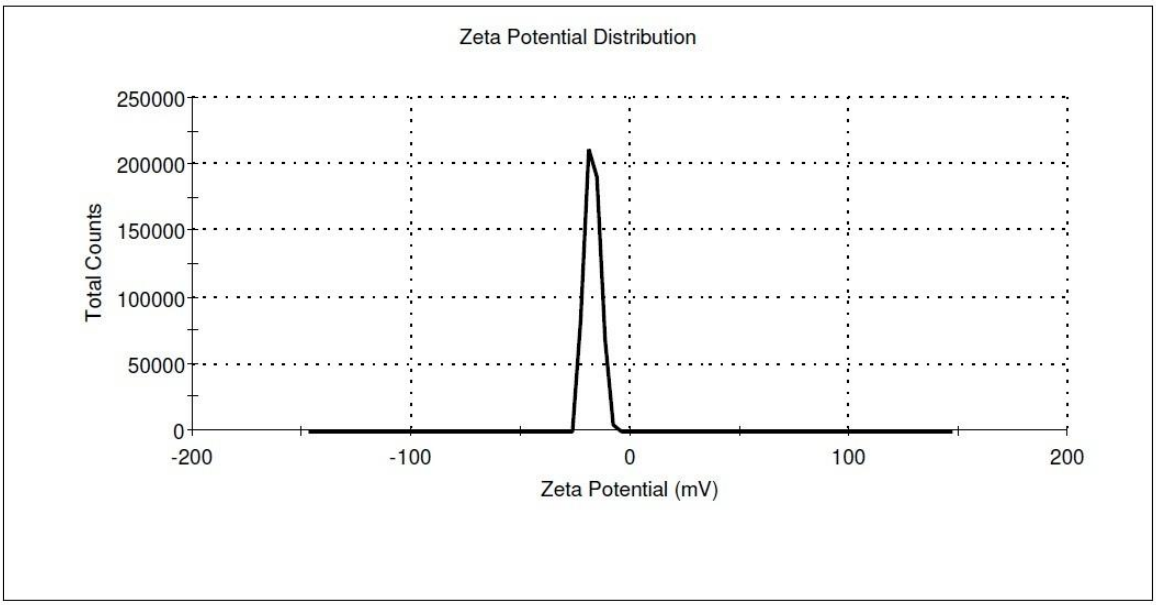


Fig 10.5. Zeta potential of Nanosuspension of Aceclofenac- F-15BYH

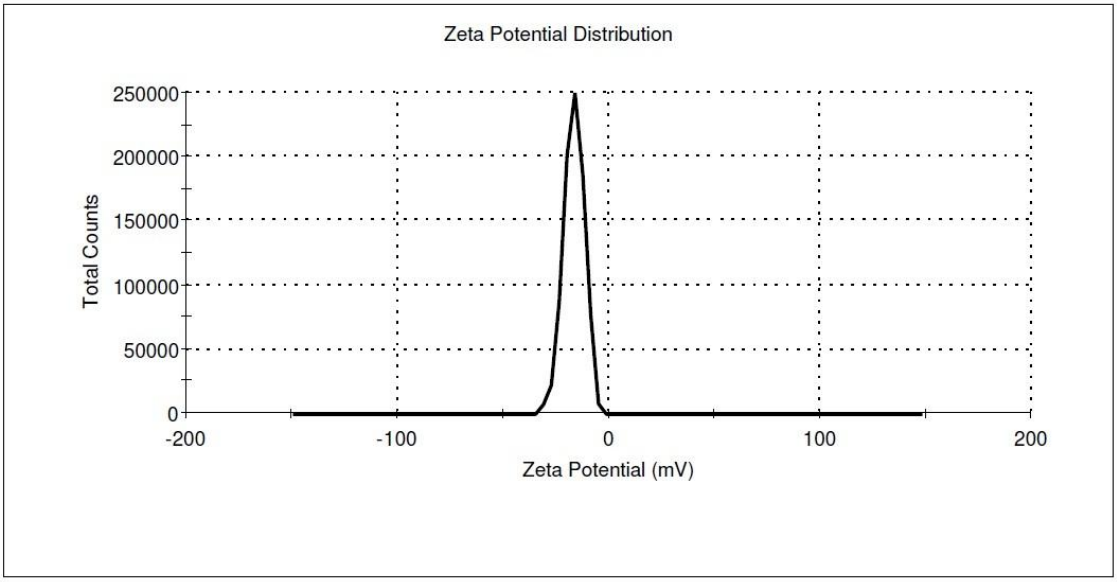


Fig 10.6. Zeta potential of Nanosuspension of Aceclofenac - F-23CYM

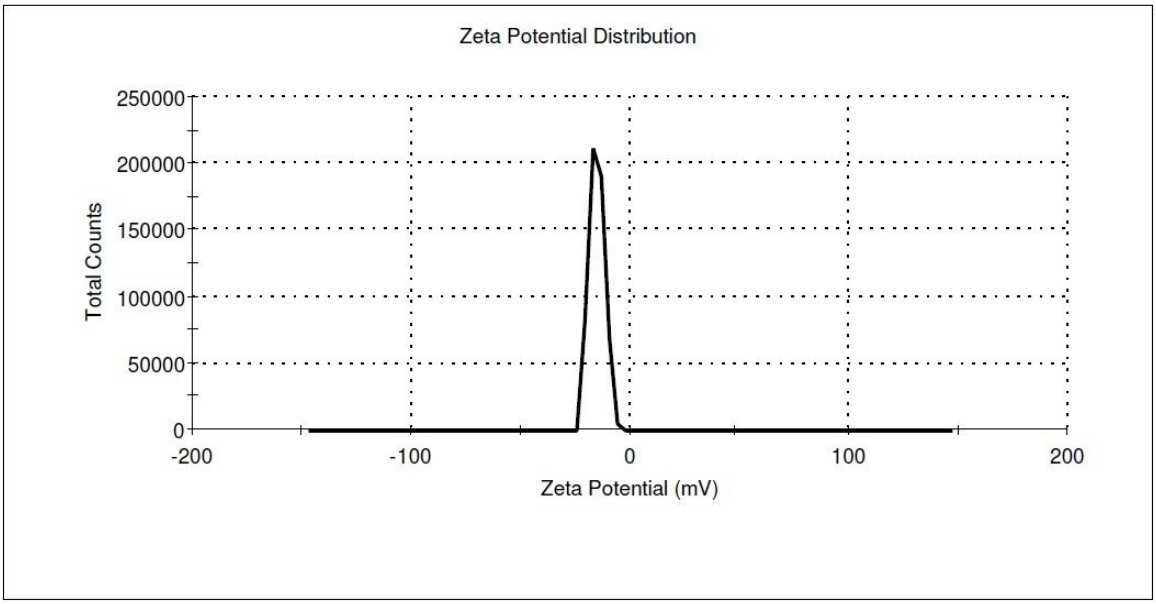


Fig 10.7. Zeta potential of Nanosuspension of Aceclofenac- - F-24CYH

Drug Content:

The drug content of formulations vary from minimum of 16±1.6% to maximum of 82±1.1%. F-1AXL showed minimum of 16±1.6% and F-14BYM exhibited maximum of 82±1.1%. The optimization studies suggested that the increase in the ratio of the polymer indicated increase in the drug content, except in formulations F-8AZM, F-15BYH, F-18BZH, F-24CYH and F-27CZH. The drug content was not increased in formulation beyond a level because of the saturation of the adsorption of drug molecules on the polymeric matrices. This further indicates that the drug is physically adsorbed on to the polymer and it correlates with results of the IR spectroscopic and DSC studies. The details were given in table 10.7 and fig.10.8.

Entrapment efficiency

The entrapment efficiencies of formulations vary from minimum of 22.2±0.6% and maximum of 89.6±0.9%. F-1AXL showed minimum of 22.2±0.6% and F-14BYM exhibited maximum of 89.6±0.9%.

The increase in polymer proportion had caused exhibited increase in the entrapment efficiency of the nanosuspensions, except in F-15BYH (86.2±1.6%), F-24CYH (88.6±1.5%), F-26CZM (74.3±1.3%), F-27CZH (72.6±1.3%). This also suggests that the drug physically adsorbs to the enteric polymer, Eudragit L100 without any chemical modifications, and this is supported the IR and DSC studies. The parallel increase in the particle size was also observed with increase in entrapment efficiency. However the entrapment efficiency increased with increased polymer proportion, it was not increased beyond 1:3, because of the linear increase in the particle size that may reduce the colloidal properties of the formulation. The details of the report were given in table 10.8 and fig. 10.9

Based on the analysis of the data obtained from the above studies, four formulations, F-14BYM, F-15BYH, F-23CYM and F-24CYH with minimal particle size and appreciable drug content and entrapment efficiency were selected for further studies.

Table 10.7. Drug content (%) of aceclofenac nanosuspension

Batches	**F-1AXL**	**F-2AXM**	**F-3AXH**	**F-4AYL**	**F-5AYM**	**F-6AYH**	**F-7AZL**	**F-8AZM**	**F-9AZH**
Drug content (%)	16±1.6	23±1.4	25±2.6	35±1.0	36±1.8	41±3.5	28±1.6	28±1.5	32±1.4
Batches	**F-10BXL**	**F-11BXM**	**F-12BXH**	**F-13BYL**	**F-14BYM**	**F-15BYH**	**F-16BZL**	**F-17BZM**	**F-18BZH**
Drug content (%)	45±1.6	58±1.6	69±2.5	64±2.5	82±1.1	79±2.3	46±1.1	48±1.6	48±1.8
Batches	**F-19CXL**	**F-20CXM**	**F-21CXH**	**F-22CYL**	**F-23CYM**	**F-24CYH**	**F-25CZL**	**F-26CZM**	**F-27CZH**
Drug content (%)	59±1.0	60±1.5	62±2.2	65±1.3	81±1.6	80±1.1	69±1.3	69±1.7	66±1.6

Values expressed as ±S.D in percentage

Fig 10.8. PERCENTAGE DRUG CONTENT OF ACECLOFENAC NANOSUSPENSION FORMULATIONS

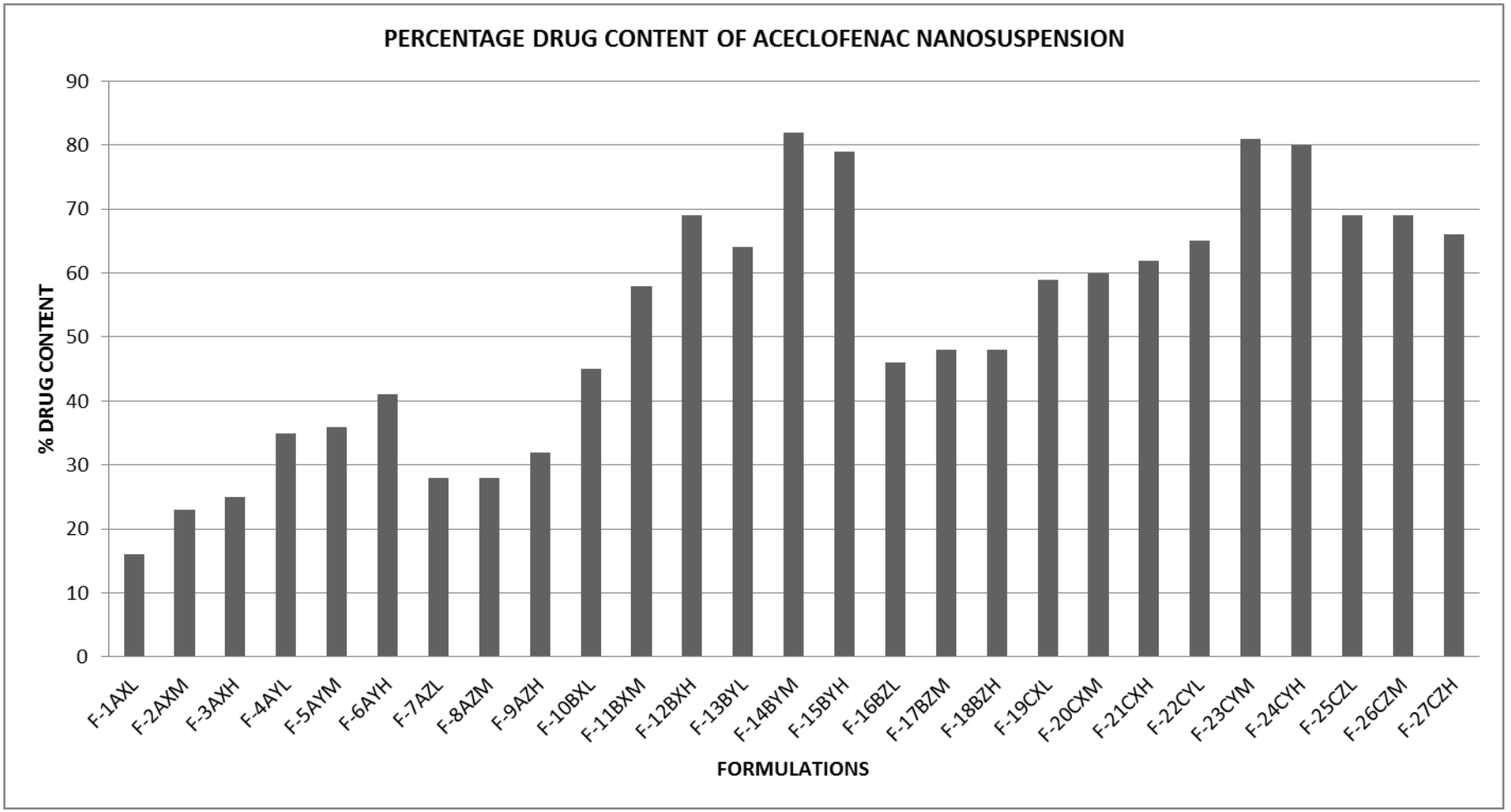
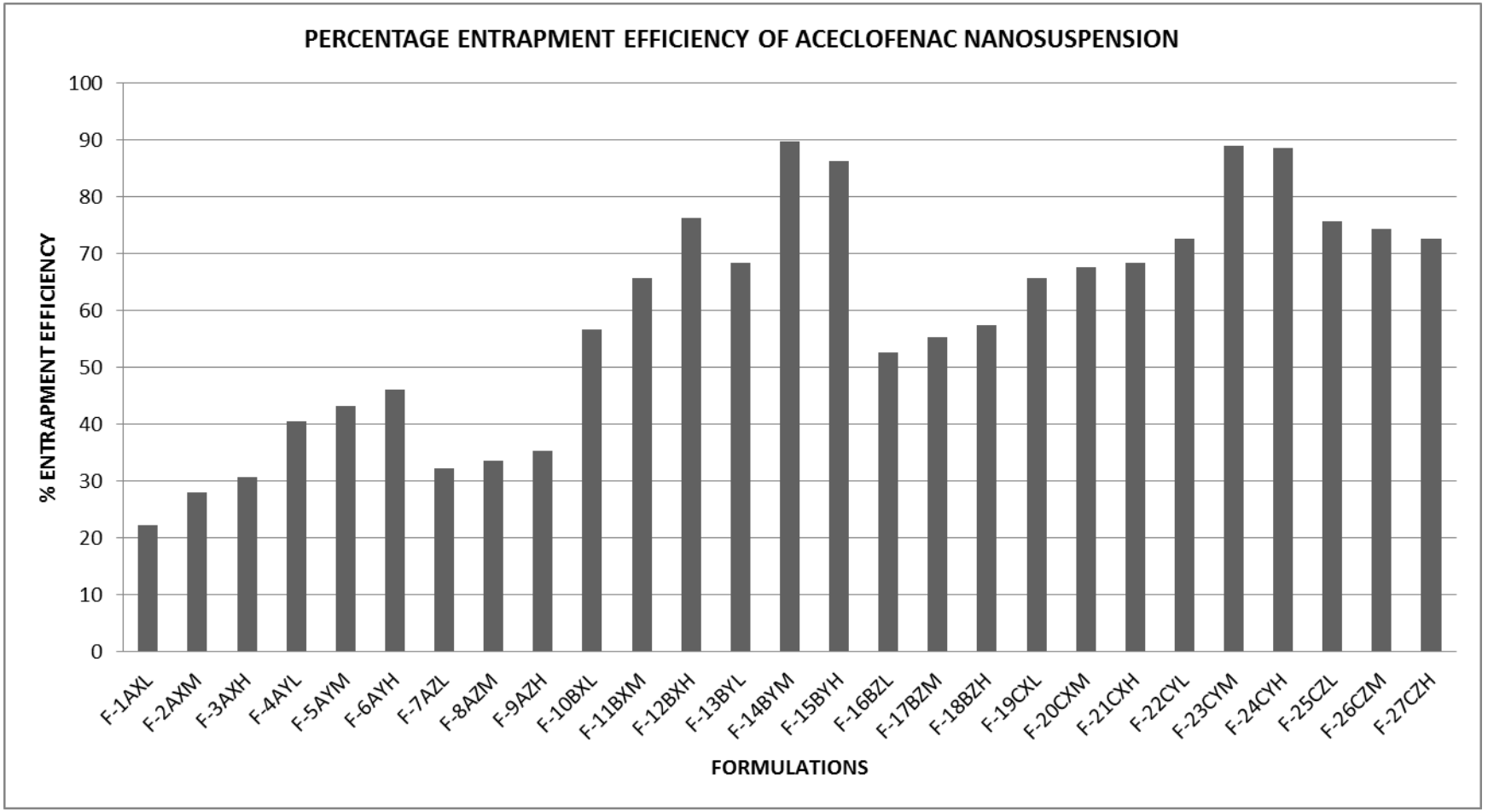


Table 10.8. ENTRAPMENT EFFICIENCY OF ACECLOFENAC NANOSUSPENSION

Batches	**F-1AXL**	**F-2AXM**	**F-3AXH**	**F-4AYL**	**F-5AYM**	**F-6AYH**	**F-7AZL**	**F-8AZM**	**F-9AZH**
Entrapment efficiency(%)	22.2±0.6	28±0.3	30.6±1.2	40.4±0.9	43.1±1.1	46.1±1.4	32.3±0.4	33.6±0.5	35.2±0.7
Batches	**F-10BXL**	**F-11BXM**	**F-12BXH**	**F-13BYL**	**F-14BYM**	**F-15BYH**	**F-16BZL**	**F-17BZM**	**F-18BZH**
Entrapment efficiency(%)	56.6±1.1	65.6±1.4	76.2±1.5	68.4±0.9	89.6±0.9	86.2±1.6	52.5±0.8	55.3±0.8	57.4±1.0
Batches	**F-19CXL**	**F-20CXM**	**F-21CXH**	**F-22CYL**	**F-23CYM**	**F-24CYH**	**F-25CZL**	**F-26CZM**	**F-27CZH**
Entrapment efficiency(%)	65.6±0.9	67.6±1.2	68.4±1.8	72.6±0.5	88.9±1.3	88.6±1.5	75.6±1.2	74.3±1.3	72.6±1.3

Values expressed as percentage ±S.D

Fig 10.9. PERCENTAGE ENTRAPMENT EFFICIENCY OF ACECLOFENAC NANOSUSPENSION FORMULATIONS



***In vitro* Release studies & Release kinetics:**

The *in vitro* release of the four formulations, F-14BYM, F-15BYH, F-23CYM and F-24CYH were 98.7±1.2, 94.1±1.4, 85.7±2.0, and 88.5±2.2% at the end of 24 hrs, respectively. The batch, F-14BYM released 20.2±0.5% of the loaded aceclofenac within 1 hr, which may be helpful in achieving quick onset of action. Thereafter steady controlled release was exhibited for 24 hrs by F-14BYM.

F-15BYH released 8.3±1.2% of drug loaded in 1hr. F-23CYM and F-24CYH releases 4.5±1% and 8.3±0.4% of drug release in 1hr. Hence it suggests that the release profile of F-14BYM is ideal to consider the controlled release formulation for performing the in vivo studies.

Table 10.9. Drug release data of Aceclofenac Nanosuspension

S.No	Time (hr)	F-14BYM(%)	F-15BYH (%)	F-23CYM(%)	F-24CYH(%)
1	0	0	0	0	0
2	0.5	8.7±0.4	5.2±0.6	3.1±0.2	5.2±0.1
3	1	20.2±0.5	8.3±1.2	4.5±1	8.3±0.4
4	1.5	30.1±0.7	11.2±1.5	7.6±0.8	9.9±0.2
5	2	32.8±0.6	14.6±1.7	8.9±0.6	12.3±0.7
6	2.5	39.6±1.0	17.8±1.5	11.3±1.4	13.6±1.2
7	3	40.1±1.1	22.9±1.8	15.3±1.5	17.5±0.8
8	4	41.6±0.9	32.2±1.8	17.5±1.8	22.3±1.0
9	6	45.6±1.2	41.7±0.8	26.8±1.8	28.3±1.8
10	8	57.6±1.4	48.3±1.3	36.2±2.0	38.6±1.3
11	10	65.4±1.4	57.3±1.5	42.7±1.6	46.3±1.6
12	12	78.2±1.4	65.6±1.4	55.6±2.5	60.1±2.4
13	16	84.3±1.8	81.8±1.7	71.2±2.0	73.2±1.9
14	20	92.8±1.2	94.1±1.4	85.1±2.2	87.1±2.4
15	24	98.7±1.2	94.1±1.4	85.7±2.0	88.5±2.2

