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FORMULATION, DEVELOPMENT AND INVITRO EVALUATION OF PINDOLOL (PDL) SUPPOSITORY FOR THE TREATMENT OF HYPERTENSION

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ABSTRACT

Pindolol(PDL) is a non selective moderately lipophillic beta – blocker (adrenergic beta-antagonists). Chemically it is [2-hydroxy-3-(1H-indol-4-yloxy) propyl](propan-2-yl)amine. It is non-cardioselective and has intrinsic sympathomimetic actions, but little membrane-stabilizing activity. The purpose of this study was to develop an immediate release rectal suppositories. Different formulation of 20mg Pindolol were prepared as immediate release rectal suppositories by fusion method PEG 4000, PEG 6000 and Polaxomer188 are hydophillic bases used as standard excipients for formulation of Pindolol (PDL)suppository. Cross carmallose sodium(CCS) was used as super disintegrant with a view to improve bioavailability.The prepared suppositories were evaluated for visual characteristion, hardness, thickness, friability, melting point, Weight variation, Disintegration time, Content uniformity, In-vitro drug release. The drug release profiles were studied in phosphate buffer P^H 7.4.The optimized formulation of Pindolol suppository containing different ratios of hydrophilic base(PEG 4000) and superdisintegrant (Cross carmallose sodium) was found to be having no interactions upon FT-IR analysis. Differential scanning colorimetry characterization of optimized formulation showed respective peaks at different temperature revealing the compatibility of the drug and hydrophillics bases formulated with the superdisintegrant. Visual characterisation revealed that any fissuring, pitting, fat bloom, exudation and migration of active ingredients were not found in any of the formulation from F_1-F_9. All the formulations were found to be within limits. Invitro drug release studies showed that among all formulations, F5 formulation was considered as optimised formulation

as it showed 99.14% of drug release within 180 mins. The data obtained in the in vitro drug release studies were fitted into various kinetic equations like Zero order, first order Higuchi, Korrs- meyer peppas equation. The kinetic data shows the values were best fitted to Higuchi model. Stability studies as per ICH guidelines on promising prepared suppository indicated that there are no significant changes in physical characterization and drug release patterns.

KEYWORDS: Pindolol(PDL), PEG 4000, PEG 6000, Polaxome188r, Cross carmallose sodium(ccs), Suppositories, melting time, in vitro residence.

AIM OF THE WORK

The present study- formulation, development and invitro evaluation of pindolol suppository for treatment of hypertension is a immediate release form. It is particularly designed to improve the therapeutic efficacy of pindolol. Scientifically it was found that the absolute bioavailability of pindolol [PDL] is75% which can be enhanced to 90-95% when formulated in the form of rectal suppository which by passes the first pass metabolism and gastric degradation is avoided.

The following experimental protocols were therefore intended to allow a systemic approach to the study.

1. Drug excipient compatibility studies usinf Fourier transform infrared spectroscopy(FTIR).
2. Analysis of the formulation excipient by Differentail scanning colorimetry.
3. Determination of λ_{max} and development of calibration curve of Pindolol(PDL) at p^H 7.4using doble beam uv spectrophotometer.
4. Preformulation studies such as API characterisation, solubility studies, determination of melting point
5. Preparation of rectal suppository by fusion method
6. Study of visual characterisation of suppositories, hardness ,weight variation, friability, melting point determination, disintegration test
7. In-vitro drug release evaluation
8. Comparison of the different formulations by kinetic models
9. Stability studies as per ICH guidelines

2.4. PLAN OF WORK

1. Literature Survey

2. Preformulation studies

a. API characterization

- Selection of Drug
- Selection of Bases
- Physical characterization
- Solubility studies
- Detremination of Wavelength
- Preparation of standard graph

b. Drug-excipients Compatibility studies

3. Formulation development

- Thorough study of drug and suitable bases for the formulation
- Estimation of the mould capacity using base and drug individually
- Estimation of displacement value of the suppository base
- Estimation of density factor for the drug as well as the different suppository bases
- Optimization of formulation

4. Evaluation studies

i. Thickness.
ii. Hardness.
iii. Weight variation.
iv. Melting point
v. Disintegration time.
vi. Content uniformity.
vii. In-vitro drug release evaluation
viii. Kinetic analysis of drug release data

5. Stability studies as per ICH guidelines

3. MATERIALS AND METHODS

3.1 MATERIALS

The following are used in the present study

- Materials

- Equipment

List of Materials Used

S.NO	INGREDIENTS	CATEGORY	Source
1.	Pindolol (USP)	Antihypertensive, API	Sigma Aldrich
2.	PEG 4000	Water miscible base	Merck Specialities Pvt Ltd, Mumbai, India
3.	PEG 6000	Water miscible base	Merck Specialities Pvt Ltd, Mumbai, India
4.	Polaxomer 188	Water miscible base	Merck Specialities Pvt Ltd, Mumbai, India
5.	Crosscarmellose Sodium	superdisintegrent	Merck Specialities Pvt Ltd, Mumbai, India
6.	Liquid paraffin	lubricant	
7.	Potassium hydrochloride	---	
8.	Sodium hydroxide	---	

3.2. EQUIPMENTS

Table. No:3.2: List of equipments used in the study

S.NO	EQUIPMENTS	MANUFACTURER	MODEL	PURPOSE
1.	Digital Balance	Wensar weighing	PGB-600	Weighing
2.	Heating Mantle		------	Melting bases
3.	Suppository Mould	Scientech , Delhi, India	------	Moulding molten bases
4.	Hardness tester	Monsanto, Mumbai, India.	-------	hardness
5.	Digital Micrometer	Mitutoyo, Japan.	-------	Determine thickness of suppository
6.	Roche Friabilator	Labindia, Mumbai, India	-------	Friability
7.	Dissolution Apparatus	Labindia, Mumbai, India	DS8000/S	% Drug release
8.	UV-Visible Spectrophotometer	Elico, PG instrument	T60 UV	
9.	pH meter	Systronics	Digital-335	
10.	FT-IR Spectrophotometer	Agilent technologies	Cary 630 FTIR	Drug- excipient compatibility
11.	Differential scanning colorimetry	hitachi	DSC7000X	Thermal analysis
12.	Dessicator	Plastic ware manufacturers	------	Protects moisture sensitive substances
13.	Distill water plant	Premier trading co.	------	Water distillation
14.	Stability and humidity chamber	Partha sartha enterprises	8050	
15.	Syringe	Hamilton	------	Sample withdrawal
16.	Tablet disintegration apparatus	Lab Hosp	------	Invitro residence time measurement

3.2. DRUG PROFILE

Accession Number **: DB00960** (APRD00678)

Name : Pindolol(PDL)-USP

Description : A moderately lipophilic beta blocker (adrenergic beta-antagonists). It is non-cardioselective and has intrinsic sympathomimetic actions, but little membrane-stabilizing activity.

Structure :

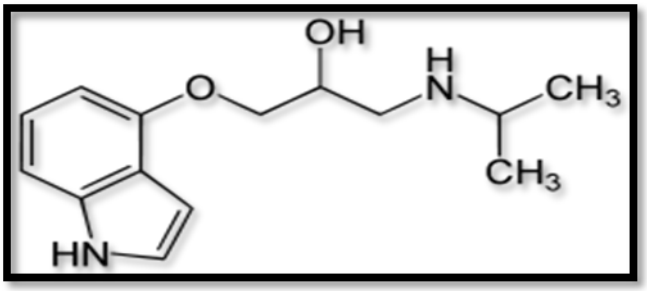


Figure:3.2.a: structure of pindolol

Chemical Name :[2-hydroxy-3-(1H-indol-4-yloxy)propyl](propan-2- yl)amine

Molecular Formula : $C_{14}H_{20}N_2O_2$

Molecular Weight : 248.3208 gram/mole

Appearance : Solid

Solubility : Water solubility-7880 mg/L

Melting Point : 171^0C

PKa : 9.67

Category : Antihypertensive, antianginal, Beta blocker

Pharmacokinetic Data

Absorption : Rapidly and reproducibly absorbed (bioavailability greater than 95%).

Protein Binding : 40%

Metabolism : Hepatic. In man, 35% to 40% is excreted unchanged in the urine and 60% to 65% is metabolized primarily to hydroxy-metabolites which are excreted as glucuronides and ethereal sulfates.

Half-life : 3 to 4 hours

Excretion : Pindolol undergoes extensive metabolism in animals and man. In man, 35% to 40% is excreted unchanged in the urine and 60% to 65% is

metabolized primarily to hydroxy-metabolites which are excreted as glucuronides and ethereal sulfates. About 6% to 9% of an administered intravenous dose is excreted by the bile into the feces.

Pharmacodynamics

Pindolol is a non-selective beta-adrenergic antagonist (beta-blocker) which possesses intrinsic sympathomimetic activity (ISA) in therapeutic dosage ranges but does not possess quinidine-like membrane stabilizing activity. Pindolol impairs AV node conduction and decreases sinus rate and may also increase plasma triglycerides and decrease HDL-cholesterol levels. Pindolol is nonpolar and hydrophobic, with low to moderate lipid solubility. Pindolol has little to no intrinsic sympathomimetic activity and, unlike some other beta-adrenergic blocking agents, pindolol has little direct myocardial depressant activity and does not have an anesthetic-like membrane-stabilizing action.

Mechanism of Action: Pindolol non-selectively blocks beta-1 adrenergic receptors mainly in the heart, inhibiting the effects of epinephrine and norepinephrine resulting in a decrease in heart rate and blood pressure. By binding beta-2 receptors in the juxtaglomerular apparatus, Pindolol inhibits the production of renin, thereby inhibiting angiotensin II and aldosterone production and therefore inhibits the vasoconstriction and water retention due to angiotensin II and aldosterone, respectively.

Uses: For the management of hypertension, edema, ventricular tachycardias, and atrial fibrillation.

Side Effects: Dizziness, drowsiness, weakness, and nausea may occur as your body adjusts to the medication. This drug may reduce blood flow to your hands and feet, causing them to feel cold.

Marketed Products

Table.no:3.2.b: marketed products of Pindolol

S No	Brand Name	Manufacturers	Dosage form	Strength
1	Visken	Novartis	Tablets	10mg, 15mg.
2.	Pindolol tablet	Sun pharma	Tablets	5mg,10mg

International brands

1. Betapindol
2. Blockin L
3. Calvisken
4. Decreten
5. Durapindol
6. Glauco-Visken

3.3. EXCIPIENT PROFILE

POLYEHYLENE GLYCOL

Synonyms **:** PEG, Macrogol

Description **:** PEG's below 700 molecular weight occur as clear to slightly hazy, colourless, slightly hygroscopic liquids with a slight characteristic odour. PEG's between 700-900 are semi-solid. PEG's over 1000 molecular weight are creamy white waxy solids, flakes, or free-flowing powders.

