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Development and In - Vitro Characterization of the Glimepiride Loaded Submicron Emulsion to Enhance the Oral Bioavailability

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ABSTRACT

Glimepiride (GMP), an oral hypoglycemic agent is extensively employed in the treatment of type 2 diabetes. To satisfy the demands of patients, oral antidiabetic medicines are being developed quickly. Diabetes is a metabolic condition characterized by hyperglycemia. As a result, numerous studies have attempted to increase its solubility, and a number of papers have been published, including those that used nanocrystals, cosolvency, spray congealing, solid self-nano emulsified, solid dispersions, micelles, and inclusion complexation. The current study involve the enhancement of the oral bioavailability by improve the dissolution of the glimepiride by employing the submicron emulsion. Therefore, submicron emulsions might be a suitable vehicle for oral administration of glimepiride and they are expected to exhibit superior oral bioavailability.

1. Introduction

The term bioavailability is defined as the rate and extent at which active drug moiety enters systemic circulation by crossing intestinal barrier, thus accessing the site of action. It depends largely on properties of a dosage form, physiochemical properties of the drug, restriction produced by intestinal barriers, several enzymes, metabolites and glycoproteins. The presence of intestinal barriers produces a major challenge for drug delivery. Various physiological factors that reduce the oral bioavailability of drugs include disease state, gastric emptying rate, circadian differences, and interaction with food, intestinal motility and presence of intestinal microflora.

➢ **Mechanisms of drug absorption through oral route** Various routes of entry of drugs through oral route are mainly divided into four processes as: passive transcellular pathway, passive paracellular pathway, carrier-mediated efflux transportation pathway and carrier mediated active transportation pathway. These pathways generally operate at brush border lining of intestinal epithelial cells and play a wider role in transport of many macromolecular drugs into the systemic circulation. The passive transcellular pathway is found to operate at apical membrane of intestinal epithelium and helps in transport of drugs from apical side to basolateral side by diffusion mechanism. The entry of drugs through this pathway largely depends upon the associated membrane structures like lipids and proteins which act as a barrier for entry of drugs. Low molecular weight drugs with moderate lipophilicity can be easily transported through this pathway. However high lipophilicity drugs show poor permeation characteristics towards the basolateral side due to being trapped inside the epithelial tight junctional cells. Low molecular weight hydrophilic drugs with small to moderate size can easily migrate through this pathway. Carrier mediated active transport (transcytosis) and carrier-mediated efflux transport are the transportation pathways employ a series of transporters that helps in active migration of drugs with expense of energy.

➢ **Major barriers to oral absorption of drugs** There are several barriers affecting the oral absorption of drugs across the intestinal route into the systemic circulation. Poor penetration and low oral bioavailability of drugs mainly depend on the obstruction created by major physiological barriers and several pharmaceutical barriers that restrict the brisk entry of drugs.

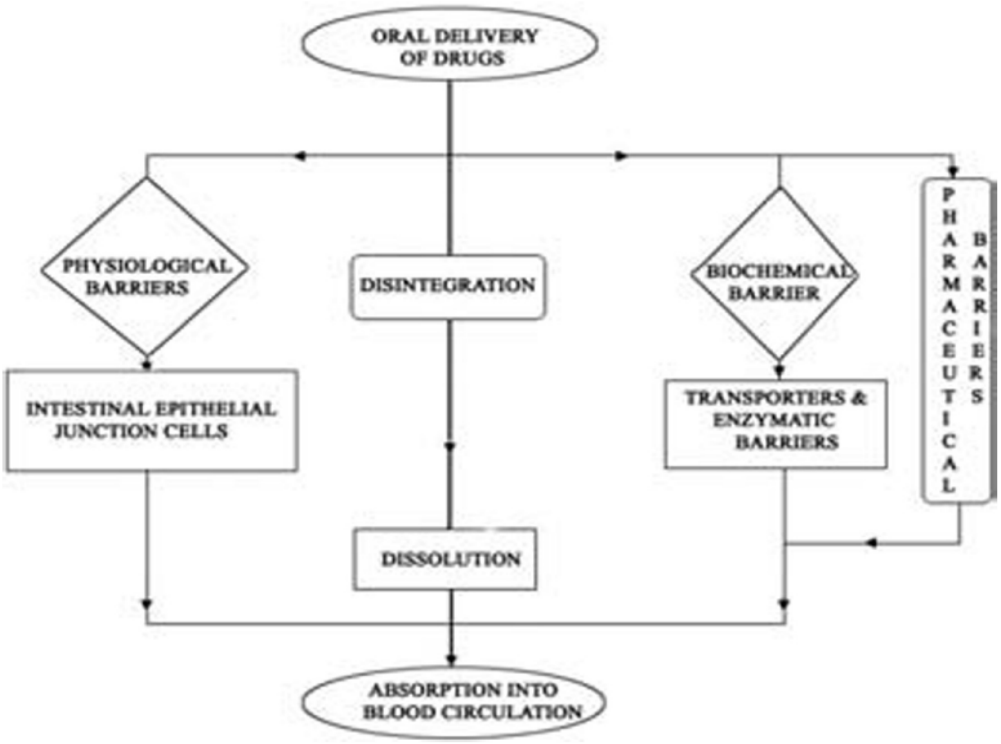


Figure: Schematic overview of different barriers to poor oral bioavailability of drugs.