Cumulative release of drug expressed in percentage ±SD of triplicate trials

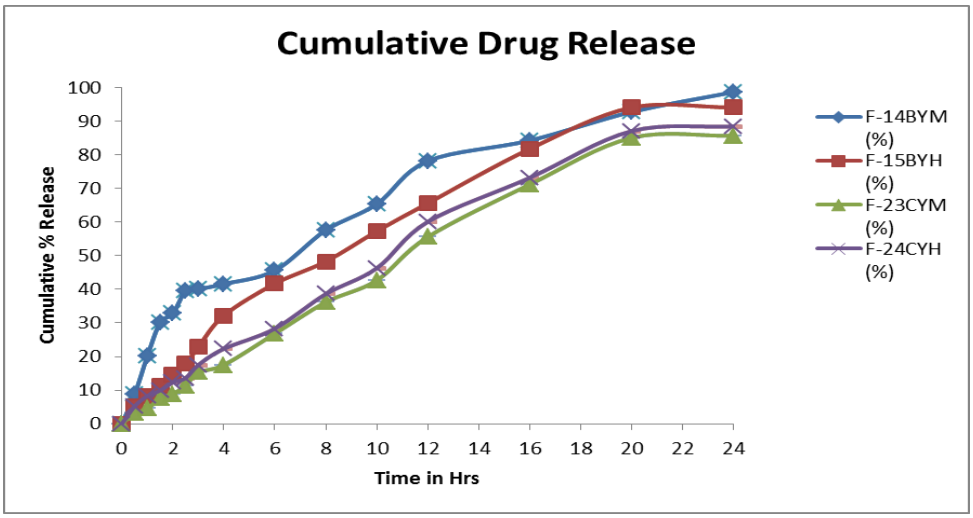


Fig 10.10 Cumulative drug release Vs Time in hrs for optimized trials

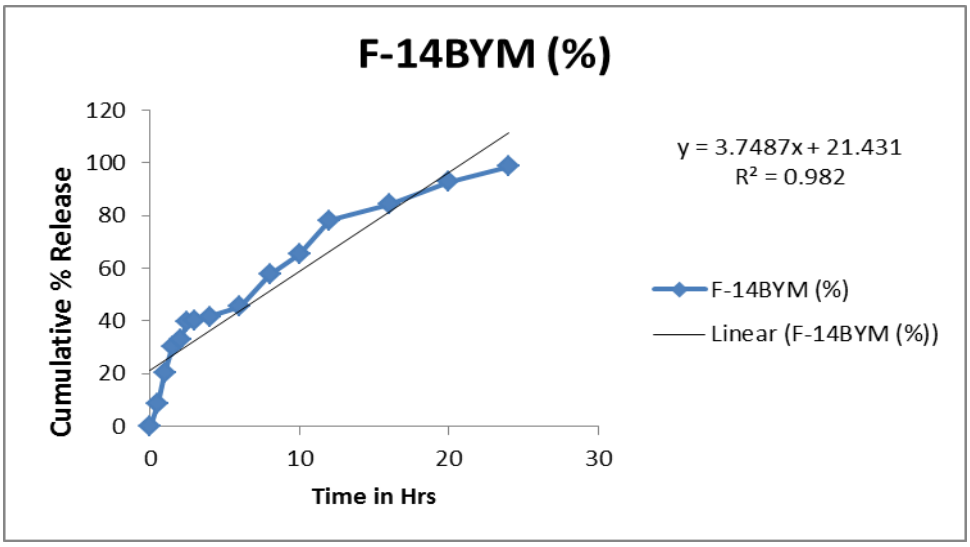


Fig 10.11 Cumulative drug release Vs Time in hrs for F-14BYM

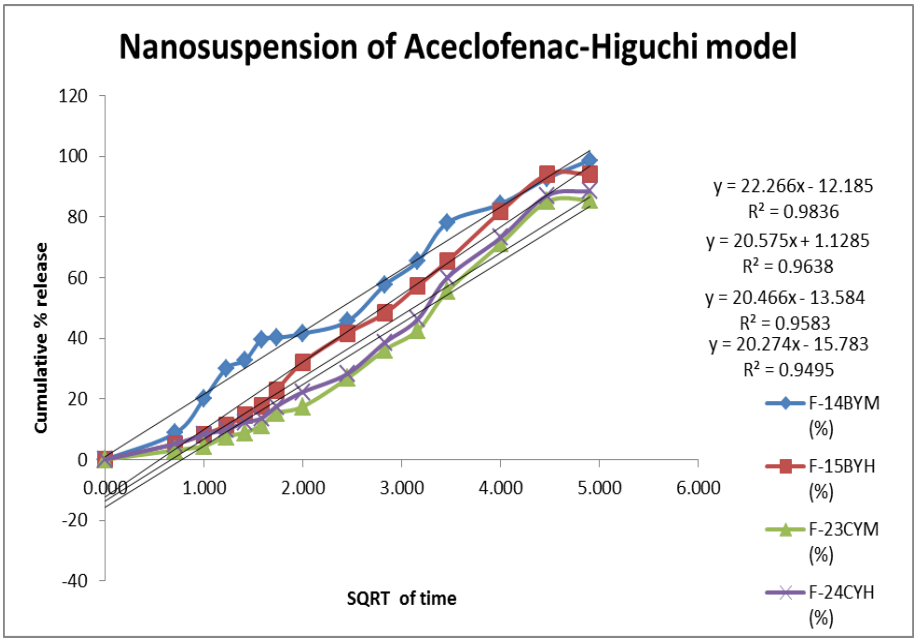


Fig10.12. Higuchi model for optimized trials

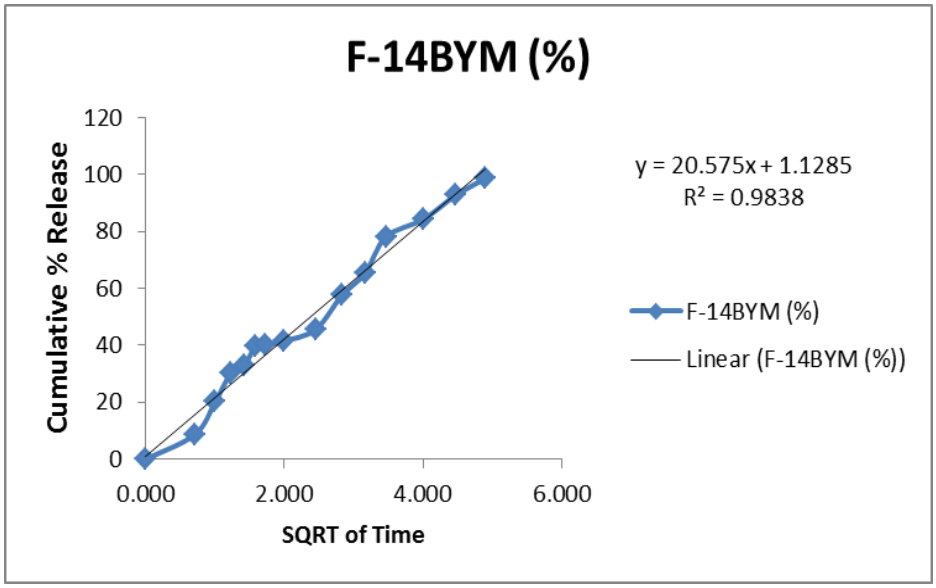


Fig.10.13. Higuchi model for F-14BYM

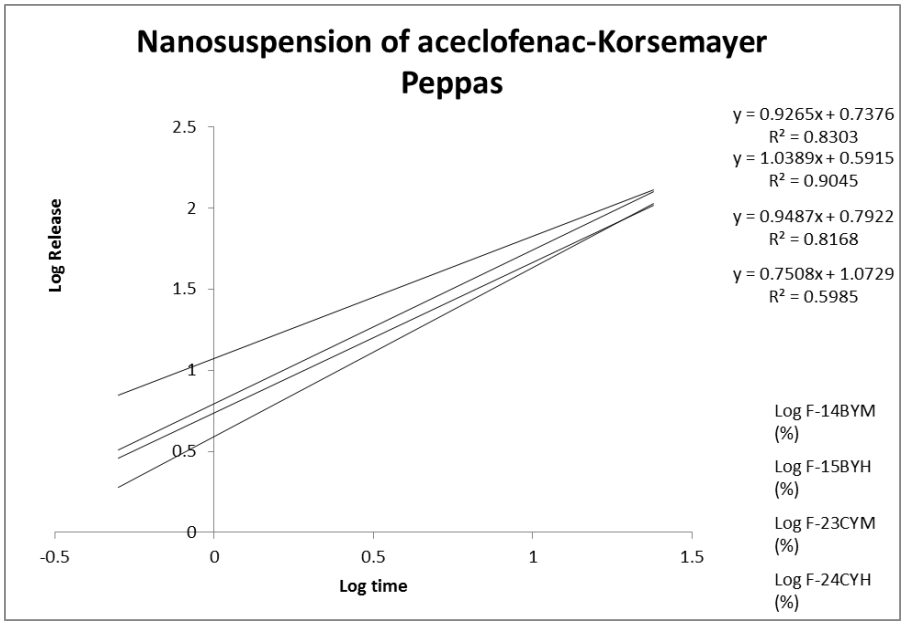


Fig. 10.14 Korsemayer Peppas

To know the mechanism of drug release from various preparations the data were treated according to zero order, first order, Higuchi and korse mayer equation. The release rate kinetic data for all the equations were shown in graph. Fig 10.10, 10.11, 10.12, 10.13. The value fitted to zero order plot and its regression value was 0.982, as its value is close to 1, it was conformed that it followed zero order release. The mechanism of drug release was further cconformed by korsemayer and peppas plot. According to this 0.5 is Fickian diffusion, $0.5 < n < 1$ is anomalous transport or Non-Fickian transpory, 1 is Case II transport, $n > 1$ is Super case II transport. The n value of the formulation F-14BYM was 0.926 and hence it suggests non fickian diffusion.

11. PHARMACOLOGICAL STUDIES

MATERIALS AND METHODS

Animals

Healthy Male Wistar rats (120 - 170 g) obtained from the animal house of Vel's College of Pharmacy, Tamil Nadu, India was used for the study. Animals were housed in polypropylene cages and were left 7 days for acclimatization to animal room maintained under controlled condition (a 12 h light–dark cycle at 22±2 ◦C) on standard pellet diet and water ad libitum. All animals were taken care of under ethical consideration as per the guidelines of CPCSEA with due approval from the Institutional Animal Ethics Committee. The approval no. was 290/CPCSEA/PH-15/13.10.2009. Animals were used for acute toxicity study and anti-inflammatory study.

EVALUATION OF ANTI-INFLAMMATORY ACTIVITY

Method:

Carrageenan induced paw edema in rats:

Wistar albino rats were used, which were divided into 5 groups (n =6). The animals were fasted overnight with free access to water. At the *lateral malleolus* portion, an ink mark was made in the left paw. On immersing the paw till the level of *lateral malleolus*, volume displaced was measured using plethysmometer that implies the basal paw volume. In the right hind paws were injected 0.1 ml of carrageenan in isotonic saline (1%w/v) in subplantar region of all group of animals, which induces edema. Reference and controlled drug delivery systems [nanosuspension (F-14BYM) & microspheres (M4)] of aceclofenac were given orally 30 minutes prior to carrageenan injection. The volume of the injected paws and contra-lateral paws were measured at 1, 2, 3, 4 and 5 hours intervals using plethysmometer[243]. The percent Inhibition was calculated using the following formula

$$\% \text{ Inhibition} = 1 - Vt / Vc \times 100$$

Where, V_c = Mean edema volume in control group,

Vt = Mean edema volume in test group.

Adjuvant induced inflammation of rat paw

Adjuvant inflammation was induced by injection of 0.1ml of heat-killed *Mycobacterium hutyricum* suspended in mineral oil (10 mg/ml) into the right hind foot pad of the rats. After 14 and 21 days, the inflammation development was evaluated. Primary and secondary arthritic lesions were scored on an arbitrary scale as follows: left and right hind feet each 0-7, left and right fore feet each 0- 4.5, tail 0-5, ears 0-2, nose and eyes each 0-l[138].

Gastrointestinal tolerability:

At the end of the study period, after 24 h of fasting, the animals were anaesthetized with anesthetic ether and killed[244]. The gastrointestinal tract was opened, the luminal content removed by washing with saline solution, the tissues carefully examined for any presence of lesions according to the standard protocol. Coprophagia was prevented during and after the experiment.

Statistical analysis:

The statistical significance was assessed by using one-way analysis of variance (ANOVA) and followed by Dunnet's comparisons test. All the data are presented as mean ± SEM and $p<0.05$ was considered as significant.

RESULTS AND DISCUSSION:

The paw edema induced by the injection of Carrageenan is the commonly used experimental model for acute inflammation. The edema induced by carageenan is a biphasic event. The first phase (1-2 h) is mediated by serotonin, histamine and due to increased synthesis of prostaglandins in damaged tissue. The late phase sustained by prostaglandin release, mediated by Polymorphonuclear (neutrophils and monocytes), bradykinin, leukotrienes and prostaglandins produced by tissue macrophages[244]. Carrageenan induced inflammation is useful in detecting orally active anti-inflammatory agents[245]. Administrations of carrageenan to normal animals showed significant ($P< 0.05$) increase in paw inflammation from 1hrs to 5 hrs when compared to control. Paw edema in rats reached its

peak at 4 hrs after carrageenan administration. Per oral treatment of nanosuspension of aceclofenac (F-14BYM) showed higher significant (P<0.01) reduction paw volume in rats from 1 hrs and maintained its effect throughout the study period. In addition, microspheres also produced a significant inhibition (P<0.05) of paw volume in rats with carrageenan administration. Among the two different drug formulations, nanosuspension (F-14BYM) treated inflammatory rats exhibited high anti-inflammatory response than the microspheres (M4) (Figure 11.1).

Daily oral administration of reference aceclofenac and nano and microsphere loaded aceclofenac at 10 mg/kg markedly and significantly inhibited arthritis development until the end of the study period. With aceclofenac nanosuspension (F-14BYM), the highest percentage of inhibition was observed from various day intervals than the microspheres (M4) and reference drug (Figure 11.2 & 11.3).

The NSAIDs are used for the relief of pain and inflammation, but it increases the risk of gastrointestinal side effects ranging from dyspepsia to symptomatic and complicated ulcer[246]. Vasoactive agents (Prostaglandins, calcitonin, nitric oxide, gene related proteins) play a role in mucosal defensive processes[247].

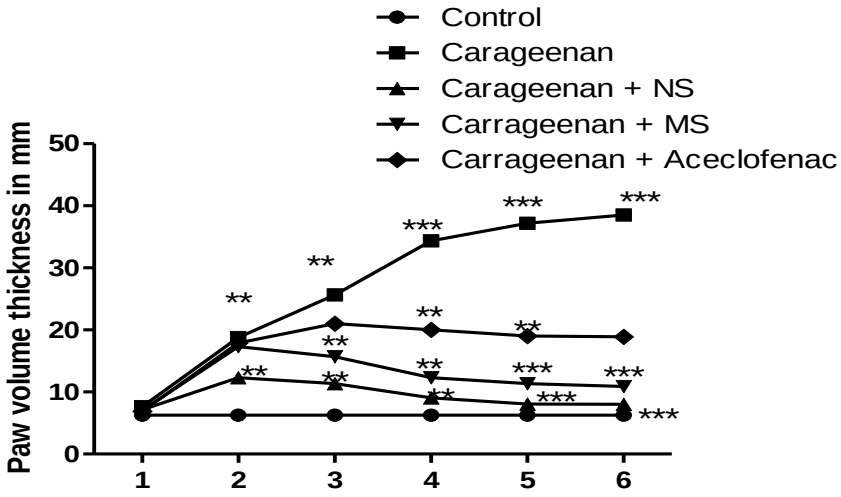


Fig. 11.1. Effect of aceclofenac loaded nanosuspension and microspheres on paw volume thickness in rats with carrageenan

Fig. 11.1 represents the effect of aceclofenac loaded nanosuspension (F-14BYM); microspheres(M4) and plain aceclofenac in rats with paw edema. It was observed from the figure that carageenan administration to rats significantly ($P< 0.05 – P < 0.001$) increases the paw volume thickness from 1 hrs to 6 hrs of time period than control. Rats treated with nanosuspension (F-14BYM) showed significant decrease in the paw volume at different time period from 1 hrs to till end of the study as compared with carrageenan treated paw volume. Further microspheres (M4) treated rats showed significant decrease in paw volume from 3 hrs to till end of the study. However the attenuation of paw volume in rats administered with plain aceclofenac sodium was noted from 4 hrs after carrageenan challenge.

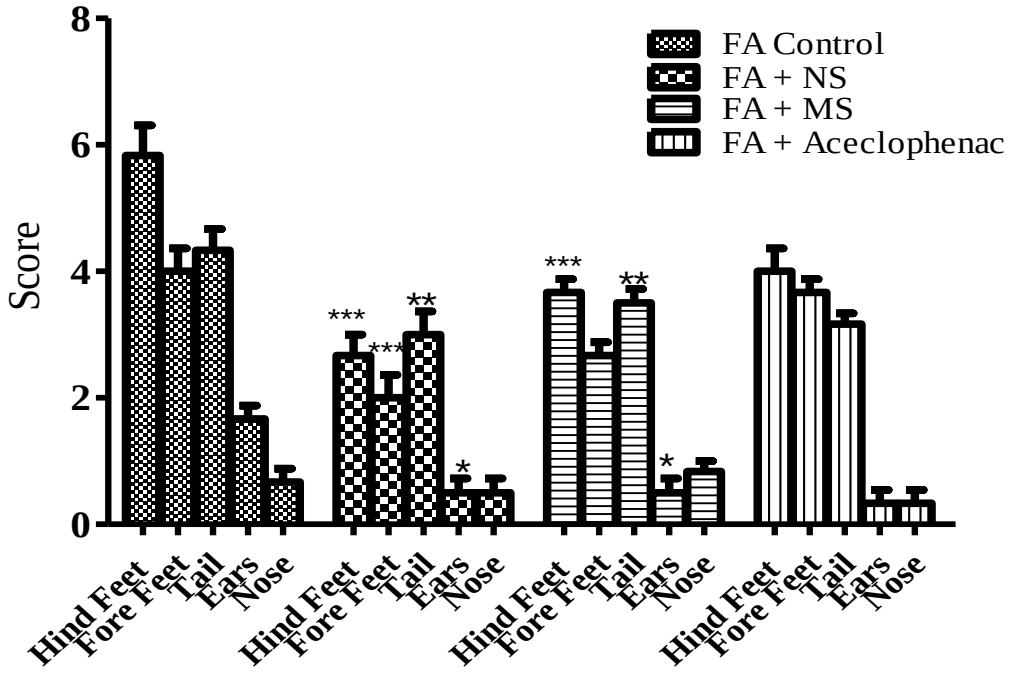


Fig. 11.2. FA induced inflammatory score day 14

Figure 11.2 represents the effect of aceclofenac loaded nanosuspension (F-14BYM); microspheres (M4) and plain aceclofenac in freund's adjuvant (FA) induced inflammation in rats. Administration of aceclofenac nanosuspension has shown significant (P<0.001)

reduction in lesion scores than the freund's adjuvant treated rats. In addition, microspheres loaded with aceclofenac also showed significant effect in reducing scores only in hind paw, tail and nose than control FA treated rats. However there is no significant reducing effect was noted in plain aceclofenac.

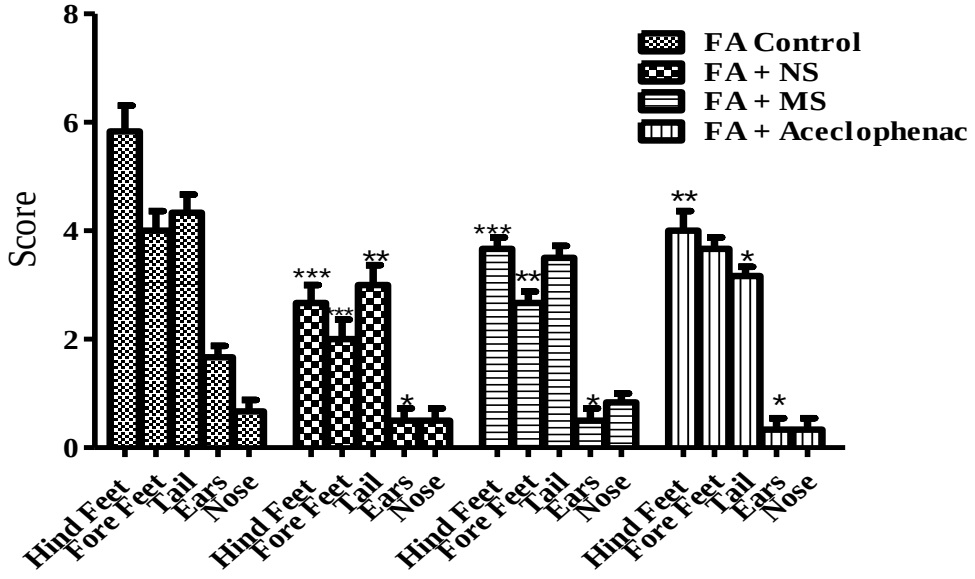


Fig. 11.3. FA induced inflammatory score day 21

Fig. 11.3 represents the effect of aceclofenac loaded nanosuspension (F-14BYM); micro sphere and plain aceclofenac in freund's adjuvant induced inflammation in rats. Administration of aceclofenac nanosuspension has shown significant ($P<0.001$) reduction in lesion scores than the freund's adjuvant treated rats. In addition, microsphere loaded aceclofenac also showed significant effect in reducing scores only in hind paw ($P<0.001$), fore paw ($P<0.01$) nose than control FA treated rats. However there was no significant reducing effect was noted in plain aceclofenac.

Gastrointestinal tolerability

Gastric ulcers are open sores in the lining of the upper GIT. They include duodenal ulcers and gastric ulcers. Gastric ulcer is one among the most serious diseases in the world. The etiology factors that may induce ulcer in human being are several they are stress, chronic

use of anti-inflammatory drugs and continuous alcohol ingestion, spicy food among others[248].

Oral administration of the test drugs F-14BYM (aceclofenac nanosuspension) and M4 (microspheres) to the different groups of animals clearly produced a mucosal damage characterized by mild to multiple hemorrhage red bands. F-14BYM showed decreased intensity of gastric mucosal damages induced by the aceclofenac compared with control group. It was represented in table 11.1 and fig.11.4, 11.5 and 11.6.

METHODOLOGY:

Scoring of ulcer will be made as follows:

Normal stomach - (0), Red coloration - (0.5), Spot ulcer - (1), Hemorrhagic streak- (1.5) Ulcers - (2), Perforation - (3)

Mean ulcer score for each animal was expressed as Ulcer Index. The percentage of Ulcer protection was determined as follows[249].

$$\% \text{ of protective} = \frac{\text{Control Mean Ulcer Index - Test Mean Ulcer Index}}{\text{Control Mean Ulcer Index}} \text{ X } 100$$

MACROSCOPICAL VIEW OF STOMACH OF THE RATS:

Fig. 11.4 (Control & Standard)

Control: Drug treated rat's shows congestion, oedema, mucosal damage.

Standard: Ranitidine 20mg/kg shows protection of mucosal layer

Fig. 11.5 (Aceclofenac Nanosuspension F-14BYM)

10mg/kg shows protection of mucosal layer.

Fig. 11.6. (Aceclofenac Microspheres M4)

10mg/kg shows protection of mucosal layer.

Table: 11.1 Influence of *Aceclofenac Nanosuspension (F-14BYM) and Microspheres (M4)* on gastric ulcer induction.

Group	Treatment	Ulcer index
I	Control	11.4± 0.05
II	Ranitidine	3.1 ± 0.4
III	F-14BYM (10mg/kg)	4.6 ± 0.03
IV	M4 (10mg/kg)	8.4 ± 0.04

In the present study both F-14BYM and M4 showed reduced gastric ulceration. When compared to M4, nanosuspension F-14BYM showed reduced gastric ulceration.

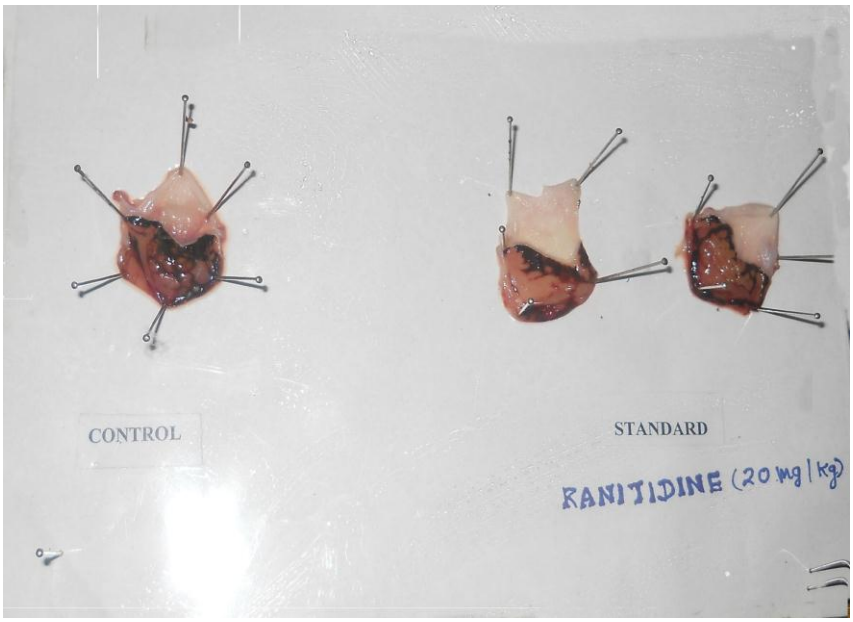


Fig. 11.4 Macroscopical view of stomach of the rats treated with control & standard

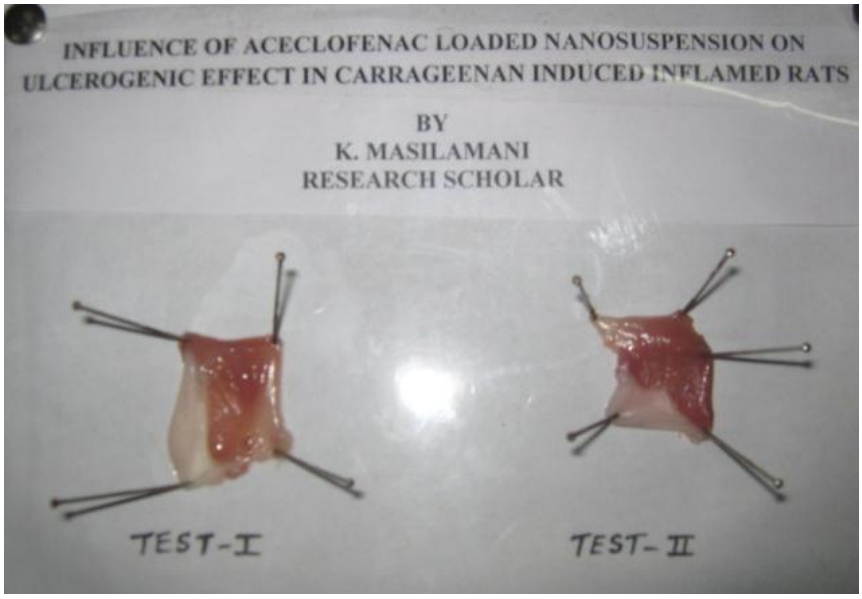


Fig. 11.5 Macroscopical view of stomach of the rats treated with Aceclofenac nanosuspension

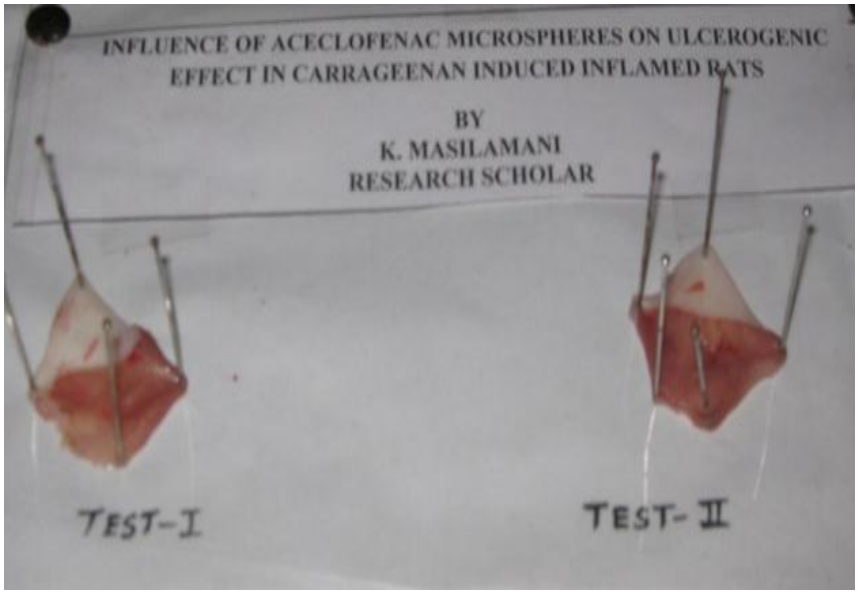


Fig. 11.6. Macroscopical view of stomach of the rats treated with Aceclofenac Microspheres

CONCLUSION:

Aceclofenac is an ideal drug for converting into the controlled release formulation and in the present investigation Aceclofenac loaded microspheres, nanosuspensions were successfully developed with desirable release profiles as per USP specifications and optimized the variables. From the above studies, the formulated controlled release dosage forms of aceclofenac showed an improved therapeutic effect and a better dosage form with decreased or no side effects (mainly the ulcerogenic potential) for the management of inflammation.