Chemical names **:** alpha-Hydro-omega-hydroxypoly (oxy-1,2-ethanediol)

C.A.S. number **:** 25322-68-3

Chemical formula **:** $(C_2H_4O)\ n+1H_2O$

Structural formula :

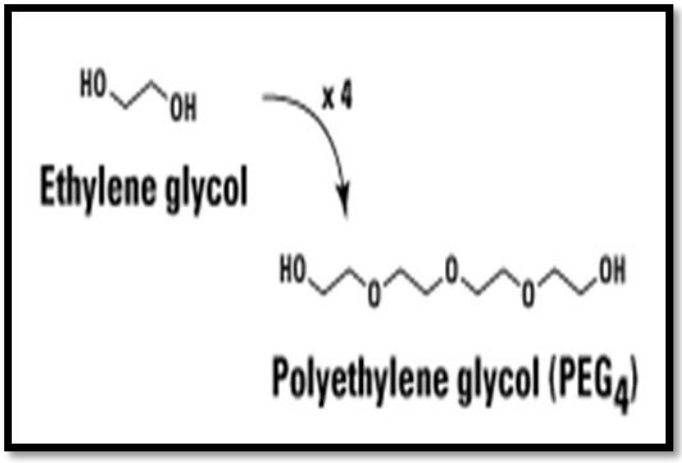


Figure.3.3.a: structure of polyethylene glycol

Molecular weight **:** 200-9500 g/mol

Functional Uses : Carrier solvent, excipient

Solubility **:** Polyethylene glycols having a molecular weight of 1000 or above are freely soluble in water; polyethylene glycols are soluble in many organic solvents, including aliphatic ketones and alcohols, chloroform, glycol ethers, esters, and aromatic hydrocarbons; they are insoluble in ether and in most aliphatic hydrocarbons; with increased molecular weight, water solubility and solubility in organic solvents decreases.

Applications

1. Components of pharmaceuticals: A variety of therapeutic molecules (proteins, peptides, nucleic acids, and small molecules) have been PEGylated by either stable or hydrolyzable linkages3. PEG acts both by improving a drug's solubility and stability and by improving a drug's circulation time while reducing its immunogenicity3,4.
2. Components of medical devices: PEG is used in a variety of medical devices such as vascular grafts, contraceptive devices, drug delivery systems, water soluble membranes, biocompatible surface coatings2. In addition, they can be used as soft tissue fillers5.
3. Tissue Engineering: PEG can be chemically modified to include cellular attachment and degradation peptide sequences to recreate an appropriate synthetic cellular niche6.
4. Microfluidics and Microstructures: PEG has been used to form microstructures and microarrays using either photolithography7 or spotting techniques8 to study cells.

POLAXOMER188

Synonyms **:** Lutrol; Monolan; Pluronic; poloxalkol; poloxamera; polyethyleneropylene glycol copolymer; polyoxyethylene–polyoxypropylene copolymer; Supronic; Synperonic

Empirical Formula **:** The poloxamer polyols are a series of closely related block copolymers of ethylene oxide and propylene oxide conforming to the general formula HO(C2H4O)a(C3H6O)b(C2H4O)aH. The grades included in the PhEur 6.0 and USP32–NF27 are shown in Table I. The PhEur 6.0 states that a suitable antioxidant may be added.

Chemical Name **:** a-Hydro-o-hydroxypoly (oxyethylene) poly (oxypropylene) poly- (oxyethylene) block copolymer

Molecular Weight **:** 4000 g/mol

Structural formula

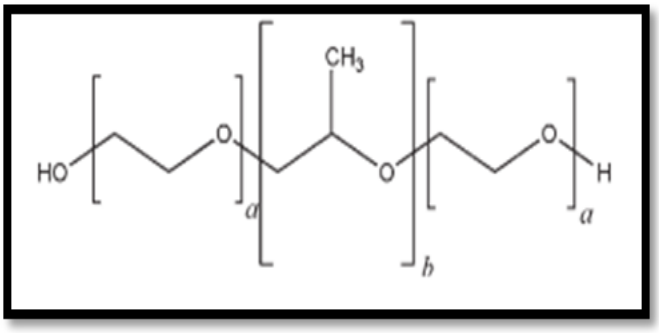


Figure.3.3.b: structure of Polaxomer188

Functional Category **:** Dispersing agent; emulsifying agent; solubilizing agent; tablet lubricant; wetting agent.

Applications **:**. Poloxamers are nonionic polyoxyethylene–polyoxypropylene copolymers used primarily in pharmaceutical formulations as emulsifying or solubilizing agents.(1-8) The polyoxyethylene segment is hydrophilic while the polyoxypropylene segment is hydrophobic. All of the poloxamers are chemically similar in composition, differing only in the relative amounts of propylene and ethylene oxides added during manufacture. Poloxamers are used as emulsifying agents in intravenous fat emulsions, and as solubilizing and stabilizing agents to maintain the clarity of elixirs and syrups. Poloxamers may also be used as wetting agents; in ointments, suppository bases, and gels; and as tablet binders and coatings. Poloxamer 188 has also been used as an emulsifying agent for fluorocarbons used as artificial blood substitutes, and in the preparation of solid-dispersion systems. More recently, poloxamers have found use in drug-delivery systems.(9–

14) Therapeutically, poloxamer 188 is administered orally as a wetting agent and stool lubricant in the treatment of constipation; it is usually used in combination with a laxative such as danthron. Poloxamers may also be used therapeutically as wetting agents in eye-drop formulations, in the treatment of kidney stones, and as skin-wound cleansers.

Table: Uses of poloxamer

Table. 3.3: different uses of polaxomer 188

Use	Concentration (%)
Fat emulsifier	0.3
Flavor solubilizer	0.3
Fluorocarbon emulsifier	2.5
Gelling agent	15–50
Spreading agent	1

Description: **:** Dispersing agent; emulsifying agent; solubilizing agent; tablet lubricant; wetting agent.

Stability **:** Microcrystalline cellulose is a stable though hygroscopic material.

Storage Conditions **:** The bulk material should be stored in a well-closed container in a cool, dry place.

Safety **:** It is widely used in oral pharmaceutical formulations is generally regarded as a relatively nontoxic and nonirritant material. Deliberate abuse of formulations containing cellulose, either by inhalation or by injection, has resulted in the formation of cellulose granulomas.

CROSCARMELLOSE SODIUM

Nonproprietary Name **:**BP: Croscarmellose Sodium, JP: Croscarmellose Sodium, PhEur: Croscarmellose Sodium USP-NF: Croscarmellose Sodium

Synonyms **:**Ac-Di-Sol; carmellosum natricum conexum; crosslinked carboxymethylcellulose sodium; Explocel; modified cellulose gum; Nymcel ZSX; Pharmacel XL; Primellose; Solutab; Vivasol.

Chemical Name **:**Cellulose, carboxymethyl ether, sodium salt, crosslinked.

Structural Formula

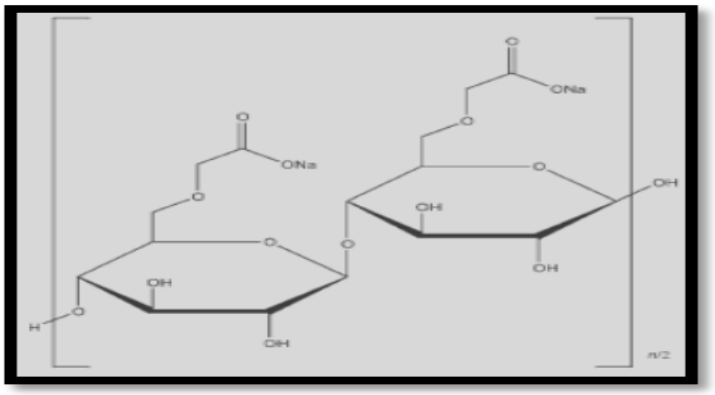


Figure.no:3.3.c: structure of crosscarmellose sodium

Functional Category **:** Tablet and capsule disintegrant.

Applications in Pharmaceutical Formulation or Technology : Croscarmellose sodium is used in oral pharmaceutical formulations as a disintegrant for capsules, tablets and granules. In tablet formulations, croscarmellose sodium may be used in both direct-compression and wet-granulation processes. When used in wet granulations, the croscarmellose sodium should be added in both the wet and dry stages of the process (intra and extra-granularly) so that the wicking and swelling ability of the disintegrant is best utilized. Croscarmellose sodium at concentrations up to 5% w/w may be used as a tablet disintegrant, although normally 2% w/w is used in tablets prepared by direct compression and 3% w/w in tablets prepared by a wet-granulation process.

Use Concentration (%)

Disintegrant in capsules 10–25

Disintegrant in tablets 0.5–5

Description **:**Croscarmellose sodium occurs as an odorless, white or grayish white powder.

Stability and Storage Conditions **:**Croscarmellose sodium is a stable though hygroscopic material. A model tablet formulation prepared by direct compression, with croscarmellose sodium as a disintegrant, showed no significant difference in drug dissolution after storage at 30^0C for 14 months. Croscarmellose sodium should be stored in a well-closed container in a cool, dry place.

Incompatibilities **:**The efficacy of disintegrants, such as croscarmellose sodium, may be slightly reduced in tablet formulations prepared by either the wet-granulation or

direct-compression process that contain hygroscopic excipients such as sorbitol. Croscarmellose sodium is not compatible with strong acids or with soluble salts of iron and some other metals such as aluminum, mercury, and zinc.

Safety **:** Croscarmellose sodium is mainly used as a disintegrant in oral pharmaceutical formulations and is generally regarded as an essentially nontoxic and nonirritant material. However, oral consumption of large amounts of croscarmellose sodium may have a laxative effect, although the quantities used in solid dosage formulations are unlikely to cause such problems. In the UK, croscarmellose sodium is accepted for use in dietary supplements. The WHO has not specified an acceptable daily intake for the related substance carboxymethylcellulose sodium, used as a food additive, since the levels necessary to achieve a desired effect were not considered sufficient to be a hazard to health.

Handling Precautions **:** Observe normal precautions appropriate to the circumstances and quantity of material handled. Croscarmellose sodium may be irritant to the eyes; eye protection is recommended.

ANALYTICAL METHOD DEVELOPMENT

3.4.1.PREPARATION OF PHOSPHATE BUFFER P^H 7.4

6.8 gm of potassium dihydrogen phosphate and 1.58 gm of sodium hydroxide in suffient water to produce 1000 ml

3.4.2. Determination of λmax of Pindolol (PDL) USP

Preparation of stock solution

10mg of pure drug was dissolved in 10ml methanol (primary stock solution - 1000 µg/ml). From this primary stock solution 1 ml was pipette out into 10 ml volumetric flask and made it up to 10ml with the media (Secondary stock solution – 100µg/ml). From secondary stock solution again 1ml was taken it in to another volumetric flask and made it up to 10 ml with media (working solution - 10µg/ml). The working solution was taken for determining the wavelength.

3.4.3.Construction of calibration graph of Pindolol (USP) P^H 7.4

10mg of pure drug was dissolved in 10ml methanol (primary stock solution - 1000 µg/ml). From this primary stock solution 1 ml was pipette out into 10 ml volumetric flask and made it up to 10ml with the media (Secondary stock solution – 100µg/ml). From secondary stock

solution required concentrations were prepared (shown in Table 7.1) and those concentrations absorbance were found out at required wavelength.

