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Formulation development and evaluation of nanoparticles of Anti-diabetic drug by Hot Homogenization technique

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ABSTRACT

Nanostructured lipid carriers of glimepiride (NLC) have been developed for the treatment of type 2 diabetes. The developed NLC consists of Compritol ATO 888 as a solid lipid, medium chain triglycerides as a liquid lipid, and Poloxamer 188 as a surfactant. NLCs were prepared using a hot homogenization method and characterized by FT-IR. All NLCs showed recording efficiency in the range of 78.52 to 90.38%. Both the capture efficiency and the release rate were affected by the lipid concentration. Formulation F5 was considered an optimized formulation based on particle size and percentage of drug release. The zeta potential value indicated good stability of the particles. The optimized formulation showed no physical/chemical changes when exposed to accelerated stability conditions. It was concluded that the developed NLC is a potential approach for controlled medication that can reduce dosing frequency and improve patient compliance.

Key words

Glimepiride, hot homogenization technique, Nanocapsule, nanopartical, homogenization

INTRODUCTION

Nanotechnology revolutionized the field of pharmacy by its application in the dosage forms containing nano sized active ingredients. National nanotechnology initiative defines nanotechnology as the study of all particles having size in the range of 0.1 to 100nm. The word nano arises from greek word ‘Nanos’ means dwarf or extremely small. Nanometer is one billionth of a meter or 10^{-9}m in size. Advantage of the small particle size is the ratio of surface atoms or molecules to the total number increases. That means surface area increases resulting in increase in their surface activity and changes in physical and biological properties. **Nanoparticles:**

Nanoparticles are colloidal particles ranging from 1nm to 100 nm in size, active ingredients (drug or biologically active material) is dissolved or encapsulated in polymeric particle. Nanoparticles are mainly of two types as shown in fig.1.1

1) **Nanosphere**: It is a matrix type structure in which a drug is dispersed in polymer matrix.
2) **Nano capsule**: In this drug is encapsulated within central volume surrounded by continuous polymeric sheath.