12. STABILITY STUDIES

OSMOTIC TABLETS

The osmotic tablets (OT10) had not shown any visible changes including colour at least for 90 days. The tablets were stored at 40^{o}C /RH75% for 90 days in stability test chamber. The formulation were subjected to various quality control test were performed at pre-determined intervals. The formulation had not indicated any significant changes in the parameters like Drug Content (%), Weight variation,Thickness (mm), Hardness (kg/cm2) and Friability (%).However, a slight alteration in the extent of release of aceclofenac was reduced by 2.74% after 90 days.

Table 12.1. Stability Studies of osmotic tablet containing Aceclofenac (OT-10) at Temp 40^{o}C /RH75%

Parameters	Days				
	15	30	45	60	90
Drug Content (%)	99.89 ±0.20	99.51 ±0.28	99.40 ±0.34	98.83 ±0.22	98.50 ±0.51
Weight variation	551.22 ± 0.25	551.22 ±0.35	551.22 ±0.42	551.22 ±0.58	551.22 ±0.65
Thickness (mm)	5.27 ± 0.29	5.27 ±0.17	5.27 ±0.31	5.27 ±0.25	5.27 ±0.20
Hardness (kg/cm^2)	7.5 ±0.37	7.5 ±0.20	7.5 ±0.38	7.5 ±0.22	7.3 ±0.41
Friability (%)	1.0 ± 0.54	1.04 ± 0.24	1.10 ± 0.15	1.05 ± 0.16	1.12 ± 0.31

Values expressed ±SD of triplicate trials

Table 12.2. Stability Studies of osmotic tablet containing Aceclofenac (OT-10) at Temp 40^{o}C /RH75%-Drug Release

S.No	Time (hrs)	Cumulative percentage release of aceclofenac(%)				
		15	30	45	60	90
1	0	0	0	0	0	0
2	2	12.23± 0.24	11.36± 0.15	11.16± 0.11	10.24± 0.28	09.12± 0.21
3	4	28.11± 0.08	27.13± 0.06	26.11± 0.24	22.69± 0.08	20.56± 0.12
4	6	46.22± 0.21	42.83± 0.86	41.14± 0.65	40.64± 0.24	40.22± 0.44
5	8	68.92± 0.17	66.32± 0.19	66.12± 0.22	63.45± 0.21	60.66± 0.13
6	12	76.57± 0.13	73.57± 0.23	71.19± 0.43	70.61± 0.29	69.15± 0.87
7	16	75.12± 0.15	86.68± 0.08	84.55± 0.10	82.74± 0.15	80.11± 0.21
8	20	92.14± 0.20	90.21± 0.47	89± 0.16	88.12± 0.32	85.28± 0.41
9	24	94.04± 0.26	93.3± 0.35	92.6± 0.65	91.61± 0.20	91.3± 0.12

Microspheres

The optimized formulation of M4 was subjected to stability studies by storing the samples at three variant temperature conditions, i.e 2-8, 25 and 40^{o}C. Samples were withdrawn at predetermined time intervals of 15, 30, 45, 60 and 90 days, and then evaluated for the particle size and drug content. The results show that there were no considerable changes in the parameters studied, however minor changes in the particle size and drug content at elevated temperature conditions. This suggests that the formulations were stable under norma conditions, the ideal storage of the microsphere formulation at temperatures is less than 25^{o}C.

Table 12.3. Stability Studies of Aceclofenac microspheres (M4)

Temp	Parameters	Days				
		15	30	45	60	90
2-8^{o}C	Particle Size (µm)	21±10	21±12	21±12	21±15	21±13
	Drug content (%)	65±0.2	65±0.5	65.±0.7	64.75±0.5	64.50±0.5
25^{o}C	Particle Size (µm)	21±10	21±22	22±12	22.5±19	23±25
	Drug content (%)	65 ±0.1	64.5±0.4	64.5±1.2	64.25±1.0	63.25±1.2
40^{o}C	Particle Size (µm)	21±8.0	21±22	22±10	23±17	24±20
	Drug content (%)	65±0.3	62±0.7	60.25±0.9	61±1.9	60.25±2.0

Values expressed ±SD of triplicate trials

Nanosuspension:

None of the NS formulations indicated any symptoms of agglomeration, sedimentation or colour change during the period of assessment. However the formulations were subjected to particle size analysis and drug content evaluation which are considered to be very critical parameters in deciding the stability of the nanosuspensions. The formulation F-14BYM stored at 2-8^{o}C retained the particle size (210nm) with minor variation (0.5%) until 90days.

Table 12.4. Stability Studies of Aceclofenac Nanosuspension (F-14BYM)

Temp	Parameters	Days				
		15	30	45	60	90
2-8^{o}C	Particle Size (nm)	210±18	210±22	210±22	210±25	210±25
	Drug Content (%)	82±0.2	82±0.2	81.50±0.4	81.50±0.5	81.50±0.7
25^{o}C	Particle Size (nm)	210±20	210±25	210±25	215±15	215±20
	Drug Content (%)	82 ±0.1	82±0.4	81.25±0.8	81.25±1.2	81.25±1.5
40^{o}C	Particle Size (nm)	210±18	210±22	212±15	218±15	218±20
	Drug Content (%)	82±0.7	82±0.9	81.25±1.0	81.25±1.6	80.25±2.0

Values expressed ±SD of triplicate trials

The formulations stored at 25^{o}C exhibited a negligible increase in particle size (5nm) with a minor decrement in the drug content (0.75%). A considerable increase in particle size (8nm) was recorded with parallel drug degradation (1.75%) with in the period of 90 days. The study suggests storage in cool condition for the NS F-14BYM, although the product is stable even above 25^{o}C. The use of steric stabilizer, tween 80 might have played a vital role in achieving the stability of this formulation.

13. SUMMARY AND CONCLUSION:

Oral drug delivery is the most common and frequently used system to deliver drugs and it is one of the most suitable, convenient, safe, economic and effective way to deliver the drug. Aceclofenac is a drug commonly used in the management of pain and inflammation in various conditions like post-traumatic, cervical and low back pain, ankylosing spondylitis, rheumatoid arthritis and osteoarthritis. This drug, on long term usage, specifically by oral route is reported to cause adverse effects including gastritis. So the situation demands development of safe and effective oral drug delivery system for aceclofenac.

Its short half-life (4- 4.3hrs), which demands frequent administration and severe gastrointestinal (GI) irritation and other side effects demands the development of a controlled release formulation. Thus the overall aim and objective of the present work was to:

- ✓ Enhance the overall therapeutic efficacy of aceclofenac by controlled release
- ✓ Minimize the adverse effects
- ✓ Reduce the overall dose and dosing frequency of aceclofenac
- ✓ Achieve improved patient compliance

To achieve the above goals oral controlled drug delivery systems like Osmotic tablets, Microspheres and Nanosuspensions of Aceclofenac were developed, optimized and evaluated *in vitro* and *in vivo.*

The preformulation studies were performed for the drug and excipients. The melting point, loss on drying, angle of repose, bulk density, tap density, Hausner's ratio compressibility index were calculated. The flow property studies indicated that the aceclofenac has poor flow properties.

Compatibility studies of IR and DSC studies were performed and these reports suggested no chemical interaction between aceclofenac and the polymers/excipients used for the development of various formulations.

Aceclofenac osmotic tablets were prepared by wet granulation method using different osmotic agents - sodium bicarbonate sodium chloride, and potassium chloride and their concentrations were optimized performing 13 trails (OT1-OT13). Cellulose acetate was used as semipermeable membrane and PEG 400 as pore forming agent. The precompressional parameters of aceclofenac granules like bulk density, tapped density, angle of repose, compressibility index, hausner ratio were evaluated. The results of pre compression parameters and post compression parameters were found to be within the limits and there was no significant change in the pre compression parameters and post compression parameters of all the formulations (OT1-OT13). The maximum drug content was obtained with the OT10 which was found to be 99.80 ± 0.58%. The invitro release studies carried out on the osmotic tablets indicated the effect of same amount of three different osmogens (sodium bicarbonate, sodium chloride and potassium chloride) and the combinations of these osmogens exhibits significant increase in rate and extent on drug release. All the prepared push pull osmotic tablets of Aceclofenac (OT9- OT13) had shown one hour delayed drug release, which may be attributed to time elapsed for imbibition of osmotic core with the release medium. After one hour, almost all the batches exhibited linear and controlled drug release profiles. Sodium chloride(25mg) and sodium bicarbonate(50mg) based push pull osmotic tablets of aceclofenac (OT10) exhibited little higher rate and extent of drug release than potassium chloride(25mg) and sodium bicarbonate(50mg) based tablets. The drug release also increased significantly with increase in the concentration of osmotic agent.

Three different medium simulating the GI pH were used to carry out the *in vitro* dissolution studies and the results indicated that the rate of release was moderately affected by pH. The invitro drug release results of formulations **(OT1- OT6)** were not found to be official limits for controlled release due to lesser concentration of osmogens. Further, the

formulations (OT7-OT13) showed profound increase in the dissolution profile due the increased concentration of osmogens in combination mode. The formulation **(OT10)** gave desired drug release profile of 94.3% in 24 hrs in which a combination of sodium chloride (25mg) and sodium bicarbonate (50mg) were used as osmogens in this formulation.

The microsphere formulations were made with varying proportion of the drug polymer ratios as well as varying concentrations of two polymers (polymer mix) Eudragit L 100 and PLGA. For this polymer mix of Eudragit L100: PLGA in the ratios of 1:1,1:2 and 1:3. The internal variation in the polymer mix were designated as a, b and c.

Aceclofenac loaded Eudragit:PLGA microspheres were prepared by using PVA (3%w/v) as stabilizer. Eudragit L100 and drug were dissolved in ethanol and PLGA was dissolved in acetone, respectively. Subsequently the Eudragit solution in ethanol was added slowly to the PLGA solution in acetone with a constant stirring and stirring is continued to remove the solvent. Then the microspheres were washed and resuspended in distilled water and lyophilized.

The prepared microspheres were characterized by determining the particle size, drug content, entrapment efficiency, invitro release and stability studies. The determination of the particle size was carried out by optical microscopy and there was an increase in particle size with increase in polymer concentration**.** The mean particle size of the formulation M4 was 20±10µm and M13was 630±36µm, indicated the lowest and the highest particle size. The entrapment efficiency of the microspheres were also found to be varying and was maximum of 86.6±0.6**%** with **M4** and minimum **25±1.1%** with **M3.** The morphology of the optimized formulation of microspheres was found to be discrete, smooth and spherical. Based on the particle size and entrapment efficiency the optimized formulations (M4, M5 and M13) were selected for further studies. The *in vitro* release profile of M4, M5 and M13 indicated controlled release of aceclofenac with maximum of 96.6±1.6, 88.6±1.8 and 76.2±1.9%, respectively. The formulation M4 was subjected for in vivo anti-inflammatory, anti-arthritic and gastro intestinal tolerability tests as it exhibited maximum release at 24 hrs along with other optimal characters. M4 was exhibited for stability studies. Samples

were withdrawn at predetermined time intervals of 15, 30, 45, 60 and 90 days, and then evaluated for the particle size and drug content.

Aceclofenac loaded Eudragit L100 nanosuspension was prepared by o/w emulsion method. The product and process parameters involved were optimized by the application of multifactorial design. The prepared NS were characterized by study of morphological characters, determination of particle size analysis, zeta potential, drug content, entrapment efficiency, invitro release and stability studies.

From the results of the optimization studies data, the formulations F-14BYM, F-15BYH, F-23CYM and F-24CYH were considered as ideal and selected for further studies. They have particle size of 210±15nm, 212±23nm, 225±30nm and 220±42nm and entrapment efficiency of 89.6±0.9%, 86.2±1.6%, 88.9±1.3% and 88.6±1.5%, respectively. The formulations with F-14BYM, F-15BYH, F-23CYM and F-24CYH were identified as the optimized formulations with respect to particle size, drug content and entrapment efficiency, and these were selected for the other studies. The SEM analysis was performed and the SEM photos of aceclofenac nanosupension were recorded. The particles of all the batches are almost spherical with smooth surface, however they shown variation in size. Increase in sonication time/agitation speed there is reduction in particle size. Entrapment efficiency increased with the increase in polymer concentration. It is well evident that the concentration of tween 80 played a significant role in achieving the particle size and entrapment efficiency. The increase in tween 80 concentration, from 0.01% to 0.02% there is an increase in entrapment efficiency and decrease in particle size. But the increase in the concentration of tween 80 from 0.02 to 0.03% the particle size increased and entrapment efficiency decreased. The zeta potential values were found to be -18.6±0.3mV, -18.3±0.4 mV, -16.3±0.4 mV and -16.4±0.3 mV for the formulations F-14BYM, F-15BYH, F-23CYM and F-24CYH respectively. These results indicate that the four batches of the nanosuspension had more stability on storage. Eudragit L100 is an anionic polymer and the hence the formulations indicated negative surface charge and the variation in the charge may be attributed by the presence of aceclofenac and tween 80. The drug content of formulations varied from minimum of 16±1.6% to maximum of 82±1.1%. F-1AXL

showed minimum of 16±1.6% and F-14BYM exhibited maximum of 82±1.1%. The optimization studies suggested that the increase in the ratio of the polymer indicated increase in the drug content, except in formulations F-8AZM, F-15BYH, F-18BZH, F-24CYH and F-27CZH. The entrapment efficiencies of formulations vary from minimum of 22.2±0.6% and maximum of 89.6±0.9%. F-1AXL showed minimum of 22.2±0.6% and F-14BYM exhibited maximum of 89.6±0.9%.

The increase in polymer proportion caused increase in the entrapment efficiency of the nanosuspensions, except in F-15BYH (86.2±1.6%), F-24CYH (88.6±1.5%), F-26CZM (74.3±1.3%), F-27CZH (72.6±1.3%). Based on the analysis of the data obtained from the above studies, four formulations, F-14BYM, F-15BYH, F-23CYM and F-24CYH with minimal particle size and appreciable drug content and entrapment efficiency were selected for further studies.

The *in vitro* release of the four formulations, F-14BYM, F-15BYH, F-23CYM and F-24CYH were 98.7±1.2, 94.1±1.4, 85.7±2.0, and 88.5±2.2% at the end of 24 hrs, respectively. F-15BYH released 8.3±1.2% of drug loaded in 1hr. F-23CYM and F-24CYH releases 4.5±1% and 8.3±0.4% of drug release in 1hr. Hence it suggests that the release profile of F-14BYM is ideal to consider the formulation for performing the in vivo studies.

None of the NS formulations indicated any symptoms of agglomeration, sedimentation or colour change during the period of assessment. However the formulations were subjected to particle size analysis and drug content evaluation which are considered to be very critical parameters in deciding the stability of the nanosuspensions. The formulation F-14BYM stored at 2-8^{o}C retained the particle size (210nm) with minor variation (0.5%) until 90days.

The formulations stored at 25^{o}C exhibited a negligible increase in particle size (5nm) with a minor decrement in the drug content (0.75%). A considerable increase in particle size (8nm) was recorded with parallel drug degradation (1.75%) with in the period of 90 days. The study suggests storage in cool condition for the NS F-14BYM, although the product is stable even above 25^{o}C.

The selected formulations of microsphere (M4) and nanosuspensions (NS F-14BYM) were subjected for the evaluation of in vivo anti-inflammatory activity in rats. The paw edema induced by the injection of Carrageenan is the commonly used experimental model for acute inflammation. Administrations of carrageenan to normal animals showed significant ($P< 0.05$) increase in paw inflammation from 1hr to 5 hrs when compared to control. Paw edema in rats reached its peak at 4 hrs after carrageenan administration. Per oral treatment of nanosuspension of aceclofenac showed higher significant ($P<0.01$) reduction paw volume in rats from 1 hrs and maintained its effect throughout the study period. In addition, microspheres (M4) also produced a significant inhibition ($P<0.05$) of paw volume in rats with carrageenan administration. Among the two different drug formulations, nanosuspension (NS F-14BYM) treated inflammatory rats exhibited high anti-inflammatory response than the microspheres. No ulcers were observed in nanosuspension treated arthritic rats, even if in some rats treated with reference and microspheres and nanosuspension of aceclofenac thin inflammatory reactions of the wall and hemorrhagic effusions were present after administration of the highest dose. From the above studies, the formulated controlled release dosage forms of aceclofenac showed an improved therapeutic effect and a better dosage form with decreased side effects (mainly the ulcerogenic potential) for the management of inflammation.

CONCLUSION:

The present work was aimed at the development of controlled drug delivery of aceclofenac for oral route. Three dosage forms were developed.

- Osmotic controlled tablets
- Microspheres
- Nanosuspension

The osmotic tablets (OT10) contain sodium bicarbonate and sodium chloride s osmogen indicated ideal controlled release of aceclofenac. It released 94.3 ± 0.2% of the drug at the pH 7.4. This formulation do not cause any gastric irritation because, the release of drug on the acidic pH is very low. The therapeutic efficacy will be improved due to the controlled release for 24 hrs.

The microspheres containing aceclofenac M4 indicated excellent flow properties when compared with pure aceclofenac. Thus the micronized particles (20±10μm) and higher entrapment efficiency (86.6±0.6%) were achieved. The formulation was stable atleast for 90 days and indicated controlled release for 24 hrs. The formulation exhibited better score in ulcerative index in the in vivo studies carried out in rats.

The nanosuspension containing aceclofenac exhibited most of the ideal characters required for an oral controlled release dosage forms. The colloid particles (F-14BYM) of lower particle size (210± 15 nm) aided with negatively charged surface charge (-18.6±0.3 mV) has been achieved. The release profile indicated an intial burst release for achieving the initial loading dose followed by continuous release up to 24 hr. The in vivo animal studies indicated

- Improved anti-inflammatory and arthritic activity
- No sign of ulcer was recorded in nanosuspension treated rats.

Although controlled release was achieved with the osmotic tablets and microspheres, nanosuspensions exhibited the ideal characters including in vitro and in vivo studies. The animal studies revealed that GI irritation of aceclofenac has been eliminated. The selected formulations of the three drug delivery systems were found to be stable. Hence it can be concluded that the newly developed oral controlled drug delivery systems of aceclofenac is considered to be ideal and effective in the management of inflammation. The research work demonstrated the capability of the drug delivery systems- osmotic controlled tablets, microspheres and nanosuspension of aceclofenac, whereas the dosage forms can serve as an effective delivery system for other drugs with similar characters.

14. BIBLIOGRAPHY

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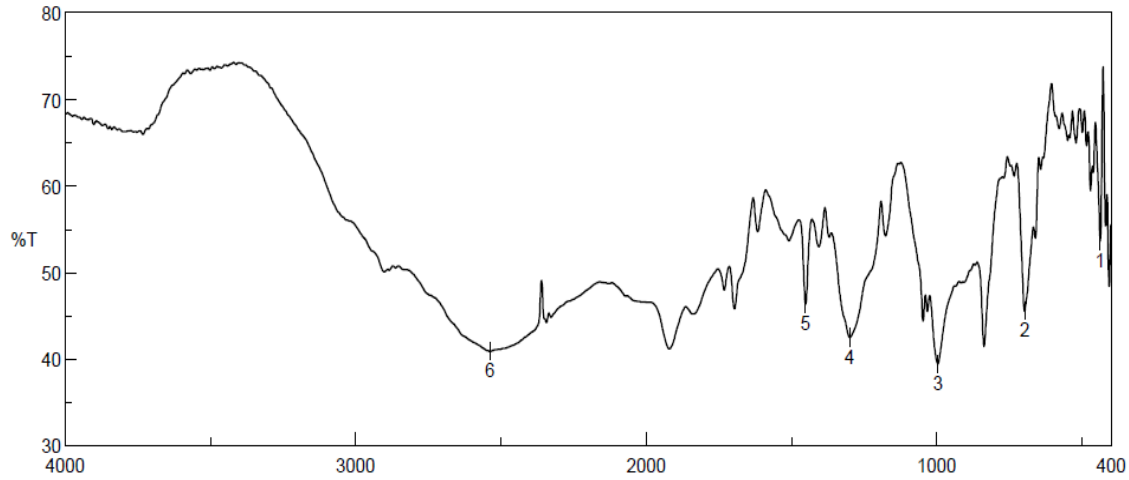
Fig. 7.2. IR spectra of Aceclofenac

Results of Peak Find

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	406.907	61.5183	2	479.224	50.2817	3	539.007	51.0536
4	626.752	49.4448	5	665.321	52.0208	6	750.174	37.9566
7	855.275	47.3555	8	898.666	45.5035	9	965.198	49.6337
10	1055.84	45.6511	11	1153.22	39.9319	12	1255.43	35.727
13	1344.14	49.9785	14	1452.14	36.1321	15	1507.1	41.2701
16	1718.26	28.8902	17	1771.3	38.9576	18	2937.06	61.127
19	3318.89	47.3747						

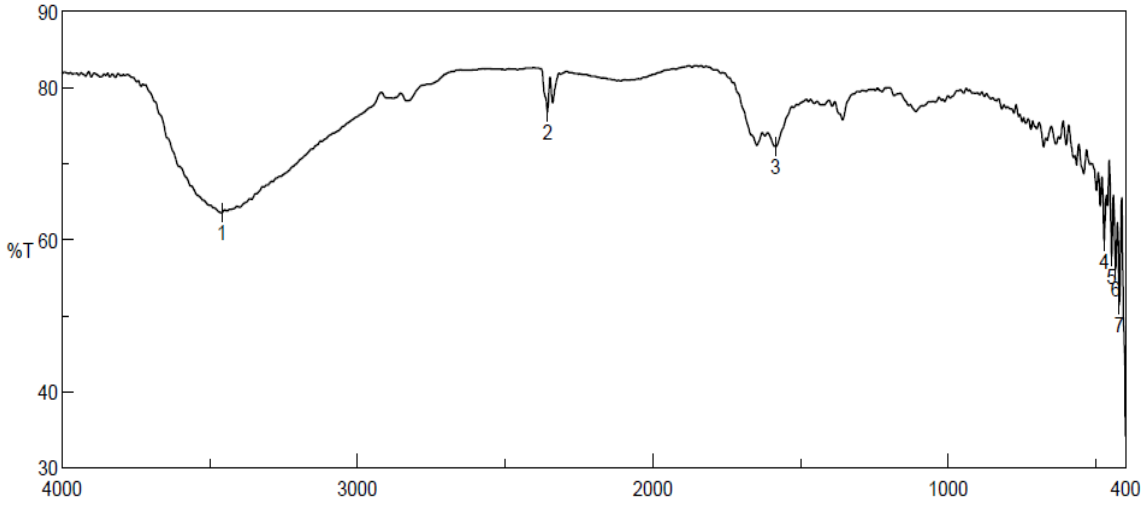
Fig. 7.3. IR spectra of Sodium bicarbonate



