

**“COMPARATIVE IN-VITRO EVALUATION
OF GLIMEPIRIDE NANOSUSPENSION
PREPARED BY DIFFERENT TECHNIQUES”**

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENT FOR THE DEGREE OF
MASTER OF PHARMACY

In

PHARMACEUTICS

By

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Under the Guidance of

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ABSTRACT

Nanosuspension is a part of nanotechnology which is a submicron colloidal dispersion of pharmaceutical active ingredients in a liquid phase having size range below 1 μm , and which is stabilized by surfactants and polymers. Most of the newly developed drugs are water insoluble, shows poor bioavailability. Glimepiride is an anti-diabetic drug belongs to sulfonylurea class, which is used to treat type II diabetes mellitus. Glimepiride increases the insulin secretion by acting on β -cells of the pancreas. Glimepiride binds to sulphonylurea receptors which are present on β -cell on the plasma membrane, which close the ATP-sensitive potassium channel leading to depolarization of cell membrane. So there is the opening of voltage-gated calcium channel due to which there is an influx of calcium ions causes secretion of the preformed insulin molecule. It is categorized under biopharmaceutical classification system class II drug, having poor solubility and high permeability. In this study different methods were used to formulate the nanosuspension of glimepiride to increase the solubility of glimepiride. Twelve formulations of Glimepiride nanosuspensions were prepared by combination method which includes antisolvent precipitation method followed by sonication in which drug was taken with polymers in ratio of 1:10, 1:20 and 1:30 and 6 formulations were prepared by nanoprecipitation method in which drug was taken with polymer in ratio of 1:10, 1:20 by using different polymers like PVPK30, PEG 6000 and PEG 400 according to literature review. Characterization of glimepiride nanosuspensions prepared by different techniques was done by optical microscopy, entrapment efficiency, particle size analysis, zeta potential, TEM and In-vitro dissolution. From the results of different characterization, G1 formulation was found best formulation having highest entrapment efficiency i.e 82.16%, particle size in range of 129-180 nm, zeta potential value 30.16 mV and PI value 0.253 and highest drug release profile 86.76% as compared to Gii formulation. From different studies it is concluded that glimepiride nanosuspension G1, prepared by combination technique shows good solubility and dissolution than Gii and the combination technique is the better technique to prepare glimepiride nanosuspension.

Keywords: Glimepiride, Nanosuspension, Anti-Diabetic, Solubility, Polymers, Drug Delivery.

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LIST OF ABBREVIATIONS

| | |
|----------------|---|
| DM | Diabetes mellitus |
| IDDM | Insulin dependent diabetes mellitus |
| NIDDM | Non-insulin dependent diabetes mellitus |
| COA | Certificate of analysis |
| TEM | Transmission electron microscopy |
| ⁰ C | Degree centigrade |
| % E.E | Percentage entrapment efficiency |
| et al. | And co-worker |
| cm | Centimeter |
| WHO | World Health Organisation |
| NDDG | National Diabetes Data Group |
| ADA | American Diabetes Association |
| IDF | International Diabetes Federation |
| FDA | Food and drug administration |
| SUR | Sulphonylurea receptors |
| GI | Gastrointestinal |
| GIT | Gastrointestinal tract |
| pH | Power of hydrogen |
| λ | Lambda |
| μg | Microgram |
| mg | Milligram |
| min | Minute |
| ml | Millilitre |
| nm | Nanometer |
| %RSD | Percentage Relative Standard Deviation |
| SCF | Super critical fluid |
| BCS | Biopharmaceutics classification system |
| KBr | Potassium bromide |
| FTIR | Fourier transform infrared spectrometer |
| UV | Ultraviolet |
| LOD | Limit of detection |
| LOQ | Limit of quantification |
| ICH | International conference on Harmonization |
| hrs | Hours |
| DCM | Di-chloromethane |
| rpm | Rotations per minute |
| PEG | Poly ethylene glycol |
| mV | Micro volt |
| PI | Polydispersity Index |