➢ **Physiological Barriers** It comprises physical barriers and biochemical barriers. Intestinal paracellular tight junction acts as a physical barrier, while transporters and enzymes act as biochemical barriers. The paracellular route is defined as the aqueous pathway along the intercellular space between adjacent epithelial cells, which is restricted by tight junction.

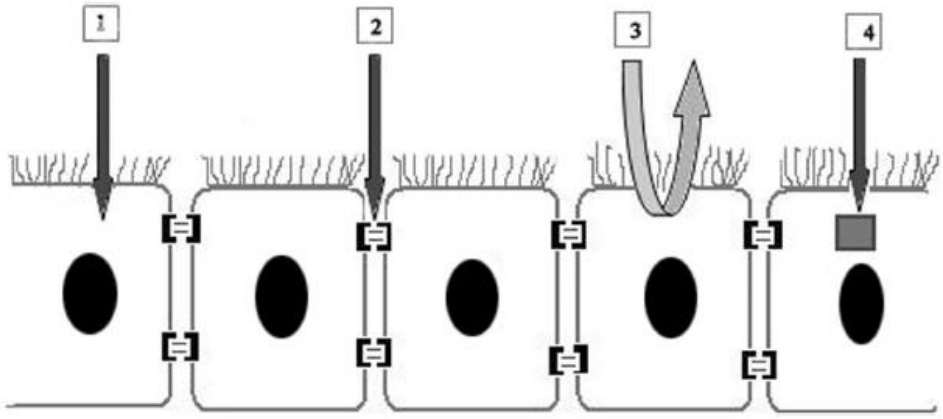


Figure: Routes of drug transport through intestinal epithelial cells.

- **Pharmaceutical Barriers** Poor solubility causes improper absorption of drugs. Solubility is an intrinsic property of the material that can be influenced by chemical modification such as salt formation, and complexation to form prodrugs, by physical modification using solid dispersion with a suitable polymeric carrier. Solubility of a drug is related to dissolution tendency, which is a characteristic measure of oral bioavailability. Solubility depends on several factors like composition of aqueous media, pH, temperature, crystal nature, ionic strength, presence of counter ions and polymorphism. New chemical entities (NCEs) synthesized by application of rapid drug discovery techniques like combinatorial chemistry and high throughput screening to speed up the drug development process leads to poor aqueous solubility. Hence for resolving the bioavailability issues, solubility of drug substances is mandatory to improve for the desired pharmacological actions.

➢ **Classification of submicron emulsions**

- The submicron emulsions are classified into the following:

Three types:

❖ Oil in water submicron emulsion

❖ Water in oil submicron emulsion

❖ Bi-continuous submicron emulsion

➢ **Advantages of submicron emulsion**

- Submicron emulsions are potential drug carrier systems for various routes of administration. These have advantages when compare to the other dosage forms.

❖ These are thermodynamically stable and require minimum energy for formation.

❖ Ease of manufacturing and scale-up.

❖ Improved drug solubilization and bioavailability.

❖ This system is reckoned advantageous because of its wide application in colloidal drug delivery systems for the purpose of drug targeting and controlled release.

➢ **Factors Affecting Choice of Excipients for Oral Submicron Emulsion**

❖ **Regulatory issues:** Irritancy, toxicity, knowledge and experience. All surfactants are potentially irritant or poorly tolerated as a result of these non-specific effects.

❖ **Solvent capacity:** Triglycerides are poor solvents for all but highly lipophilic compounds, so most submicron emulsions contain polar oils, surfactants and/or cosurfactant to improve the solvent capacity of anhydrous formulation.

❖ **Miscibility:** Mutual miscibility of excipients is necessary to produce a clear, stable, submicron emulsion.

❖ Morphology at room temperature.

❖ **Digestibility:** Fate of digested products.

❖ **Capsule compatibility:** Low molecular weight polar molecules present in capsule formulations are able to penetrate and plasticise gelatin capsule shells, which restricts the concentration of cosurfactants that can be used in capsule fills.

❖ Purity, chemical stability.

❖ Cost of goods.

➢ **Techniques of Submicron Emulsion Preparation**

There are two methods of submicron emulsion preparation-

(a) High-energy Emulsification Methods

(b) Low-energy Emulsification Methods

➢ **Application of submicron emulsion**

❖ Cosmetics

❖ Antimicrobial

❖ Mucosal vaccines

❖ Non-toxic disinfectant cleaner

- ❖ Cell culture technology
- ❖ Cancer therapy

2. Material and Methodology

To satisfy the demands of patients, oral antidiabetic medicines are being developed quickly. Diabetes is a metabolic condition characterized by hyperglycemia. The third-generation sulfonylurea drug glimepiride (GM), which lowers blood sugar levels by activating pancreatic beta-cells to make more insulin, is helpful for treating type 2 diabetes mellitus (BGL). 4,5 GM has demonstrated a number of benefits, including being strongly protein-bound, having a long half-life, and enabling simultaneous usage with insulin. However, GM's low water solubility and sluggish dissolution rate, which result in limited oral bioavailability, are a barrier to using it as an oral dose form. As a result, numerous studies have attempted to increase its solubility, and a number of papers have been published, including those that used nanocrystals, solvency, spray congealing, solid self-nano emulsified, solid dispersions, micelles, and inclusion complexation. These strategies have had only patchy success. Due to their advantages in terms of increased physicochemical stability, higher drug loading capacity, and appropriateness for large-scale production, submicron emulsions have recently been drawing more interest as oral delivery vehicles for poorly soluble medicines. These carriers could also protect the drug from coming into direct contact with human fluids and tissues, reducing irritation and toxicity, increasing solubility and stability, and possibly allowing for sustained release. Therefore, submicron emulsions might be a suitable vehicle for oral administration of glimepiride and they are expected to exhibit superior oral bioavailability.

