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INSULIN NANOPARTICLES LOADED SUPPOSITORES INTENDED FOR THE SYSTEMIC DRUG DELIVERY

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ABSTRACT

The main objective of the present work was to develop colon targeted insulin nanoparticle loaded suppositories intended for systemic delivery of the active. This delivery provides sustained release delivery of the drugs through the rectal route. Nanoparticles based on PLGA were loaded with insulin using w/o/w solvent evaporation technique. The prepared nanoparticles were characterized for various in vitro properties. Particle size and charge were measured using zeta sizer and SEM. Integrity of the drug at the end of formulation development was determined using FTIR, DSC and XRPD. The duration of drug release was determined using in vitro release testing methods. After selecting suitable nanoparticle formulation, they were incorporated into cocoa butter as suppositories. The drug release was also determined at the end of formulation development and a suitable formulation was proposed. The results demonstrated that sustained released of insulin was observed over one week with improved stability of insulin. These suppository formulations loaded with insulin nanoparticles are intended for colon delivery so as to achieve systemic levels of insulin as an alternate route of delivery of this drug.

KEYWORDS: Colon drug delivery, insulin, lyophilization, suppository.

INTRODUCTION

For many a years the treatment of an acute disease or a chronic disease has been mostly accomplished by the delivery of drugs using various dosage forms such as tablet, capsules, pills, suppositories, ointments, liquids, aerosols, and injectables. All these are the conventional drug delivery systems. These systems are the primary pharmaceutical products

commonly seen in the prescriptions and they will be available as over the counter. The release of this drug delivery system shows significant fluctuation in drug levels in the body. To avoid these several technical advancements have resulted in the development of new techniques for drug delivery. These techniques are capable of controlling the rate of drug delivery, sustaining the duration of therapeutic activity and the most important one targeting the delivery of drug to a tissue. These are known as novel drug delivery systems and they have revolutionized the method of medication which provides a number of therapeutic benefits.[1]

Advantages of novel drug delivery systems: These systems: Improves the therapy by increasing the duration of action and reducing the side effects. Increases the patient compliance and provides convenient route of administration. Achieve the targeting of drugs to a specific site which reduces the unwanted side effects and obtain maximum efficacy. Reduces the dose and thus reduces the side effects of drugs.

Types of novel drug delivery systems: There are number of novel drug delivery systems are available.[3] They are: a). Hydrogels, b). Colloidal drug carrier systems: Micelles Microspheres Nanoparticles Liposomes and neosomes, c) Mucoadhesives, d). Transdermal drug elivery, e). Ocular drug delivery f). Nasal drug delivery.

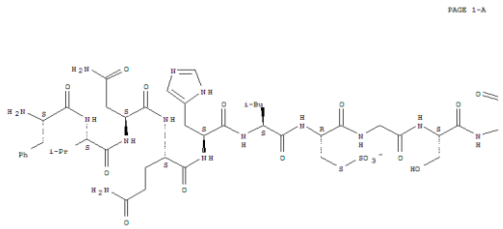
Materials And Methods: Proposed methodology: Preparation of nanoparticles and freeze drying by solvent evaporation method, Preparation of nanoparticles loaded suppositories by mould method.

Evaluation: Characterization of formulation: a). particle size analysis: by SEM (scanning electron method) b). X-ray powder diffraction method (XRPD) c). Zeta potential d)FT-IR e). DSC (differential scanning calorimetry), f). In vitro release studies.

List of materials: Insulin Human insulin, PLGA-MSN Laboratories Hyderabad, India, Chitosan- Sea food Cochin, Kerala, Dichloromethane-SD Fine chemical limited, Mumbai, Poly vinyl alcohol- SD Fine chemical limited, Mumbai, Cocoa butter- SD Fine chemical limited, Mumbai, Sodium chloride- MSN laboratories ltd. Hyderabad, Sodium di hydrogen phosphate- MSN laboratories lmtd Hyderabad, Di sodium hydrogen phosphate- MSN laboratories lmtd Hyderabad.

List Of Equipments Or Instruments: Digital balance, Probe soncator, Magnetic stirrers, Centrifuge, Freeze dryer, UV spectrophotometer, SEM, FTIR, DSC, XRPD, Zeta Potential, Dissolution Tester.