CHAPTER-1

INTRODUCTION

Diabetes mellitus (DM) is chronic, life-long endocrine and metabolic disorder which occurs due to the defect in insulin secretion and insulin action. Insulin is hormone which is produced by a specialized cell called as β -cells present on organ pancreas. Normally our body breakdown the carbohydrates and sugars which convert into glucose molecule and act as fuel for our body, but for utilization of glucose, hormones insulin is required. Deficiency of insulin leads to increase blood glucose level in a body along with disturbances in the metabolism of carbohydrates, fats, and proteins. If diabetes is uncontrolled then it leads to severe diabetic complications like retinopathy, neuropathy, and various cardiovascular complications. Diabetes is most common disease by the year 2010 and more than 200 million people are suffering from DM. The diagnostic criteria and types of diabetes were first forwarded by World Health Organization (WHO) in 1965, after that, it was forwarded to National Diabetes Data Group (NDDG), and this was followed according to WHO in 1980. The WHO Recommendations were modified in 1985. And the latest recommendation was published by American Diabetes Association (ADA) in 1997 and by WHO in 1999. (Bastaki .S, 2005)

India is having the huge population of diabetes in all over the world. And according to international diabetes federation (IDF), so many people with diabetes in India will reach 80 million by the year 2025. One survey represents that 4% of adults in India will suffer from diabetes in the year 2000 and it is expected to increase up to 6% from the year 2025. (Piero, 2015)

1.1 Types of DM

There are three major types of DM

- Type-1 (IDDM)
- Type-2 (NIDDM)
- Gestational diabetes

1.1.1 Type -1

Type -1 DM is also termed as insulin-dependent DM or juvenile onset diabetes because it is started from childhood. In this type, the pancreas is damaged so there is no production of insulin due which type-1 diabetes is caused.

1.1.2 Type-2

Type-2 DM is also known as non-insulin dependent DM or adult onset diabetes because it mainly occurs in adult people. In this type pancreas normally produces some insulin but the amount of insulin produced is not enough for body or body cells are resistant to insulin. This type of diabetes mainly occurs in obese patient.

1.1.3 Gestational diabetes

This is the type of diabetes which occurs during pregnancy. (Bastaki, 2005)

Table 1.1

Clinical characteristics of patients with type-1 and type 2 diabetes mellitus. (Ozougwu et al., 2013)

| Features | Type-1 | Type-2 |
|---------------------|--------------------------|---|
| Body mass | Low to normal | Obese |
| Plasma glucagon | High , can be suppressed | High , resistant to suppression |
| Plasma insulin | Low or absent | Normal to high initially |
| Plasma glucose | Increased | Increased |
| Insulin sensitivity | Normal | Reduced |
| Therapy | Insulin | Thiozolidenediones,sulphonylureas,metformin,insulin |

Table 1.2

Suggested blood sugar levels

| Age (years) | mg/dl | Mmol/l |
|--------------------|---------|----------|
| Below – 5 years | 80-200 | 4.5-11.1 |
| 5-11 years | 70-180 | 3.9-10.0 |
| 12 and above years | 70 -150 | 3.9-8.3 |

1.2 Contributing factors

1.2.1 Type -1 DM

- DM may be caused due to genetics
- May be caused due to any infection or other stress

1.2.2 Type-2 DM

- Obesity
- Age
- Lack of activity
- Genetic predisposition
- Condition associated with insulin resistance.(polycystic ovary syndrome).(Ozougwu et al., 2013)