Plan of Work

- ➢ Selection of drugs and excipients
- Preformulation studies
- ❖ Organoleptic characterization
- ❖ Melting Point
- ❖ Determination of λ max and Standard curve

- ❖ Solubility of drug
- ❖ Partition Coefficient
- ❖ FTIR Spectra

➢ Preparation of glimepiride-loaded Submicron emulsion

➢ Optimization of the glimepiride-loaded Submicron emulsion

➢ Evaluation of Submicron Emulsion

- Visual appearance and pH
- Drug content
- Particle size and zeta potential
- TEM
- Viscosity
- In vitro drug release study
- In vitro drug release kinetic

➢ **Experimental Work**

- **Preformulation study**

❖ **Organoleptic properties (API):** Glimepiride was identified by examining its external characteristics, including colour, odour, and taste. During formulation, it was noted and stored as a reference for comparison with other batches.

❖ **Melting point** A melting point apparatus and the capillary technique were used to ascertain the drug's melting point. A glass capillary melting point tube that has one end sealed and the other unsealed. Transfer an amount of glimepiride to the tube's exposed end. The solid will fall to the closed end of the capillary tube if you invert it and lightly tap it against the benchtop. After that, lower the capillary tube multiple times through a long, narrow tube

with the closed side down. The solid will compress into the bottom of the capillary tube as it bounces off the benchtop.

❖ **Partition coefficient** Even though a drug molecule is easily soluble at physiological pH levels, its capability to cross membranes may be greatly influenced by its ability to partition into and cross lipophilic substrates, such as parts of cell walls.

The samples were centrifuged to aid in the development of the bilayer. At least two separate tests were conducted.

Glimepiride's partition coefficient was determined using the formula below:

$$\text{Partition coefficient} = \frac{Concentration\ of\ drug\ in\ organic\ phase}{Concentration\ of\ drug\ in\ aqueous\ phase}$$

❖ **Determination of absorption maxima** For the spectrophotometric examination of glimepiride, methanol was chosen as the optimum solvent. Typically, a plot of absorbance versus wavelength is used to represent the UV spectrum.

❖ **Preparation of standard calibration curve** A Stock Solution of glimepiride 100 µg/mL was prepared by dissolving 10 mg of glimepiride in 1 ml of DMF and then diluting with methanol to the mark of the volumetric flask. In order to generate several clear working standard dilutions of 1, 2, 3, 4, 5, 6, 7, 8, and 9 g/mL, various aliquots of working stock solutions were transferred to 10 mL volumetric flasks, and the volume was made up to mark with methanol.

❖ **Drug and excipients compatibility studies** Studies on the compatibility of drugs and excipients were carried out to ascertain how well the excipients worked with the drugs in the formulation-preparation process. The produced samples were scanned in the 400cm^{-1}-4000cm^{-1} range from which the drug glimepiride spectra and an optimized submicron emulsion were recorded.

❖ **Solubility Studies** In order to assess the solubility of glimepiride in different solvents, such as water, methanol, ethanol, 0.1NHcl, and phosphate buffer Ph6.8, an excess amount of glimepiride was dissolved in 1ml of each of the chosen solvents in stoppered vials. To achieve equilibrium, the mixtures were constantly swirled in a vortex mixer for 10 minutes

and maintained at 37°C ± 0.5°C in a water bath shaker for 24 hours Using a UV-VIS spectrophotometer set to measure substances at 224 nm, the amount of drugs was determined.

➢ **Preparation of glimepiride-loaded Submicron Emulsion** At 55°C, the glimepiride, phospholipid, and alpha-tocopherol (0.3% w/w) was dissolved in soybean oil. At 55 °C, glycerol (2.5%w/w), water, and F68 were combined. To create a coarse emulsion, the water phase was slowly introduced into the lipid phase while the lipid phase was being swirled at 55°C and 2,000 rpm by a high-speed stirrer. The coarse emulsion was processed by a probe sonication to create a fine emulsion. Typical sonication conditions included a 60% amplitude pulse for 1 minute at 25°C. The formulation was then put through a new membrane filter with a 0.45 m pore size. After filtering, the emulsion was placed in plastic bottles and autoclaved for 15 minutes at 121°C to sterilize it.

❖ **Screening of amount of soybean oil** The glimepiride-loaded submicron emulsion was made using various amounts of soybean oil, including 5%w/w, 10%w/w, 15%w/w and 20%w/w. The physical characteristics and glimepiride percentage drug content of the prepared submicron emulsions were examined.