Results of Peak Find

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	436.798	53.5662	2	697.141	45.4927	3	996.053	39.3907
4	1298.82	42.45	5	1451.17	46.2865	6	2536.9	40.8427

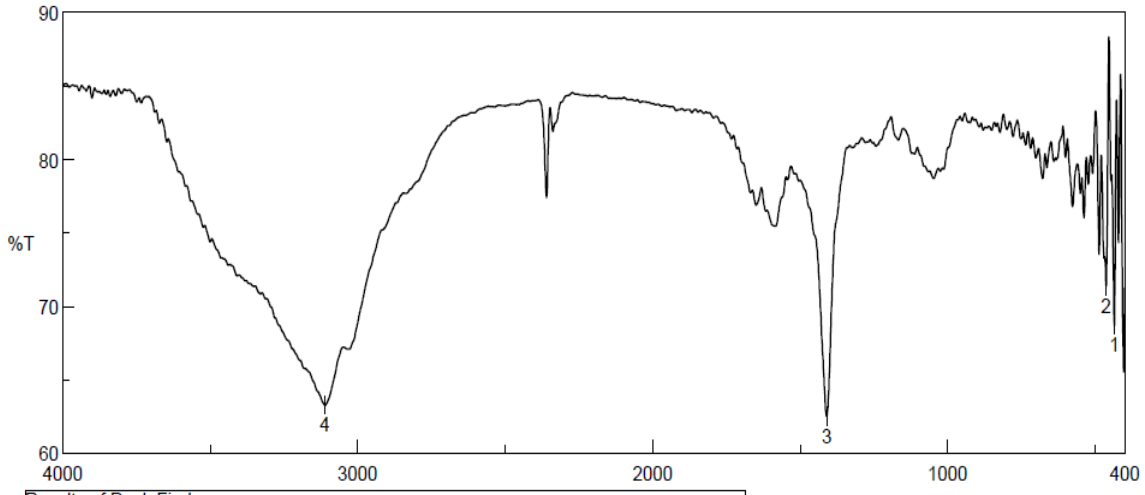
Fig. 7.4. IR spectra of Sodium chloride



Results of Peak Find

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3459.67	63.5175	2	2356.59	76.7753	3	1585.2	72.2312
4	471.51	59.8232	5	446.44	57.7864	6	432.941	56.1165
7	420.406	51.4038						

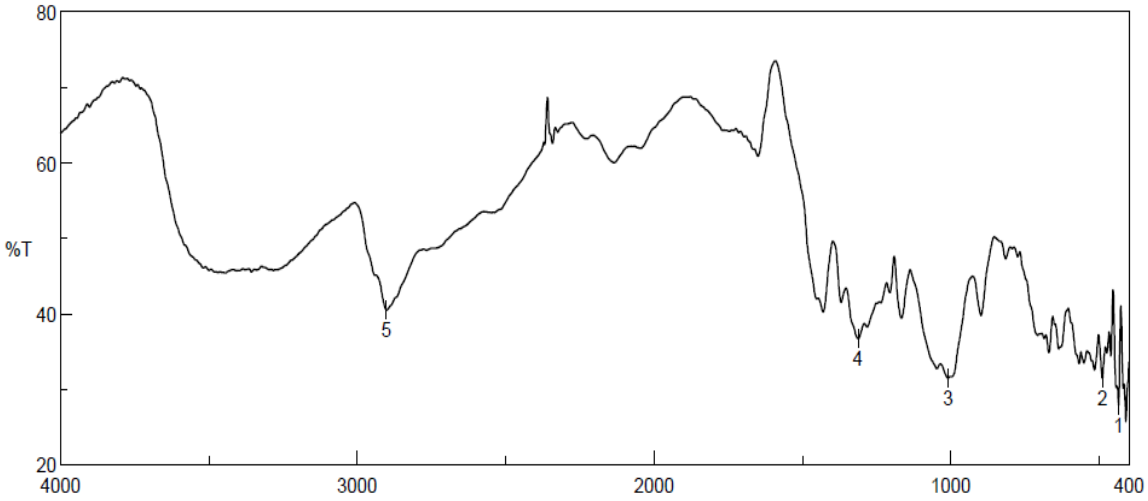
Fig. 7.5. IR spectra of Potassium chloride



Results of Peak Find

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	434.869	68.6634	2	462.832	71.3781	3	1409.71	62.4895
4	3110.62	63.2514						

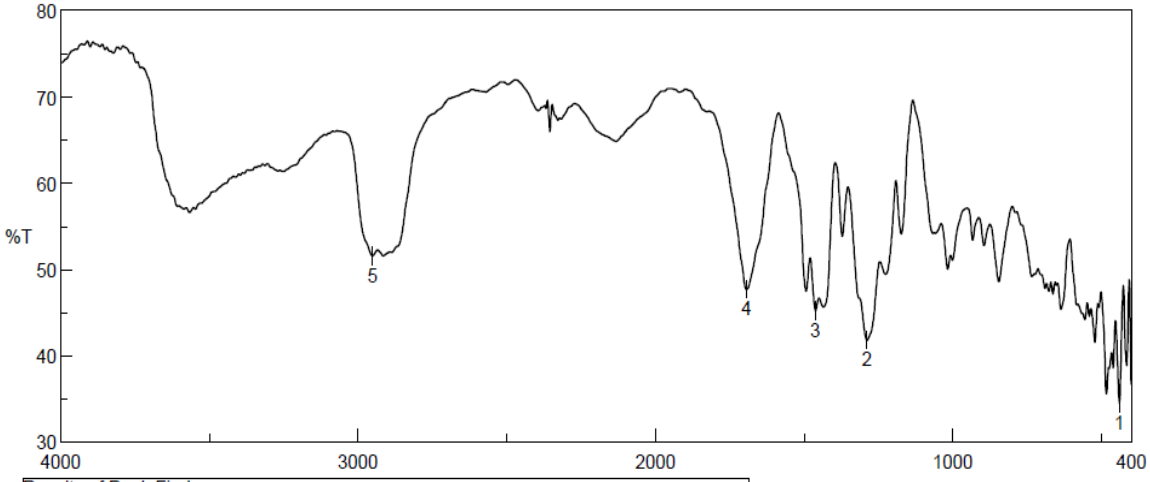
Fig. 7.6. IR spectra of MCC (microcrystalline cellulose)



Results of Peak Find

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	434.869	27.8443	2	489.831	31.4158	3	1009.55	31.4295
4	1312.32	36.675	5	2901.38	40.4419			

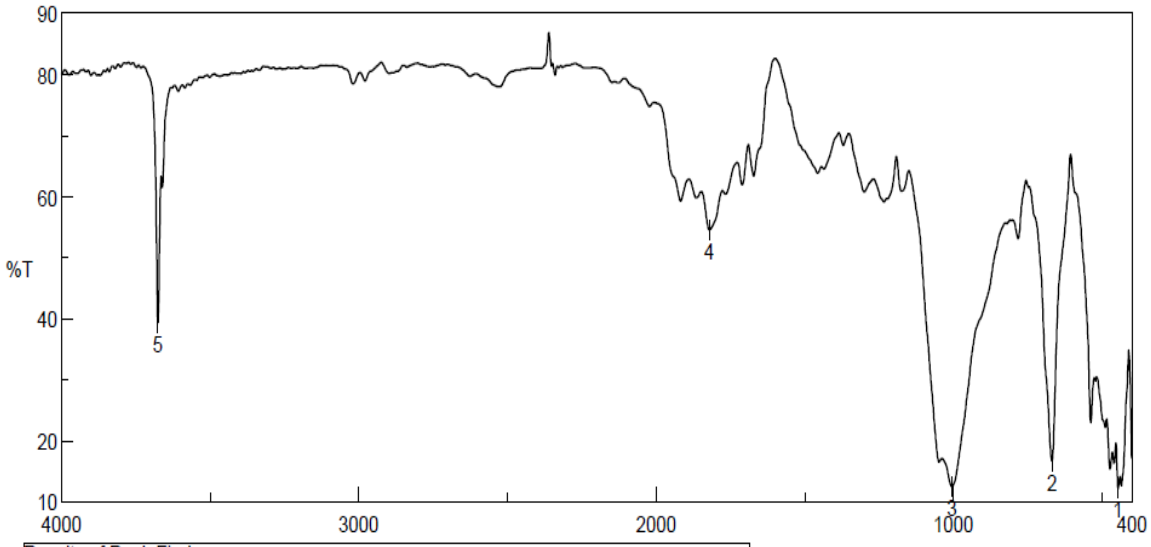
Fig. 7.7. IR spectra of PVP K30



Results of Peak Find

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	439.69	34.4481	2	1288.22	41.7435	3	1461.78	45.1716
4	1694.16	47.6379	5	2951.52	51.5212			

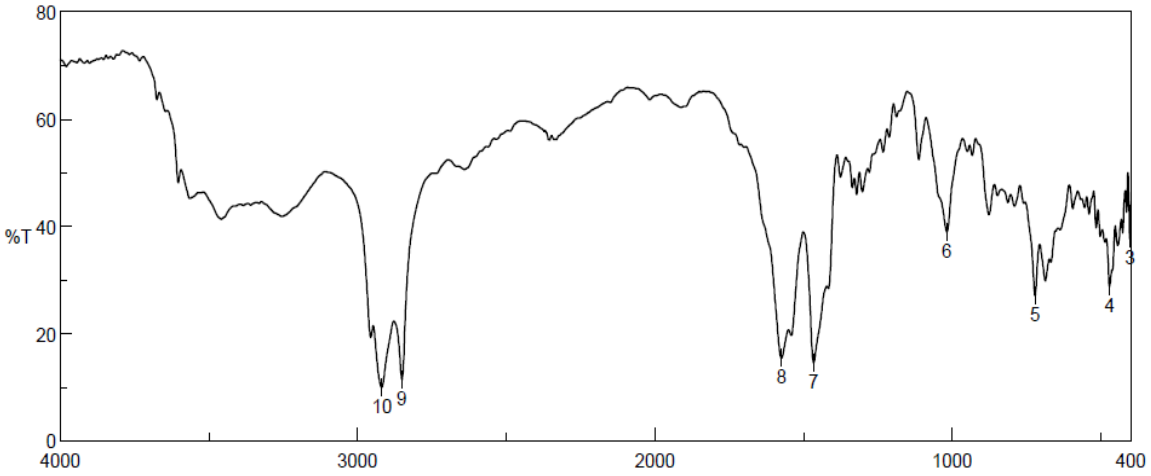
Fig. 7.8. IR spectra of Talc



Results of Peak Find

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	446.44	12.1754	2	669.178	16.6611	3	1004.73	12.4285
4	1820.47	54.523	5	3675.66	39.2987			

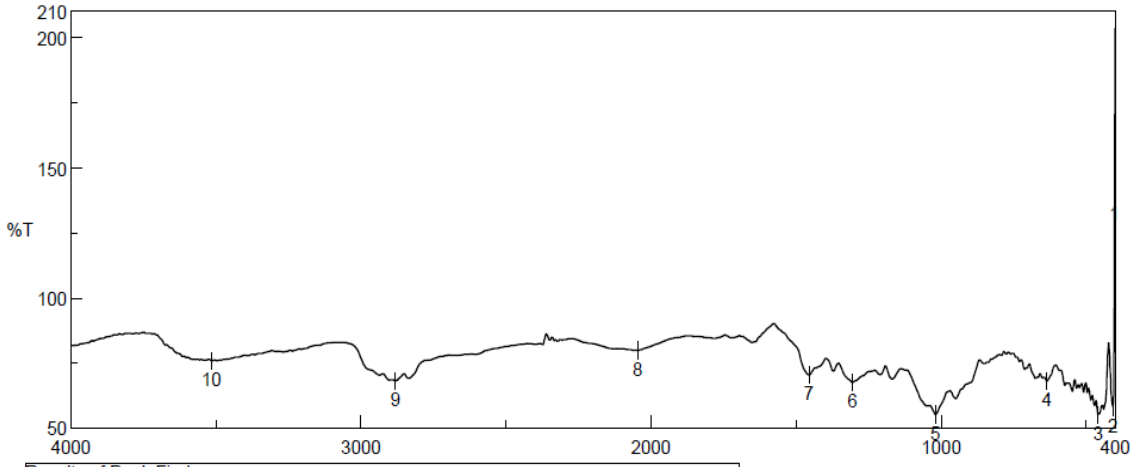
Fig. 7.9. IR spectra of Magnesium stearate



Results of Peak Find

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	369.301	29.3396	2	380.872	22.5307	3	402.085	37.6601
4	470.546	28.7643	5	721.247	27.0554	6	1018.23	38.9795
7	1466.6	14.554	8	1574.59	15.3723	9	2851.24	11.4507
10	2918.73	9.88513	11	4324.59	56.5945			

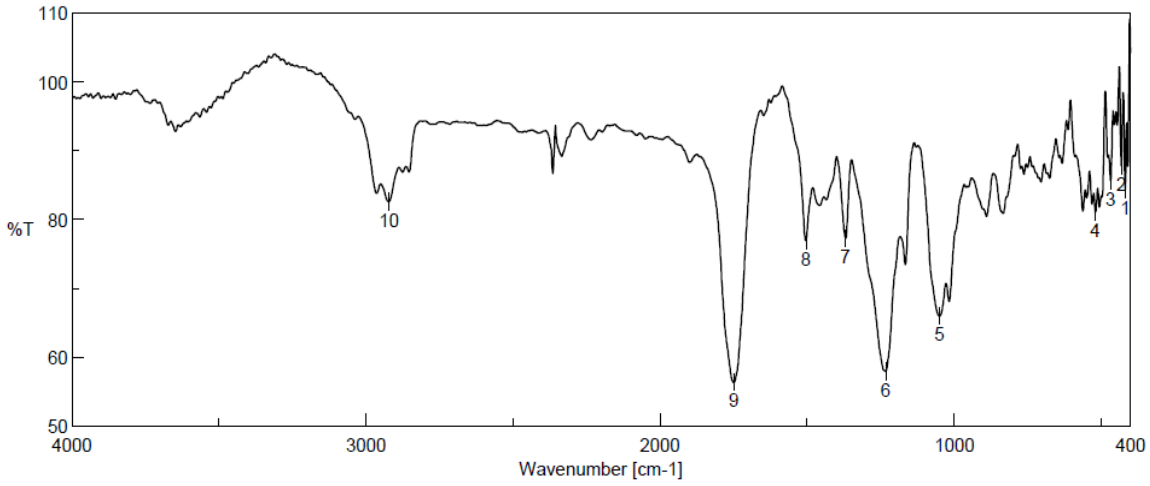
Fig. 7.10. IR spectra of HPMC K4M



Results of Peak Find

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	401.121	139.682	2	406.907	58.0292	3	458.975	55.0486
4	634.466	68.0211	5	1018.23	55.1518	6	1305.57	67.4228
7	1455.03	70.2968	8	2046.1	79.6881	9	2881.13	68.0132
10	3514.63	75.7477						

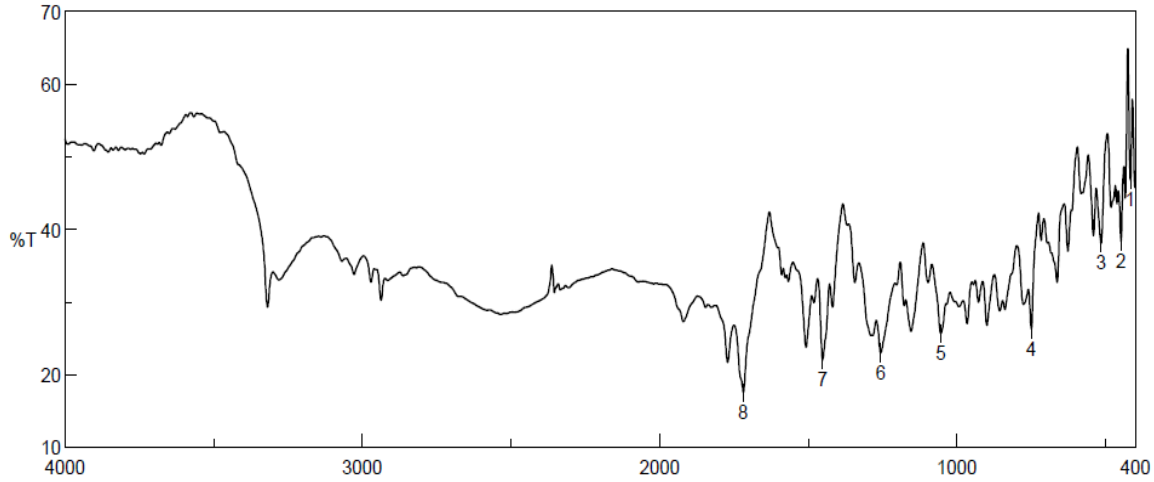
Fig. 7.11. IR spectra of Cellulose acetate



Results of Peak Find

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	417.513	84.3801	2	431.012	87.7707	3	467.653	85.6051
4	519.722	80.9991	5	1050.05	65.9519	6	1233.25	57.93
7	1369.21	77.2382	8	1504.2	76.9172	9	1749.12	56.2934
10	2922.59	82.5561						

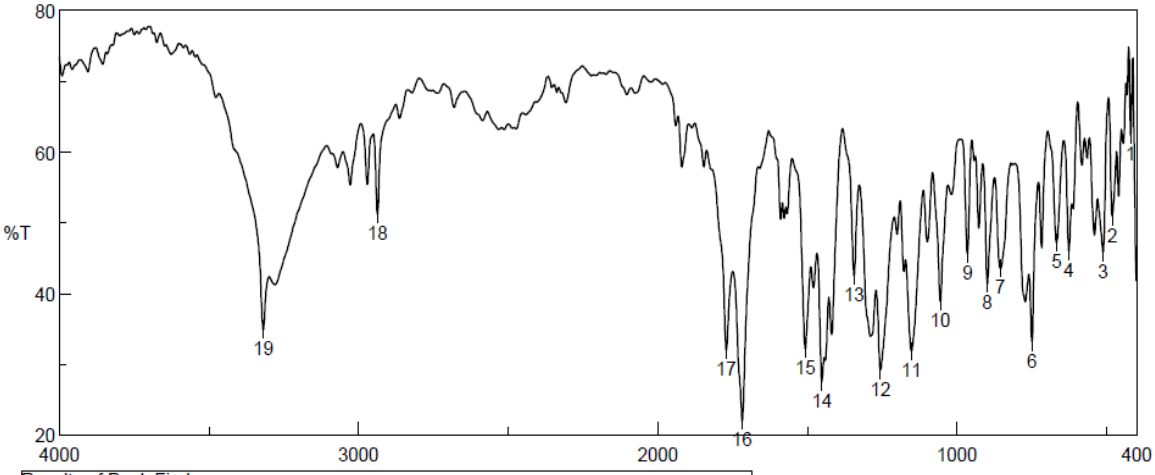
Fig. 7.12. IR spectra of Aceclofenac + sodium bicarbonate



Results of Peak Find

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	416.549	46.83	2	447.404	38.3079	3	514.901	38.0358
4	749.209	26.2432	5	1053.91	25.6282	6	1256.4	22.9348
7	1452.14	21.9466	8	1718.26	17.5162			

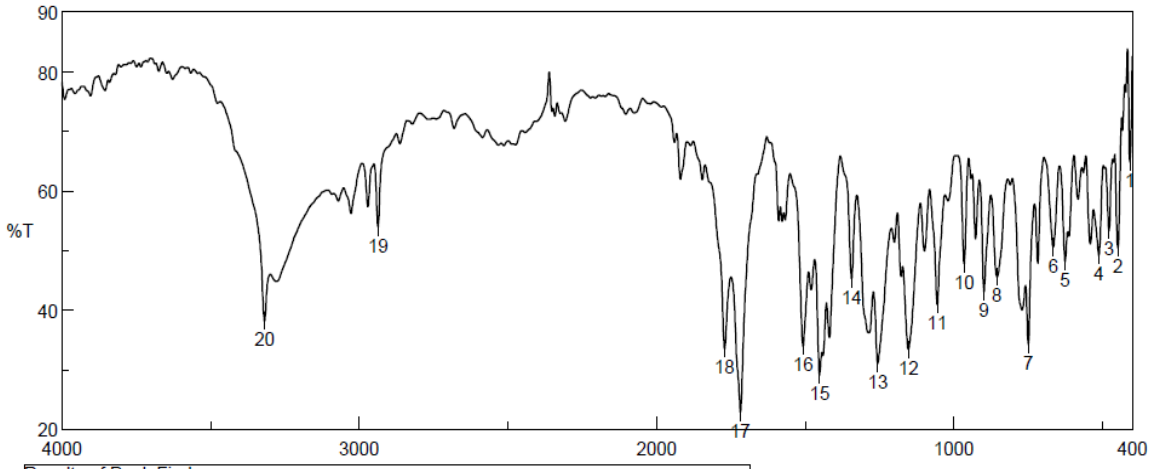
Fig. 7.13. IR spectra of Aceclofenac + Sodium chloride



Results of Peak Find

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	419.442	62.413	2	481.153	50.9191	3	512.008	45.8447
4	625.788	45.9736	5	667.25	47.2133	6	749.209	33.1281
7	855.275	43.5527	8	898.666	41.3071	9	965.198	45.5996
10	1055.84	38.849	11	1152.26	31.8549	12	1256.4	29.1509
13	1344.14	42.5541	14	1452.14	27.4769	15	1507.1	32.1755
16	1718.26	21.9825	17	1771.3	31.988	18	2937.06	51.1884
19	3318.89	34.8444						

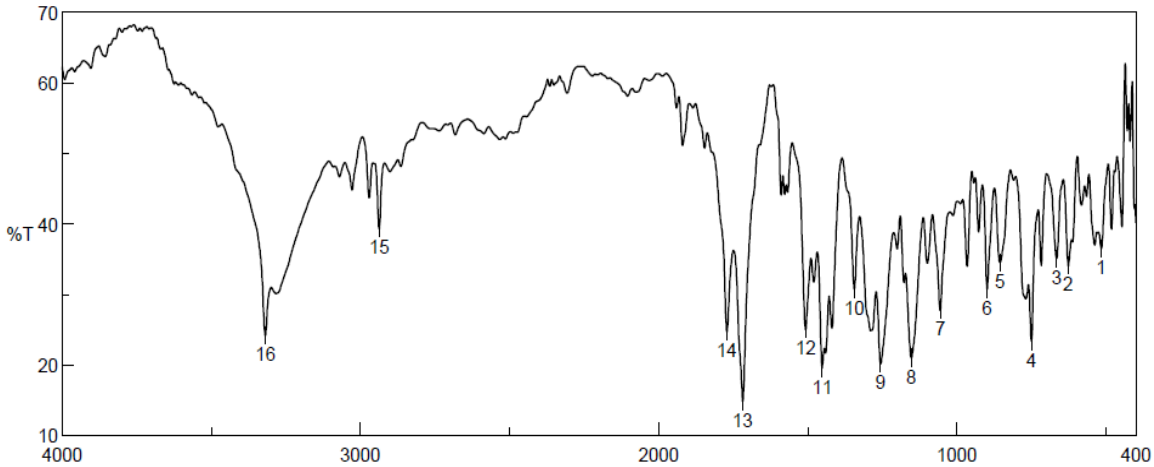
Fig. 7.14. IR spectra of Aceclofenac + Potassium chloride



Results of Peak Find

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	407.871	64.7789	2	448.369	50.3743	3	478.26	53.3135
4	512.008	49.2253	5	625.788	48.1098	6	665.321	50.5465
7	750.174	34.1735	8	855.275	45.6066	9	898.666	43.0269
10	965.198	47.7845	11	1055.84	40.9376	12	1153.22	33.3935
13	1256.4	31.0234	14	1344.14	45.3073	15	1452.14	29.0837
16	1507.1	33.9156	17	1718.26	22.8784	18	1771.3	33.4856
19	2937.06	53.9778	20	3318.89	38.1256			