1.3 Pathophysiology of DM

1.3.1 Pathophysiology of type-1 DM

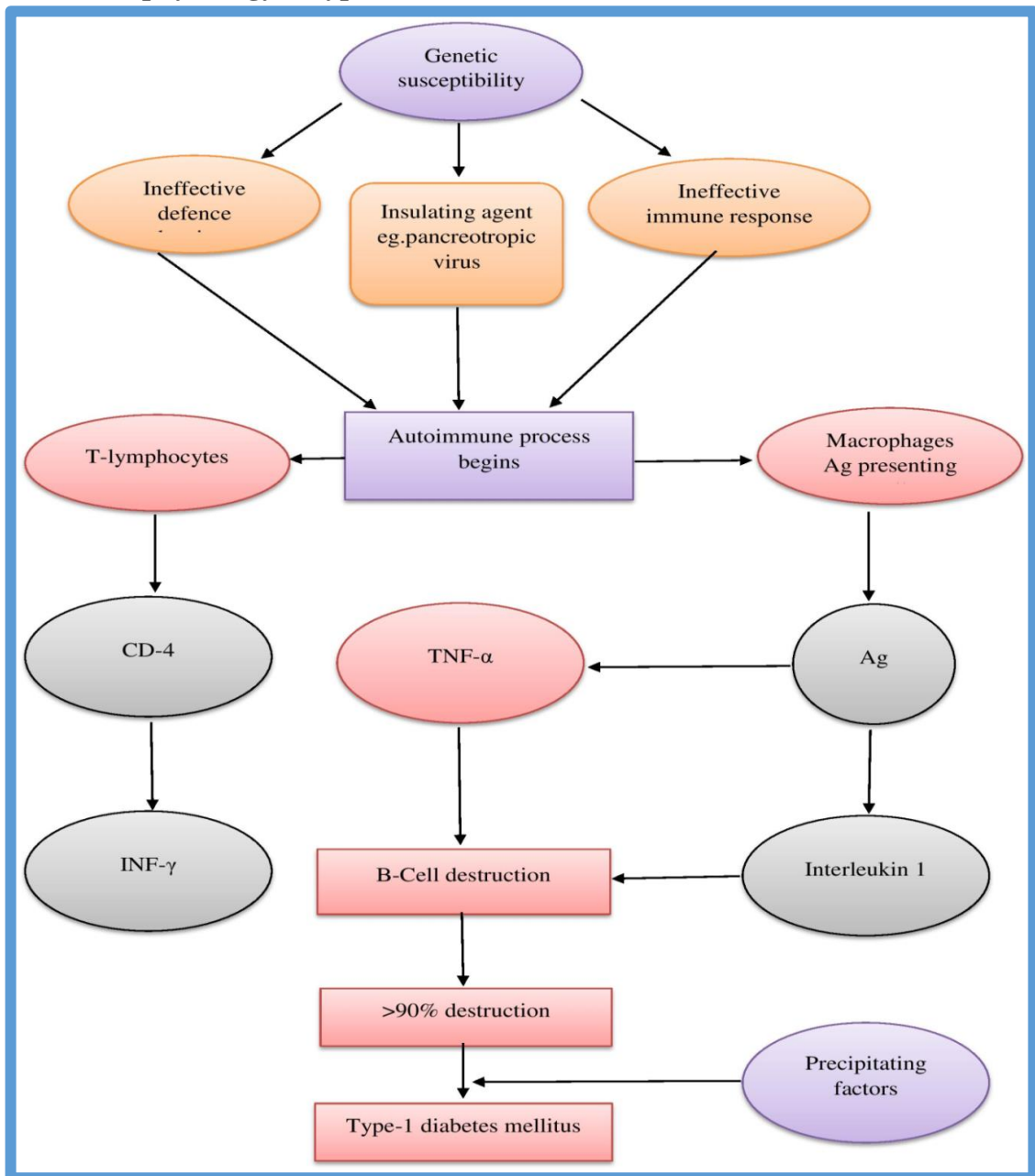


Fig.1.1 Pathophysiology of type-1 DM

1.3.2 Pathophysiology of type-2 DM

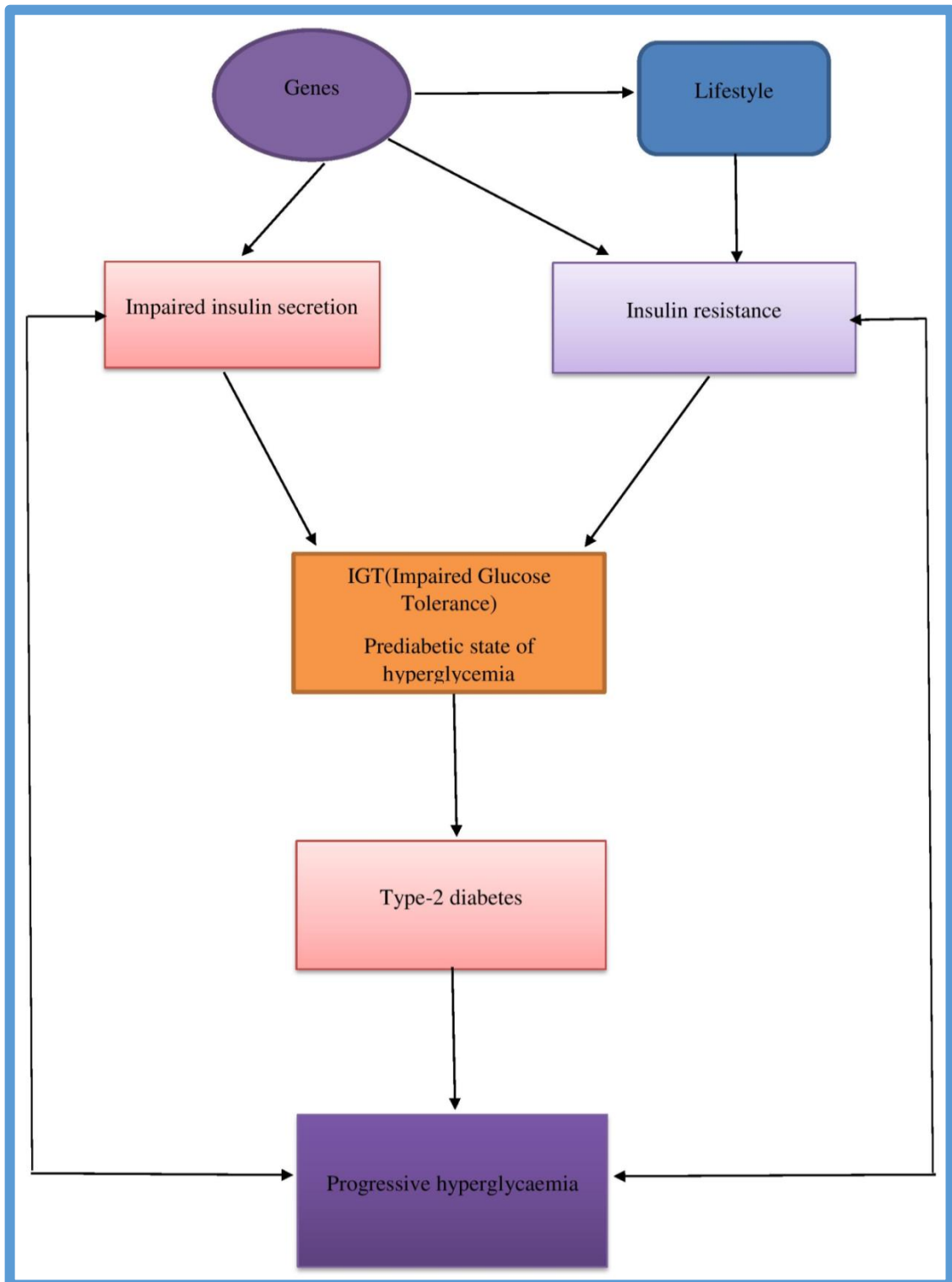


Fig. 1.2 Pathophysiology of type-2 DM (Ozougwu et al., 2013)

- Type 2 DM is the most common type of diabetes which is indicated by a disorder of insulin secretion and insulin resistance (Bastaki, 2005)

- DM having different characteristic symptoms like polyuria, thirst, blurring of vision and weight loss

1.4 Treatment of Diabetes

1.4.1 Oral Anti-Diabetic Drugs (Tripathi .KD)

| | |
|--|--|
| <ul style="list-style-type: none"> • <u>SULPHONYLUREAS:-</u> | |
| First generation: <ul style="list-style-type: none"> ❖ Chlorpropamide ❖ Tolbutamide | Second generation: <ul style="list-style-type: none"> ❖ Glimpiride ❖ Glibenclamide ❖ Gliclazide ❖ Glipizide |
| <ul style="list-style-type: none"> • <u>BIGUANIDES:-</u> | |
| <ul style="list-style-type: none"> ❖ Metformin | |
| <ul style="list-style-type: none"> • <u>MEGLITINIDE:-</u> | |
| <ul style="list-style-type: none"> ❖ Nateglinide ❖ Repaglinide | |
| <ul style="list-style-type: none"> • <u>THIAZOLIDINEDIONES:-</u> | |
| <ul style="list-style-type: none"> ❖ Rosiglitazone ❖ Pioglitazone | |
| <ul style="list-style-type: none"> • <u>ALPHA-GLUCOCIDASE INHIBITORS:-</u> | |
| <ul style="list-style-type: none"> ❖ Acarbose ❖ Miglitol | |

Fig.1.3 Classification of anti-diabetic drugs

Since 1995 there are so many anti-diabetic medications for the management of type two DM which are approved by FDA. Traditionally in oral hypoglycemic therapy, the class sulphonylurea has always been the agent of the first choice. (Kannan et al., 2011)

Sulphonylureas were discovered in 1942, and these are structurally related to sulphonamides when it was noted that sulphonamides caused hypoglycaemia in experimental animals.