3.5. PREFEORMULATION STUDIES

Preformulation testing is an investigation of physical and chemical properties of a drug substance alone and when combined with excipients. It is the first step in the rationale development of dosage form. For any drug substances to formulate into a dosage form, it is necessary to study the physicochemical properties of the drug like solubility studies, melting point determination and drug – excipient compatibility studies were determine.

SOLUBILITY AND MELTING POINT OF METFORMIN HCL

Water solubility-7880 mg/L,Soluble in 0.1 M NaOH (0.2 mg/ml), DMSO (100 mM), methanol, and ethanol (with heating, 25 mM)..Melting point ranges between 171^0C

3.6 DRUG EXCIPIENT COMPATIBILITY STUDY FT- IR

Excipients are integral components of almost all pharmaceutical dosage forms. The successful formulation of a stable and effective solid dosage form depends on the careful selection of the excipients, which are added to facilitate administration, promote the consistent release and bioavailability of the drug and protect it from degradation.

Infra Red spectroscopy is one of the most powerful analytical techniques to identify functional groups of a drug.

DRUG-EXCEPIENTS COMPATIBILITY STUDIES BY I.R.

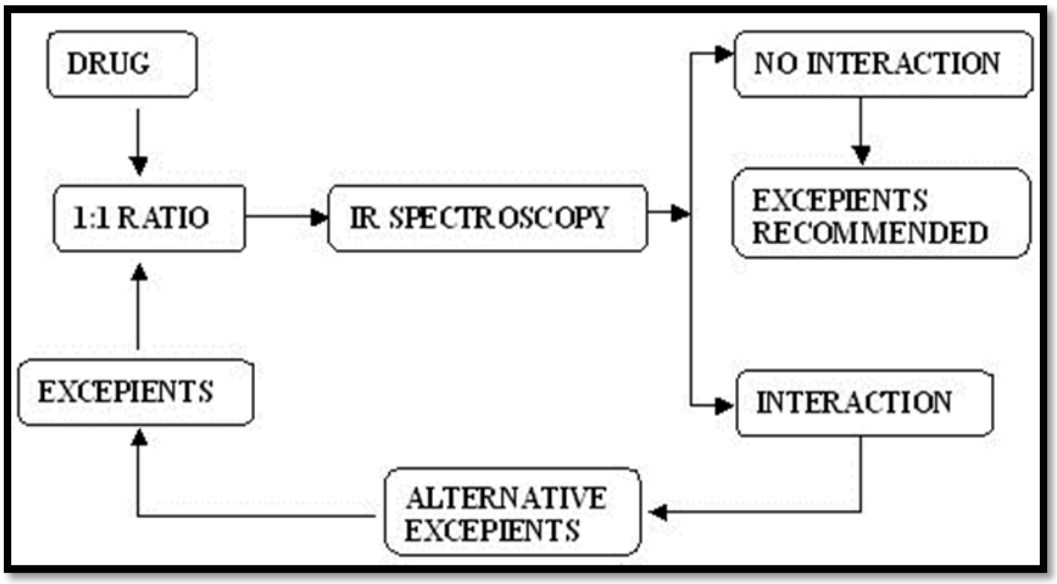


Fig: 3.6.a Schematic representation of compatibility studies

FTIR studies were performed on drug and the optimized formulation using Shimadzu FTIR (Shimadzu Corp., India). The samples were analyzed between wavenumbers 4000 and 400 cm^{-1}.

Method

The pure drug and its formulation were subjected to IR studies. In the present study, the potassium bromide disc (pellet) method was employed.

3.6.1 DIFFERENTIAL SCANNING COLORIMETER OR DSC[62]

It is a thermoanalytical technique in which the difference in the amount of heat required to increase thetemperature of a sample and reference is measured as a function of temperature. Both the sample and reference are maintained at nearly the same temperature throughout the experiment. Generally, the temperature program for a DSC analysis is designed such that the sample holder temperature increases linearly as a function of time. The reference sample should have a well-defined heat capacity over the range of temperatures to be scanned.

Types of DSC

- Power compensated DSC, keeps power supply constant
- Heat flux DSC, keeps heat flux constant

POLYMERS

DSC is used widely for examining polymeric materials to determine their thermal transitions. The observed thermal transitions can be utilized to compare materials, although the transitions do not uniquely identify composition. The composition of unknown materials may be completed using complementary techniques such as IR spectroscopy. Melting points and glass transition temperatures for most polymersare available from standard compilations, and the method can show polymer degradation by the lowering of the expected melting point, T_m,

3.7 FORMULATION DEVELOPMENT

FUSION METHOD/HEAT MOLDING METHOD

Required amount of Drug and Excipient were weighed after calculating the displacement values. For the preparation of suppositories 2gms mould was used which was pre calibrated. The base was melted and drug and required exipients were dispersed in base which was shown in table

METHOD

Moulds

The suppository and pessary moulds are made of metals and have four, six or twelve cavities. By removing a screw, they can be opened longitudinally for lubrication, extraction of the suppositories and cleaning.

Capacity of moulds*: The nominal capacities of the common moulds are 1g, 2g, 4g and 8g.*

Calibration

The nominal capacity of a mould varies with the base selected. Each mould should be calibrated before use by preparing a set of suppositories or pessaries using the base alone, weighing the products and taking the mean weight as the true capacity. This procedure is repeated for each base.

Displacement value

The volume of a suppository from a particular mould is uniform but its weight will differ with the density of the base.

Definition

It is the quantity of the drug that displaces one part of the base. e.g. Zinc oxide, D = 5.

Calculation of displacement value

Formula for calculation of the amount of base required in each mould

$$\text{Amount of base required for each suppository (gm)} = \text{Capacity of each mould (gm)} - \frac{\text{Dose of drug (gm)}}{\text{Displacement value of the drug}}$$

Lubrication of mould

If the cavities are imperfect, i.e. poorly polished or scratched, it may be difficult to remove the suppositories without damaging their surfaces. So lubrication of the moulds is necessary. In case of greasy or oily base water soluble lubricants are required.

For water soluble /miscible bases oily lubricant may be used. e.g. For glycero-gelatin base liquid paraffin or arachis oil may be used as lubricant.

Figure:3.7.a :stainless steel suppository mould with 6 cavity

Table. 3.7.a: composition of rectal suppository of Pindolol(PDL) U.S.P

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Drug (mg)	20	20	20	20	20	20	20	20	20
PEG 4000(%)	100	-	-	95	90	-	-	-	-
PEG 6000 (%)	-	100	-	-	-	95	90	-	-
Polaxomer 188 (%)	-	-	100	-	-	-	-	95	90
CCS (%)	-	-	-	5	10	5	10	5	10
Total weight	2g	2g	1.8	2g	2g	2g	2g	1.8g	1.8g

3.8. Evaluation studies[44]

Subsequently suppositories were made to undergo through some simple tests in order to ascertain their adherence to quality. Visual inspection for fissuring, pitting, fat blooming, exudation, migration of active ingredients; physical features such as length, width, weight variation, hardness (mechanical strength), breaking strength, liquefaction time, melting time, were determined.

Visual characterization[44,45]

Two suppositories from each batch were randomly selected, longitudinally cut and examined through naked eyes for the assessment of physical characters like absence of fissuring, pitting, fat blooming, exudation and migration of active ingredients.[47]

Length and width[43,44]

Two suppositories were selected randomly from each batch, their length and width was measured using micrometer respectively.[48]

Hardness (Breaking strength)[43]

Hardness of suppositorys is defined as the force applied across the diameter of the tablet in order to break the suppository. The resistance of the suppository to chipping, abrasion or

breakage under condition of storage transformation and handling before usage depends on its hardness. For each formulation, the hardness of three tablets was determined using Monsanto hardness tester and the average is calculated.

Figure.3.8.b : Monsanto hardness tester

Weight variation[43,44]

Ten suppositories were weighed and average weight was found out. After that each suppository was weighed individually on electronic balance. Not more than 2 individual suppositories deviate from average by 5%.[49]

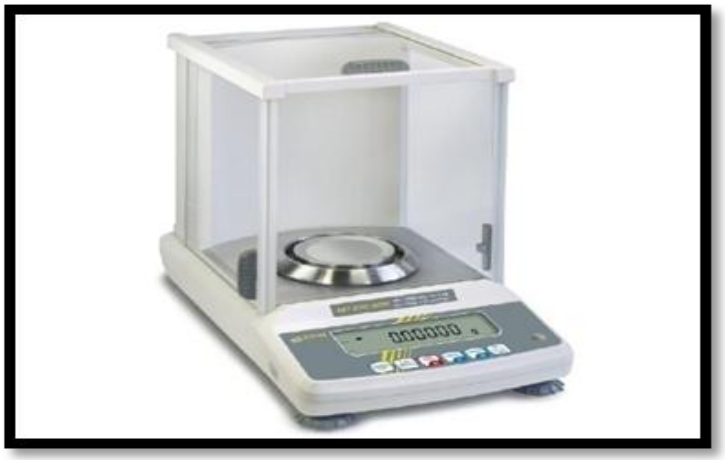


Figure.3.8.c: Digital weighing balance

Friability[43,44]

Ten suppositories were weighed and placed in the chamber of the friabilator. The friabilator was operated at 25 rpm for 4 min. After completion of the cycle the friability is calculated using formula

$$\frac{W_o - W_f}{W_o} \times 100$$

Where W_o is initial weight of six suppositories and W_f is the final weight of suppositories after testing.[50]

Melting point[43]

Macro melting range test is performed with the whole suppository. A suppository from each formulation was placed in a beaker with Phosphate Buffer pH 6.8 maintained at constant temperature 37± 0.5℃. The time required by the whole suppository to melt or disperse in the media was noted. The melting time plays a crucial role in the release of active ingredient.[51,52]

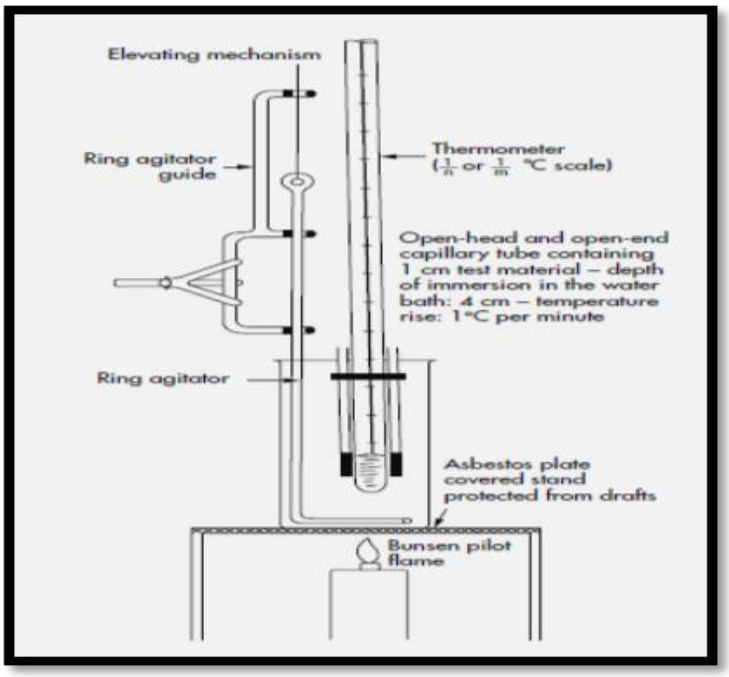


Figure. 3.8.e: Schematic representation of the melting point determination as per USP specifications

Drug Content[42,43,45]

Drug Content was determined spectrophotometrically. Suppositories were melted individually subsequently dissolved in phosphate buffer pH 7.4. After necessary dilutions the solutions were subjected to UV spectroscopy (Lab india UV3000+) at respective wavelength.[53]

Dissolution study[42,43]

Dissolution test was carried out in USP rotating basket dissolution apparatus. Employing the stirrer speed at 100 rpm and Phosphate buffer pH 7.4 as dissolution medium (900ml), at fixed time intervals 5 ml of the aliquot was withdrawn and same quantity was replaced by fresh buffer. The withdrawn samples were spectrophotometrically analysed at respective wavelength on Lab india UV3000+.[54]

3.9. KINETIC ANALYSIS OF DISSOLUTION DATA

Application of Release Rate Kinetics To Dissolution Data

Various models were tested for explaining the kinetics of drug release. To analyze the mechanism of the drug release rate kinetics of the dosage form, the obtained data were fitted into zero-order, first order, Higuchi, and Korsmeyer-Peppas release model.