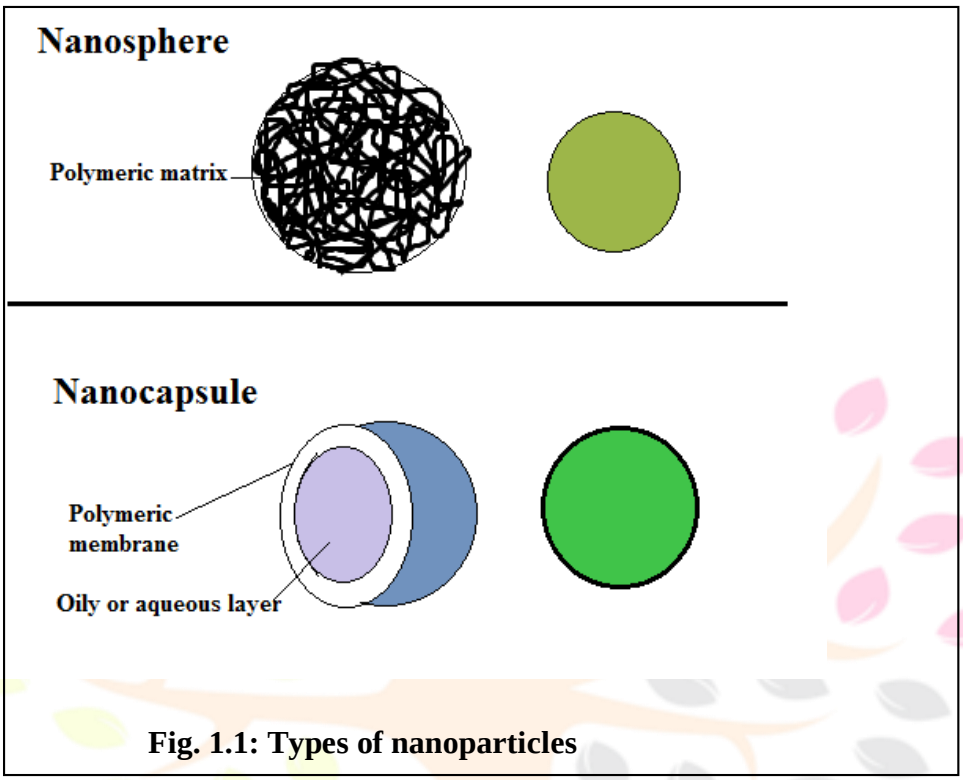

Fig. 1.1: Types of nanoparticles

AIMS OF SOLID LIPID NANOPARTICLES:

- Possibility of controlled drug release
- Increased drug stability
- High drug pay load
- No biotoxicity of the carrier
- Avoidance of organic solvents
- Incorporation of lipohilic and hydrophilic drugs.

ADVANTAGES OF SOLID LIPID NANOPARTICLES

1. SLNs have better stability and ease of upgradibility to production scale as compared to liposome.
2. In SLNs the lipid matrix is made from physiological lipid which decreases the danger of acute and chronic toxicity.
3. Very high long-term stability.
4. It is easy to manufacture than polymeric nanoparticles.
5. Better control over release kinetics of encapsulated compound.
6. SLNs enhances the bioavailability of entrapped bioactive.
7. Chemical protection of labile incorporated drug.
8. Raw materials used are same as that of emulsion.

9. Large scale production is possible.
10. High concentration of functional compounds can be achieved.
11. Lyophilization possible.

1.5 Disadvatages

1. Poor drug loading capacity.
2. Drug expulsion after polymeric transition during storage.
3. Relatively high-water content of the dispersions (70-99.9%).
4. The low capacity to load hydrophilic drugs due to partitioning effects.

MATERIALS USED IN PREPARATION OF SOLID LIPID NANOPARTICLES:

Lipids:

i) saturated triglycerides: Glyceryl tristearate, Glyceryl trimyristate, Glyceryl tripalmiatate, medium chain triglycerides, Glyceryl trioleate.

ii) Partial monoglycerides: Glyceryl monostearate, Glycryl beheneate, Glyceryl palmitostearate.

iii) Free fatty acids: Behenic acid, Stearic acid, Palmitic acid, Myristic acid, Oleic acid.

iv) Free fatty alcohols: Stearyl alcohol, Cetyl alcohol, Myristyl alcohol, Lauryl alcohol.

v) Waxes: Cetyl palmitate, Bee's wax, Carnauba wax.

vi) Others: Castor oil, Hydrogenated palm oil, Cocoa butter, Milk fat, Goat fat.

Surfactants/Emulsifiers

i) Nonionic surfactants:

a) Polyoxyethylene sorbitan fatty esters: Polysorbate 20, Polysorbate 60, Polysorbate 80, Polysorbate 85.

b) Ethoxylated castor oils: PEG-35 castor oil, PEG 40 hydrogenated castor oil.

c) Poloxamers: Poloxamer 188, Poloxamer407.

ii) Anionic surfactants: Sodium dehydrocholate, sodium taurocholate, Sodium glycocholate, sodium lauryl sulphate.

iii) Cationic: Cetrimonium bromide, DOTAP, DOTMA.

iv) Amphoteric: Soy lecithin. Egg lecithin.

v) Co-surfactants: 1-Butanol, Ethanol, Propylene glycol, Sorbitan monostearate, low molecular weight PEG.

METHODS OF PREPARATION OF SOLID LIPID NANOPARTICLES

1) High pressure homogenization

High pressure homogenization is reliable technique for the preparation of solid lipid nanoparticles. High pressure homogenizers push a liquid with high pressure (100-2000 bar) through a narrow gap in the range of few microns. The fluid accelerates on a very short distance to very high velocity (over 1000km/h).

a) Hot homogenization technique

Hot homogenization is carried out at temperatures above the melting point of lipid. A pre-emulsion is obtained by stirring the drug loaded lipid melt and the aqueous surfactant solution at the same temperature in a high shear mixing device (eg. Ultraturrax).

b) Cold homogenization technique

Cold homogenization is carried out with solid lipid and involves high pressure milling of a suspension. It needs high effective temperature control and regulation in order to ensure the unmolten state of the lipid due to increase in the temperature during homogenization.