DRUG PROFILE: INSULIN: is 51 residue peptide hormone composed of two amino acid chain covalently linked by disulfide bounds. Insulin human produced by recombinant technology in sacchoromyces cerevecisiae produces insulin for human.



Mechanism of Action: the primary activity of insulin regulation of glucose metabolism. insulin promotes glucose and amino acid up take into muscle and adipose tissue and other expect brain and livers. it also this as antibiotic role in glycogen, fatty acid and protein insulin inhibits gluconeogenesis in the liver. Insulin binds to the receptor, a heterotetrameric protein consisting of two extra cellular alpha units and two trans membranes beta units. The binding insulin to the alpha submit of IR stimulates the tyrosin kinase activity intrinsic to the beta sub unit of the receptor. The bounds receptors is able to autophosphorylate and phosphorylate numerous intracellular substrate such as insulin receptor substrate (IRS) proteins.

Excipients Profile: Excipients used in the formulation are: PLGA, Chitosan, Dichloromethane, Poly vinyl alcohol, Cocoa butter.

Preformulstion Studies: Pre-formulation testing is the first step in the rational development of dosage forms of a drug. It can be defined as an investigation of Physical and chemical properties of drug substance, alone and when combined with excipients. The overall objective of pre-formulation testing is to generate information useful to the formulator in developing stable and bio available dosage forms, which can be produced at large scale. A thorough understanding of physic -chemical properties may ultimately provide a rationale for formulation design or support the need for molecular modification or merely confirm that there are no significant barriers to the compounds development. The goals of the program therefore are.

1. To establish the necessary physic -chemical characteristics of a new drug substance.
2. To determine its kinetic release rate profile.
3. To establish its compatibility with different excipients.

Hence, pre-formulation studies on the obtained sample of drug include physical tests and compatibility studies.

Analytical Method Development

Preparation of standard solution of insulin: Procedure: Accurately measured 1ml of insulin was dissolved in 100 mL of water (Conc. 0.014 µg/mL). From this solution, 1 mL, 2Ml, 3Ml, 4Ml, 5mL was pipetted out into 10 mL volumetric flask and volume was made up to with water (Conc. 1 ng/mL). And after dilution absorbe the standard.

Determination of absorption maxima (λ_{max}) for insulin: The solution containing 0.014 µg /mL insulin was scanned over the range of 200 to 400 nm against suitable blank using double beam UV spectrophotometer. The maximum obtained in the graph was considered as λ_{max} for the pure drug.

Proposed Methodology Of Nanoparticles: Preparation Of Nanoparticles**:** Insulin nanoparticles were prepared by solvent- evaporation method.

Media-1: Required quantity of insulin and polymer was dissolved in 10 ml of Dichloromethane.

Media-2: 1% Pva solution was taken in another beaker. Dichloromethane was used as solvent and polymer was used as carrier of drug. Media 1 and media 2 was emulsified by using probe sonicator. The emulsion was stirred on magnetic stirrer plate to allow organic solvent to be evaporated. The nanoprticle suspension was centrifuged at 6000rpm for 10minute. The precipitated nanopaticles were washed three times with distilled water to remove residual PVA. The obtained nanoparticles were centrifuged at 6000rpm for 10minute to remove aggregated nanoparticles and the supernatants were collected. The nanoparticles were freeze dried at -50°c by using freeze dryer.

Characterization Of Nanoparticle Loaded Suppositories

Particle size and zeta potential: The size, size distribution and zeta potential of the nanoparticles were analyzed by Zeta sizer(ZS 90 malvern). During analysis of size these

samples were first kept in an another clean cubet and put it on to the zeta size analysis chamber to get various peak and next to find its average zeta size. On for analysis surface charge potential or zeta potential samples were kept into the zeta sizer analysis chamber observe for its peak to get an data of zeta potential. During analysis of these data monodisperse nature are always took in to consideration rather than polydisperse character.