Sulphonylureas are the first class of Anti-diabetic Drugs which is generally used in the treatment of type -2 DM. They increase the insulin secretion by acting on β -cells of pancreas. These agents are not used in type – 1 DM because in type -1 DM there is a lack of insulin secretion.(Bastaki, 2005)

1.4.2 Mechanism of action of Sulphonylureas

Sulphonylureas increase the insulin secretion by acting on β -cells of the pancreas. They bind to sulphonylurea (SUR) receptors which are present on β -cell on the plasma membrane, which closes the ATP-sensitive potassium channel leading to depolarization of cell membrane, by which there is the opening of voltage-gated calcium channel due to which there is an influx of calcium ions causes secretion of preformed insulin molecule.(Bastaki, 2005)

Among the various drugs from class sulphonylurea, which are used, Glimepiride (GMP) which is third generation sulphonylurea is one of the first-line drug of choice for management of NIDDM. (Mahalaxmi et al., 2010)

Glimepiride is an anti-diabetic drug belongs to class sulphonylurea which is widely used for the treatment of type – 2 DM. Glimepiride is only the drug which lowers the blood glucose level in the healthy subject as well as patient with type-2 DM. Glimepiride belongs to BCS (Biopharmaceutics classification system) Class-II, having low solubility and high permeability.(KAKU, 2010)

BCS is a system used for classifying drugs based on its solubility and permeability.It is used for the prediction of in vivo pharmacokinetics of oral immediate release product by classifying drugs into for classes.

1.4.3 Table 1.3

Biopharmaceutics classification system: - (Basanta Kumar Reddy & Karunakar, 2011)

| Class | Solubility | Permeability | Examples |
|-------|------------|--------------|---------------------|
| I | High | High | Metoprolol |
| II | Low | High | Glimepiride |
| III | High | Low | Cimetidine |
| IV | Low | Low | Hydrochlorothiazide |

Glimepiride is insoluble in water so its oral absorption is dissolution rate limited due to it shows poor solubility in GI Fluid as a result having poor bioavailability. There are different techniques used to enhance the solubility of the drug. As solubility increases leads to increase in bioavailability. The major problem faced by oral administration is bioavailability and we can increase bioavailability by increasing the solubility of that particular drug. (Tiwari et al., 2016)

1.5 Solubility profiles

1.5.1 Need of solubility

Drug absorption from GIT is poor if drug having poor solubility. When a drug is administered orally then it must first dissolve in the gastric or intestinal fluid before permeating a membrane of GIT to reach in the systemic circulation. Pharmaceutical research focus on improving the bioavailability of drug includes:

- Enhancement of solubility of poorly water soluble drug
- Enhancement of dissolution of poorly water soluble drug

1.5.2 Solubility

Solubility may be defined as the maximum quantity of solute dissolves in a particular quantity of solvent at a particular temperature. Solubility is one of the important parameters to achieve the desired concentration of the drug in systemic circulation to get desired pharmacological response. The poor solubility and dissolution of the drug in GIT fluid result in poor bioavailability. (Patel et al., 2012)

1.5.3 Process of solubilisation

- Separation of the solute molecule which provides space for the solvent molecule
- Breakdown of the intermolecular ionic bond of solute molecule
- Then interaction of solute and solvent molecule

1.5.4 Table 1.4 Solubility may be defined as: (Kadam et al., 2013)

| Descriptive term | Parts of solvent required for one part of solute |
|-----------------------|--|
| Very soluble | <1 |
| Freely soluble | 1-10 |
| Soluble | 10-30 |
| Sparingly soluble | 30-100 |
| Slightly soluble | 100-1000 |
| Very slightly soluble | 1000-10,000 |
| Insoluble | >10,000 |

1.6 Techniques used to increase Solubility

There are different techniques which are used to increase solubility which are as follows: - (Savjani et al., 2012)