2) Ultrasonication or high-speed homogenization technique

Solid lipid nanoparticles were successfully prepared by high-speed homogenization or ultrasonication method to enhance the oral bioavailability of poorly watersoluble drug. Absorption is enhanced significantly by employing SLN formulation reared by this method. SLNs can also be reared by high-speed stirring or sonication. It is very simle and advantageous over other methods like hot and cold homogenization method as the equipments used in this technique easily available in every lab.

3) Microemulsion technique

This is a new technique for preparation of solid lipid nanoparticles based on the dilution of microemulsion. They are prepared by stirring an optical transparent mixture at 65-700 which is typically composed of a low melting fatty acid, an emulsifier, coemulsifiers and water.

4) Solvent emulsification –Evaporation technique

In solvent emulsification-evaporation method, the lipophillic material and hydrophobic drug were dissolved in a water immiscible organic solvent (eg. Cyclohexane,

5) Solvent emulsification –diffusion technique

In solvent emulsification diffusion technique, the solvent used (e.g., benzyl alcohol, butyl lactate, ethyl acetate, isopropyl acetate, methyl acetate) must be partially miscible with water and this technique can be carried out in aqueous phase or in oil.

6) Adsorption onto solid carriers

This is simple and economical process. In this method, a liquid lipid formulation is adsorbed onto solid carriers like calcium silicate, silicon dioxide or magnesium alumino-metasilicate. The liquid lipid formulation is added to the carrier by mixing in a blender.

7) Melt granulation

This is also referred as pelletization which transforms a powder mixture with drug into granules or pellets. In this method, a meltable binder is blended with powder mixture and due to the friction of particles during the high shear mixing, the binder melts.

8) Melt extrusion/spheronization:

Extrusion is a process of converting raw material with plastic properties into a product of uniform shape and density by forcing it through a die under controlled temperature, product flow and pressure condition.

9) Supercritical fluid-based methods

Lipids can be used in supercritical fluid-based methods for coating of drug particles or for producing solid dispersions. The coating process results in diseprsing the drug particles (as powder) in a supercritical fluid containing one or more coating materials dissolved therein at elevated pressure and temperature conditions.

TYPES OF SOLID LIPID NANOPARTICLES

a) Type I or Homogeneous matrix model

The SLN Type I is derived from a solid solution of lipid and active ingredient. A solid solution can be obtained when SLN are produced by the cold homogenization method. A lipid blend can be produced containing the active ingredient in a molecularly dispersed form.

b) Type II or drug enriched shell model

It is achieved when SLN are produced by hot homogenization technique, and the active ingredient concentration in the melted lipid is low. During the cooling process of the hot o/w nanoemulsion, the lipid will precipitate first leading to a steadily increasing concentration of active molecules in the remaining melt; an outer shell will solidify containing both active and lipid.

c) Type III or drug enriched core model

Core model can take place when the active ingredient concentration in the lipid melt is high & relatively close to its saturation solubility. Cooling down of the hot oil droplets will in most cases reduce the solubility of the active in the melt.

CHARACTERIZATION OF SOLID LIPID NANOPARTICLES

1. Particle size and zeta potential
2. Dynamic light scattering
3. Static light scattering/ Fraunhofer diffraction
4. Electron microscopy
5. Atomic force microscopy (AFM)
6. Acoustic methods
7. Nuclear Magnetic Resonance
8. Differential Scanning Calorimetry
9. Powder X-ray Diffractometry (PXRD)
10. Storage Stability of SLN
11. In vitro and ex vivo methods for the assessment of drug release from SLN

APPLICATIONS OF SOLID LIPID NANOPARTICLES

Oral delivery

Oral administration of SLNs is possible as aqueous dispersion or after transforming in to dosage form i.e. tablets, pellets, capsules or powder in sachets. For the production of the tablets the aqueous SLN dispersion can be used instead of a granulation fluid in the granulation process.