Drug Entrapment Efficiency: The nanoparticle were evaluated for percentage drug entrapment as follows. The nanoparticle percentage drug entrapment was calculated by observing the percentage of un entrapment freely present in supernatant nanoparticulate suspension after centrifuging using a remi centrifuge. The amount of drug entrapped was determined by using formula.

$$\% \text{ Drug entrapment} = \frac{\text{Total amount of drug- free drug present in supernatant}}{\text{Total amount of drug}} \text{ X } 100$$

Scanning Electron Microscopy (SEM): The most widely used procedures to visualize microparticles are conventional light microscopy (LM) and scanning electron microscopy (SEM). It images the sample surface of a solid specimen by using a focused beam of high-energy electrons. The signal contains information about surface topography, texture, external morphology of fractured or sectioned surface, chemical composition, crystallographic information, and electrical conductivity. In order to examine the particle surface morphology and shape, Scanning Electron Microscopy (SEM) was used. Nanoparticles were scanned and examined under Electron Microscope. Dry Nanoparticles were spread over a slab. The sample was shadowed in a cathodic evaporator with gold layer 20 nm thick. Photographs were taken using an S-3700N Scanning Electron Microscope (Hitachi) operated at 20 kV.

Fourier Transformation Infra-Red (FTIR) Analysis: FTIR spectrum of drug, polymer and physical mixture of drug with polymers were obtained on FTIR instrument. Sample about 5 mg was mixed thoroughly with 100 mg potassium bromide IR powder and compacted under vacuum at a pressure of about 12 Psi for 3 minutes. The resultant disc was mounted in a suitable holder in Perkin Elmer IR spectrophotometer and the spectrum was scanned over the wave number range of 4000-400 cm^{-1}. IR helps to confirm the identity of the drug and to detect the interaction of the drug with the carriers.

X-Ray Powder Difraction Method (Xrpd): X-ray powder difraction method of the drug and pure and formulation conducted. It is determine the crystalinity of the formulation The

powder surfaces were pressed and smoothed with a glass slide. The total time of the diffraction scan was 19 min, and each sample was examined in three separate experiments. The voltage and current generator were set at 40 kV and 30 mA, respectively. The obtained data were analyzed by EVA software. Relative crystallinity was determined from the XRD results by the ratio of the intensity of a characteristic crystalline peak to that of the amorphous halo for each powder sample. If the ratio of the peak to the amorphous halo around it decreases, then the crystallinity is proportionately lower.

Differential Scanning Calorimetry (Dsc): Differential Scanning colorimetry is used to determine drug excipient compatibility studies, and also used to observe more phase changes such as glass transition, crystallization, amorphous forms of drugs and polymers. The physical state of drugs and polymer was analyzed by Differential Scanning calorimeter (Schimadzu). Approximately 10 mg of sample was analyzed in an open aluminum pan, and heated at scanning rate of 10°C/min between 0°C and 400°C. Magnesia was used as the standard reference material.

In-Vitro Release Study: The drug release rate from nanoparticle loaded suppositories was determined by using USP dissolution apparatus Type II (basket-type). A weighed amount of nanoparticle loaded suppositories was weighed and placed in media. Dissolution medium used was phosphate buffer saline for first 2 hours and maintained at 37 ± 0.5°C at a rotation speed of 100 rpm. 1 ml of sample was withdrawn at each 60min interval for the first hour followed by 1hour interval, later this interval was extended to 24 hour.

Sample was analyzed spectrophotometrically at 276 nm present in the dissolution medium respectively. The initial volume of dissolution medium was maintained by adding 1 ml of fresh dissolution media after each withdrawal. The dissolution study was continued with using phosphate buffer saline for next 10 hours. The cumulative % drug release was calculated using standard calibration curve.

Details of dissolution testing: Apparatus: Electro lab USP Type II, Dissolution media: Phosphate buffer saline, Speed: 100 rpm, Volume of medium: 1mL, Aliquots taken at each time interval: 1mL, Temperature: 37±0.5°C, Wavelength: 276 nm.

RESULTS AND DISCUSSION

In the present investigation an attempt has been made to formulate nanoparticles loaded suppositories of insulin by using biocompatible polymer like PLGA as carrier for sustained release and for enhancement of bioavailability after rectal administration. Nanoparticles were prepared by do solvent evaporation method. Prepared nanoparticle loaded suppositories are subjected for characterization and evaluation studies.

Preformulation Studies: Pre formulation study insulin has been performed to know the drug physical properties so as to design it to a suitable formulation.