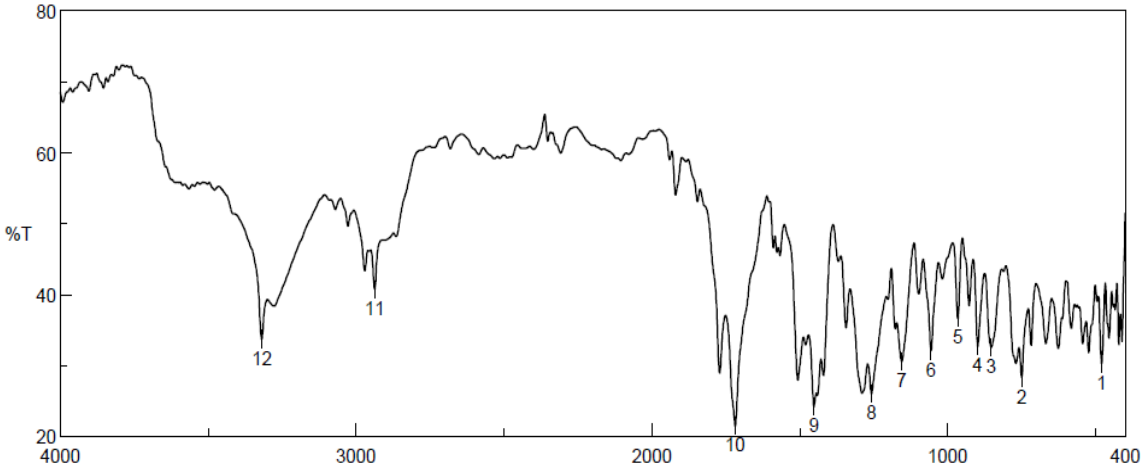
Fig. 7.15. IR spectra of Aceclofenac + MCC (microcrystalline cellulose)



Results of Peak Find

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	515.865	36.4886	2	626.752	34.0614	3	665.321	35.0471
4	750.174	23.2879	5	855.275	34.5015	6	898.666	30.7583
7	1055.84	27.739	8	1152.26	20.9659	9	1255.43	20.0749
10	1344.14	30.7136	11	1452.14	19.5336	12	1507.1	25.0158
13	1718.26	14.7956	14	1771.3	24.7152	15	2937.06	39.2732
16	3318.89	24.1407						

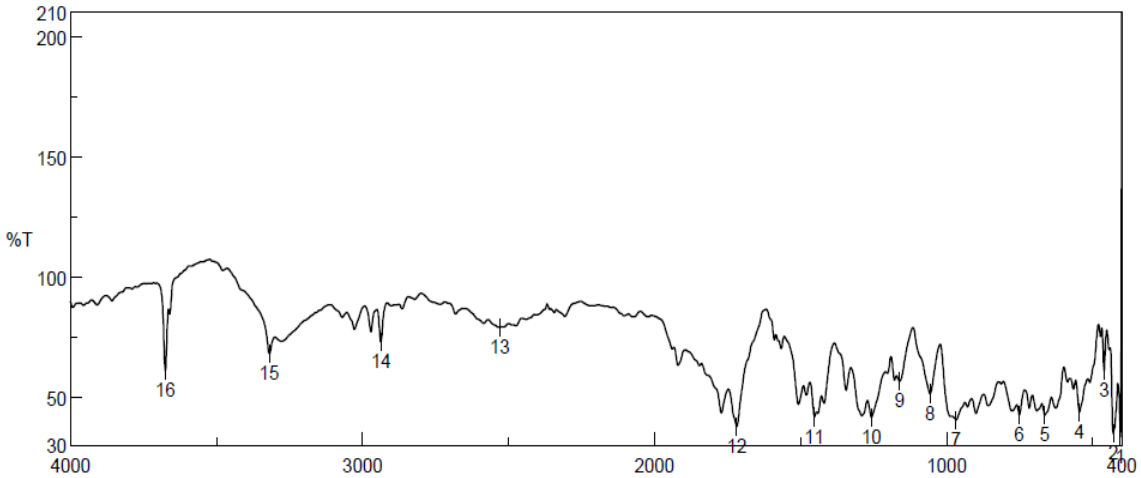
Fig. 7.16. IR spectra of Aceclofenac + PVP K30



Results of Peak Find

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	479.224	30.2524	2	750.174	28.2694	3	852.382	32.5068
4	898.666	32.654	5	966.162	36.588	6	1056.8	32.0578
7	1155.15	30.5056	8	1257.36	25.9702	9	1452.14	24.1436
10	1718.26	21.3669	11	2937.06	40.7489	12	3318.89	33.6863

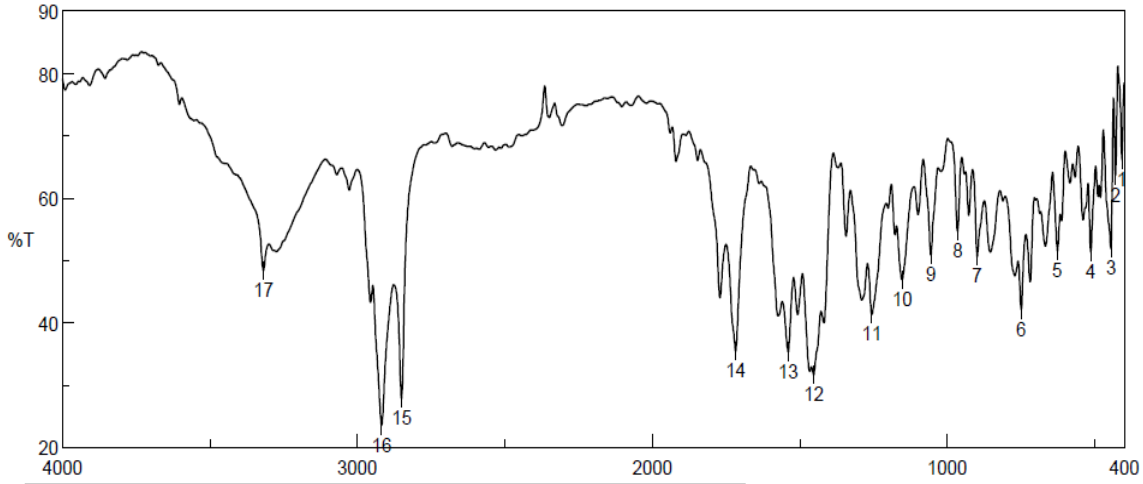
Fig. 7.17. IR spectra of Aceclofenac + Talc



Results of Peak Find

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	404.014	33.4657	2	429.084	34.7945	3	459.939	60.8013
4	544.792	43.6931	5	663.393	42.487	6	750.174	42.5766
7	967.126	40.587	8	1055.84	51.4916	9	1159.97	56.7467
10	1256.4	41.6437	11	1452.14	41.7486	12	1718.26	37.8287
13	2531.11	78.9432	14	2937.06	72.9756	15	3318.89	68.0309
16	3675.66	61.0967						

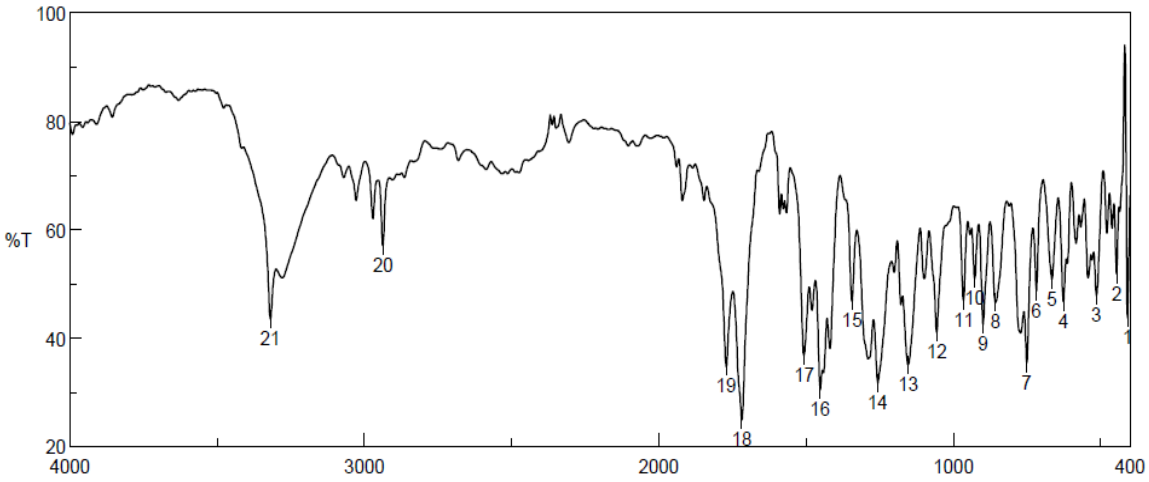
Fig. 7.18. IR spectra of Aceclofenac + Magnesium stearate



Results of Peak Find

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	407.871	66.1416	2	430.048	63.6613	3	445.476	51.8506
4	512.972	51.3108	5	626.752	51.5369	6	749.209	42.0717
7	898.666	50.6465	8	965.198	54.6586	9	1055.84	50.8306
10	1153.22	46.8805	11	1255.43	41.2804	12	1453.1	31.6107
13	1539.88	35.3577	14	1717.3	35.4516	15	2850.27	27.7907
16	2917.77	23.5244	17	3318.89	48.383			

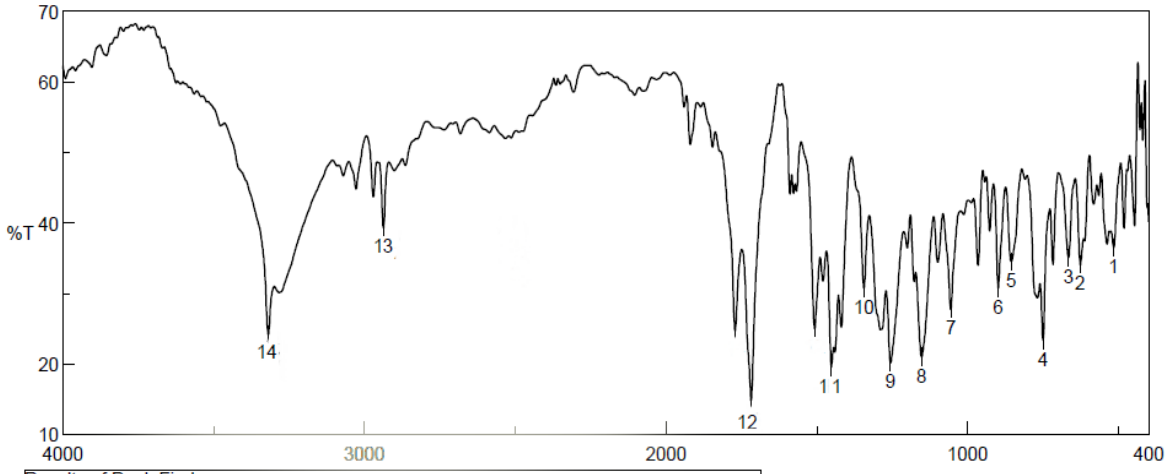
Fig. 7.19. IR spectra of Aceclofenac + HPMC K4M



Results of Peak Find

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	407.871	43.6928	2	445.476	51.821	3	513.936	47.8035
4	625.788	46.7093	5	665.321	50.806	6	717.39	48.6388
7	750.174	35.2927	8	856.239	46.5563	9	898.666	42.5227
10	927.593	50.9213	11	965.198	46.8168	12	1055.84	41.0014
13	1153.22	35.0179	14	1256.4	31.6736	15	1344.14	46.7596
16	1452.14	30.3779	17	1507.1	36.7776	18	1718.26	24.8524
19	1771.3	34.7126	20	2937.06	57.0008	21	3318.89	43.4908

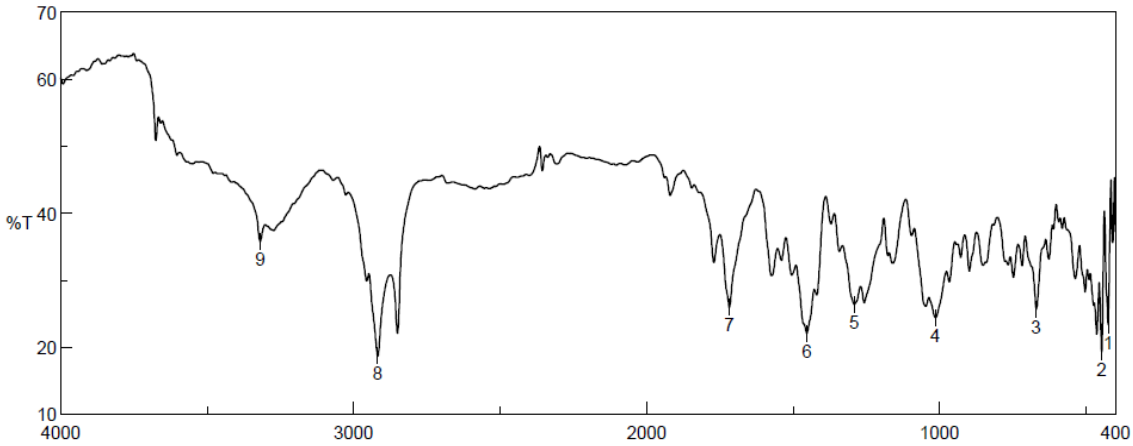
Fig. 7.20. IR spectra of Aceclofenac + Cellulose acetate



Results of Peak Find

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	515.865	36.4886	2	626.752	34.0614	3	665.321	35.0471
4	750.174	23.2879	5	855.275	34.5015	6	898.666	30.7583
7	1055.84	27.739	8	1152.26	20.9659	9	1255.43	20.0749
10	1344.14	30.7136	11	1452.14	19.5336	12	1507.1	25.0158
13	2937.06	39.2732	14	3318.89	24.1407			

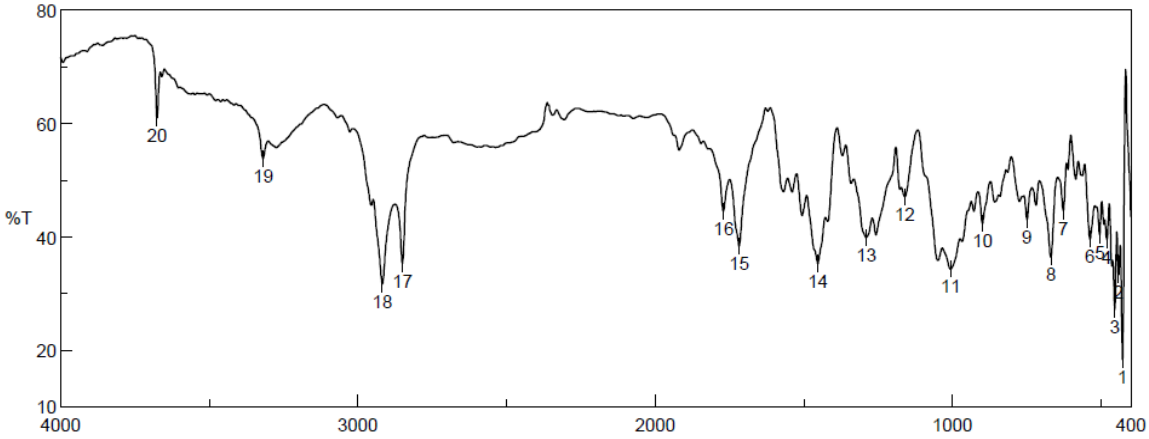
Fig. 7.21. IR spectra of Aceclofenac + sodium bicarbonate + MCC + PVPK30 +talc + Magnesium stearate + HPMC K4



Results of Peak Find

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	424.263	23.278	2	446.44	19.2616	3	670.142	25.6384
4	1013.41	24.3832	5	1291.11	26.3722	6	1453.1	22.0261
7	1717.3	25.9251	8	2917.77	18.6096	9	3318.89	35.7619

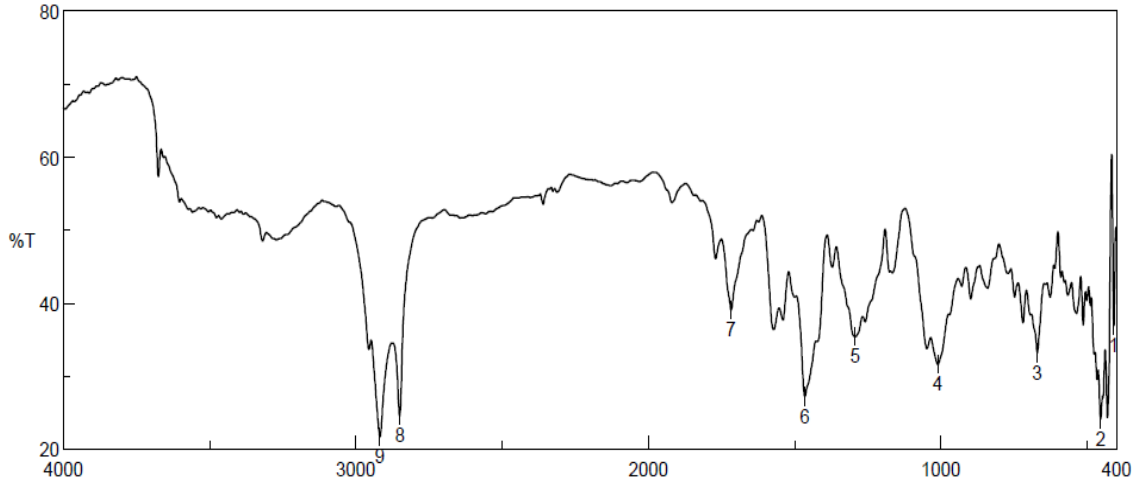
Fig. 7.22. IR spectra of Aceclofenac + sodium bicarbonate +sodium chloride+ MCC + PVPK30 + talc + Magnesium stearate + HPMC K4



Results of Peak Find

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	428.12	18.2599	2	441.619	33.2797	3	454.154	27.1554
4	480.188	39.4486	5	505.258	40.324	6	537.078	39.5073
7	626.752	44.5545	8	669.178	36.3865	9	749.209	43.0416
10	899.63	42.3316	11	1006.66	34.2751	12	1159.97	47.0778
13	1290.14	39.8699	14	1453.1	35.2485	15	1717.3	38.3596
16	1770.33	44.5273	17	2850.27	35.3013	18	2917.77	31.6452
19	3318.89	53.8279	20	3675.66	61.03			

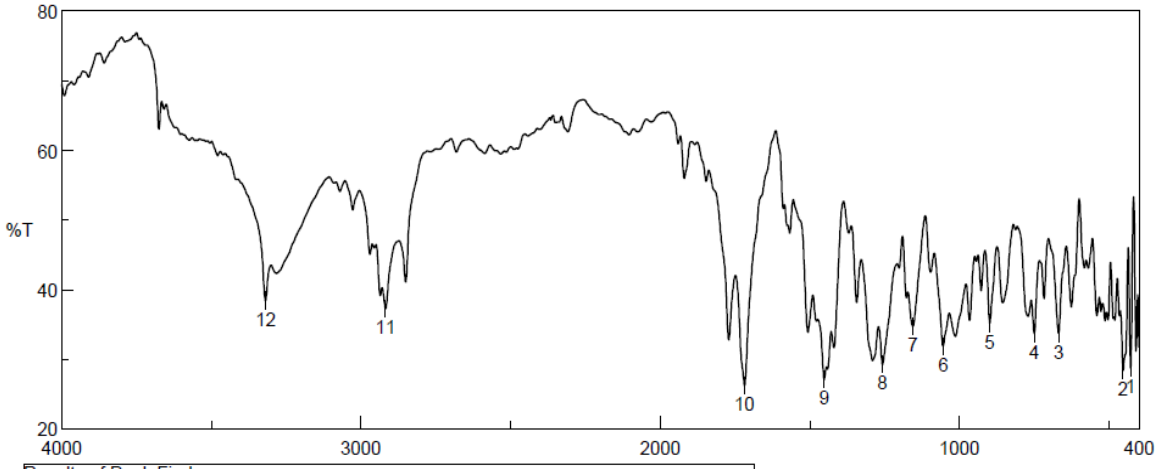
Fig. 7.23. IR spectra of Aceclofenac + sodium bicarbonate +potassium chloride+ MCC + PVPK30 +talc + Magnesium stearate + HPMC K4



Results of Peak Find

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	408.835	36.9032	2	453.19	24.0589	3	670.142	33.172
4	1010.52	31.571	5	1293.04	35.3404	6	1465.63	27.178
7	1717.3	39.0361	8	2850.27	24.4858	9	2917.77	21.6513

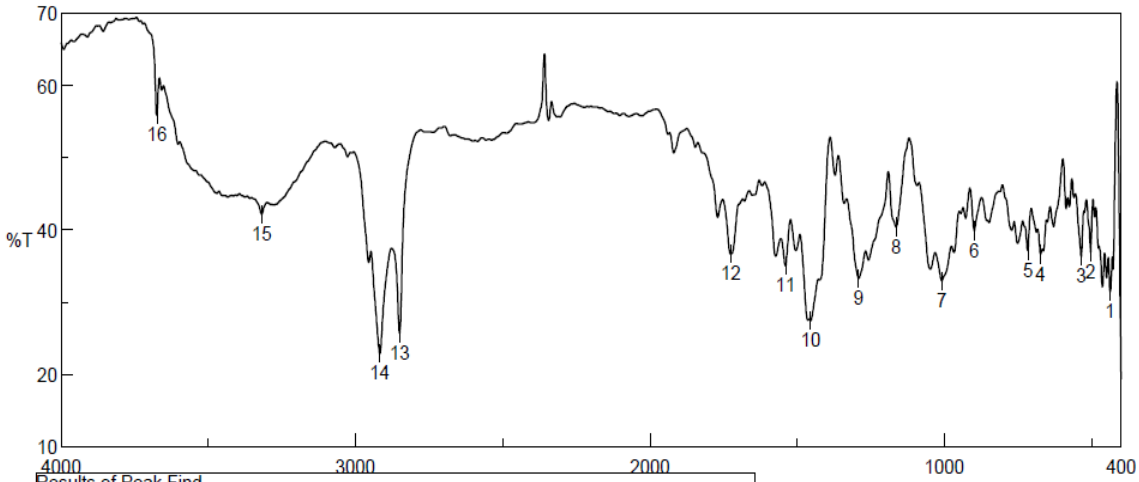
Fig. 7.24. IR spectra of Aceclofenac + sodium chloride +potassium chloride+ MCC + PVPK30 + talc + Magnesium stearate + HPMC K4



Results of Peak Find

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	428.12	28.7016	2	453.19	28.3207	3	669.178	33.7006
4	750.174	33.6499	5	898.666	35.1763	6	1054.87	31.8186
7	1156.12	34.6727	8	1257.36	29.225	9	1452.14	27.0554
10	1718.26	26.1589	11	2917.77	37.2279	12	3318.89	38.3778

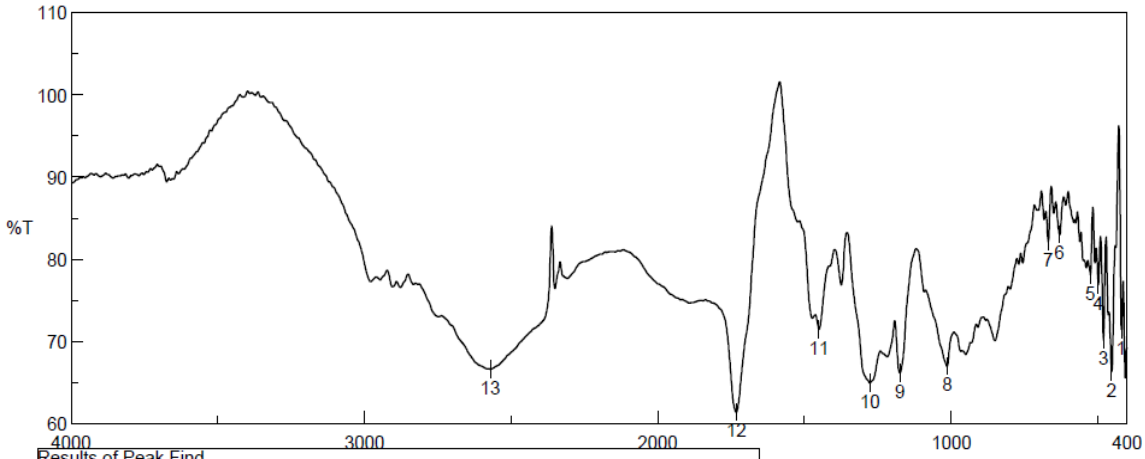
Fig. 7.25. IR spectra of Aceclofenac + sodium bicarbonate + Sodium chloride + Potassium chloride +MCC (microcrystalline cellulose) + PVPK30 +talc+Magnesium stearate + HPMC K4 + Cellulose acetate



Results of Peak Find

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	437.762	31.3822	2	504.294	36.8839	3	536.114	36.3186
4	673.999	36.5657	5	717.39	37.1178	6	898.666	39.8629
7	1009.55	32.9462	8	1164.79	40.3764	9	1291.11	33.2304
10	1454.06	27.4155	11	1539.88	35.0011	12	1725.98	36.6128
13	2850.27	25.6645	14	2917.77	22.8405	15	3318.89	42.146
16	3674.69	55.8173						