Table: Glimepiride-loaded submicron emulsion containing different amount of soybean oil

S.No.	Formulation code	Glimepiride (%w/w)	Soybean oil (%w/w)	Soybean Phospholipids (%w/w)	Poloxamer F68 (%w/w)	Sonication amplitude (%)
1	GSM1	0.002	5	1.2	0.1	60
2	GSM2	0.002	10	1.2	0.1	60
3	GSM3	0.002	15	1.2	0.1	60
4	GSM4	0.002	20	1.2	0.1	60

❖ **Screening of amount of soya lecithin** To prepare the glimepiride loaded submicron emulsion, different amounts of soya lecithin 0.6%w/w, 1.2%w/w, and 1.8%w/w were used. The physical characteristics and glimepiride percentage drug content of the prepared submicron emulsions were examined.

Table Glimepiride loaded submicron emulsion containing different amplitude

S.No.	Formulation code	Glimepiride (%w/w)	Soybean oil (%w/w)	Soybean Phospholipids (%w/w)	Poloxamer F68 (%w/w)	Sonication amplitude (%)
1	GSM5	0.002	15	0.6	0.1	60
2	GSM3	0.002	15	1.2	0.1	60
3	GSM6	0.002	15	1.8	0.1	60

❖ **Central composite design for optimization**

The screening was organized using a central composite design in order to minimize the number of trials and obtain the most data on product attributes. It has been observed that the qualities of submicron emulsion are influenced by the amount of soybean oil in the formulation (oil%, w/w), and the amplitude. To examine the major effects and interactions of the two factors on the single answer, a two-factor, five-level CCD was used (Table). The design consists of 4 replicated runs (center points) and 9 runs (4 factorial points, 4-star points, and 1 centre point), producing a total of 13 experiments.

Table: Summary of the central composite design

Factor	Name	Units	Low Actual	High Actual	Low Coded	High Coded	Mean
X1	Amount of Soybean oil	%	10	15	-1	1	12.5
X2	Amount of soy lecithin	%	0.6	1.8	-1	1	1.2
Y1	Percentage drug content (%)						

❖ **Desirability function** In order to optimize the glimepiride submicron emulsion, research responses should be assessed. As a result of its incongruence and potential for conflict, it is nearly impossible to concurrently optimize all of the objectives. The region corresponding to the best values for the percentage drug content of glimepiride was identified from a three-dimensional graph of the response against the two factors (X1, X2) in order to determine the condition on the design variables that maximize the percentage drug content of glimepiride was yielded.

➢ **In vitro characterization**

❖ **Physical appearance** To check for the presence of drug particles and the conversion of solid ingredients into liquid solutions, all of the prepared formulations underwent visual inspection.

❖ **pH analysis** The drug-loaded DESs sample weights were determined, and the corresponding volume of deionized water was added to dissolve the samples. The prepared solutions were maintained at room temperature. The pH electrode was dipped into each solution and record the pH of the each solution.

➢ **Percentage drug content** The glimepiride-loaded submicron emulsion was centrifuged at 18,000 g for 30 minutes at 4°C in a centrifuge to separate the incorporated drug from the non-incorporated drug. In order to calculate the percentage of entrapment, the UV spectroscopic analysis of the glimepiride was used to measure the amount of unincorporated drug. After being diluted with methanol, glimepiride concentrations in the emulsion (n1) and free drug in the aqueous (the unincorporated drug) (n2) were measured by UV. The following equation could be used to get EE%.

➢ **Percentage drug content** The glimepiride-loaded submicron emulsion was centrifuged at 18,000 g for 30 minutes at 4°C in a centrifuge to separate the incorporated drug from the non-incorporated drug. In order to calculate the percentage of entrapment, the UV spectroscopic analysis of the glimepiride was used to measure the amount of unincorporated drug. After being diluted with methanol, glimepiride concentrations in the emulsion (n1) and free drug in the aqueous (the unincorporated drug) (n2) were measured by UV. The following equation could be used to get EE%:

$$Percentage\ drug\ content = \frac{Initial\ drug - Drug\ in\ supernatent}{Initial\ drug} \times 100$$

➢ **Viscosity** The viscosity of the produced formulations was measured using a Brookfield digital viscometer based on the parameters and spindle speed. It was reported that the viscosity was directly measured in mPas. During this study, the viscosity of the formulations was evaluated using spindle number 18. The formulations' resistance was tested using the viscometer at 100rpm while the spindle was immersed in the formulations.

➢ **In vitro drug release study** A dialysis approach was used to carry out an in vitro drug release test using submicron emulsion. In a separate beaker, 100 mL of 0.1NHcl and PBS (pH=6.8) were pre-maintained at 100 rpm and 37 °C over the magnetic stirrer, and an accurately measured volume of the drug-loaded submicron emulsion equivalent to 2 mg of glimepiride was added. 5 mL of sample was taken under magnetic stirring conditions, and at each time point, an equal volume of fresh media was added (0.5, 1, 2, 3, 4, 6, 8, 10, 12, and 24h). The withdrawn sample was filtered, and the UV spectrophotometers were used to scan the sample between 200 and 400 nm to calculate the concentration of glimepiride in each sample at 224 nm.

➢ **Drug release kinetics**

❖ **Zero-order kinetics** Drug release rate is independent of concentration during drug dissolution from a dosage form that does not disaggregate and releases the drug slowly, which is represented as:

$$A_0 - A_t = k_t \text{---------- (I)}$$

Where A_0 is initial amount of drug in the dosage form; At is the amount of drug in the dosage form at time‘t’ and ‘k’ is the proportionality constant. Dividing this equation by A0;

$$1-(A_t / A_0) = kt/A_0$$

$$\text{or } 1-A_t / A_0 = k_0 t \text{---------- (II)}$$

Where $1- (A_t / A_0)$ represents the fraction of drug dissolved in time and k0 the zero order release constant. Linear graphs will be used to show how much of the drug dissolves over time.