Description data of insulin

Physical property	Pure Insulin
Empirical Formula	$C_{257}H_{383}N_{65}O_{77}S_6$
Molecular Weight	5808doltons
Color	White
ʎmax	276nm

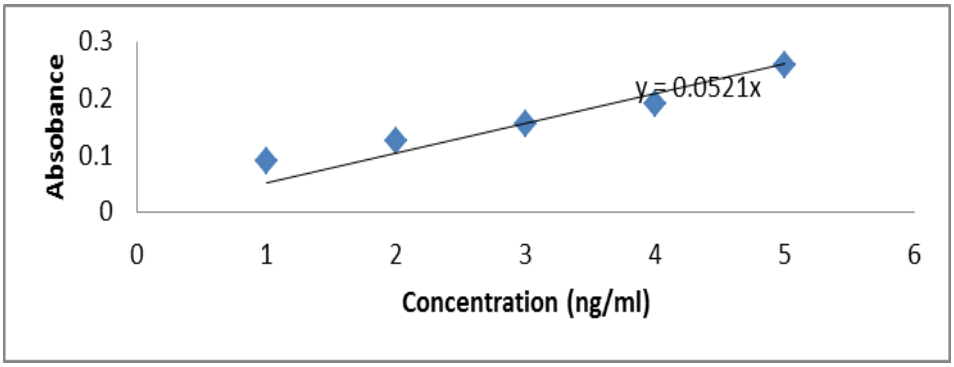
Preformulation of Nanoparticles Loaded Suppositories: Different batches of insulin loaded nanoparticles suppository preparation.

Ingredients	Formulation 1	Formulation 2	Formulation 3
INSULIN	2µg	2µg	2µg
DCM	10mL	10mL	10mL
PVA SOLUTION	0.1%	0.1%	0.1%
PLGA 8515	50mg	-	-
PLGA5050	-	-	50mg
CHITOSEN	-	50mg	-

Insulin nanoparticle loaded suppositories were prepared by solvent evaporation method and mould method. In this a mixed solvent system of polymer and dichloromethane as internal organic phase and PVA solution is aqueous phase.

Analytical Method Development: Standard graph for insulin has been prepared by calibration curve method as per the procedure discussed in the section 3.4.4. The drug was scanned for the ʎ max and found to be 276nm. Absorbance was taken for the standard concentration as resulted in the table 4.3. and graph has been plotted as shown in fig .4.6. From the standard graph, concentration for nanoparticles loaded suppositories formulation pure % drug releases were calculated. (**Insulin standard graph).**

Concentration(ng/ml)	Absorbance
1	0.088
2	0.125
3	0.153
4	0.190
5	0.256



Standard graph of insulin

ZETAPOTENTIAL AND ZETA SIZE: The size, size distribution and zeta potential of the nanoparticles were analyzed by Zeta sizer(ZS 90 malvern).

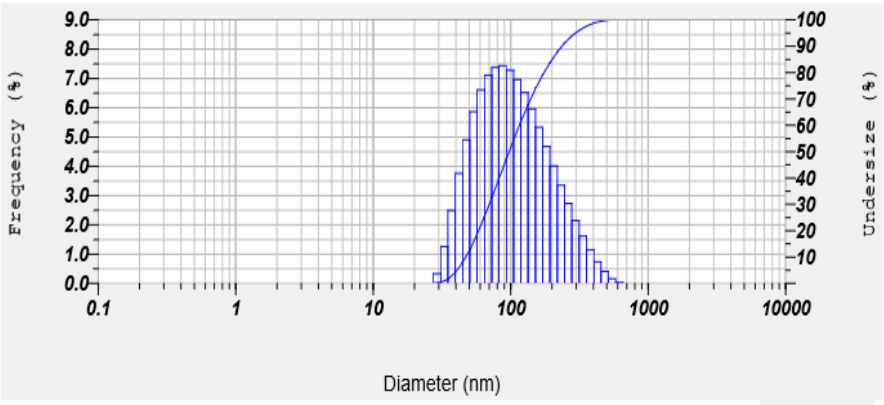
Measurement Type	:	Particle Size
Sample Name	:	F3-Size
Scattering Angle	:	90
Temperature of the holder	:	25.1 deg. C
T% before meas.	:	26566
Viscosity of the dispersion medium	:	3.084 mPa.s
Form Of Distribution	:	|Standard|
Representation of result	:	Scattering Light Intensity
Count rate	:	927 kCPS