Zero order release rate kinetics

To study the zero–order release kinetics the release rate data are fitted to the following equation.

$F = K_o t$

Where, 'F' is the drug release at time't', and 'K_o' is the zero order release rate constant. The plot of % drug release versus time is linear.

First order release rate kinetics: The release rate data are fitted to the following equation

Log (100-F) = kt

A plot of log cumulative percent of drug remaining to be released vs. time is plotted then it gives first order release.

Higuchi release model: To study the Higuchi release kinetics, the release rate data were fitted to the following equation.

F = k t1/2

Where, 'k' is the Higuchi constant.

In higuchi model, a plot of % drug release versus square root of time is linear.

Korsmeyer and Peppas release model

The mechanism of drug release was evaluated by plotting the log percentage of drug released versus log time according to Korsmeyer- Peppas equation. The exponent 'n' indicates the mechanism of drug release calculated through the slope of the straight Line.

$M_t/ M_\infty = K t^n$

Where, M_t/ M_∞ is fraction of drug released at time 't', k represents a constant, and 'n' is the diffusional exponent, which characterizes the type of release mechanism during the dissolution process. For non-Fickian release, the value of n falls between 0.5 and 1.0; while in case of Fickian diffusion, n = 0.5; for zero-order release (case I I transport), n=1; and for supercase II transport, n > 1. In this model, a plot of log (M_t/ M_∞) versus log (time) is linear.

The following plots were made using the in-vitro drug release data

Cumulative % drug release vs. time (Zero order kinetic model);

Log cumulative of % drug remaining vs. time (First order kinetic model);

Cumulative % drug release vs. square root of time (Higuchi model);

And cube root of initial concentration minus the cube root of percentage of drug remaining in the matrix vs. time (Hixson-Crowell cube root law).

3.10. STABILITY STUDIES AS PER ICH GUIDELINES

Stability of a drug has been defined as the ability of a particular formulation, in a specific container, to remain within its physical, chemical, therapeutic and toxicological specifications. In any rational design and evaluation of dosage forms for drugs, stability of the active component must be a major criterion in determining their acceptance or rejection**.**

The accelerated stability studies were carried out according to ICH guidelines.

Optimized formulation F_5 was packed in strips of aluminum foil this packed formulation was stored in ICH certified stability chambers (Thermo labs, Mumbai) maintained at 25^oC and 75% RH (zone III conditions as per ICH Q_1 guidelines) for 3 months. The suppositories were evaluated beforeand after one month of stabilization for the drug content, Friability, hardness, disintegration visual characterisation and *in vitro* release.

4. RESULTS AND DISCUSSION

4.1. Analytical method development: Determination of λmax

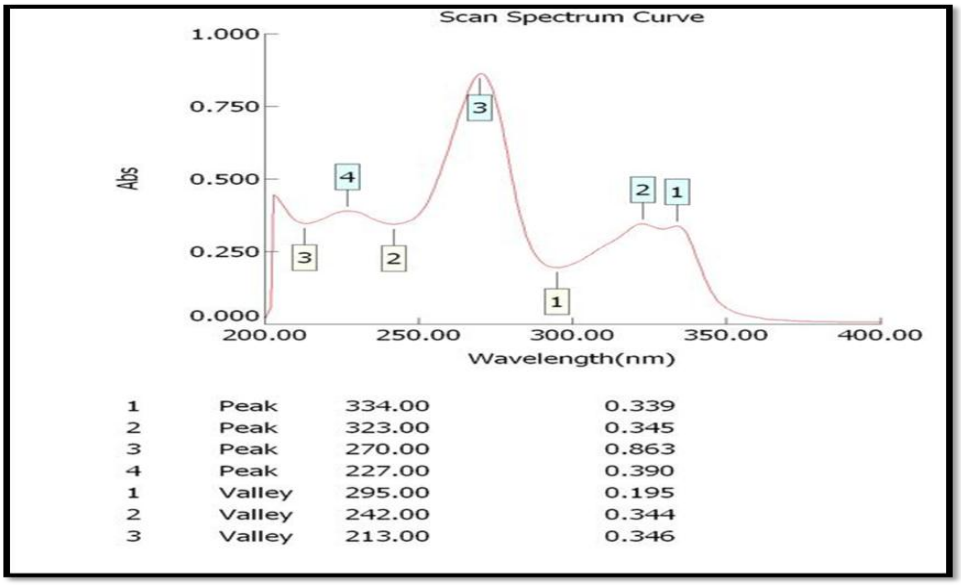


Figure: 4.1.a : Determination of λ_{max} of Pindolol (USP)

PREPARATION OF STANDARD CALIBRATION CURVE OF PINDOLOL IN p^H 7.4 PHOSPHATE BUFFER

Table 4.1.a: Observations for graph of Pindolol in pH 7.4 phosphate buffer

Conc [µg/mL]	Abs
0	0
2	0.188
4	0.384
6	0.523
8	0.733
10	0.896

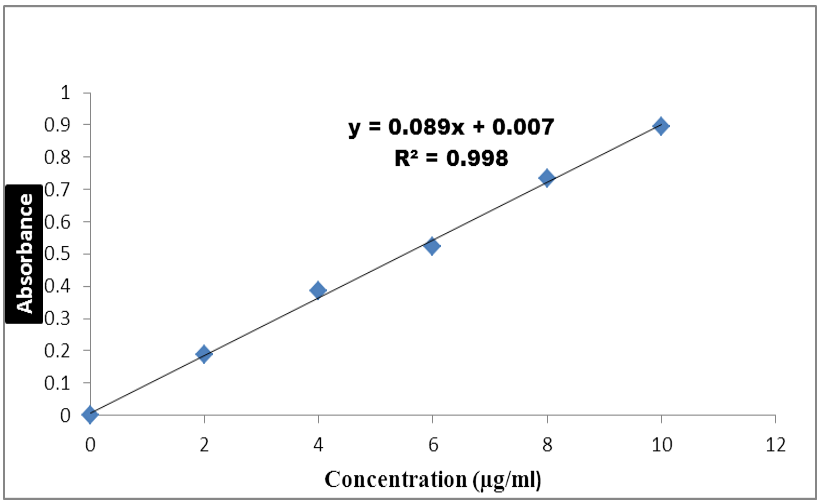


Figure:4.1.b: Standard graph of Pindolol in pH 7.4 phosphate buffer

DISCUSSION

It was found that the estimation of pindolol by UV spectrophotometric method at λ_{max} 227 nm in pH 7.4 phosphate buffer had good reproducibility and this method was used in the study. The correlation coefficient for the standard curve was found to be closer to 1, at the concentration range, 2- 10µg/ml. The regression equation generated was y = 0.089x + 0.007.