3. Result and Discussion

➢ **Preformulation studies**

❖ **Organoleptic properties**

Organoleptic properties of drug (API) was found to be as per I.P. monograph.

Table: Observation of Organoleptic properties of glimepiride

S.No.	Test	Specification	Observation
1.	Colour	White to yellowish-white	White to yellowish-white
2.	Odour	Odorless	Odorless
3.	Appearance	Crystalline	Crystalline

❖ **Melting point**

The capillary method was used to determine the melting point of glimepiride. It might be found in table. It was nearly identical to the reference value demonstrating the drug's purity.

Table: Value of Melting point of glimepiride

Drug	Specification	Observation
Glimepiride	207°C(89)	202.33^{0}C±1.15-208±100°C

Standard Calibration curve of glimepiride in Methanol

Concentration (µg/ml)	Absorbance at 224nm
0	0±0
1	0.106±0.001
2	0.224±0.004
3	0.314±0.008
4	0.415±0.004
5	0.522±0.003
6	0.625±0.004
7	0.727±0.002
8	0.821±0.002
9	0.938±0.008

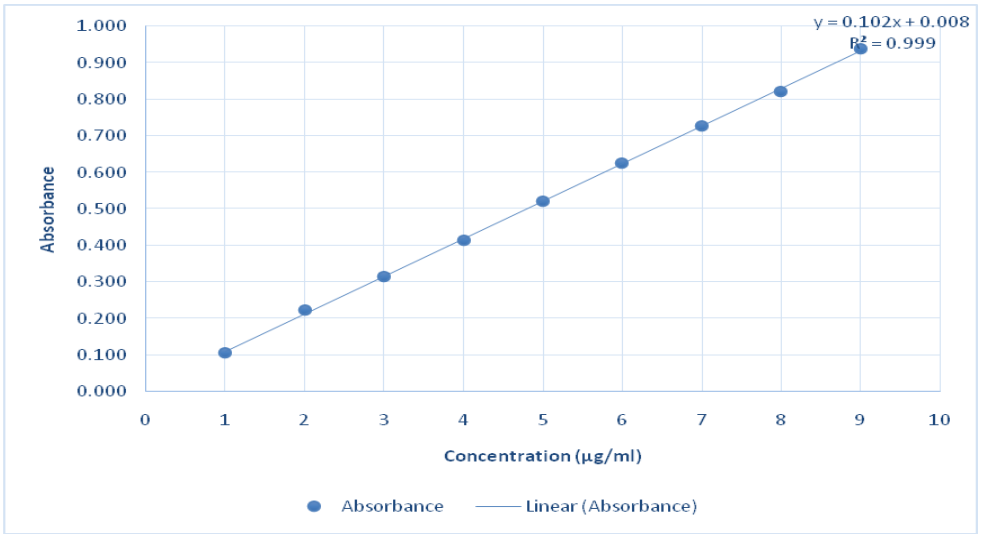
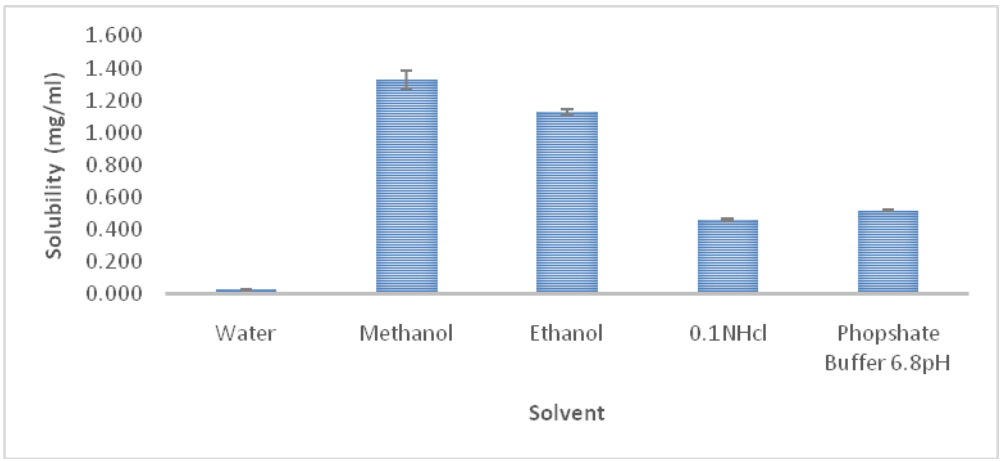


Figure: Graph of Standard Calibration curve between absorbance and concentration of glimepiride in methanol

❖ **Solubility studies of the drug:** The solubility of the drug was determined in solvents of varying polarity are shown in the table.

Table: Value of Solubility (mg/ml) of glimepiride in different solvents.