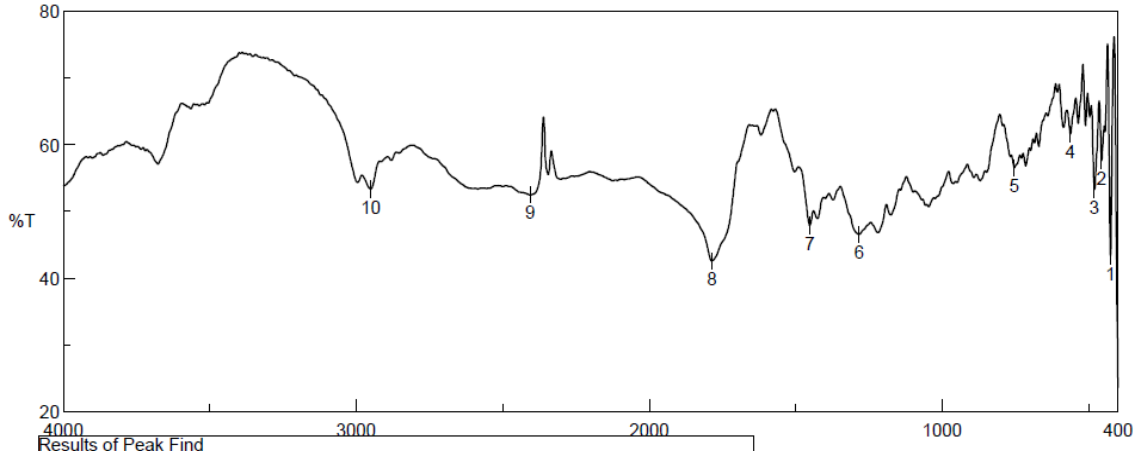
Fig. 7.26. IR spectra of Eudragit L100



Results of Peak Find

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	419.442	71.3932	2	452.225	66.2807	3	480.188	70.1027
4	497.544	76.8346	5	524.543	78.0752	6	629.644	82.9012
7	669.178	82.1192	8	1013.41	66.9333	9	1174.44	66.1297
10	1276.65	64.9054	11	1450.21	71.4265	12	1732.73	61.334
13	2571.61	66.5939						

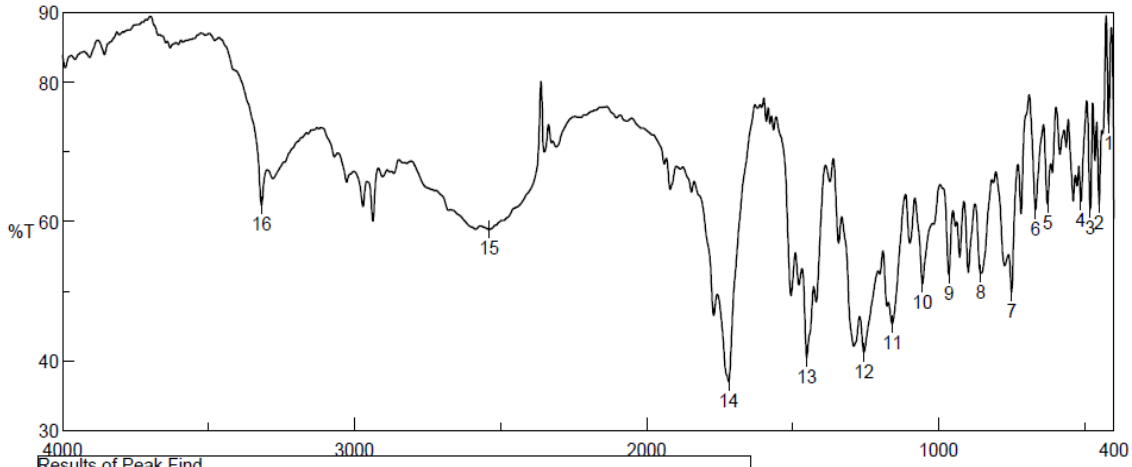
Fig. 7.27. IR spectra of PLGA



Results of Peak Find

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	425.227	43.2957	2	456.082	57.5646	3	480.188	53.2194
4	562.148	61.5218	5	754.031	56.5067	6	1284.36	46.5103
7	1452.14	47.8445	8	1786.72	42.5446	9	2405.76	52.4175
10	2951.52	53.3174						

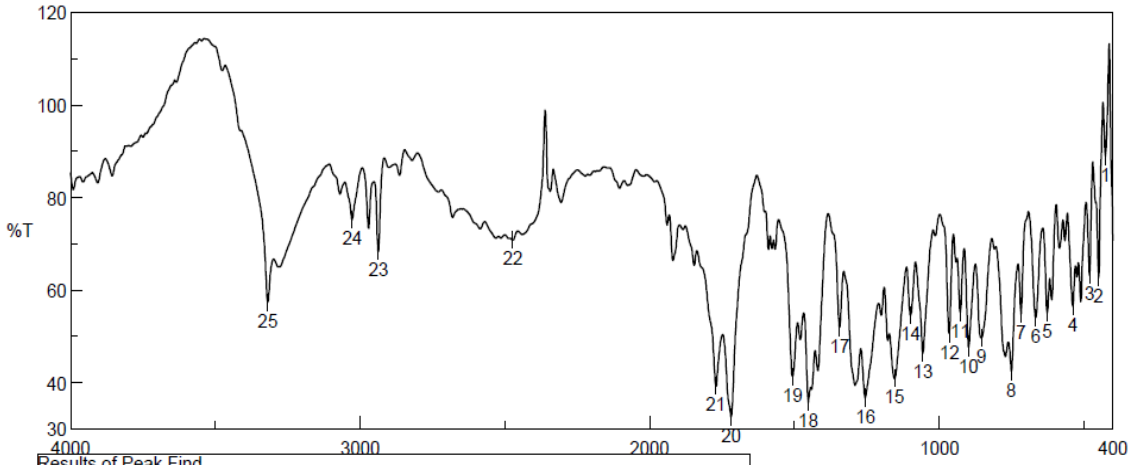
Fig. 7.28. IR spectra of Aceclofenac + Eudragit L100



Results of Peak Find

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	418.477	73.8467	2	450.297	62.4492	3	480.188	61.6663
4	512.972	62.8761	5	627.716	62.4215	6	668.214	61.6007
7	750.174	49.8675	8	856.239	52.5532	9	965.198	52.3541
10	1055.84	51.0325	11	1159.01	45.2611	12	1256.4	41.1958
13	1452.14	40.4522	14	1719.23	36.9576	15	2539.79	58.7797
16	3318.89	62.3025						

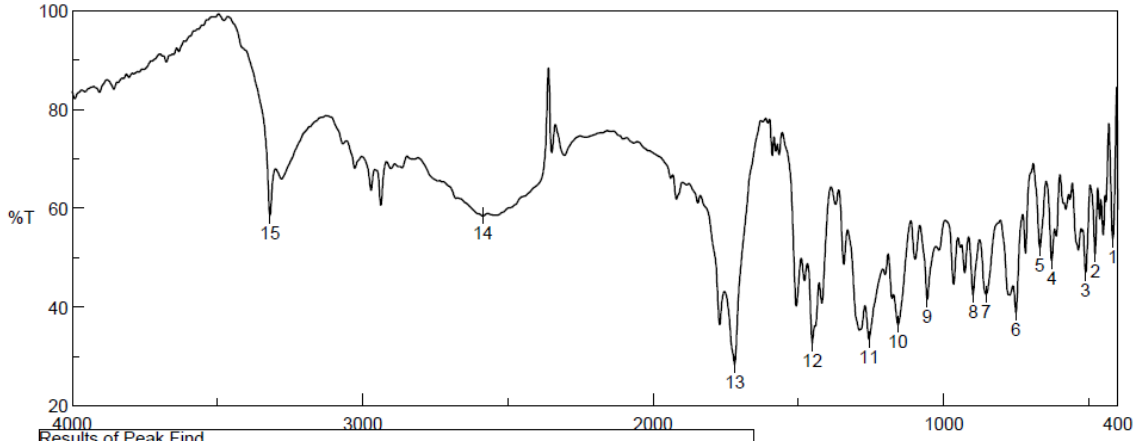
Fig. 7.29. IR spectra of Aceclofenac + PLGA



Results of Peak Find

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	426.191	88.8167	2	449.333	62.584	3	480.188	63.1278
4	539.007	56.439	5	626.752	55.0538	6	666.285	54.005
7	717.39	54.7436	8	750.174	42.3314	9	855.275	49.6519
10	898.666	47.5307	11	927.593	55.0979	12	965.198	50.5788
13	1056.8	46.295	14	1099.23	54.4962	15	1154.19	40.8327
16	1255.43	36.6492	17	1344.14	51.9291	18	1452.14	35.7222
19	1506.13	41.2694	20	1719.23	32.5411	21	1771.3	39.1631
22	2473.26	70.7161	23	2937.06	68.276	24	3027.69	75.186
25	3318.89	57.3561						

Fig. 7.30. IR spectra of Aceclofenac + Eudragit L100 + PLGA



Results of Peak Find

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	417.513	53.6502	2	478.26	50.6933	3	511.044	46.9355
4	627.716	49.2866	5	669.178	51.9633	6	751.138	38.809
7	854.311	42.5329	8	898.666	42.3874	9	1055.84	41.429
10	1157.08	36.4227	11	1256.4	33.3824	12	1452.14	32.5738
13	1719.23	28.2928	14	2586.07	58.3354	15	3318.89	58.4884

Fig. 7.31. DSC of Aceclofenac

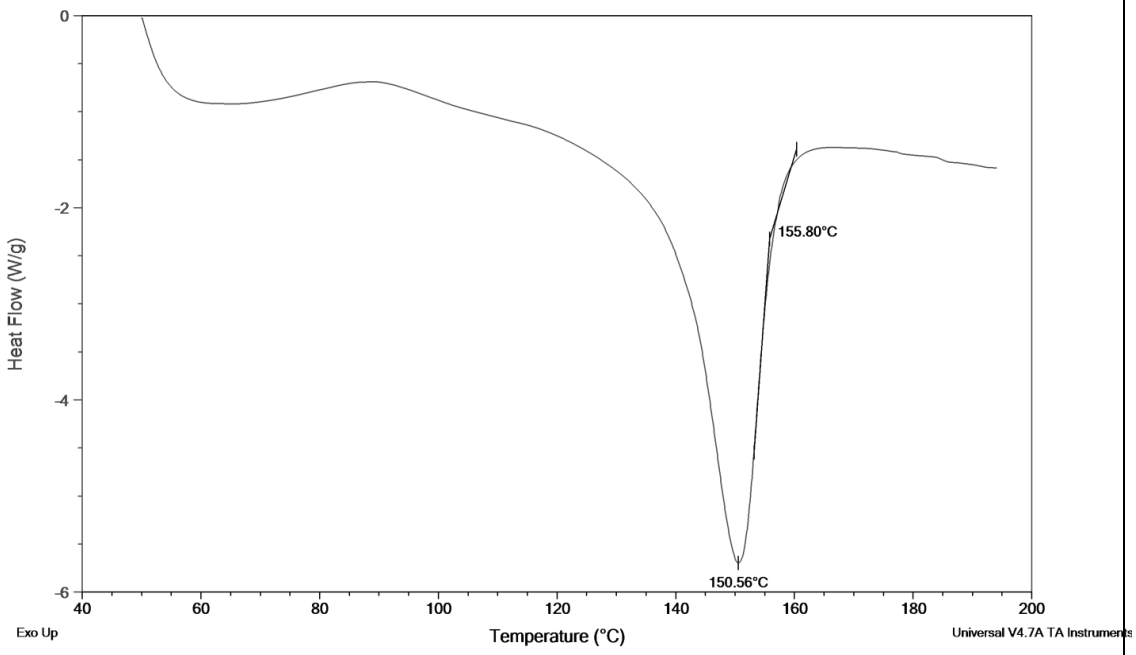


Fig. 7.32. DSC of Sodium bicarbonate

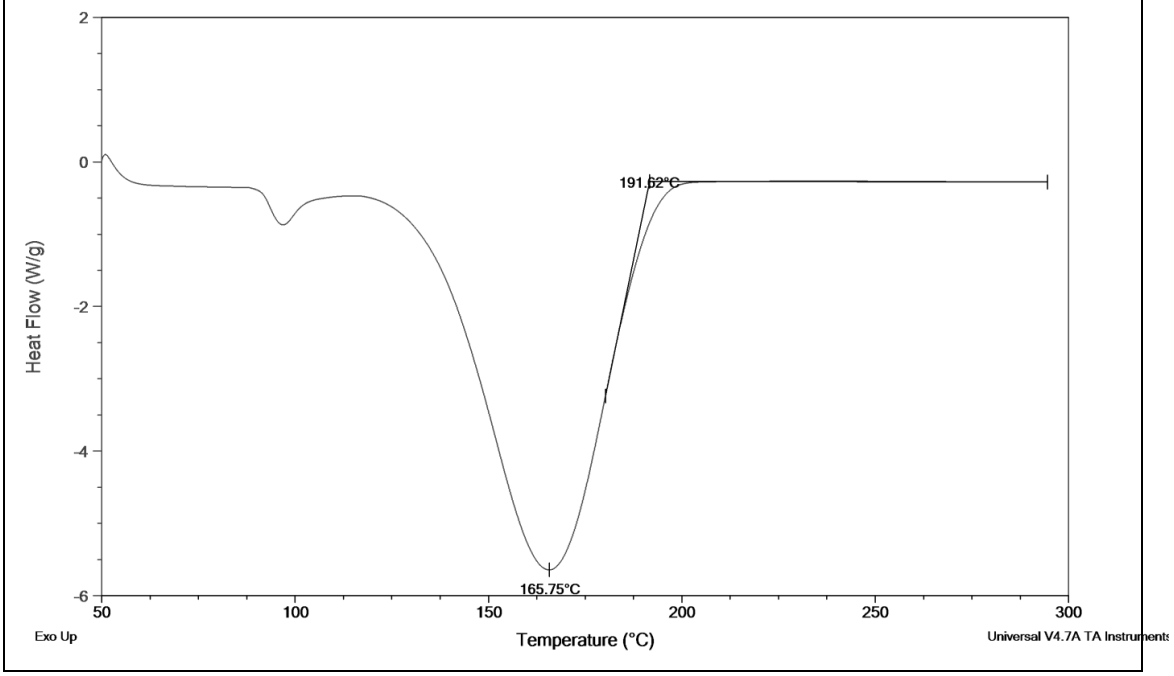


Fig. 7.33. DSC of Sodium chloride

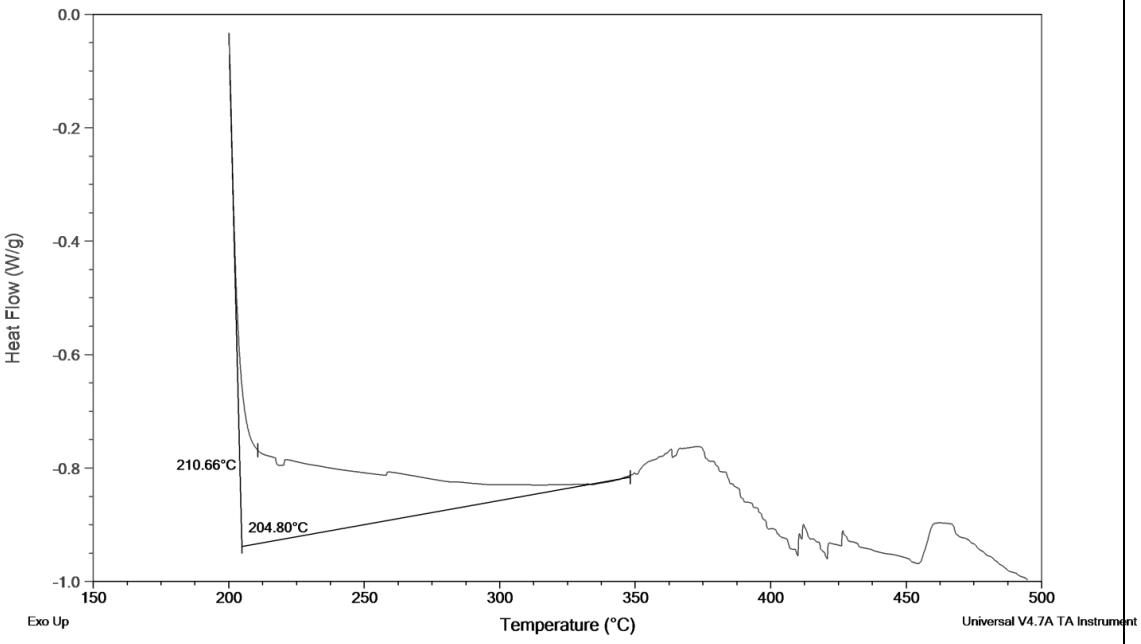


Fig. 7.34. DSC of Potassium chloride

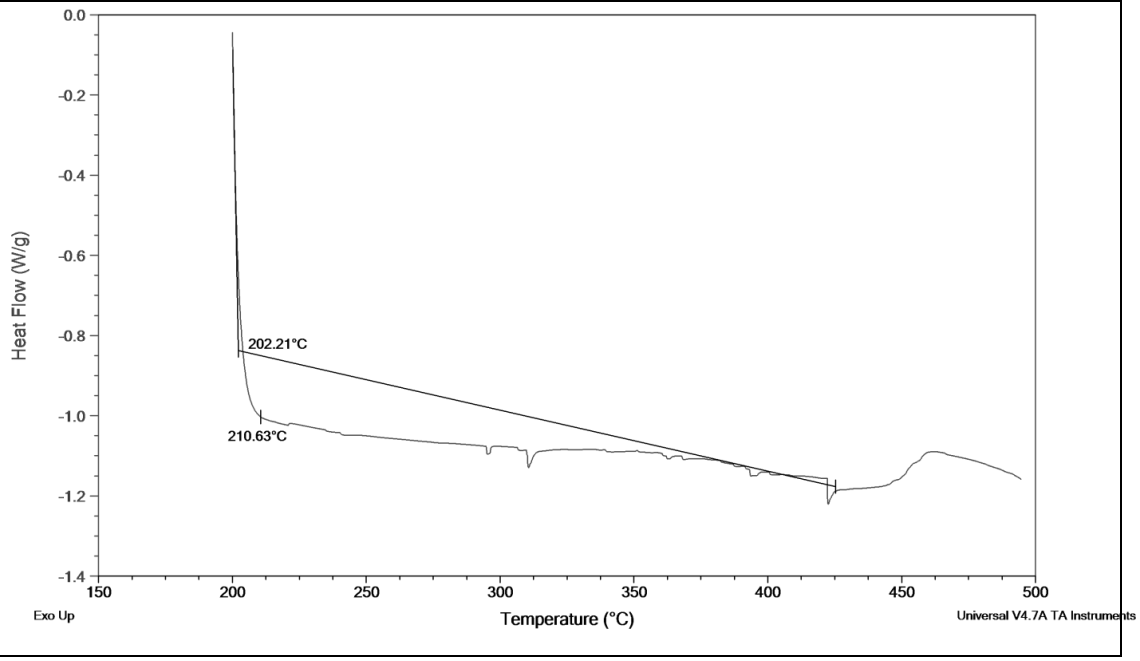


Fig. 7.35. DSC of MCC (microcrystalline cellulose)