DRUG – EXCIPIENT COMPATIBILITY STUDIES BY FT-IR

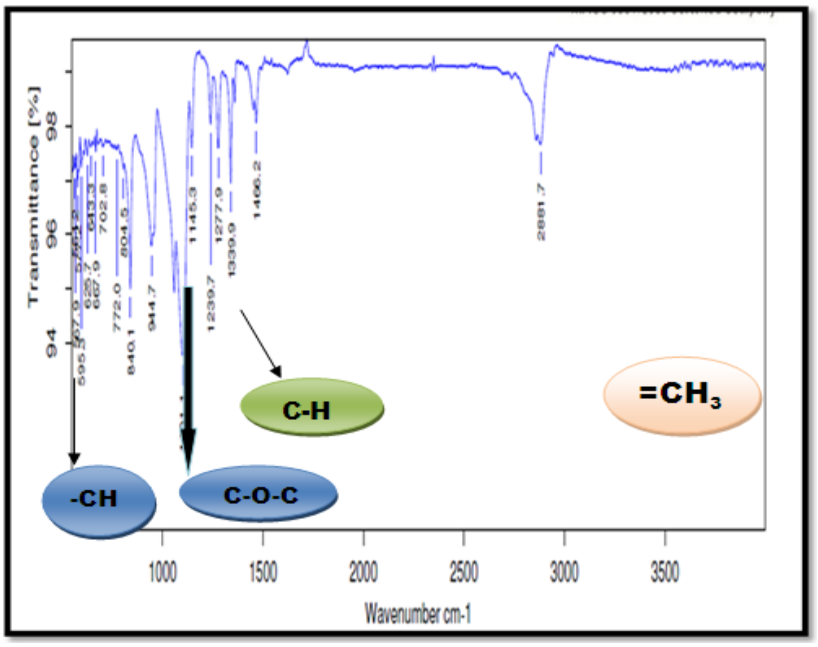


Figure:4.2.a: FT-IR spectrum of pure drug Pindolol (PDL)-USP

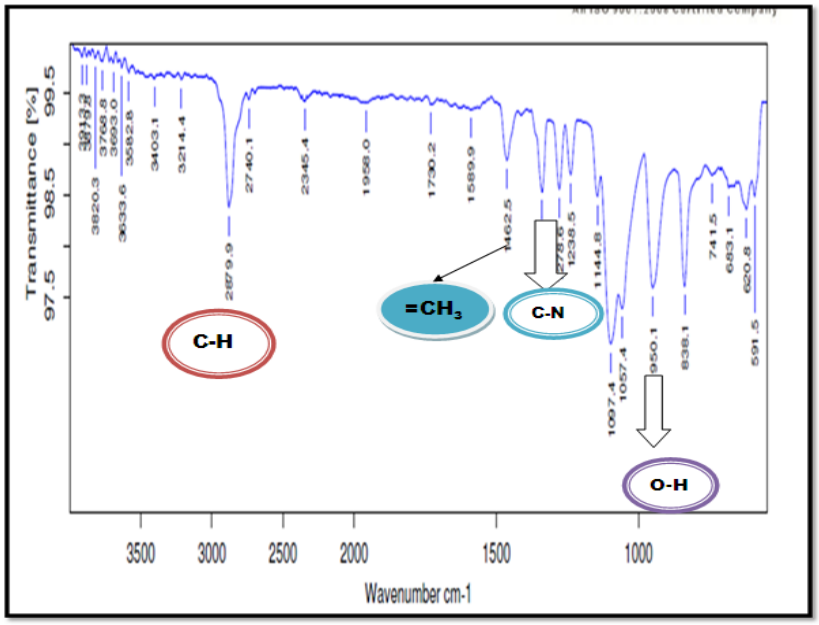


Figure:4.2.b: FT-IR Spectrum of PEG 4000

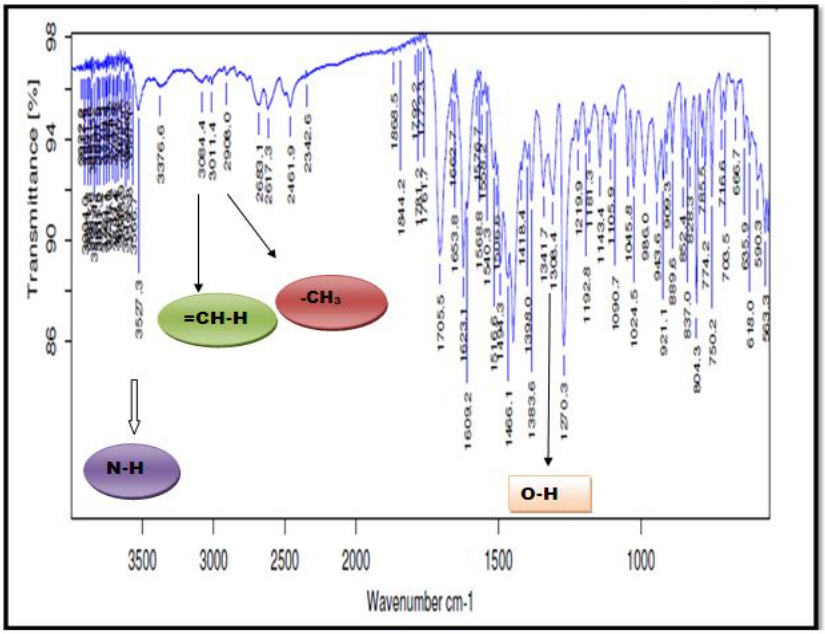


Figure:4.2.c: FT-IR spectrum of optimised formulation of Pindolol suppository containing pure drug Pindolol , PEG 4000 and cross carmallose sodium.

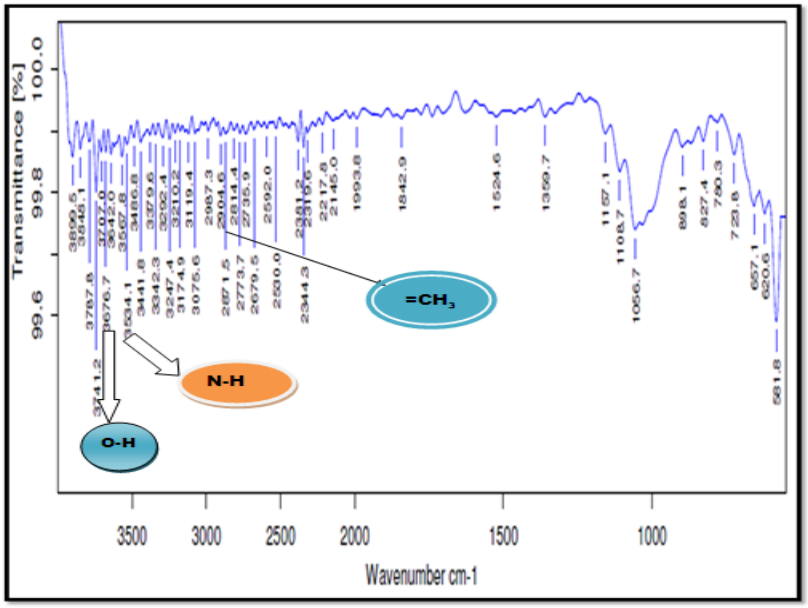


Figure:4.2.d: FT-IR spectrum of Cross carmallose sodium(CCS)

Table: 4.2.a: FT-IR spectrum of pure drug Pindolol(USP)

Wave numbers (cm-1) Pindolol	Functional group
2881.7	CH_3 Stretching
1339.9	C-H Stretching
3345.03	O-H Bending
3056.07	N-H Stretching
772.0	-CH Bending

There is no significant change in the shift of major peaks of in the above graph ,hence there is no drug and excipient interactions was found.

DIFFERENTIAL SCANNING COLORIMETRY

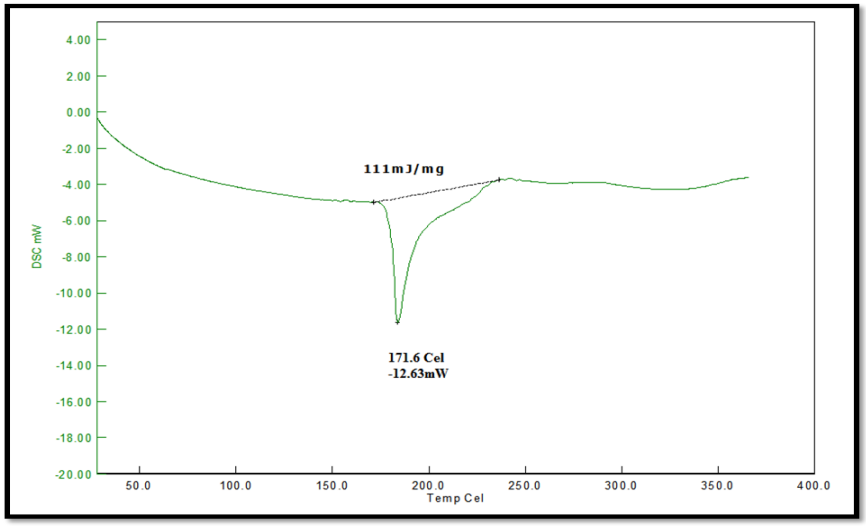


Figure:4.3.a: DIFFERENTIAL SCANNING SPECTRA OF PINDOLOL

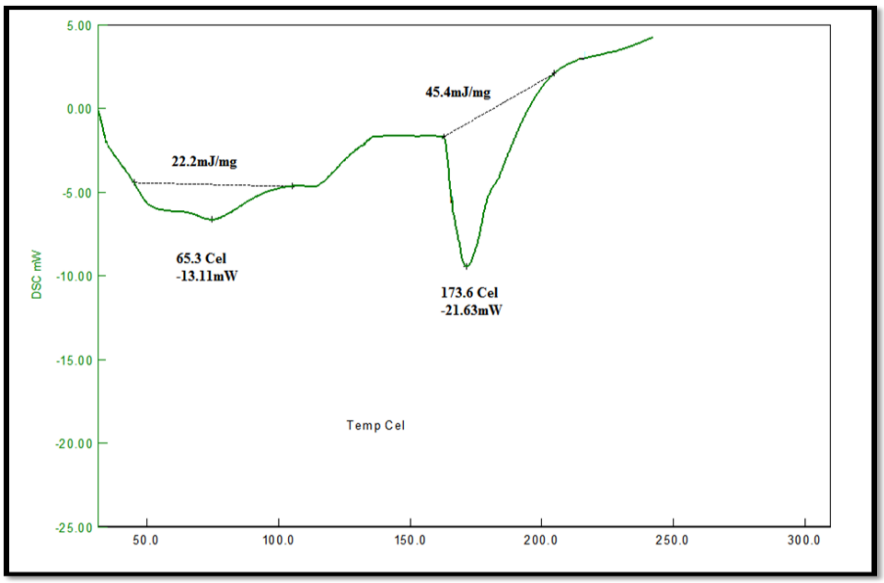


Figure :4.3.b : Differential scanning spectra of optimised formulation F_5 containing PEG 4000 and 10% cross carmallose sodium along with the pure drug

DISCUSSION

The differential scanning spectra of pure drug Pindolol showed differential peaks at 111 mJ/mg and the optimised formulation conyaining PEG 4000 along with the 10% cross carmallose sodium showed different peaks at22.2mJ/mg and 45.4mJ/mg respectively.

4.4 Evaluation of Suppositories

4.4.1.Visual Characterisation

Table 4.4.1.a: Visual characterization of Suppositories

Parameters	F1	F2	F3	F4	F5	F6	F7	F8	F9
Fissuring	No	No	No	No	No	No	No	No	No
Pitting	No	No	No	No	No	No	No	No	No
Fat blooming	No	No	No	No	No	No	No	No	No
Exudation	No	No	No	No	No	No	No	No	No
Migration of active ingredient	No	No	No	No	No	No	No	No	No

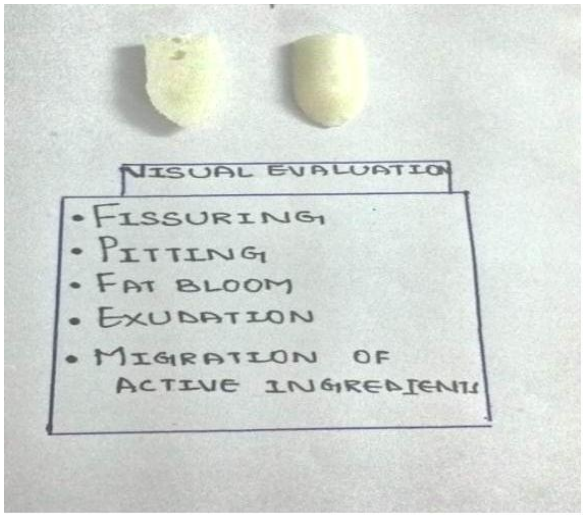


Figure:4.4.1.a: Visual characterization of F4 formulation

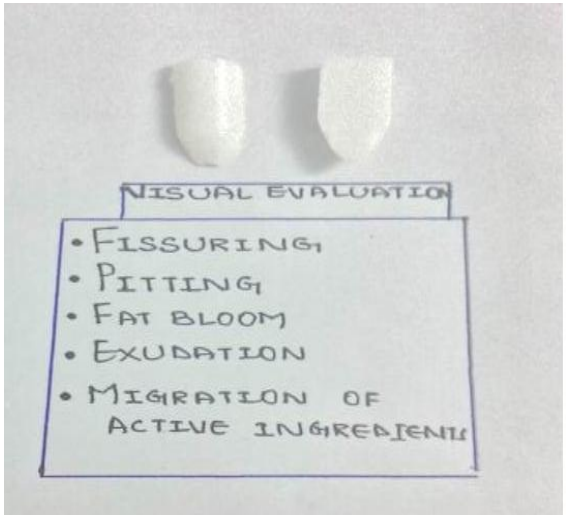


Figure :4.4.1.b: Visual characterization of F7 formulation

Figure:4.4.1.c: Visual characterization of F3 formulation

4.4.2. Physico-chemical evaluation of Suppositories.

Table:4.4.2.a: Physico-chemical evaluations of Suppositories

Formulation codes	Weight variation(g)	Hardness (kg/cm2)	Friability (%loss)	Length (cm)	Width (cm)
F1	1.91	3.4	0.52	1.2	2.5
F2	1.97	3.3	0.54	1..3	2.4
F3	1.83	4.5	0.51	1.1	2.5
F4	1.85	3.4	0.55	1.2	2.5
F5	2.00	3.0	0.56	1.5	2.4
F6	1.85	3.2	0.45	1.4	2.5
F7	1.84	3.1	0.51	1.1	2.6
F8	1.87	3.3	0.49	1.4	2.5
F9	1.88	3.5	0.55	1.2	2.5

4.4.2.1 Weight variation

Suppositories of each batch were subjected to weight variation test, difference in weight was calculated for each tablet and was shown in the Table 7.3. The average weight of the suppository is approximately in range of 1.83 to 2.00 gms.