Parenteral delivery

SLNs can be administered intravenously, intramuscularly, subcutaneously or to the target organ, because of their small size. The particles are cleared from the circulation by the liver and the spleen. SLN formulations can be used for systemic body distribution with a minimized risk of blood clotting and aggregation.

Topical delivery

Topical applications of lipid nanoparticles have been used with promising results either for therapeutic or cosmetic purposes. SLNs have shown some protective activity on skin surface, such as a UV-blocking potential. SLNs may be formulated in creams, gels, sprays.

SLNs as gene vector carrier:

SLN can be used in the gene vector formulation. In one work, the gene transfer was optimized by incorporation of a diametric HIV-1 HAT peptide (TAT 2) into SLN gene vector. The lipid nucleic acid nanoparticles were prepared from a liquid nanophase containing water and a water miscible organic solvent where both lipid and DNA are separately dissolved by removing the organic solvent, stable and homogeneously sized lipid-nucleic acid nanoparticle (70-100 nm) were formed.

SLNs as cosmeceuticals:

The SLNs have been applied in the preparation of sunscreens and as an active carrier agent for molecular sunscreens and UV blockers. The in vivo study showed that skin hydration will be increased by 31% after 4 weeks by addition of 4% SLN to a conventional cream.

SLNs as a targeted carrier for anticancer drug to solid tumors:

SLNs have been reported to be useful as drug carriers to treat neoplasms. Tamoxifen, an anticancer drug incorporated in SLN to prolong release of drug after i.v. administration in breast cancer and to enhance the permeability and retention effect. Tumour targeting has been achieved with SLNs loaded with drugs like methotrexate and camptothecin.

Aim: Formulation development and evaluation of nanoparticles of Anti-diabetic drug by Hot Homogenization technique.

NEED FOR STUDY:

The poor dissolution characteristics of relatively insoluble drugs have long been a problem to the pharmaceutical industry. The solubility characteristics of a drug is a good indicator of gastrointestinal absorptivity. Poorly soluble drugs such are characterized by low absorption and weak bioavailability. For such drugs there is need to find appropriate formulation approaches to improve aqueous solubility and thus bioavailability.

Solid lipid nanoparticles are submicron sized colloidal drug carriers composed of physiological lipid, dispersed in an aqueous surfactant solution." It enhances the bioavailability of drug with better stability and increased drug stability. Generally, 699ipophilic drugs are incorporated into these colloidal carriers. Surfactants used acts as emulsifying agent thereby reduces the interfacial tension of drug and air at their surfaces' therefore entrapped drug is going to enhance the solubility and bioavailabilty.

OBJECTIVES:

The main objectives of this study are

i. To increase the solubility and bioavailability of poorly water-soluble antidiabetic Glimepiride drug by formulation of solid lipid nanoparticles using lipid Glyceryl behenate and surfactant poloxamer and tween 80 as a co-surfactant.

ii. To formulate solid lipid nanoparticles of glimepiride drug with lipid glyceryl behenate by novel method i.e. hot homogenization method.

iii. To characterize lipid interactions of drug formulated solid lipid nanoparticles by FT-IR, DSC and XRD and to verify surface morphology by SEM and TEM.

iv. To prepare SLN dispersion and evaluate parameters like pH, Viscosity and conductivity.