S.No.	Media	Solubility (mg/ml)
1	Distilled water	0027±0.002
2	Methanol	1.330±0.054
3	Ethanol	1.129±0.014
4	0.1N Hcl	0.461±0.006
5	Phosphate Buffer pH7.4	0.519±0.005

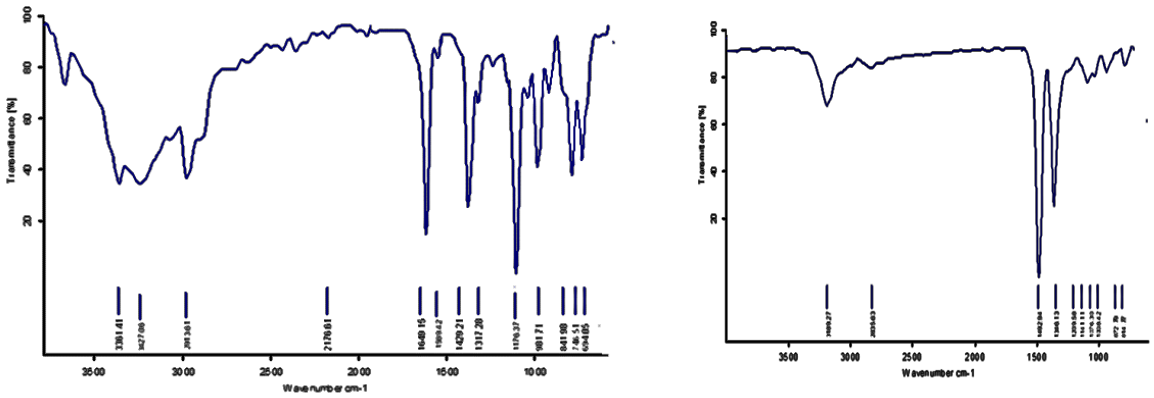


❖ **The partition coefficient of drug** Table: displays the glimepiride partition coefficient in a mixture of n-octanol and water. Drugs having partition coefficients less than one are indicative of hydrophilic drugs, while those with log P greater than one are lipophilic in nature.

Table: Value of Partition coefficient of glimepiride

S. No.	Drug	Reference partition coefficient	Observed Partition coefficient (Log P)	Nature of the drug
1.	Glimepiride	3.81(89)	3.74±0.031	Lipophilic

❖ **Identification of pure drug (FT-IR spectra):-**



❖ **Preliminary screening of the process parameters**

❖ **Effect of the amount of the oil** Investigations into the effects of adding soybean oil in amounts ranging from 5-20%w/w on characterization criteria such appearance, pH, and percentage drug content.

❖ **Physical appearance** The Physical appearance of the all investigated formulation were shown in the table.

Table: Physical appearance of the all-prepared formulation

S.No.	Formulation code	Physical appearance
1	GSM1	Uniform, homogenous, milky emulsion
2	GSM2	Uniform, homogenous, milky emulsion
3	GSM3	Uniform, homogenous, milky emulsion
4	GSM4	Phase separation was observed

❖ **pH** pH of the all prepared formulations is shown in table

Table: pH of the all prepared formulations

S.No.	Formulation code	pH
1	GSM1	6.420±0.024
2	GSM2	6.497±0.086
3	GSM3	6.360±0.043
4	GSM4	6.450±0.045

pH of all prepared formulation was found to be in a range of the 6.360±0.043 to 6.497±0.086.

❖ **Percentage drug contentThe** Percentage drug content of the all prepared formulations were shown in table:

Table: Percentage of drug content of the all prepared formulations

Formulation code	Percentage drug content (%)
GSM1	42.739±1.289
GSM2	76.308±0.250
GSM3	95.248±0.128
GSM4	83.471±0.225

❖ **Effect of amount of the soya lecithin** Investigated were the effects of soy lecithin amounts ranging from 0.6% to 1.8 %w/w on characterization factors as colour, PH, and percentage drug concentration.

❖ **Physical appearance** Physical appearance of the all investigated formulations is shown in table.

Table: Physical appearance of the all-prepared formulation

S.No.	Formulation code	Physical appearance
1	GSM5	Uniform, homogenous, milky emulsion
2	GSM3	Uniform, homogenous, milky emulsion
3	GSM6	Uniform, homogenous, milky emulsion

❖ **pH** pH of all prepared formulation were shown in table.

Table: pH of the all prepared formulations

S.No.	Formulation code	pH
1	GSM5	6.577±0.035
2	GSM3	6.507±0.025
3	GSM6	6.410±0.026

pH of all prepared formulation was found to be in a range of 6.410±0.026 to 6.577±0.035.

➢ **Optimization of the glimepiride submicron emulsion using the central composite design**

Formulation code	Factor 1 X1: Amount of Soybean oil (%w/w)	Factor 2 X2: Amount of soy lecithin (%w/w)	Response 1 Percentage drug content (%)
GSME1	16.03	1.2	95.48
GSME2	10.0	0.6	74.42
GSME3	12.5	0.35	77.77
GSME4	12.5	1.2	93.59
GSME5	12.5	2.04	84.04
GSME6	8.96	1.2	74.29
GSME7	12.5	1.2	94.1
GSME8	12.5	1.2	94.78
GSME9	10	1.8	76.14
GSME10	12.5	1.2	94.05
GSME11	15	1.8	94.09
GSME12	15	0.6	86.14
GSME13	12.5	1.2	93.1

The second-order polynomial equations were fitted as follows:

❖ **Percentage drug content** = $93.924+7.454X1+2.317X2+1.557X1X2-4.568X1^2-6.6558X2^2$.