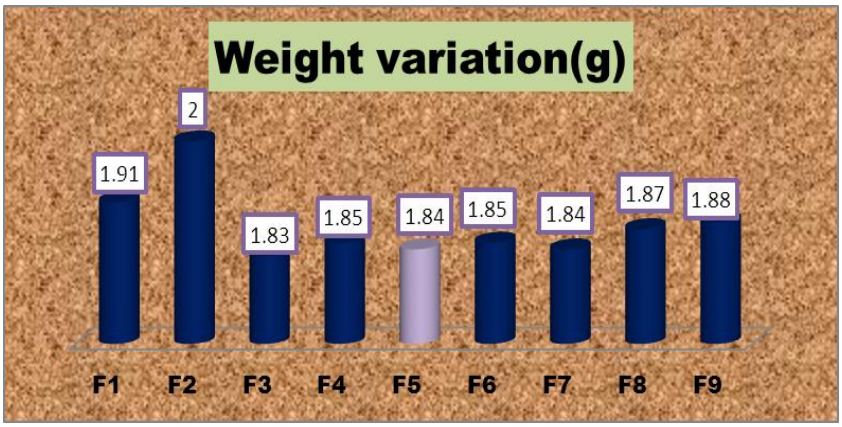


Figure:4.4.2.2: comparison of weight variation of all the formulation $F_1 – F_9$

4.4.2.2Hardness

Hardness of the suppositories of each batch was checked using Monsanto hardness tester and the data were shown in Table 4.4.2a. The results showed that the hardness of the suppositories is in range of 3.1 to 3.5 kg/cm2

4.4.2.3 Friability

Suppositories of each batch were evaluated for percentage friability and the data were shown in the Table 7.3. The average friability of all the formulations lies in the range of 0.45 to 0.55% which was less than 1% as per official requirement of IP indicating a good mechanical resistance of suppositories.

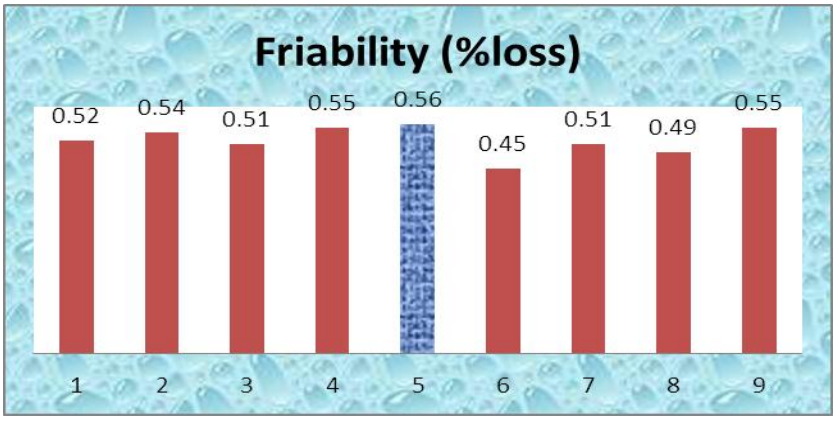


Figure:4.4.2.3: comparison of friability of all the formulation $F_1 – F_9$

4.4.2.4 Length and Width

Suppositories of each batch were evaluated for Length and width and the data were shown in the Table 7.3. The average length and width of all the formulations found to be between 1.4 to 1.6 cm and 2.4 to 2.6 cm.

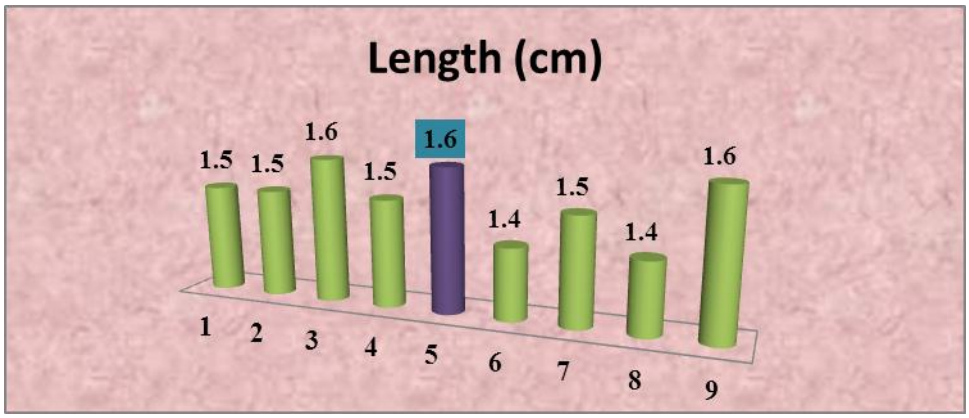


Figure:4.4.2.4: comparison of length of all the formulation $F_1 – F_9$

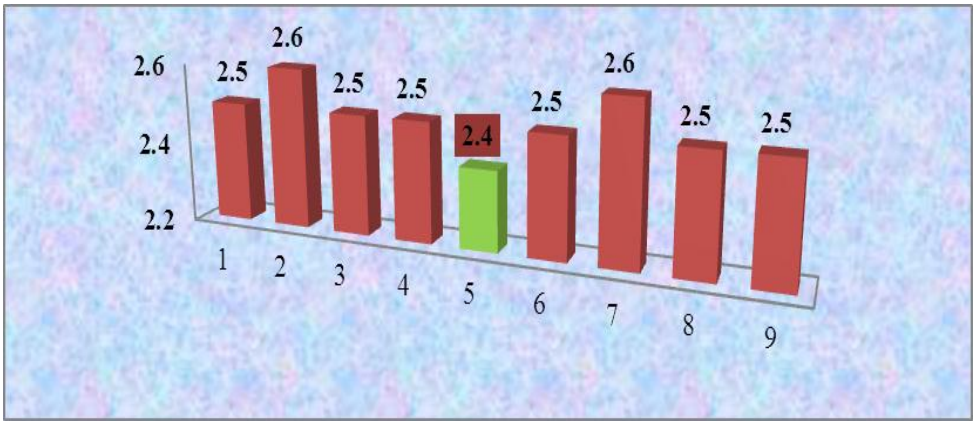


Figure:4.4.2.5: comparison of width of all the formulation $F_1 – F_9$

Table 4.4.2.b: Physico-chemical evaluations of Suppositories

Formulation codes	Drug content (%)	Disintegration Time (min)	Melting Time (Min)
F1	99.76	16	15
F2	97.45	20	18
F3	98.34	28	25
F4	99.87	28	26
F5	**99.14**	**15**	**14**
F6	97.56	19	16
F7	98.42	18	15
F8	99.65	34	30
F9	99.12	36	32

4.4.2.5 Drug content

Suppositories of each batch were evaluated for drug content and the data were shown in the Table **4.4.2.b**. The drug content of all formulations was found to be 97.45 to 99.87%.

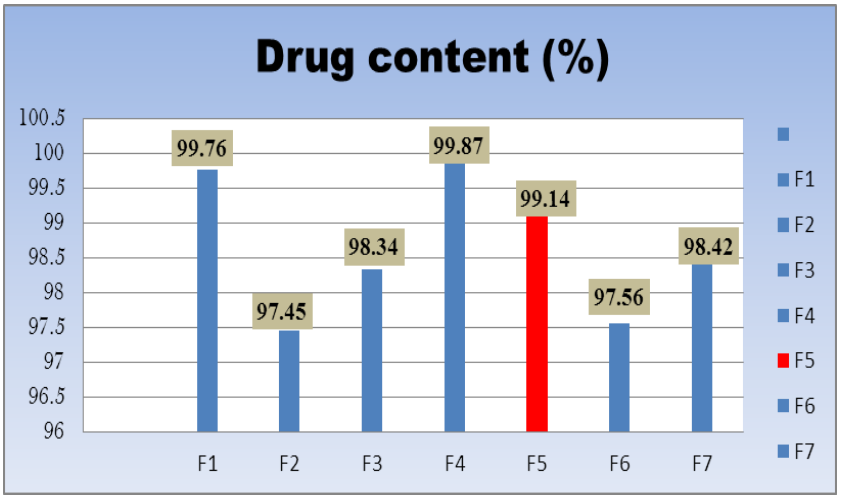
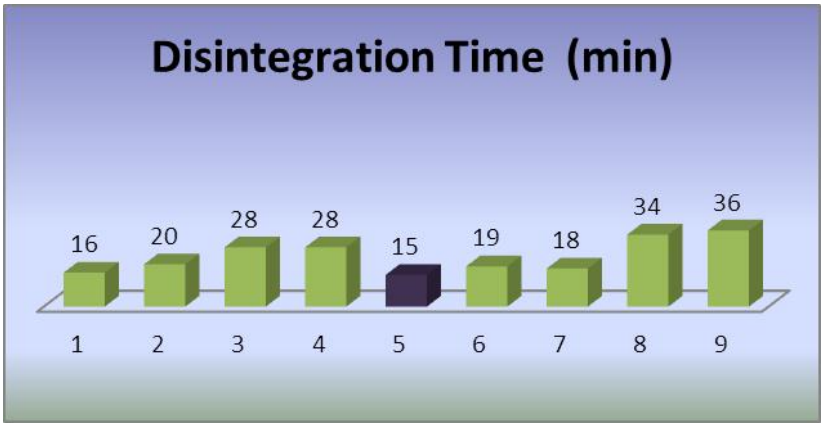


Figure: 4.4.2.5 comparison of drug content of all the formulation $F_1 – F_7$

4.4.2.6. Disintegration time

Suppositories of each batch were evaluated for disintegration time and the data were shown in the Table 4.4.2.b. The disintegration time of all formulations were found to be 15 to 36 sec.



4.4.2.7. Melting time

Suppositories of each batch were evaluated for melting time and the data were shown in the Table 7.4. The melting time of all formulations were found to be 14 to 32 sec.