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	123.5 nm	83.5 nm	87.4 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	123.5 nm	83.5 nm	87.4 nm

Cumulant Operations

Z-Average	:	69.7 nm
PI	:	0.576



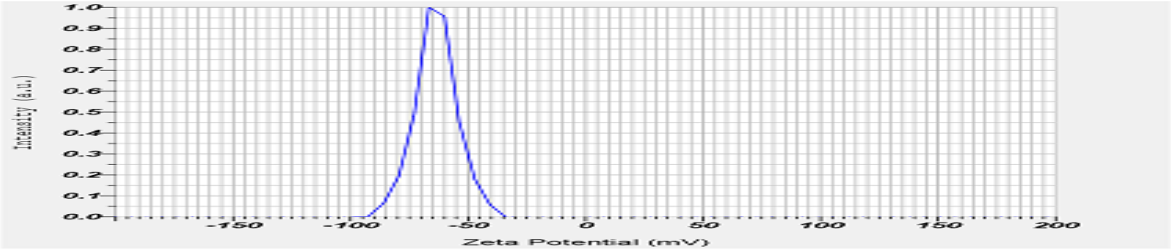
Zeta size of formulation

Measurement Type	:	Zeta Potential
Sample Name	:	F3
Temperature of the holder	:	25.1 deg. C
Viscosity of the dispersion medium	:	0.893 mPa.s
Conductivity	:	0.081 mS/cm
Electrode Voltage	:	3.9 V

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-63.6 mV	-0.000494 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs

Zeta Potential (Mean)	:	-63.6 mV
Electrophoretic Mobility mean	:	-0.000494 cm^2/Vs



Zeta potential of formulation

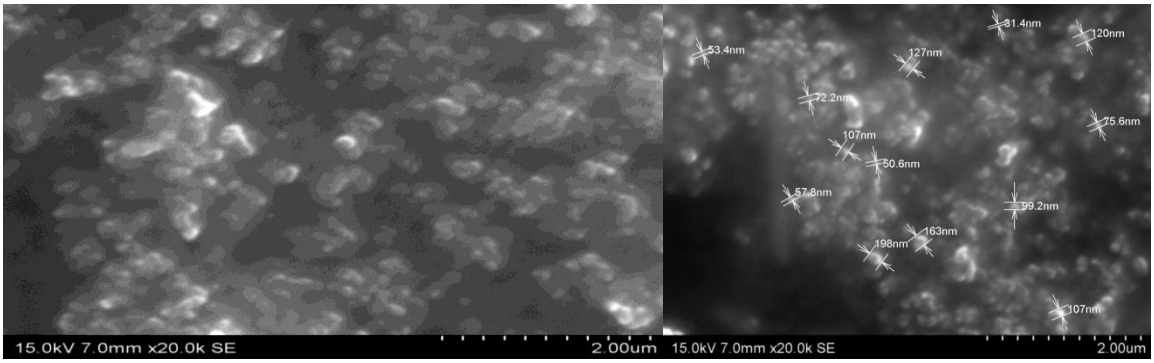
Percentage drug entrapment efficiency: The drug entrapment efficacy of insulin nanoparticle loaded suppositories for F1 to F3 was in the range of e. Highest entrapment efficacy was observed with F3 formulation, with a percentage entrapment of 66.1%. The results of percentage drug entrapment efficiency are shown in the table 23.