❖ **Validation of Model Optimization** Submicron emulsions was made utilizing the best process variable settings, where X1 and X2 were equal to 14.28 %w/w and 1.11%w/w, respectively, in order to assess the models' capacity for optimization. The table displays the drug content percentage determined using projected models. The findings demonstrated good concordance between preparation features and theoretical predictions.

Table: Optimized formulation with predicted value of percentage drug content

Formulation code	Amount of Soybean oil (%w/w)	Amount of soy lecithin (%w/w)	Predicted Percentage drug content	Observed Percentage drug content
GSME14	14.28	1.11	96.244	95.606±0.245

➢ **Evaluation of glimepiride-loaded Submicron emulsion**

❖ **Visual Appearance**

Table: Visual appearance of all prepared Submicron emulsion formulations

S. No.	Formulation code	Appearance
1	GSME1	Uniform, Homogenous, no phase separation, Non-precipitation of drug and milky appearance
2	GSME2	Uniform, Homogenous, no phase separation, Non-precipitation of drug and milky appearance
3	GSME3	Uniform, Homogenous, no phase separation, Non-precipitation of drug and milky appearance
4	GSME4	Uniform, Homogenous, no phase separation, Non-precipitation of drug and milky appearance
5	GSME5	Uniform, Homogenous, no phase separation, non-precipitation of drug and milky appearance
6	GSME6	Uniform, Homogenous, no phase separation, non-precipitation of drug and milky appearance
7	GSME7	Uniform, Homogenous, no phase separation, Non-precipitation of drug and milky appearance
8	GSME8	Uniform, Homogenous, no phase separation, Non-precipitation of drug and milky appearance
9	GSME9	Uniform, Homogenous, no phase separation, Non-precipitation of drug and milky appearance
10	GSME10	Uniform, Homogenous, no phase separation, Non-precipitation of drug and milky appearance
11	GSME11	Uniform, Homogenous, no phase separation, Non-precipitation of drug and milky appearance
12	GSME12	Uniform, Homogenous, no phase separation, non-precipitation of drug and milky appearance
13	GSME13	Uniform, Homogenous, no phase separation, non-precipitation of drug and milky appearance
14	GSME14	Uniform, Homogenous, no phase separation, non-precipitation of drug and milky appearance

❖ **pH of prepared formulation**

pH of all prepared formulation is shown in table.

Table: Value of pH of all prepared formulations

S. No.	Formulation code	pH
1	GSME1	6.443±0.040
2	GSME2	6.380±0.010
3	GSME3	6.427±0.038
4	GSME4	6.520±0.026
5	GSME5	6.187±0.042
6	GSME6	6.157±0.049
7	GSME7	6.273±0.025
8	GSME8	6.683±0.091
9	GSME9	6.500±0.030
10	GSME10	6.343±0.031
11	GSME11	6.190±0.050
12	GSME12	6.553±0.055
13	GSME13	6.417±0.057
14	GSME14	6.583±0.031

❖ **Percentage drug content**

Percentage drug content of all prepared formulations is shown in table.

Table: **Value and states of percentage drug content of all prepared formulations**

Formulation Code	Percentage drug content
GSME1	95.476±0.196
GSME2	74.423±0.101
GSME3	77.770±0.122
GSME4	93.592±0.194
GSME5	84.040±0.146
GSME6	74.293±0.190
GSME7	94.111±0.219
GSME8	94.777±0.074
GSME9	76.145±0.128
GSME10	94.046±0.122
GSME11	94.095±0.108
GSME12	86.135±0.097
GSME13	93.104±0.120
GSME14	95.606±0.245

❖ **Globule size and Zeta Potential**

Table: Average Globule size, PDI, and Zeta Potential of GSME14 formulation

S.No.	Formulation code	Globule size (nm)	PDI	Zeta Potential (mv)
1	GSME14	301.27	0.114	-26.4

❖ **Percentage drug release**

Time (Hr.)	% Drug release of pure drug suspension in 0.1NHCl	% Drug release of pure drug suspension in phosphate buffer pH 6.8	% Drug release of formulation GSME14 in 0.1NHCl	% Drug release of formulation GSME14 in phosphate buffer pH 6.8
0	0±0	0±0	0±0	0±0
0.5	7.432±00.207	6.360±0.069	7.212±0.103	6.287±0.172
1	42.836±0.172	37.914±0.179	16.813±0.241	14.059±0.138
2	86.988±1.378	69.201±0.172	22.076±0.172	20.980±0.140
3	93.324±0.689	94.298±0.988	27.339±0.034	24.586±0.069
4	96.491±1.723	95.517±0.245	38.109±0103	36.062±0.034
6			46.491±0.245	43.494±0.075
8			69.201±0.345	60.185±0.689
10			88.450±0.680	79.922±1.034
12			93.567±1.034	88.207±0.345
24			95.760±0.689	91.862±0.378

4. Conclusion The current study involves the enhancement of the oral bioavailability by improve the dissolution of the glimepiride by employing the submicron emulsion.