4.4.2.8.Invitro dissolution study

Dissolution conditions

Medium - pH 7.4 phosphate buffer

Volume - 900 ml

RPM - 50

Apparatus - USP II Paddle

Temperature - 37.0 ℃ ± 0.5℃

Time intervals - 180 Min

Table :4.4.2.8.a : invitro drug release

Time (Min)	F1	F2	F3	F4	F5	F6	F7	F8	F9
15	20.14	16.32	8.69	26.47	32.69	18.39	22.63	10.88	14.38
30	32.18	28.33	15.22	34.48	56.38	31.66	40.51	19.36	28.21
60	48.36	36.47	21.68	52.69	64.82	49.63	52.36	28.25	41.26
90	61.38	51.20	36.91	74.82	79.42	61.44	68.92	39.42	59.66
120	79.28	63.39	44.29	89.18	96.72	76.27	79.98	53.64	69.12
180	86.44	78.62	59.31	98.26	99.14	95.32	96.33	63.47	82.14

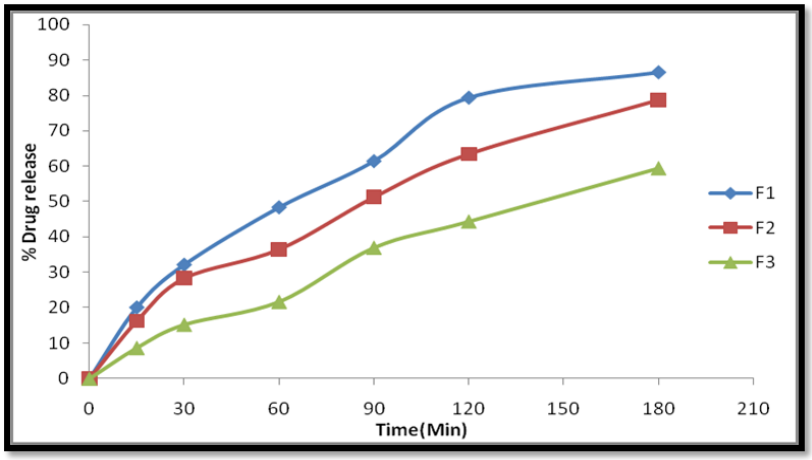


Figure 4.4.2.8.a Drug release study of F1, F2, F3 formulations containing only drug and base.

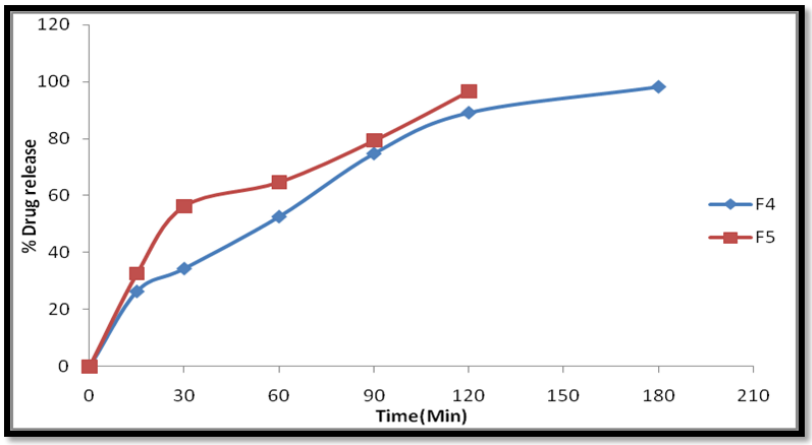


Figure 4.4.2.8.b Drug release study of F4, F5 formulations containing Drug and PEG 4000 along with CCS

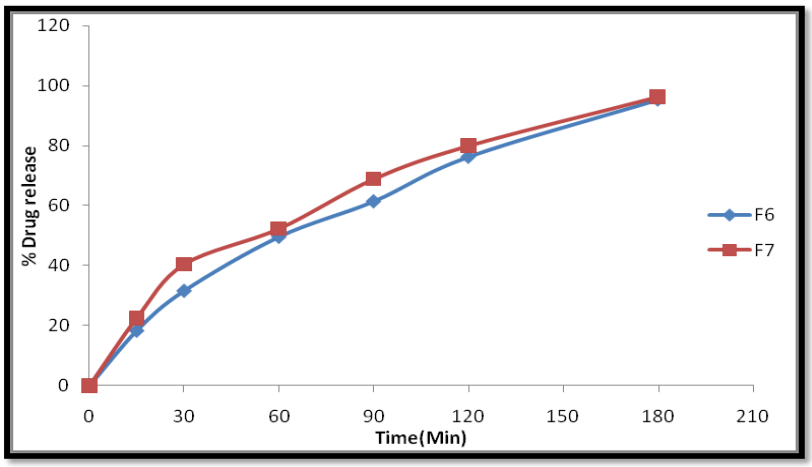


Figure 4.4.2.8.c Drug release study of F6, F7 formulations containing Drug and PEG 6000 along with CCS.

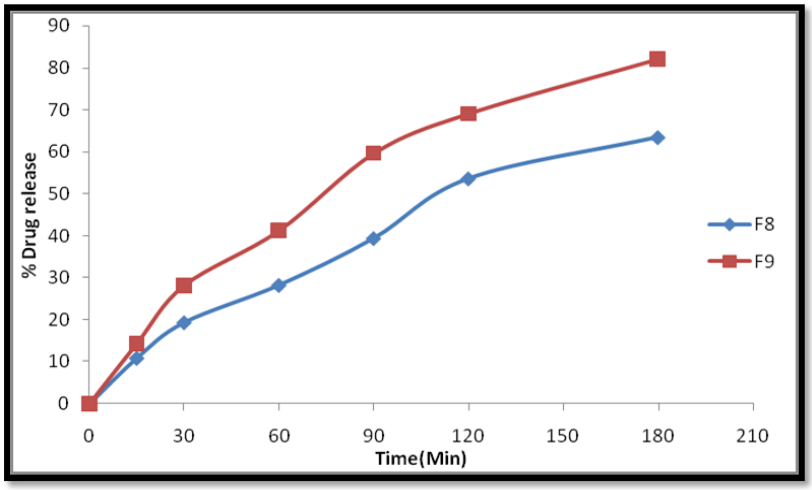


Figure 4.4.2.8.d Drug release study of F8, F9 formulations containing Drug and Polaxomer along with CCS.

From the table: 4.4.2.8.a, invitro drug release studies revealed that the formulations prepared without super disintegrant did not show maximum drug release with in 180min. When formulations prepared with super disintegrant showed good drug release compared with formulations without super disintegrant (F1, F2, F3).

Formulations F8 and F9 containing base (polaxomer) along with CCS5% and 10% did not show good release within 180 min hence those formulations did not take into considerations.

Formulations F6 and F7 containing PEG 6000 along with CCS 5% and 10% showed good drug release at 180 min. When increase the CCS concentration in these formulations shown maximum release at 180 min only.

Formulations F4 and F5 containing PEG 4000 along with CCS 5% and 10% showed good drug release. F4 formulation was shown maximum drug release at 180 min. F5 formulation was shown maximum drug release at 120 min. It was revealed that increase in the concentration of CCS reduce the time for drug release.

Among all formulations F5 formulation was considered as optimised formulation.

4.4.2.9..KINETIC ANALYSIS OF DISSOLUTION DATA OF OPTIMISED FORMULATION OF PINDOLOL SUPPOSITORY

Table 4.4.2.9.a: Drug release kinetics data

Kinetics	F1	F2	F3	F4	F5	F6	F7	F8	F9
Zero Order	0.963	0.976	0.990	0.961	0.883	0.977	0.951	0.990	0.976
First Order	0.113	0.115	0.106	0.065	0.420	0.069	0.080	0.118	0.110
Kars mayer peppas	0.457	0.445	0.370	0.472	0.596	0.443	0.472	0.401	0.423
Higuchi	0.985	0.968	0.923	0.984	0.984	0.973	0.988	0.952	0.975

	ZERO	FIRST	HIGUCHI	PEPPAS
	% CDR Vs T	Log % Remain Vs T	%CDR Vs √T	Log C Vs Log T
Slope	0.5005	-0.011	7.7156	0.888
Intercept	25.92	2.09	5.196	0.2054
R 2	0.808	0.948	0.966	0.925

DISCUSSION

From the release kinetics data, Optimised formulation F5 was following Higuchi release kinetics.it follows non –fickian diffusion with supercase II transport mechanism.