Drug entrapment efficiency Data of all the formulations

Formulation	Entrapment efficiency of formulations (%)
F1	65%
F2	24%
F3	76.1%

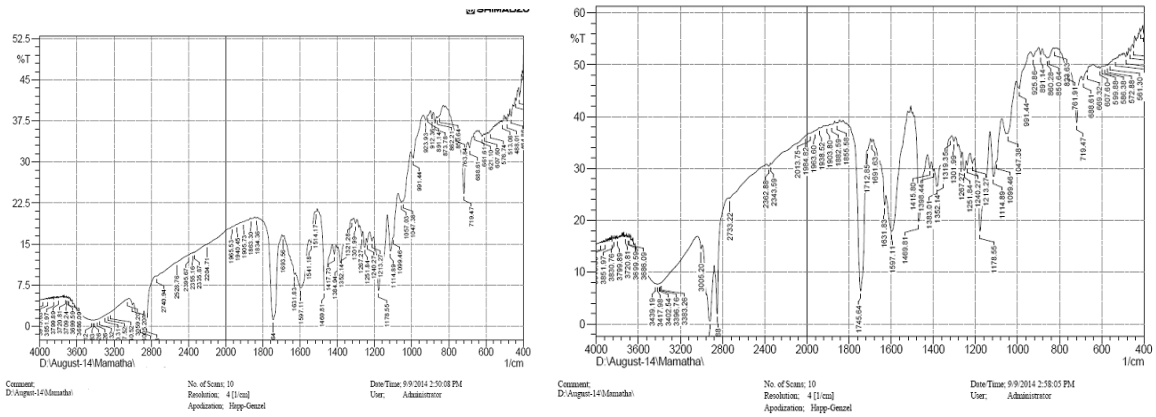
Characterization of nanoparticles loaded suppositories.

Particle size analysis by SEM: SEM was used to determine the particle size. fig. it was concluded that the average size particle size was found to be in a nano range (<1µ). Surface morphology and shape were visualized. The particle were appeared as spheres and crystal concluded from fig 4.8 and 4.9. From this study it has been concluded that there is a size reduction of particles.



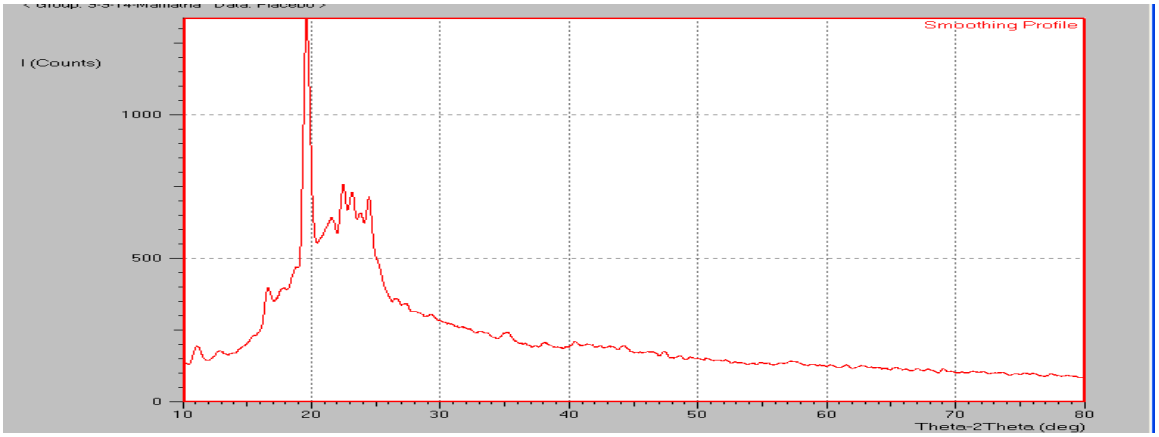
Insulin spherical particle nanoparticle particle in nanometer

Compatibility studies by FTIR: Drug polymer compatibility studies were carried out by using FTIR spectral studies to establish the possible interaction in the formulations. The FTIR spectram. The following characteristic peaks were observed with insulin. C-C- (stretching in aromatic) 1597.71, C=0 (stretching) 1745.64, C-H- (stretching) 2908.88. As the identical principle peaks were observed in all the cases, hence it shall be confirmed that interactions do not exist between the drug and polymer. The physical mixture retained the integrity of drugs and as a reason these polymer was selected for further studies.