PLAN OF WORK

- Extensive literature survey.
- Selection of raw materials (polymers) and drug.
- Preformulation study
  a. Solubility study.
  b. Melting point determination.
  c. Calibration curve of Glimepiride in water, 0.1 N HCl, Phosphate buffer pH 6.8.
  d. Drug excipient interaction study by Fourier transform Infrared spectroscopy study.
- Formulation of solid lipid nanoparticles by hot homogenization method using high pressure homogenizer and lyophilizer.
- Optimization of solid lipid nanoparticles based on solubility and dissolution Study.
- Evaluation of solid lipid nanoparticles.
  a. FTIR study.
  b. Differential scanning calorimetry (DSC) study.
  c. Powder X-ray diffraction (PXRD)
  d. Entrapment efficiency.
  e. Particle size and zeta potential.
- Particle morphology and particle size analysis of optimized solid lipid nanoparticles.
  - SEM
  - TEM
- Preparation of suitable dosage form i.e. dispersion and evaluation.
- Statistical Analysis, Data Interpretation and Conclusions.

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2. **V. Vijayan et al. (2010)**
3. **Vijaykumar Nekkanti et al. (2013)**

4. **E. B. Souto et al. (2011)**
5. **N. Silpa and Naveen Chakravarthy et al. (2012)**

MATERIALS AND EQUIPMENTS

MATERIALS:

The following drug, excipients and chemicals were selected for the formulation and evaluation of Solid lipid nanoparticles by hot homogenization technique.

Table 5.1: List of equipments

Sr. No.	Name	Category	Supplier
1	Glimepiride	Antidiabetic drug	……..
2	Glyceryl beheneate	Lipid	Himedia Laboratories Pvt Ltd.
3	Poloxamer 188	Surfactant	S. D. Fine chemicals, Mumbai.
4	Tween 80	Cosurfactant/ Stabilizer	S. D. Fine chemicals, Mumbai.
5	Mannitol	Cryoprotectant	S. D. Fine chemicals, Mumbai.
6	Sodium dihydrogen Phosphate	Chemical	Rankem Pvt Ltd., Mumbai.
7	Disodium hydrogen phosphate	Chemical	Rankem Pvt Ltd., Mumbai.
8	HCl	Solvent	S. D. Fine chemicals, Mumbai.
9	Methanol	Solvent	S. D. Fine chemicals, Mumbai.
10	Chloroform	Solvent	S. D. Fine chemicals, Mumbai.

EQUIPMENTS

Table 5.2: List of Equipments

Sr. No.	Equipment	Manufacturers	Model
1	UV Visible spectrophotometer	Shimadzu, Japan.	UV-1800
2	FT-IR Spectrophotometer	Shimadzu, Japan.	DR-8031
3	Dissolution apparatus	Electrolab, Mumbai	TDT-08L
4	Magnetic Stirrer	REMI Motors, Mumbai.	IML
5	High shear homogenizer	Kinematica.	Polytron, PT 3100 D
6	Lyophilizer	Lark	Penguin classic plus
7	Digital balance	Shimadzu, Japan.	BL-220H
8	Digital ultrasonic bath sonicator	HMG India Ltd.	CD-4820
9	pH meter	Systronics	MK VI
10	High pressure homogenizers	Panda	Homogenius

DRUG PROFILE

1. GLIMEPIRIDE

5.2.1 Description: Glimepiride is the first III generation sulphonyl urea it is a very potent sulphonyl urea with long duration of action.

Chemical name: 3 –ethyl -4 –methyl –N -{2-[4-({[(4methylcyclohexyl) carbamoyl] amino} sulphonyl) phenyl] ethyl} 2-oxo-2,5-dihydro-1H-pyrole-1-carboxamide.

Molecular formula: $C_{24}H_{34}N_4O_5S$

Molecular weight : 490.61 g/mol

Absorption: Completely (100%) absorbed following oral administration.

Protein binding: Over 99.5% bound to plasma protein.

Half-life: Approximately 5 hours following single dose.

Indications and usage: For concomitant use with insulin for the treatment of noninsulin dependent (type 2) diabetes mellitus.

Contraindications: Hypersensitivity with Glimepiride or other sulphonyl urea agents.

Excipient's profile

2. GLYCERYL BEHENATE

Synonyms: Compritol 888 ATO, 2,3-dihydroxypropyl docosanoate, docosanoic acid, glyceryl dibehenate.