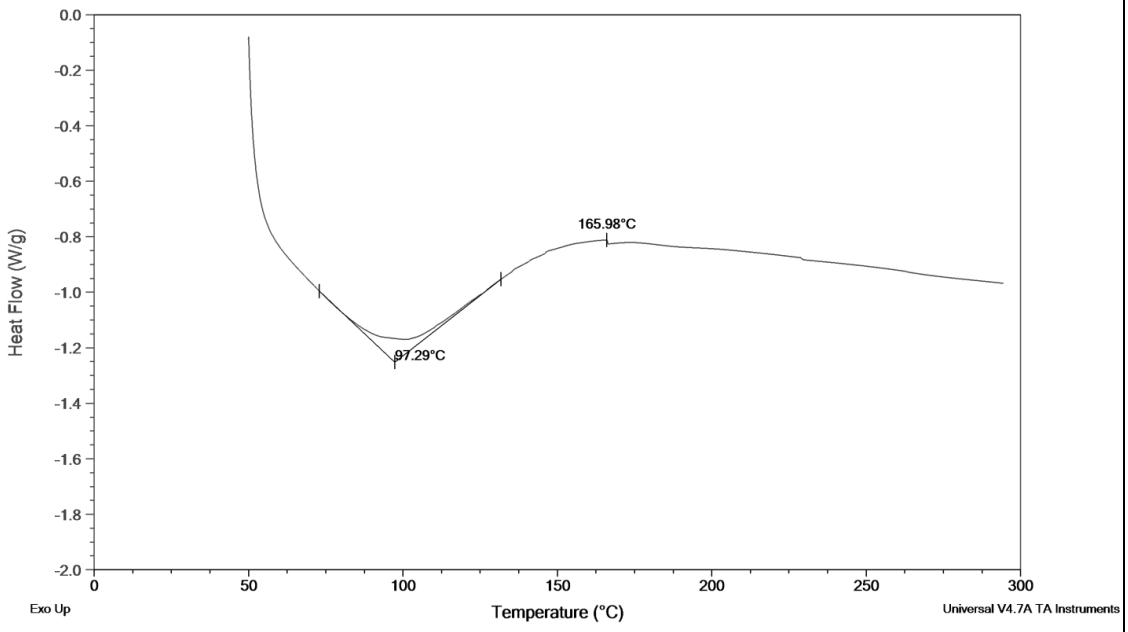


Fig. 7.36. DSC of PVP K30

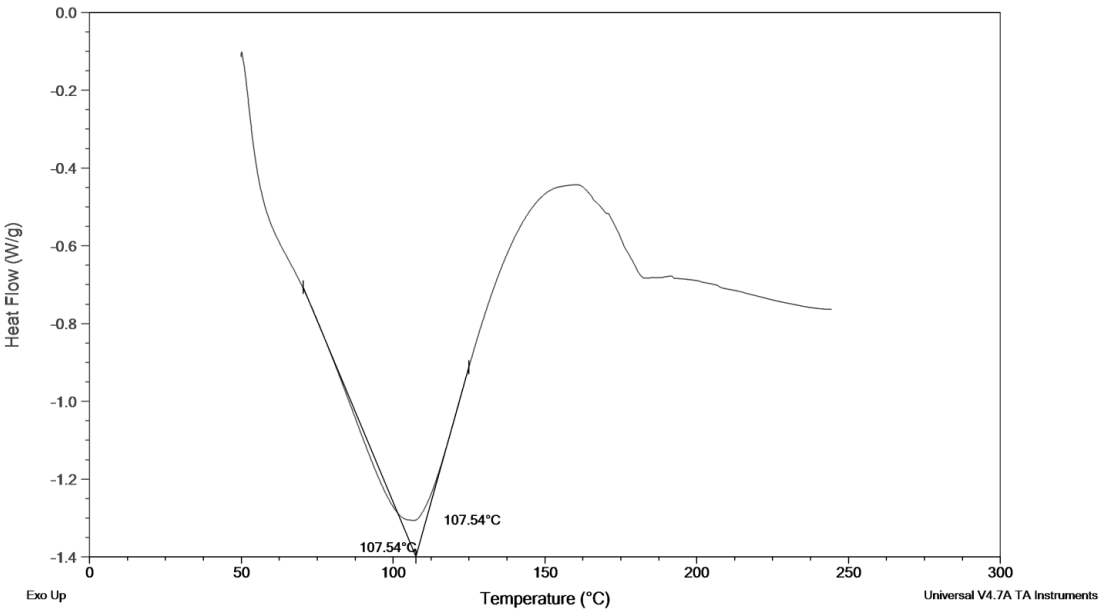


Fig. 7.37. DSC of Talc

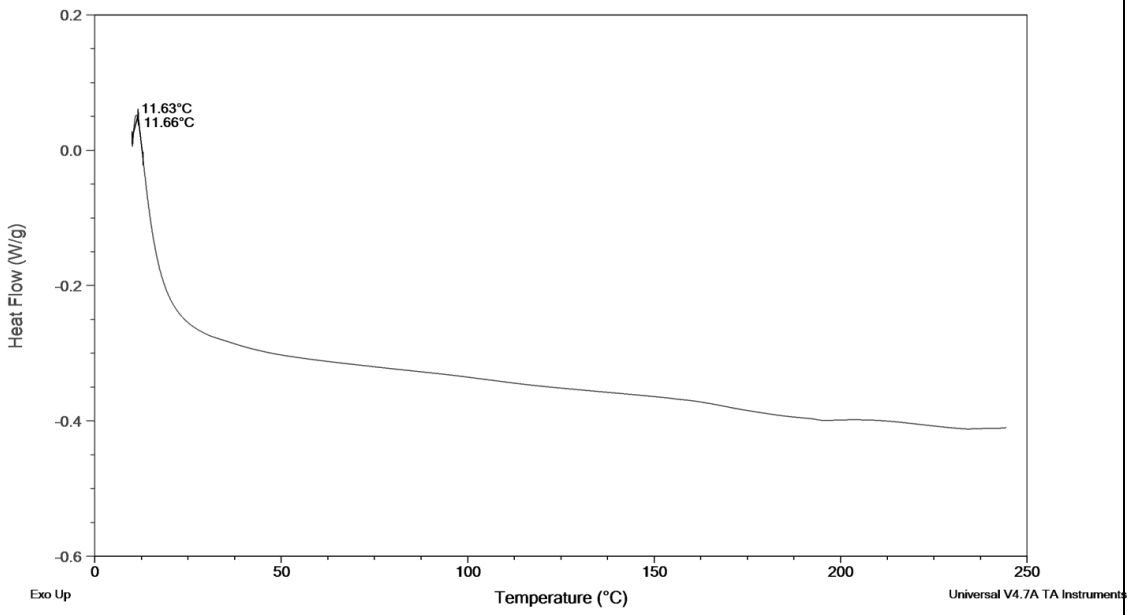


Fig. 7.38. DSC of Magnesium stearate

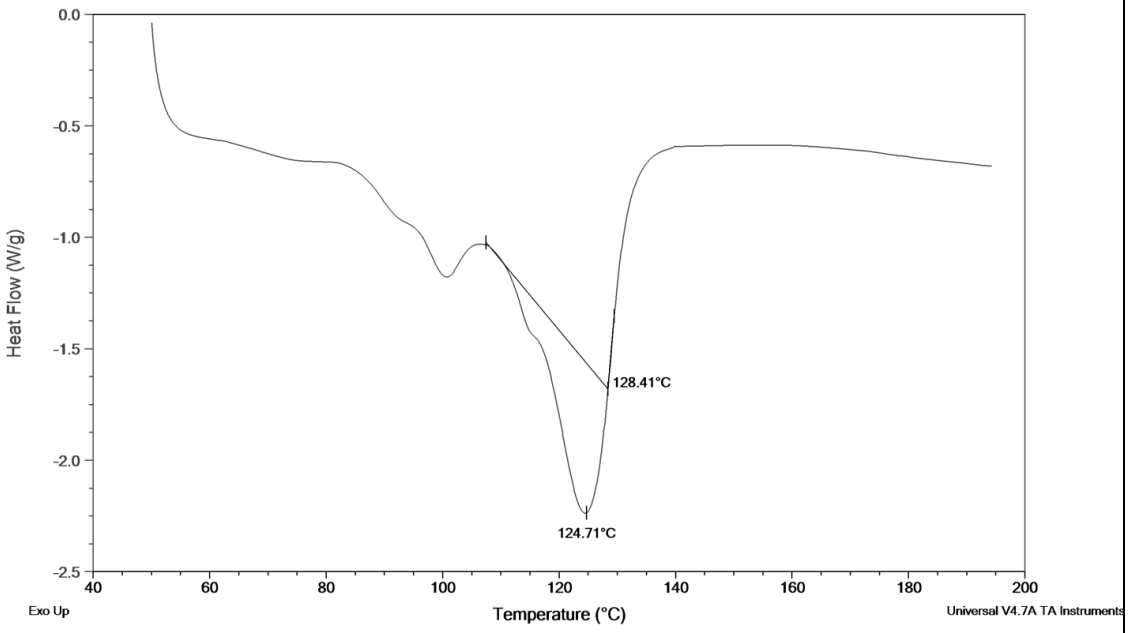


Fig. 7.39. DSC of HPMC K4M

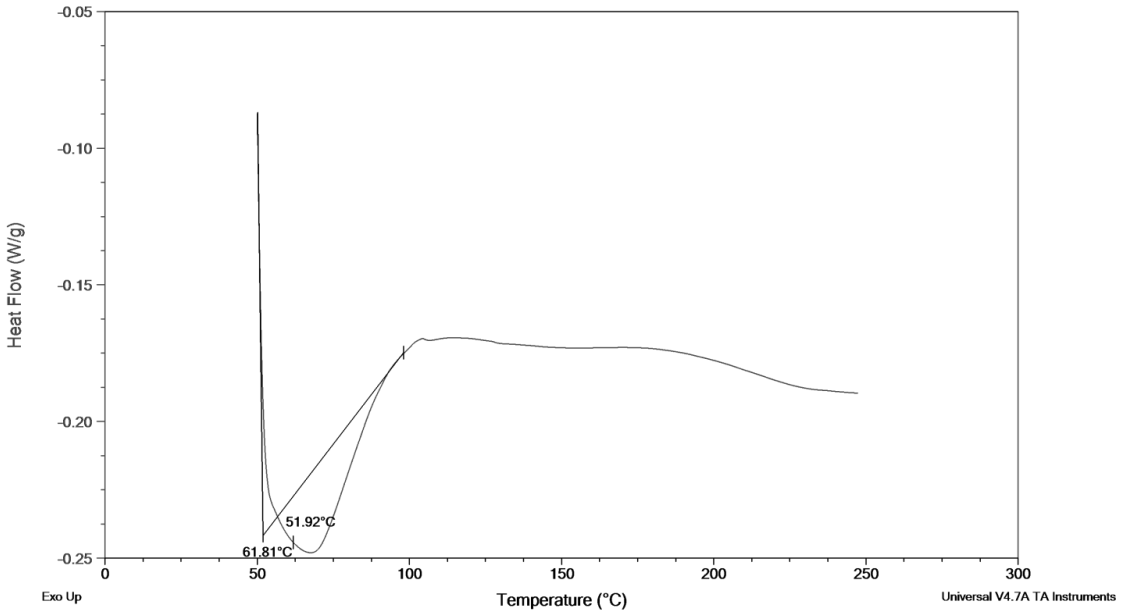


Fig. 7.40. DSC of Aceclofenac + sodium bicarbonate

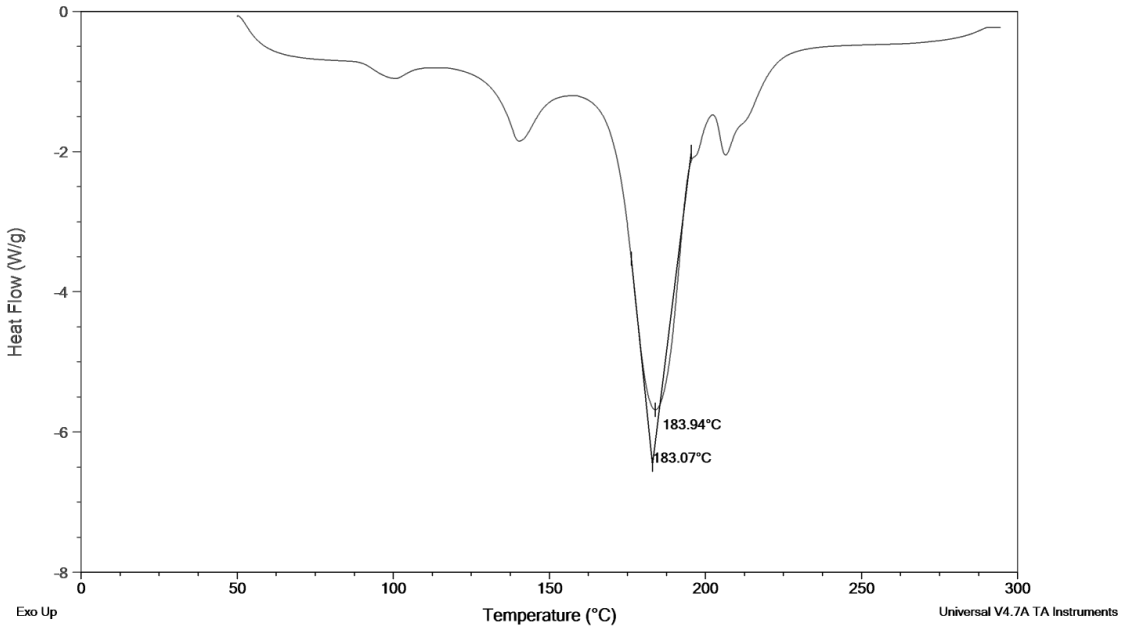


Fig. 7.41. DSC of Aceclofenac + Sodium chloride

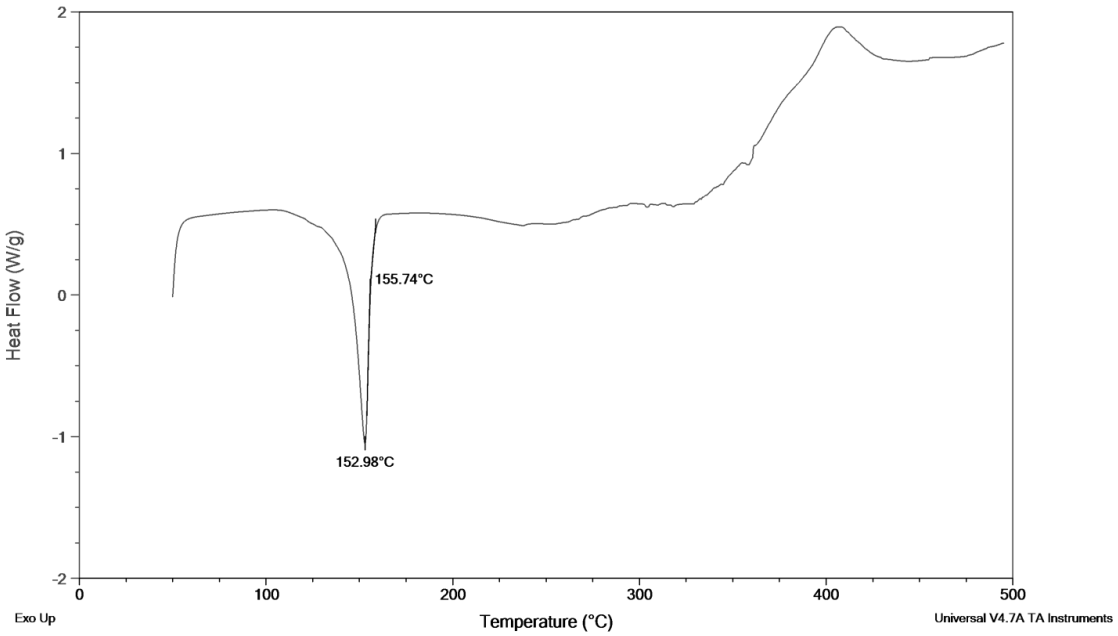


Fig. 7.42. DSC of Aceclofenac + Potassium chloride

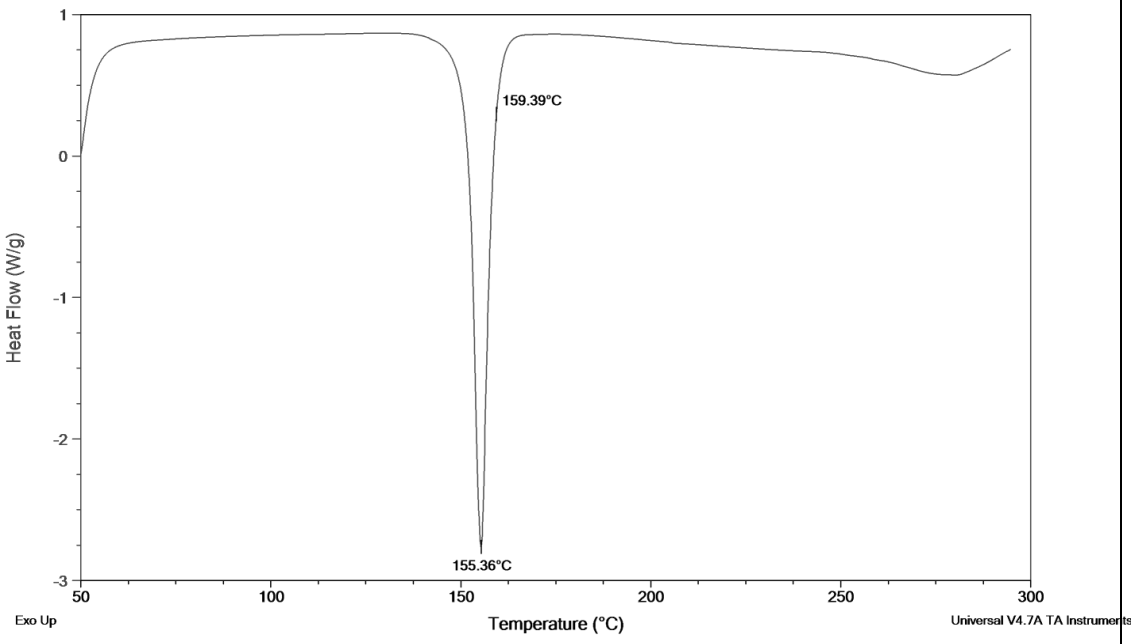


Fig. 7.43. DSC of Aceclofenac + MCC (microcrystalline cellulose)

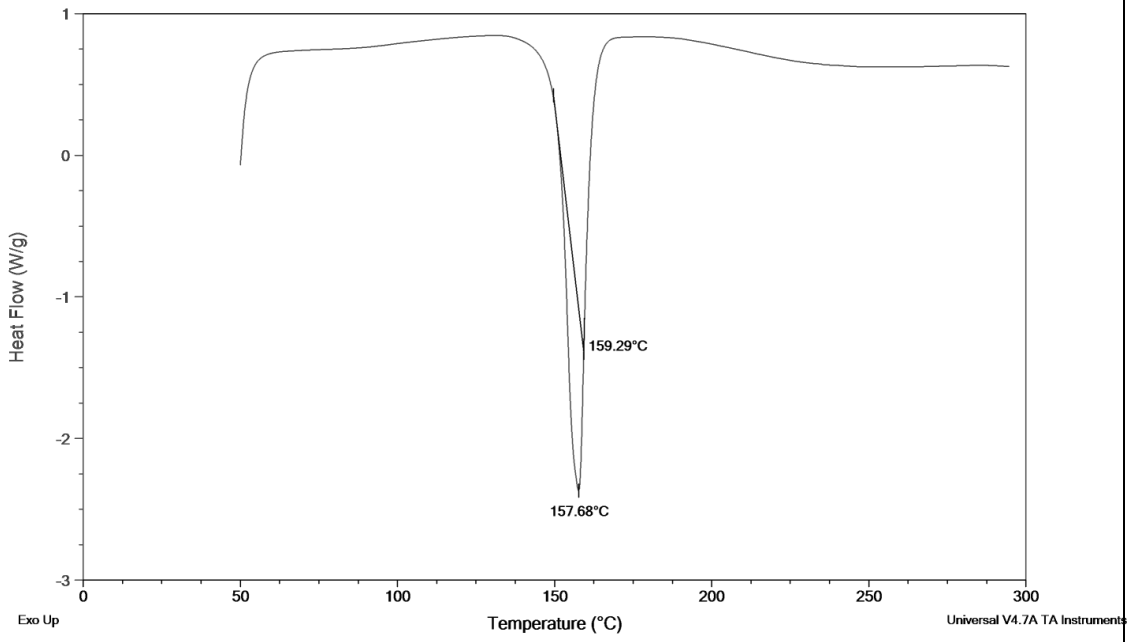


Fig. 7.44. DSC of Aceclofenac + PVP K30

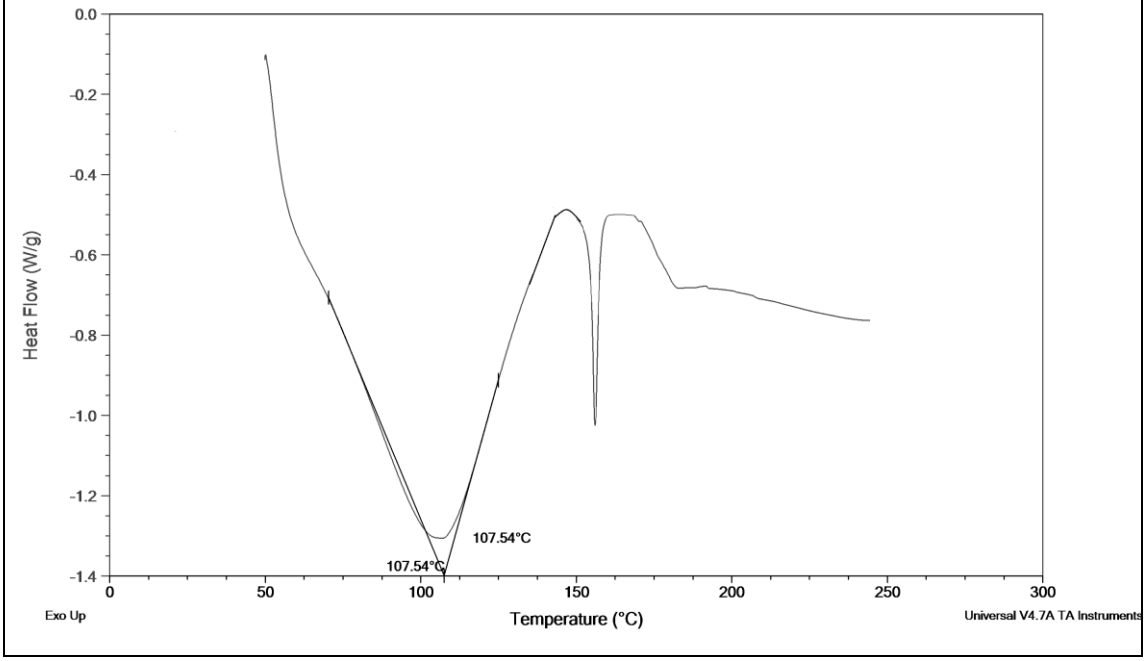


Fig. 7.45. DSC of Aceclofenac + Talc

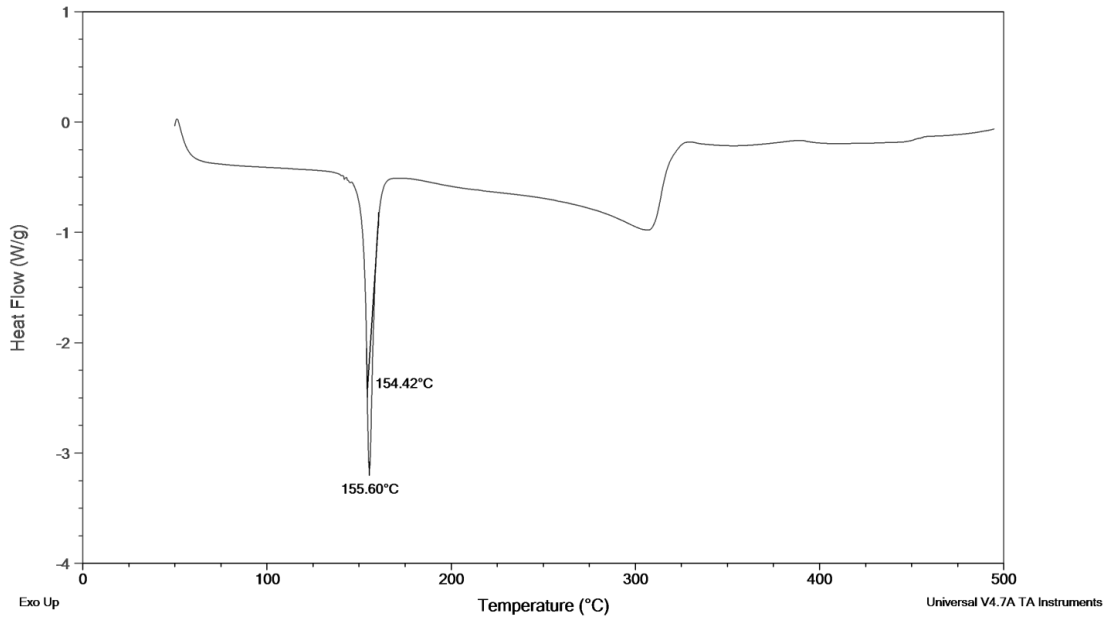


Fig. 7.46. DSC of Aceclofenac + Magnesium stearate

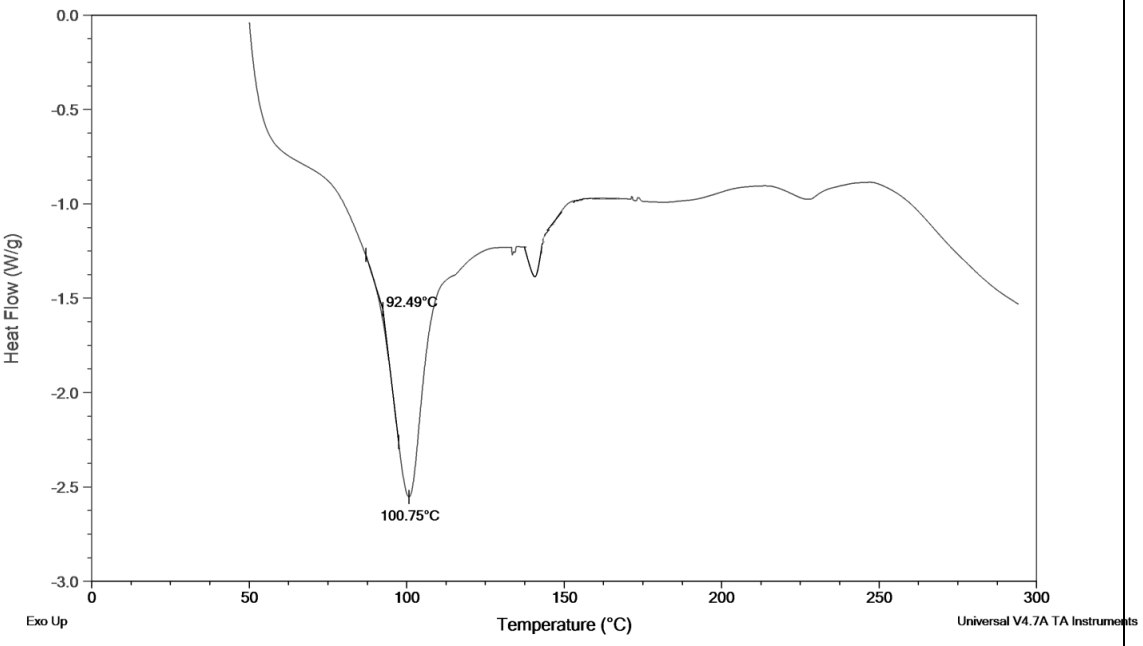


Fig. 7.47. DSC of Aceclofenac + HPMC K4M

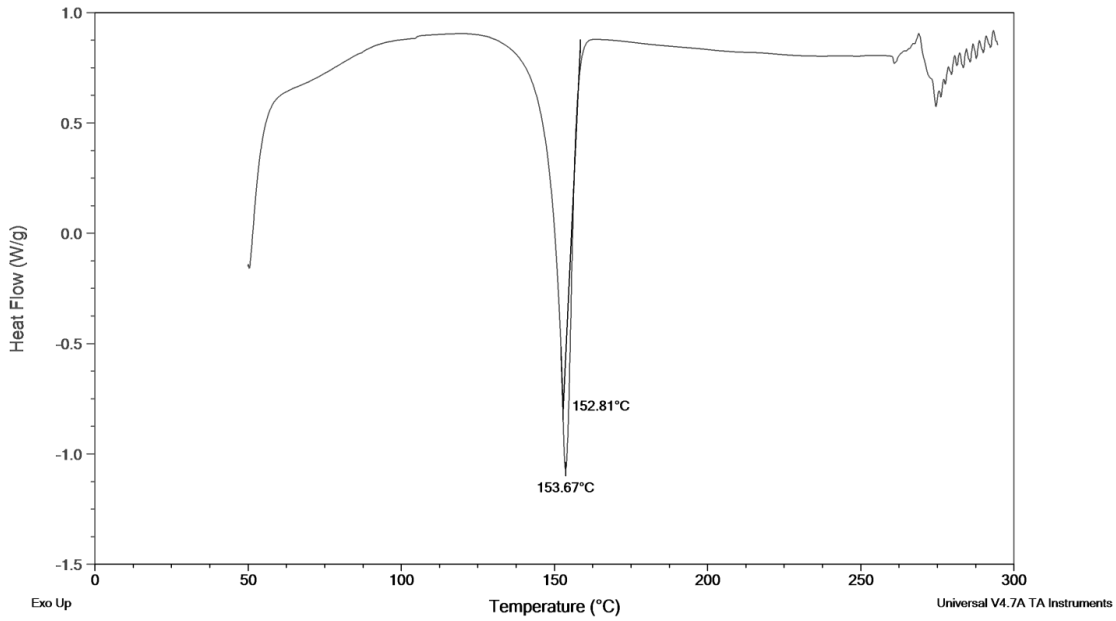


Fig. 7.48. DSC of Aceclofenac + Cellulose acetate

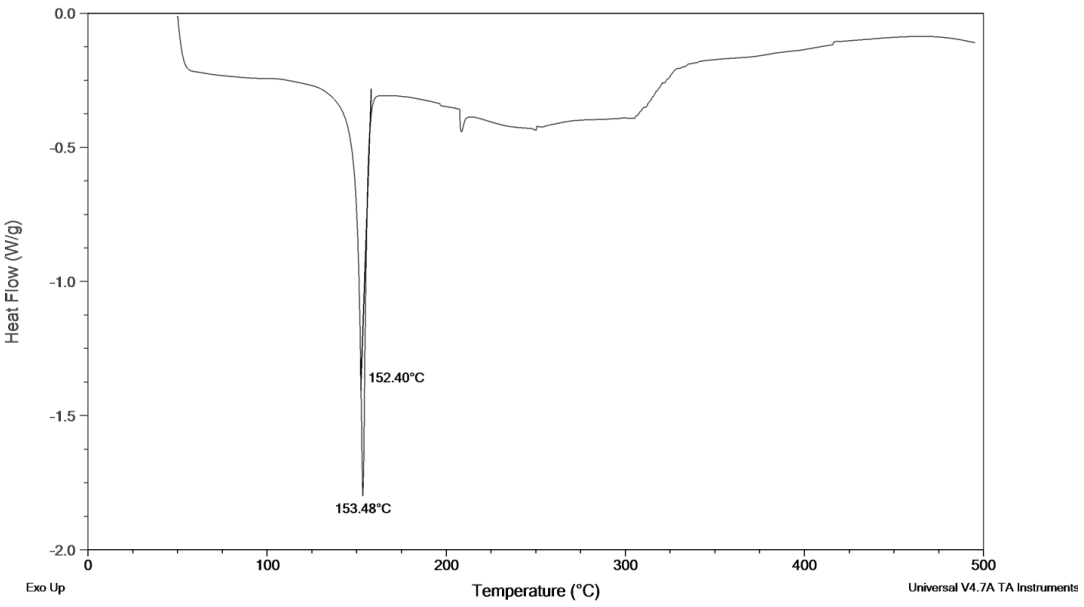


Fig. 7.49. DSC of Aceclofenac + sodium bicarbonate + MCC + PVPK30 +talc + Magnesium stearate + HPMC K4