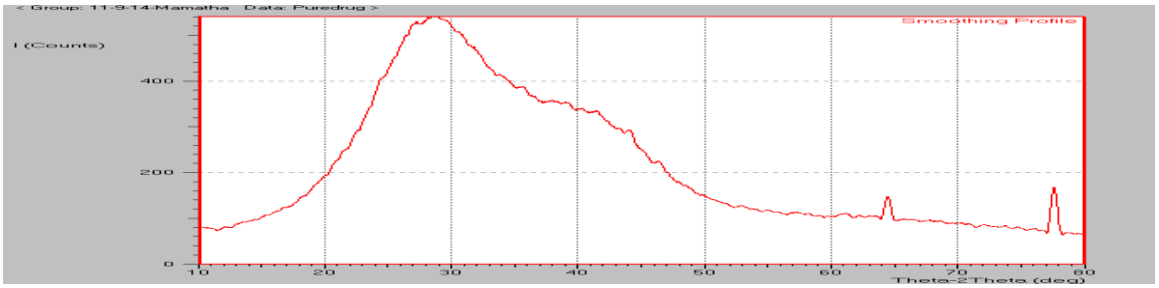


FTIR of drug **FTIR of formulation**

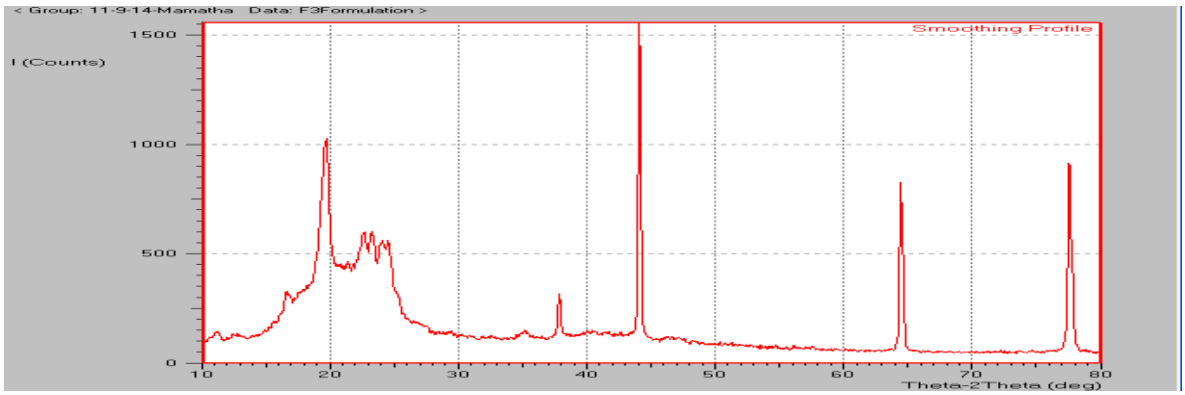
XRPD RESULTS**:** XRPD was used to investigate the physical nature of the encapsulated drug, the powder X-ray. XRPD was used for analysis of a variety of transformations during pharmaceutical processing and storage such as: poly meric transformations alterations in crystalinity it was observed that the crystallinity of the drug was changed in the nanopaticles loaded suppositories. The peak obtained for pure drug was very clear and sharp the intensity of the peaks was very high when compared to the formulation and placebo .Reduction in the peak intensity indicates the change in crystal structure. From this we can conduct the there was reduction in the crystalinity and change into amorphous stracture.