Chemical name and CAS number: Docosanoic acid, triester with glycerin [18641-57-1] (glyceryl tribehenate)

Empirical formula: Glyceryl dibehenate is a mixture of glycerol esters. The PhEur 6.0 describes glyceryl tribehenate as a mixture of triacylglycerols, mainly tribehenoylglycerol, together with variable quantities of mono- and triacylglycerols

(Molecular formula: C_{69} H_{134} O_6).

Molecular weight: 1059.9.

Functional category: Coating agent; tablet binder; tablet and capsule lubricant; thickening agent; viscosity-increasing agent.

Description: Glyceryl behenate occurs as a fine white-yellow powder, as a hard waxy mass or pellet, or as white or almost white unctuous flakes. It has a faint odor.

Pharmaceutical applications: Glyceryl behenate is used in oral enteric-coated pellets, powders and suspensions. It is also used in controlled, extended-release and orally disintegrating tablets. For oral preparations, glyceryl behenate forms a lipidic matrix for sustained-release formulations. In cosmetics, glyceryl behenate is used as a skin conditioning agent, emollient and viscosity-increasing agent in emulsions. For topical formulations, it is used as a thickening agent for oily phases.

Typical properties:

Solubility: Soluble, when heated, in chloroform and dichloromethane and in many organic solvents; slightly soluble in hot ethanol (96%); practically insoluble in cold ethanol (95%), hexane, mineral oil, and water.

Melting point: 65-77^0 c

HLB value: 2

Saponification value: 145-165

Acid value: ≤ 4.0

Iodine value: ≤ 3.0

Stability and Storage Conditions: Glyceryl behenate should be stored in a tightly closed container, at a temperature less than 35 ^{0}C.

3.POLOXAMER 188

Synonyms: Lutrol, Monolan, Pluronic, polyethylene propylene glycol copolymer.

Chemical name CAS registry numbers: a-Hydro-ω hydroxypoly(oxyethylene) poly (oxypropylene) poly-(oxyethylene) block copolymer [9003-11-6].

Empirical formula: The poloxamer polyols are a series of closely related block copolymers of ethylene oxide and propylene oxide conforming to the general formula $HO(C_2H_4O)_a(C_3H_6O)_b(C_2H_4O)_aH$.

Molecular weight: 7680- 9510

4TWEEN 80

Synonyms: Polysorbate 80, Atlas, Capmul, Crempophor, Drewmulse, emrite, emulgin, Glycosperse, Liposorb, Montanox, Polyoxyethylene 20 oleate.

Chemical name and CAS number: Polyoxyethylene 20 sorbitan monooleate [9005-65-6].

Empirical formula: C_{64} H_{124} O_{26}

Molecular weight: 1310

UV spectrophotometric analysis

UV spectrophtometry is widely employed for routine drug analysis. Glimepiride was estimated spectrophotometrically by using UV-VIS spectrophotometer.

PREPARATION OF CALIBRATION CURVE

Accurately weighed 100 mg of drug was dissolved in 100 ml of distilled water in 100 ml volumetric flask to give solution of concentration 1000 µg/ml. This solution was further diluted suitably with methanol to obtain a series of dilution of various concentration ranges.

Melting point determination

Melting point determination was carried out to check the purity and identification of sample. The melting point was determined by using melting point apparatus. The substance under test was reduced to a very fine powder. Capillary was sealed at one end and filled with tapping with sufficient drug powder.

FT-IR study:

Drug Excipient Compatibility was studied by FT-IR (Fourier Transform Infrared) Spectroscopy. The pure drug Glimepiride (GLP), physical mixture of GLP and Glyceryl behenate, physical mixture of GLP and Poloxamer, were mixed separately with infrared (IR) grade KBr in the ratio of 1:100 and corresponding pellets were prepared by applying 15000 lb of pressure in a hydraulic press.