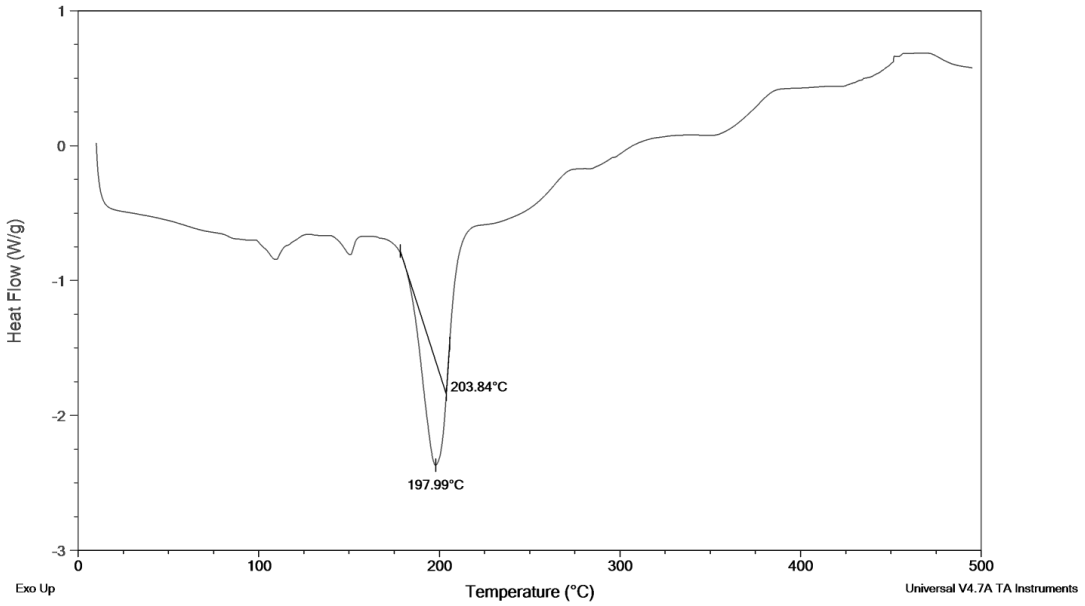


Fig. 7.50. DSC of Aceclofenac + sodium bicarbonate +sodium chloride+ MCC + PVPK30 + talc + Magnesium stearate + HPMC K4

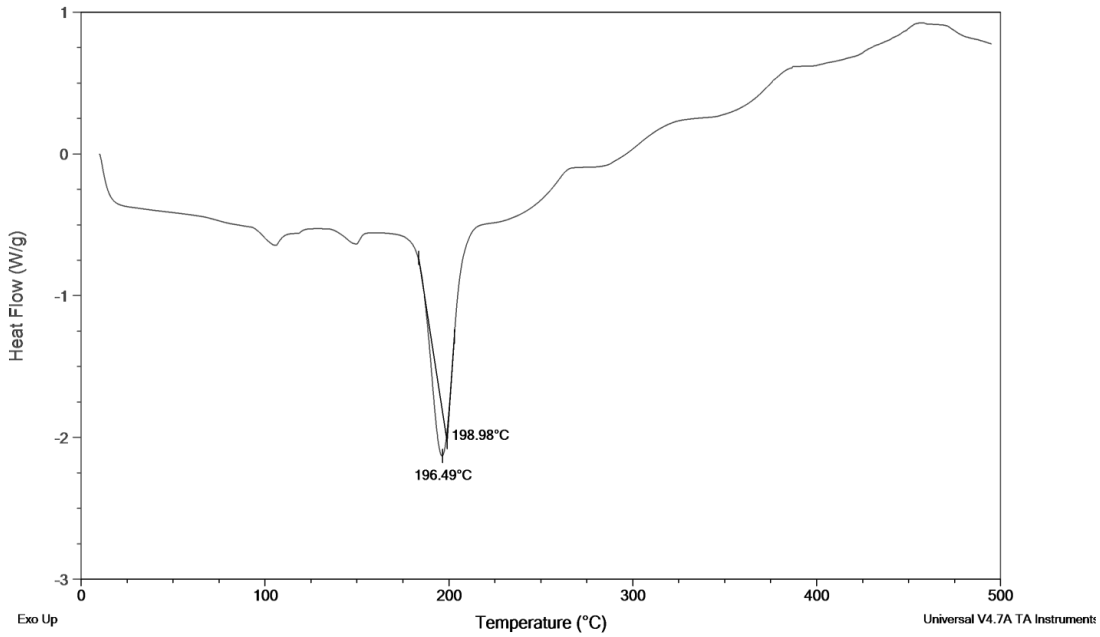


Fig. 7.51. DSC of Aceclofenac + sodium bicarbonate +potassium chloride+ MCC + PVPK30 +talc + Magnesium stearate + HPMC K4

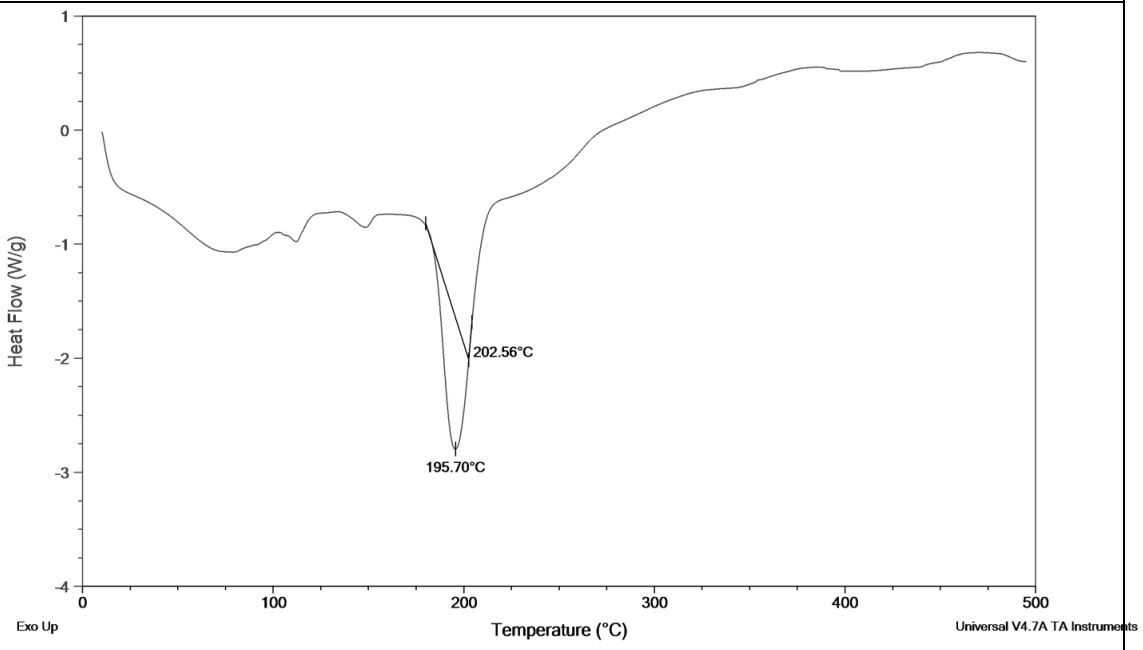


Fig. 7.52. DSC of Aceclofenac + sodium chloride +potassium chloride+ MCC + PVPK30 + talc + Magnesium stearate + HPMC K4

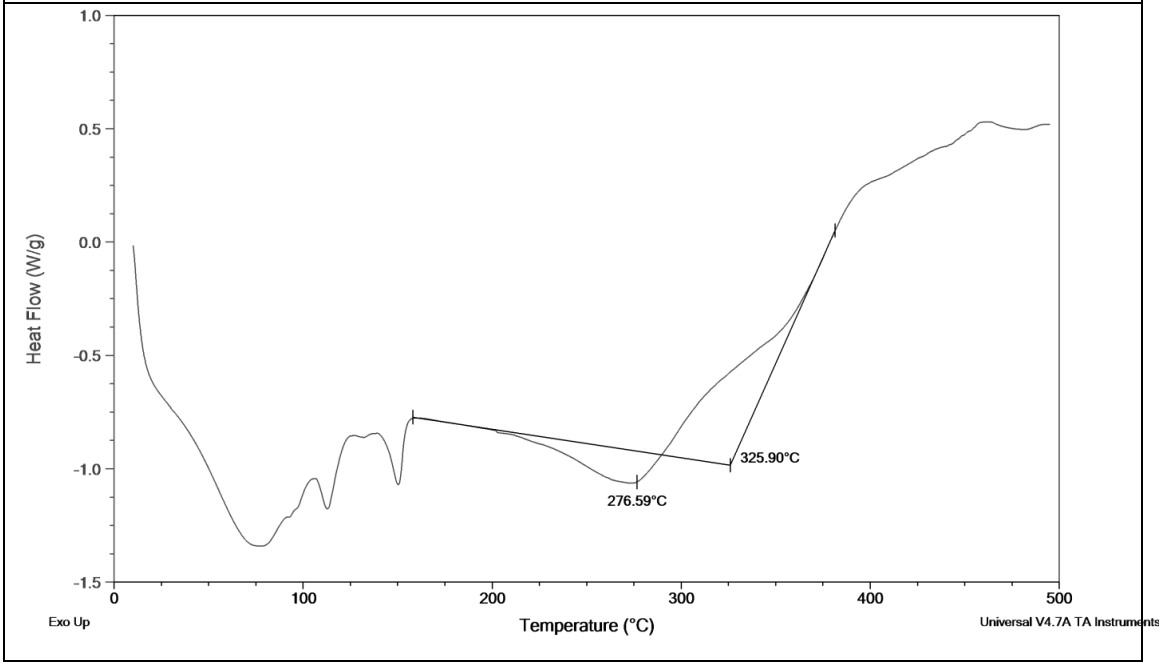


Fig. 7.53. DSC of Aceclofenac + sodium bicarbonate + Sodium chloride + Potassium chloride +MCC (microcrystalline cellulose) + PVPK30 +talc+Magnesium stearate + HPMC K4 + Cellulose acetate

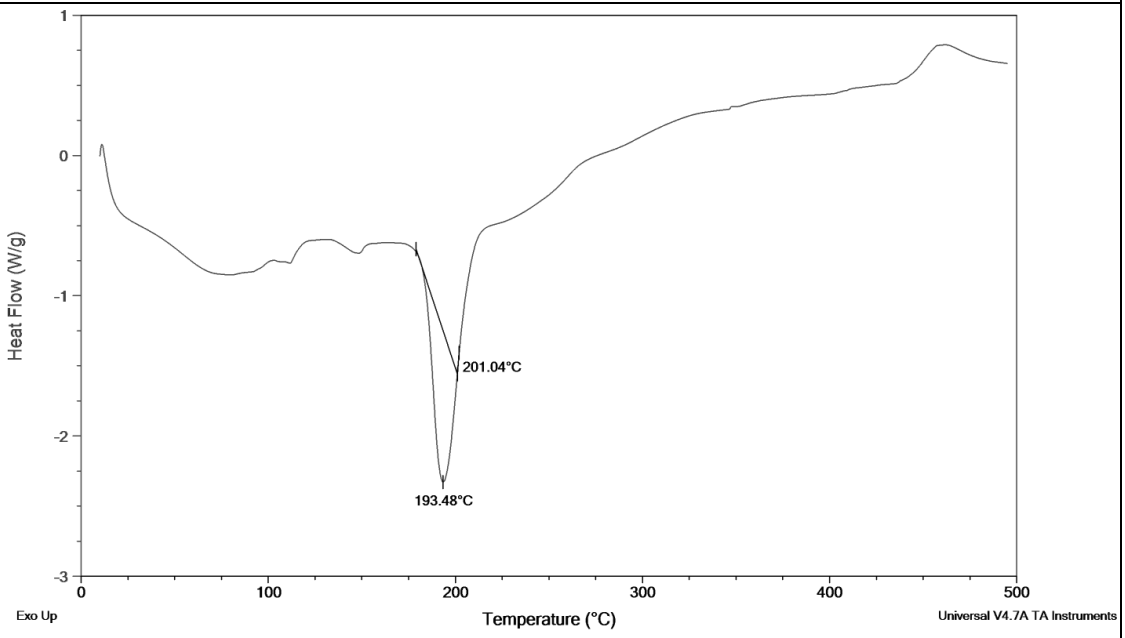


Fig. 7.54. DSC of Eudragit L100

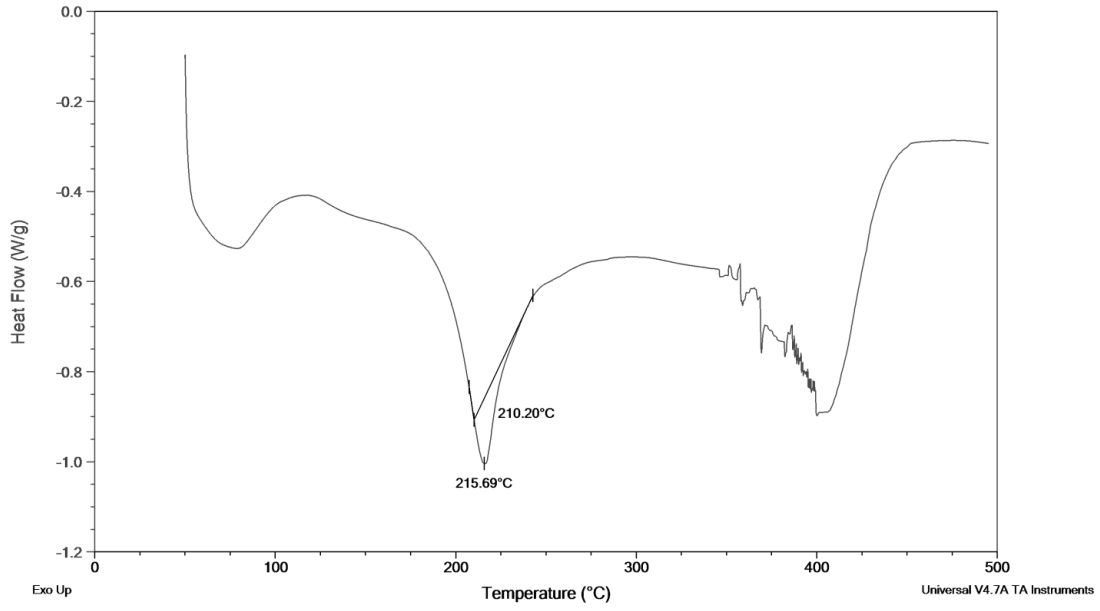


Fig. 7.55. DSC of PLGA

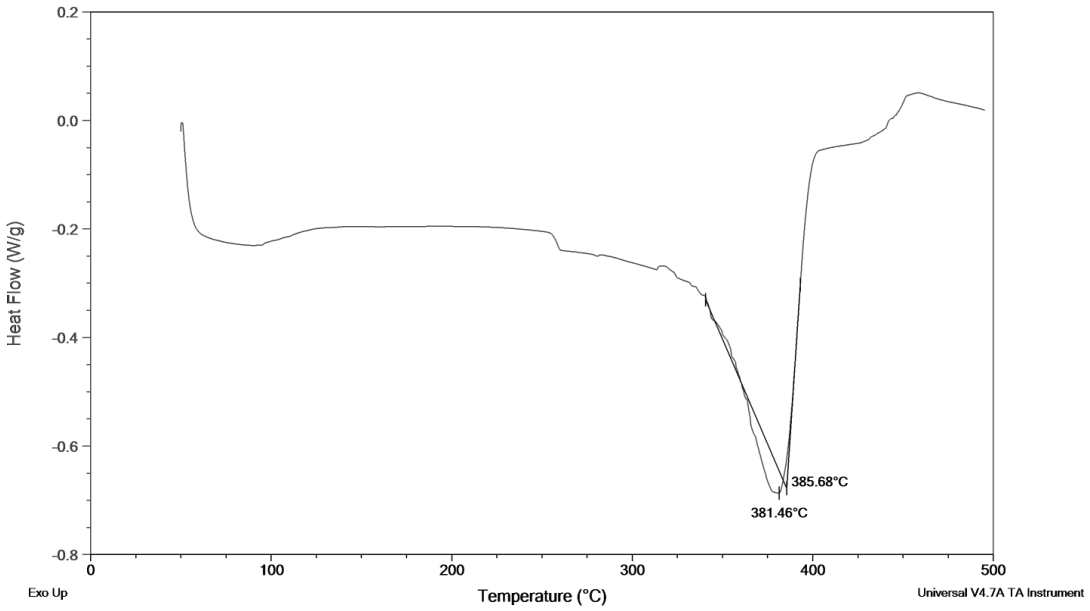


Fig. 7.56. IR spectra of Aceclofenac + Eudragit L100

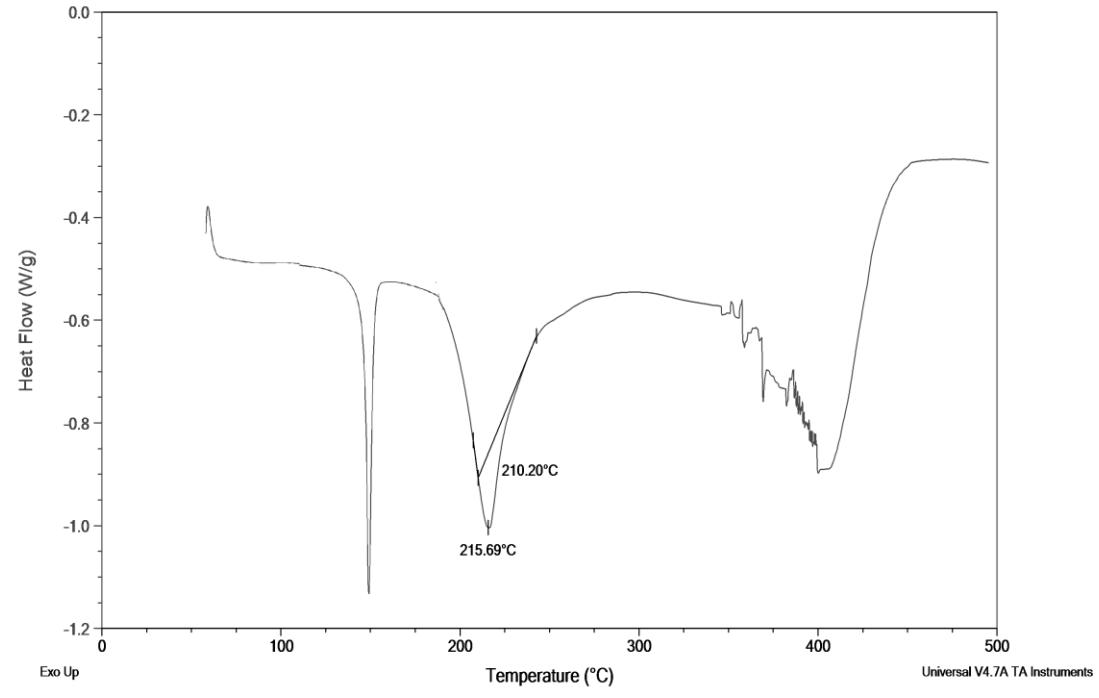


Fig. 7.57. DSC of Aceclofenac + PLGA

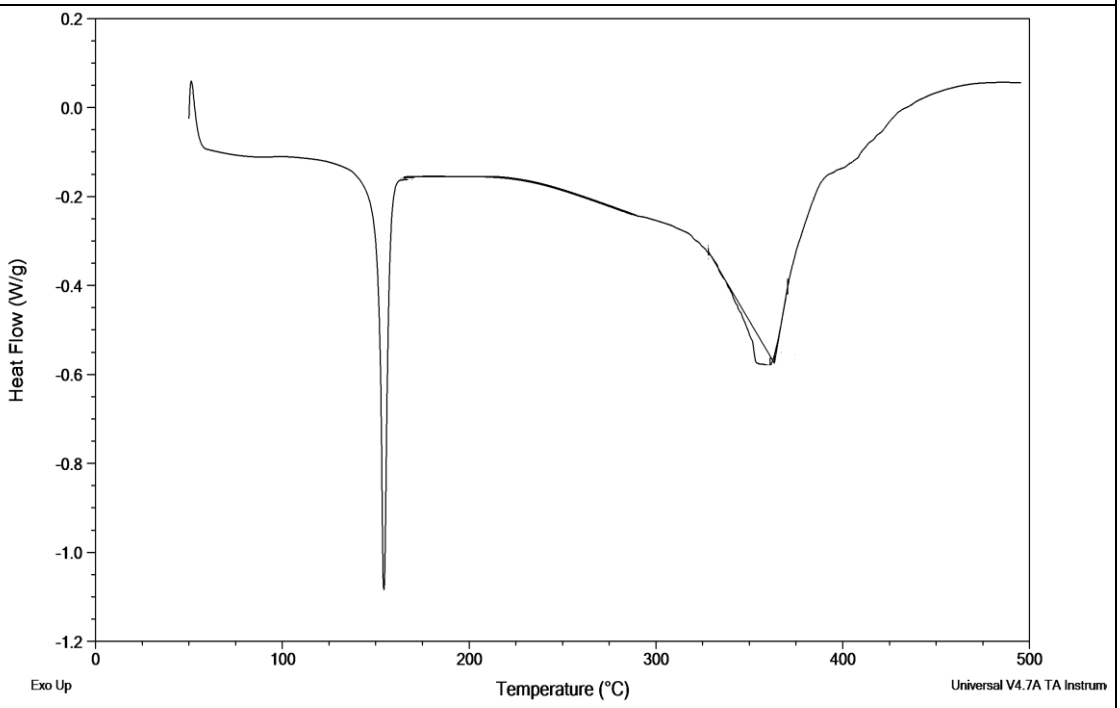


Fig. 7.58. DSC of Aceclofenac + Eudragit L100 + PLGA