In preformulation study the capillary method was used to determine the melting point of glimepiride and it was found to be 202.33^{0}C±1.15-208±100 C .The concentration of (8µg/ml) glimepiride showed maximum absorbance at 224 nm. By drawing a graph between the absorbance and concentration, a standard calibration curve was constructed in the range of 1

to 9µg/ml. The absorbance of glimepiride solutions at various concentrations, as displayed in the table. The glimepiride calibration curve, which is depicted in graph 6, revealed the value of the regression equation Y = 0.1026x + 0.0085 and an R^2 value of 0.999, demonstrating good linearity. **G**limepiride was most soluble in alcohol, followed by other alcohols, while it was least soluble in distilled water. Peaks in the IR spectrum were seen at 3361.41 and 3235.15 cm^{-1}, which were caused by the N-H stretch for urea. N-H bending caused a large peak to appear at 1649.15 cm^{-1}. The sulfonamide group was associated with the peaks at 1317.28 and 1176.31 cm^{-1}, while the carbonyl group was related to the peaks at 1649.50 cm^{-1}. The formation of submicron-sized lipid emulsions involved the use of a high-speed mixer and an ultrasonication procedure. This study found that glimepiride was sufficiently soluble in soybean oil but only marginally soluble in water. The findings of the preliminary study suggested Amount of Soybean oil (%w/w) and amount of soya lecithin have e significant effect on the percentage of drug content. For this central composite design was employed to study the effect of both variables on the percentage drug content. In the validation step of the optimization, Submicron emulsions were made utilizing the best process variable settings, where X1 and X2 were equal to 14.28 %w/w and 1.11%w/w, respectively, in order to assess the models' capacity for optimization. All prepared submicron emulsion loaded with glimepiride was uniform, homogenous, free of phase separation, devoid of drug precipitation, and had a milky appearance. The range of the glimepiride drug content was determined to be between 74.423±0.101percent to 94.476±0.196% in all manufactured submicron emulsion formulations. The percentage drug content of glimepiride increases with an increase in oil and soy lecithin amounts. The improved formulation's drug content as a percentage was found to be 95.606±0.245%.The GSME14 formulation was selected for further evaluation based on the aforementioned evaluation criteria. The measured PDI value and globule size were 301.27nm and 0.114, respectively. The arrangement of these emulsifiers at the oil-water interface and the distinct polar head groups in lecithin's structure confer negative (26.4 mV) zeta potential in addition to each other. In comparison to the instant release pure drug suspension in 0.1NHcl and phosphate buffer pH 6.8, glimepiride releases from the improved suspension formulation at 24 hours at a rate of 95.760±0.689%, 91.862±0.378 %.The results of an in vitro drug release kinetic study showed that the formulation GSME14 follows Higuchi's order release kinetic in both the 0.1NHcl and the phosphate buffer pH 6.8 media because it has a higher regression coefficient in each of those media, which is 0.9102 and 0.9252, respectively.

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REFERENCES

1. Thomas VH, Bhattachar S, Hitchingham L, Zocharski P, Naath M, Surendran N, et al. The road map to oral bioavailability: An industrial perspective. Vol. 2, Expert Opinion on Drug Metabolism and Toxicology. 2006.
2. Fahr A, Liu X. Drug delivery strategies for poorly water-soluble drugs. Vol. 4, Expert Opinion on Drug Delivery. 2007.
3. Rathbone MJ, Tucker IG. Mechanisms, barriers and pathways of oral mucosal drug permeation. Vol. 12, Advanced Drug Delivery Reviews. 1993.
4. Stenberg P, Luthman K, Artursson P. Virtual screening of intestinal drug permeability. Journal of Controlled Release. 2000;65(1–2).
5. Karlsson J, Ungell AL, Gråsjö J, Artursson P. Paracellular drug transport across intestinal epithelia: Influence of charge and induced water flux. European Journal of Pharmaceutical Sciences. 1999;9(1).
6. Artursson P, Ungell AL, Löfroth JE. Selective Paracellular Permeability in Two Models of Intestinal Absorption: Cultured Monolayers of Human Intestinal Epithelial Cells and Rat Intestinal Segments. Pharmaceutical Research: An Official Journal of the American Association of Pharmaceutical Scientists. 1993;10(8).
7. Norris DA, Puri N, Sinko PJ. The effect of physical barriers and properties on the oral absorption of particulates. Vol. 34, Advanced Drug Delivery Reviews. 1998.
8. Tavelin S, Hashimoto K, Malkinson J, Lazorova L, Toth I, Artursson P. A New Principle for Tight Junction Modulation Based on Occludin Peptides. Mol Pharmacol. 2003;64(6).
9. Cano-Cebrian M, Zornoza T, Granero L, Polache A. Intestinal Absorption Enhancement Via the Paracellular Route by Fatty Acids, Chitosans and Others: A Target for Drug Delivery. Curr Drug Deliv. 2005;2(1).
10. Gasbarrini G, Montalto M. Structure and function of tight junctions. Role in the intestinal barrier. Ital J Gastroenterol Hepatol. 1999;31(6).
11. Gumbiner B. Structure, biochemistry, and assembly of epithelial tight junctions. Vol. 253, American Journal of Physiology - Cell Physiology. 1987.
12. Cereijido M, Shoshani L, Contreras RG. Molecular physiology and pathophysiology of tight junctions. I. Biogenesis of tight junctions and epithelial polarity. Vol. 279, American Journal of Physiology - Gastrointestinal and Liver Physiology. 2000.