Preparation of solid lipid nanoparticles:

Solid lipid nanoparticles were prepared by hot homogenization technique. In this method, lipid Glyceryl behenate of quantity 1.5, 2, 2.5, 3, 3.5, 4, 4.5 taken for seven different batches F1, F2, F3, F4, F5, F6, F7. The lipid was melted at 10^0 c above the melting point of lipid, the drug glimepiride (100 mg) and poloxamer (2gm) as co-surfactant/emulsifier was dispersed in the melted lipid and the dispersion was kept at the same temperature until it appeared optically clear. An aqueous phase was prepared by dissolving Tween 80 (2.5% w/w of total weight of SLN dispersion) as a surfactant/ stabilizer was dissolved in double distilled water and heated to the same temperature as that of melted lipid phase. `The preheated aqueous phase was added to melted lipid phase and homogenized by high shear homogenizer (polytron PT 3100, Kinematica) at 12000 rpm for 20 min. The formulation was cooled down in an icebath and diluted with deionised water upto 100ml and lyophilized in lyophilizer (Penguin classic plus, Lark) to form SLN and stored at 4^0 c for further analysis.

Solubility study of solid lipid nanoparticles

The solubility of solid lipid nanoparticles was determined by taking an excess amount of SLNs and adding them to 10 ml of solvent, in Teflon-facing screw-capped vials. The samples were kept at equilibrium for a period of 48 HR on an orbital shaking incubator (CIS-24; Remi Instruments, Mumbai, India) at 37±0.5°C and 50 RPM. The supernatant liquid was collected and filtered through 0.2µ membrane filter and analysed by UV visible spectrophotometer at wavelength 234 nm for glimepiride.

In Vitro Dissolution Study of Glimepiride solid lipid nanoparticles

In vitro dissolution studies Glimepiride solid lipid nanoparticles were carried out by USP type XXIV rotating basket type dissolution apparatus (Electrolab, Mumbai). Optimization of formulation batches was estimated on the basis of cumulative percentage drug release with respect to time. The dissolution carried out in three different media deionized water, 0.1 N HCl, Phosphate Buffer 6.8 each of 900ml. SLNs were placed in each vessel and the medium was allowed to maintain at 100 RPM at $37^0 \pm 0.5^0$ c. Samples of 5ml were sink condition.

Entrappment Efficiency

The entrapment efficiency of SLN calculated by measuring the concentration of free drug in the dispersion medium. The samples of 5ml were centrifuged at 6000 rpm in REMI motor centrifuge for 30 min. The supernatant was separated, filtered, diluted suitably and measured spectrophotometrically by UV visible spectrophotometer to calculate the amount of unentrapped drug. The concentration of entrapped drug determined by substracting the concentration of free unentrapped drug from amount of initial compound used. The entrapment efficiency was calculated by the following equation:

$$\textbf{Entrappment efficiency} = \frac{\text{WInitial drug} - \text{Wfinal drug}}{\text{WInitial drug}} \times 100$$

Differential scanning calorimetry:

Thermal characteristics of drug Glimepiride (GLP) and optimized batch of solid lipid nanoparticles (SLN3) were studied by using a differential scanning calorimeter. DSC thermograms of the drug and lyophilized solid lipid nanoparticles was recorded using Mettler-Toledo DSC822e Grinfensee, Switzerland equipped with Star SW10 computer program at a heating rate of 10^0c/min from 0 to 300^0c. Then sample size was 3-5 mg for each measurement.

X-ray diffractometry

Powder X-ray diffractometric (PXRD) pattern of pure drug Glimepiride (GLP) and GLP loaded SLN were obtained by employing X-ray diffractometer (3000, Seifert); Ni-filtered Cu-K radiation, voltage of 40 kV, and current of 30mA radiation scattered in the crystalline regions were used and measured with a vertical goniometer. Patterns were obtained by using a step size of 0.045∘C with a detector resolution in 2θ (diffraction angle) between 5^0 and 80^0 at 25^0 C temperature.

EVALUATION OF SLN DISPERSION:

1.pH measurement

The pH value of the formulation was measured by immersing the electrode into the dispersion using calibrated pH meter (Systronics MK VI, Mumbai).