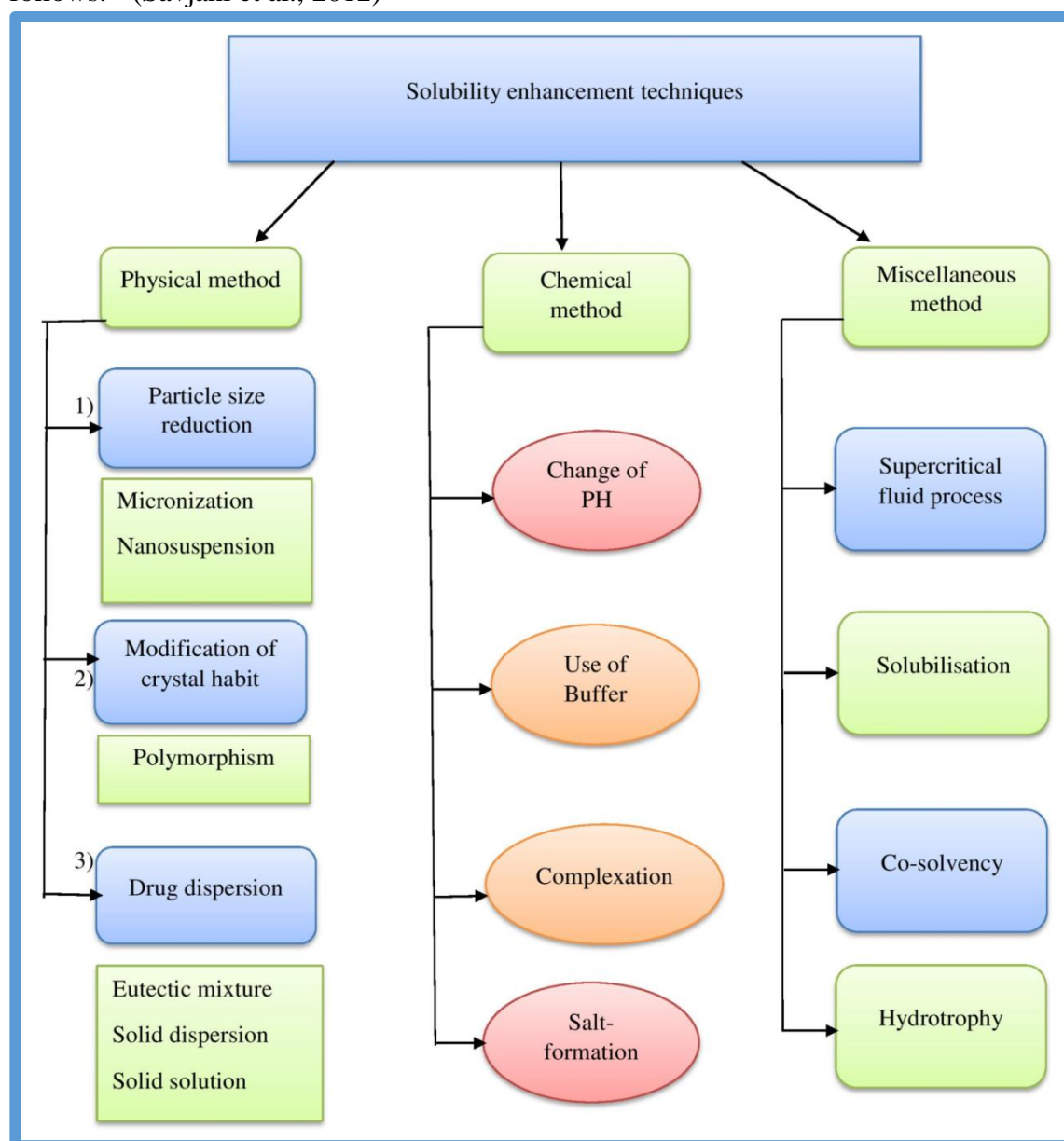


Fig.1.4 Methods for solubility enhancement

Methods which are used for solubility enhancement are as follows

1.6.1 Particle size reduction

Solubility is related to particle size of the drug, smaller the particle size greater will be the surface area and larger the surface area greater will be interaction with solvent hence greater will be the solubility. So by particle size reduction we can increase the solubility of poorly soluble drugs. (Sheetal Z Godse and Patil, Swapnil M Kothavade, 2013)

Techniques for particle size reduction**1.6.1.1 Micronization**

Micronization is a process of reducing the particle size due to which there is an increase in surface area of that drug. Greater the surface area greater will be the interaction with solvent molecule hence more soluble the drug. micronization can be done by milling techniques like jet milling, colloid milling etc.