GRAPHS INDICATINGDRUG RELEASE KINETICS OF OPTIMISED PINDOLOL SUPPOSITORY

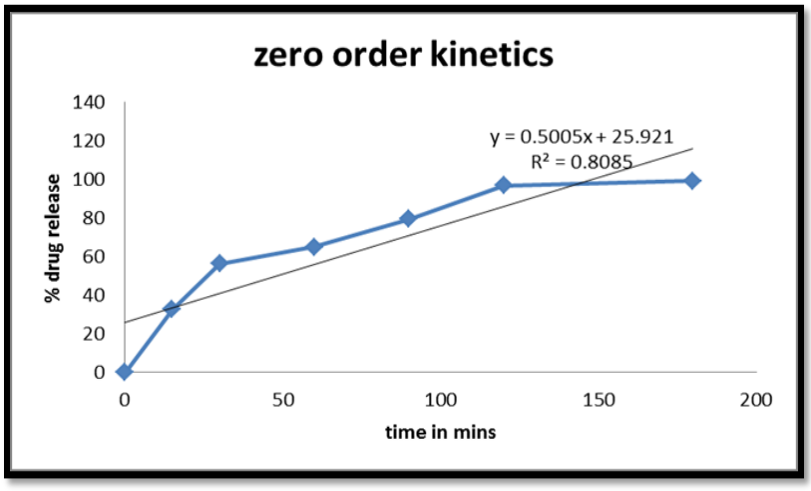


Figure 4.4.2.89.a: zero order kinetics

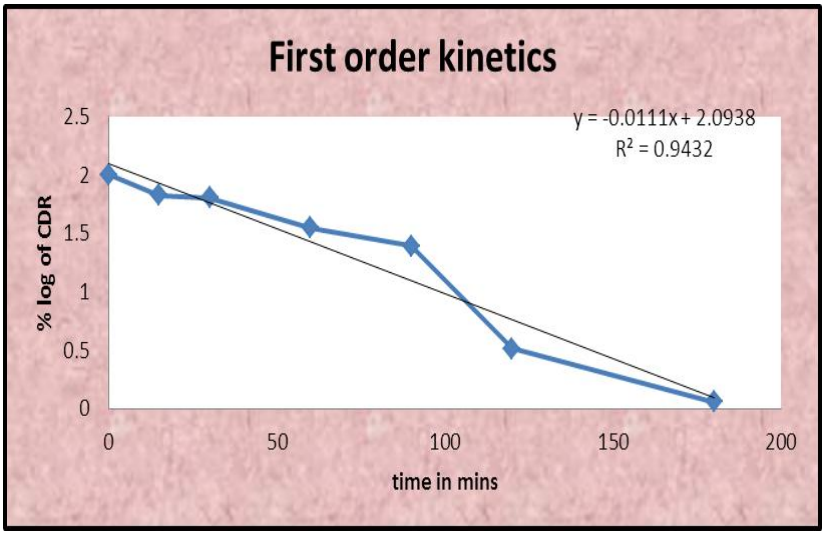


Figure. 4.4.2.9.b. First order kinetics

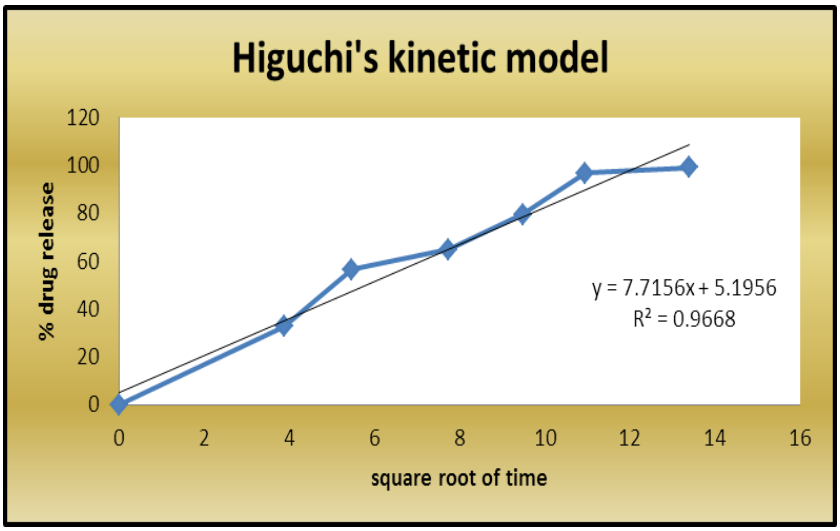


Figure: 4.4.2.9.c: Higuchi kinetics

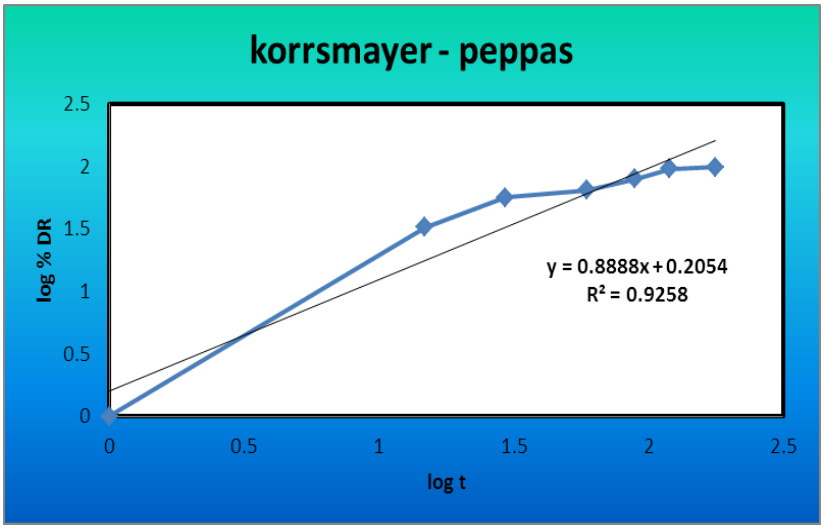


Figure: 4.4.2.9.d: Korrsmayers - peppas model

4.4.2.10. STABILITY STUDIES OF OPTIMISED PINDOLOL SUPPOSITORY

Table: 4.4.2.10: stability studies of optimised pindolol suppository as per ICH guidelines.

Time	Colour	Assay.		Cumulative % drug release at 3 hrs	
		25±2^{0}c and 65±5%RH	40±2^{0}c and 75±5%RH	25±2^{0}c and 65±5%RH	40±2^{0}c and 75±5%RH
First day	White	99.13	99.12	99.25	98.09
30 days	White	99.12	99.12	98.67	98.10
60 days	White	99.12	99.14	98.38	98.13
90 days	White	99.14	98.14	98.11	99.14

DISCUSSION

Results from stability studies indicate that the formulated pindolol suppository are stable for a period of 3 months under 2 different conditions 25±2^{0}c and 65±5%RH , 40±2^{0}c and 75±5%RH. There were no remarkable changes observed during the period of storage.

5. SUMMARY AND CONCLUSION

The present study was carried out on pindolol suppositories for treating the anti hypertension. Pindolol is a non-selective beta adrenergic receptor blocker that is widely used for the therapy of hypertension and angina pectoris. It belongs to BCS Class I which is highly soluble and permeable. The present formulations were developed to prevent the first pass metabolism thereby increase the bioavailability.

Pindolol wavelength was determined in pH 7.4 phosphate buffer using UV-Visible spectrophotometer. After developing the method, calibration curve was plotted. R^2 Value was found to be 0.998 which obeyed beer lamberts law.

Pindolol suppositories were developed with various bases such as PEG 4000, PEG 6000, Polaxomer. F1, F2 and F3 formulations containing only bases. F4 - F9 formulations containing bases along with the super disintegrant (CCS).

Visual characterisation for suppositories were shown no fissuring, pitting, Fat blooming, Exudation, Migration of Active ingredients.

Weight variation test was shown the average weight of the tablet is approximately in range of 1.83 to 2.00 gms.

Hardness of the suppositories of each batch was checked using Monsanto hardness tester and the results showed that the hardness of the suppositories is in range of 3.1 to 3.5 kg/cm^2

Suppositories of each batch were evaluated for percentage friability and the data was less than 1% as per official requirement of IP indicating a good mechanical resistance of suppositories.

Suppositories of each batch were evaluated for Length and width and the results were shown found to be between 1.4 to 1.6 cm and 2.4 to 2.6 cm.

Suppositories of each batch were evaluated for drug content and the data was found to be 97.45 to 99.87%.

Suppositories of each batch were evaluated for disintegration time and the results were found to be 15 to 36 sec.

Suppositories of each batch were evaluated for melting time and results were found to be 14 to 32 sec.

Invitro drug release studies revealed that the formulations prepared without super disintegrant did not show maximum drug release with in 180min. Formulations F8 and F9 containing base (polaxomer) along with CCS5% and 10% did not show good release within 180 min hence those formulations did not take into considerations.

Formulations F6 and F7 containing PEG 6000 along with CCS 5% and 10% showed good drug release at 180 min. When increase the CCS concentration in these formulations shown maximum release at 180 min only.

Formulations F4 and F5 containing PEG 4000 along with CCS 5% and 10% showed good drug release. F4 formulation was shown maximum drug release at 180 min. F5 formulation was shown maximum drug release at 120 min. It was revealed that increase in the concentration of CCS reduce the time for drug release. Among all formulations F5 formulation was considered as optimised formulation.

FUTURE SCOPE

- The scope of the present study is that it can be further carried out for the invivo study.
- Analytically it can studies by various method like HPLC

6. REFERENCES

1. https://en.wikipedia.org/wiki/Antihypertensive_drug
2. Law M, Wald N, Morris J; Wald; Morris (2003)."Lowering blood pressure to prevent myocardial infarction and stroke: a new preventive strategy"(PDF). *Health Technol Assess.*, 7(31): 1–94. doi:10.3310/hta7310. PMID 14604498.
3. Nelson, Mark. "Drug treatment of elevated blood pressure". *Australian Prescriber* (33): 108–112. Archived from the original on 26 August 2010. Retrieved August 11, 2010.
4. Nelson MR; McNeil JJ; Peeters A et al. (Jun 4, 2001). "PBS/RPBS cost implications of trends and guideline recommendations in the pharmacological management of hypertension in Australia, 1994–1998". *Med J Aust.*, 174(11): 565–8.PMID 11453328.
5. N.K.Jain, "Progress in controlled and Novel drug delivery system" Ist edition, CBS Publication, 2008; 96-118.
6. Boylan J. C., Swarbrick J., "Encyclopaedia of pharmaceutical technology" Vol-I, 2nd edition, 2009; 32- 940.
7. Nishihata T., Kate J., Kobayaahi M., Kamada A., "Formation and hydrolysis of enamine in aqueous Solution", Chem. Pharm. Bull., 1984; 32: 4545-4550.
8. Okamura Y., Knmada A., Higuchi T., Yagi T., "Enhanced bioavailability of insulin after rectal administration with emetine as adjuvant in depans cratered dogs". J. Pharmacol., 1985; 37: 22-36.
9. Nishihata T., Rytting J.H., Higuchi T. L., Selk S. J.,"Enhancement of rectal absorption of water soluble antibiotics in dogs", int. J. Pharm., 1984; 21: 239-245.
10. Muranishi S., "Modification of intestinal absorption of drugs by lipoid adjutants", Pharm. Res., 1985; 2: 108-I 18.
11. Kanamoto I, Nakagawa T, Horikoshi I, "Pharmacokinetics of two rectal dosage forms of ketoprofen in patients after anal surgery", J Pharmaco biodyn, 1988; 11: 141- 145.
12. Abd-el-maeboud K.H., El-naggar T, El-hawi em, Mahmoud SA, "Rectal suppositories: commonsense and mode of insertion", Lancet., 1991; 338: 798-800
13. D'haens G, Breysem Y, Rutgeerts P, Van besien B, "Proctitis and rectal steno sis induced by nonsteroidal anti-inflammatory suppositories" J Clin Gastroenterol, 1993; 17: 207-212.
14. Rejman F. "Use of Roinal suppositories in patients with ano rectal complaints Duodecim", 1962; 78: 727-729.

15. De Boer A.G., Hoogdalem E.J., Breimer D.D., "Rate controlled rectal peptide absorption enhancement, In Penetration enhancement for polypeptides through epithelia", Adv. Drug Delivery Reviews, 1992; 8: 237-253.
16. Vim Hoogdalem E.J., De Boer A.G., Bremer D. D.,"Pharmacokinetics of rectal drug administration, part I general considerations and clinical applications of centrally acting drugs". Clin. Pharmacokinet, 2006; 21(I): I l-26.
17. Vermani, K. & Garg S., The scope of potential of vaginal drug delivery. Research Focus. 2000; 3: 359-64.
18. Hussain A. & Ahsan F., The vagina as a route of systemic drug delivery, J Controlled Release, 2005; 103: 301-13.
19. Alexander N.J., Banker E., Kaptein M., Karck U., Miller L., & Zampaglione E., Why consider vaginal drug administration?, Fertility and Sterility., 2004; 82: 1-12
20. Woolfson A.D., Melcolm R.K. & Gallagher R., Drug delivery by the intravaginal route, Crit. Rev. Ther. Drug Carr. Syst, 2000; 17: 509-55.
21. Washington N., Washington C. & Wilson C.G., Vaginal and intrauterine drug delivery in Physical pharmaceutics: barriers to drugabsorption, (N. Washington, C.Washington & C.G. Wilson, eds.) Taylor and Francis, Landon, 2001; 271-81.
22. Richter K. & Frick H., Anatomy of visceral fascia of the pelvis from the didactical viewpoint (In German), Geburtshilfe frauenheilkd, 1985; 45: 282-87.
23. Funt M.I., Thompson J.D. & Brich H., Normal vaginal axis, South Med. J., 1978, 71: 1534-35.
24. Richardson J.L. & Illum L., Preparation enhancement for polypeptides through epithelia, Adv. Drug Deliv., 1992; 8: 341-66.
25. Platzorer W., poisel W. & hafez E.S.E.,Functional anatomy of human vagina, in Human reproductive medicine: The human vagina,(E.S.E. Hafez, T.N. Evans, eds.) Nath Wolland Publishing, 1978; New York, 39-54.