2.Viscosity

The viscosity of the formulation was determined by using Brookefield Viscometer. All the experiments were performed at 25^0C and in triplicates

3.Drug content

About 1 ml of SLN dispersion was diluted using solvent methanol and drug content was determined spectrophotometrically at 234 nm.

RESULTS AND DISCUSSION

1 UV Spectrophotometric analysis

The ultraviolet spectrum of drug is obtained by scanning from 200 to 400 nm. The absorption maximum (λ max) of Glimepiride (GLP) was found to be 234 nm in distilled water, methanol, 0.1 N HCl and Phosphate buffer pH 6.8. Hence all the further analysis was carried out at 234 nm

2 Preparation of standard curve of Glimepiride in distilled water

Accurately weighed 10 mg of Glimepiride and transferred to 100ml volumetric flask. This was dissolved in water and volume made up to 100ml. this solution was treated as the stock solution and contains 100 µg/ml of Glimepiride solution. Further dilutions made with distilled water to obtain the concentration of 2, 4, 6, 8, 10 µg/ml. Absorbance of these solutions were measured at 234 nm against blank solution i.e. distilled water. Same procedure was followed for the preparation of standard curve in 0.1 N HCl, Phosphate buffer pH 6.8. The coefficient of correlation and equation for the line are determined.

3 Melting point determination

The melting point of Glimepiride was found to be in the range of 208^0c – 209^0c which is in good agreement with reported values.

4 Solubility determination

Solubility Studies were performed to check the solubility enhancing property of solid lipid nanoparticles. Solubility study of drug was carried out in in different conditions and media at 37^0c the result are shown in table 9.3.

Table 7.3 Solubility of glimepiride in different media

Sr. No.	Solvents/ Media	Solubility in mg/ml
1	Distilled water	0.0336
2	Methanol	0.391
3	0.1 N HCl	0.156
4	Phosphate buffer pH 6.8	0.147

FT-IR studies

The FT-IR studies are done to check the what changes are done of drug with polymer. FT-IR studies of pure Glimepiride drug (GLP), physical mixtures GLP and Glyceryl beheneate (GB), GLP and Poloxamer (PLX) and Optimized formulation batch (SLN5) were performed.

In vitro dissolution of solid lipid nanoparticles

In vitro dissolution of solid lipid nanoparticles was carried out by using USP apparatus type XXIV (TDT 08L Electrolab, Mumbai, India) at 100 rpm in three different media water, 0.1 N HCl, Phosphate buffer pH 6.8.

XRD Studies

Powder X-Ray Diffraction (XRD) of GLP and SLN are shown in figure 7.14, 7.15. The pure GLP exhibited intense crystalline peak between 10^0 and 30^0. Characteristic diffraction peaks at 13.71^0, 17.22^0, 21.29^0, 23.15^0 were observed with intense peak at 21.29^0 indicating the crystalline nature of GLP. On the other hand, in SLN it is observed that peak intensity is reduced indicating reduction in crystallinity. Reduced peak intensity of SLN may be due to reduction in size of drug to nano level.

CONCLUSIONS-

From this study, it is concluded that Hot homogenization technique is effective and economical method to develop solid lipid nanoparticles. Glimepiride was successfully incorporated in solid lipid nanoparticles and revealed enhancement of solubility and dissolution. This is due to reduction of drug particle size to nano level due to High shear homogenization followed by lyophilization. Lyophilization is useful for stability of solid lipid nanoparticles and lyophilizates possess good redispersibility upon sonication for 2 min. SEM and TEM images given the information regarding size, shape and surface morphology of SLN. Particle size was found to be below 1000 nm and entrapment efficiency shown that drug is successfully entrapped in the lipid matrix. The characterization studies such as FT-IR, DSC, XRD confirmed the compatibility between the drug and excipients. All the characterization studies confirm the formation of solid lipid nanoparticles. The solubility and in vitro dissolution study confirmed the application of solid lipid nanoparticles in enhancement of solubility and extended release of drug.

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