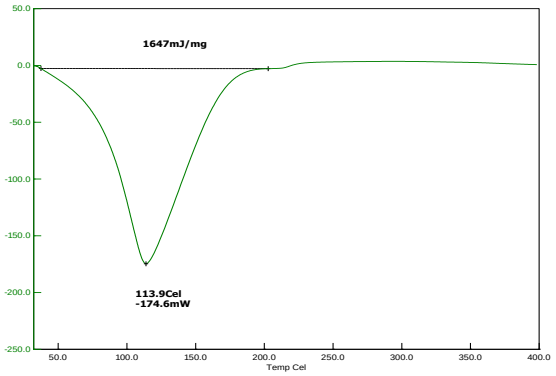
XRPD of placebo

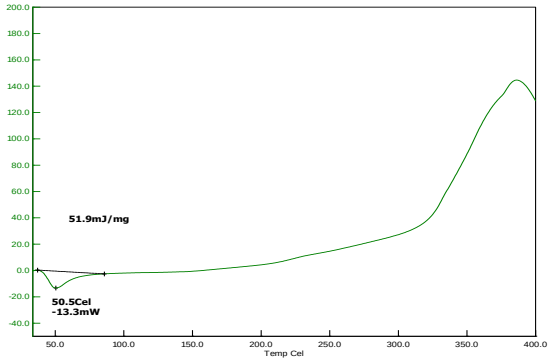


XRPD of pure drug



XRPD of formulation





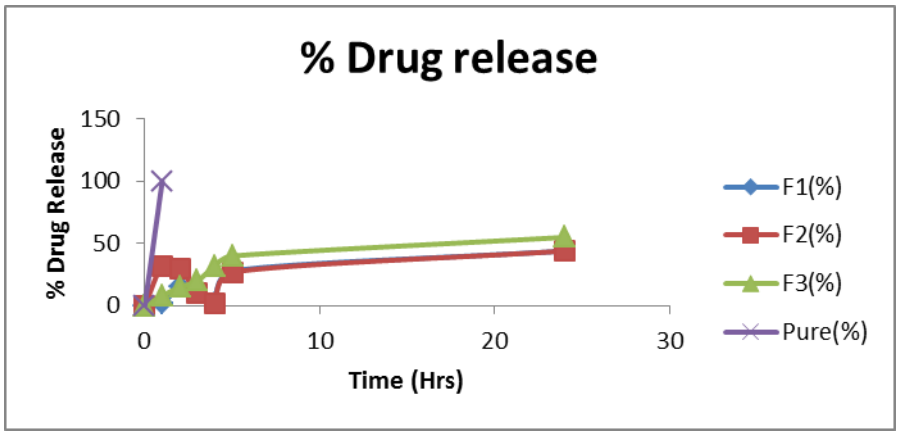
DSC RESULTS: DSC studies were performed to understand the nature of the encapsulated drug in the matrix. The physical state of drug in the polymer matrix would also influence its release characteristics. To probe this effect, DSC analysis was performed on a) pure b) formulation F3 as shown below.

DSC of formulation

DISSOLUTION STUDIES OF FORMULATION AND PURE DRUG: The cumulative present drug release of F1 to F3 formulations at various time intervals was calculated and tabulated in table no: 24 and 25, the cumulative present drug release in all formulations was plotted against time in figure no: 22 and 23, among all the batches slow and constant release was observed with F-3 formulation. It was observed that insulin nanoparticle loaded suppositories, release was sustained when compared to that of pure drug.

Time(in hours)	Formulation 1(%)	Formulation 2(%)	Formulation 3(%)	Pure(%)
1	2	32	8	100
2	15	30	15	
3	10	10	20	
4	2	2	32	
5	28	27	40	
24	44	44	**55**	

Cumulative %drug release of pure drug and formulations



CONCLUSION: Insulin is anti diabetic drug medication which is used to lower the blood glucose levela in the blood. It inhibit the gluconeogenesis.

- In this work a new drug delivery system "suppositories loaded nanoparticles" were developed, present work explain the mechanism of nanoparticles release from the suppositories in five steps.

- 1. Delivery of suppositories to rectal cavity 2. Dissolution (release of nanoparticles) by the rectal fluids 3. Nanoparticles entry in fenestrated capillaries 4. Diffusion of drug from nanoparticles. 5. Nanoparticles whose size is restricted to the entry of fenestrated capillaries due to the mucoadhesive character nanoparticles will adhere to mucosa of rectum for a prolonged period of time and release the drug in controlled manner.
- Suppositories which are designed to melt at the physiological temperature compare to subcutenious administration the rectal or vaginal drug delivery has gain importance therapeutically by avoiding the first pass metabolism. Drugs may be administered in suppository form for either local or systemic effects. Conventional suppositories have advantages 1. They exert local action on rectum 2. To Promote Evacuation of the Bowel 3. To Provide a Systemic Effect.
- The finding of the present study indicate that a method for the preparation of nanoparticle loaded suppositories formulation of insulin with an average particle size <1µhas been successfully employed by solvent evaporation method. It was found to be fesible method in laboratory scale. %drug release of insulin nanoparticle loaded suppositories was shown in increase.
- PXRD studies were done for solid state characterization where crystallinity was redused
- Compatibility study carried to examine compatibility issue if any via examining the mixture of insulin and excipients by differential scanning calorimeter (DSC) and fourier transform infra red spectroscopy (FTIR).Compatability was retained at the end of preparation.

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