1.6.1.2 Nanosuspension

Nanosuspension is a biphasic system composed of the finely divided insoluble solid drug of size range 10^{-9} m in a liquid medium which is stabilised by surfactants. Because of reduced size of the drug, there is an increase in solubility. So by the formation of Nanosuspension of poorly soluble drug we can increase the solubility of that drug. This technique is used for that drugs which are insoluble in both water and oil. Various methods are utilized for the formation of Nanosuspension like-precipitation technique, media milling etc.

Technologies used for preparation of nanosuspensions

Precipitation methods (Bottom-up methods)

Dispersion methods (Top-down techniques)

Combination methods (Andhale et al., 2016)

1.6.2 Hydrotrophy

Hydrotrophy is one of solubilization technique in which there is the addition of a large amount of solute which results in an increase in solubility of existing solute.

1.6.3 Cosolvency

This is solubilization technique in which solubility of the poorly soluble drug in water can be increased by mixing the drug with water miscible solvent in which drug become soluble. This process is known as solvency and solvent used is known as a cosolvent. Cosolvent act by decreasing interfacial tension between the hydrophobic solute and aqueous solution. (Parve et al., 2014)

1.6.4 Solubilization by using surfactants

Surfactants are the substances which are used to reduce the surface tension between two liquids or between solid and liquid. These are the molecules having different polar and nonpolar group. Polar groups may be anionic, cationic, and non-ionic. Surfactants decrease the surface tension and help in increasing the solubility.

Surfactants are also used to stabilize the drug formulation. If the concentration of surfactant is more than its critical micelles concentration which in range 0.05- 0.10% then micelles formation occurs in which drug is entrapped, this process is known as micellization which is generally used to increase the solubility of poorly soluble drugs. Most common surfactants used are-tween-80 and SLS.

1.6.5 Solid dispersion

The Solid dispersion may be defined as a dispersion of one or more active ingredient in an inert matrix which is in solid state and prepared by melting method, solvent method and melting- solvent method. Solid dispersion consists of two different components one is hydrophilic matrix and second is a hydrophobic drug. And the hydrophilic matrix may be crystalline and amorphous. Most commonly used hydrophilic carriers are polyvinylpyrrolidone, PEG.

Techniques for preparing solid dispersion

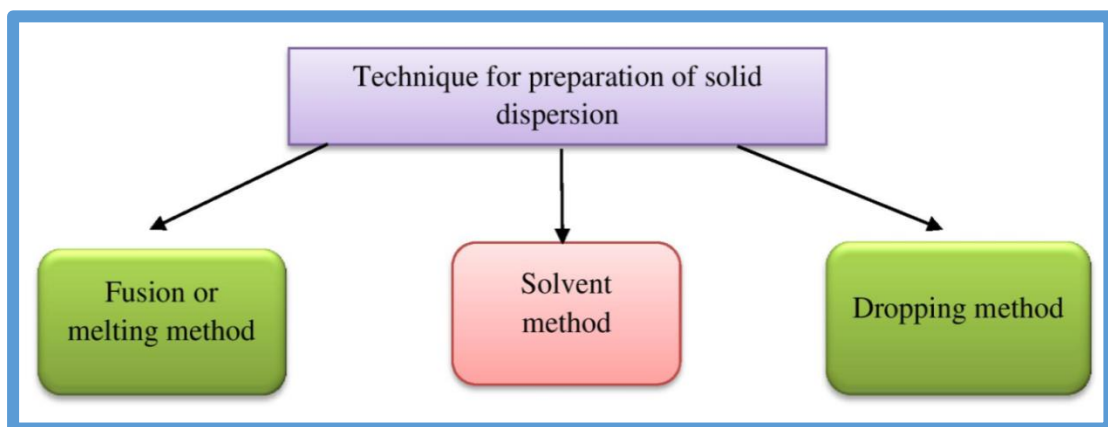


Fig.1.5 Methods for Preparation of Solid Dispersion (Patil et al., 2013)

1.6.5.1 Fusion melting technique

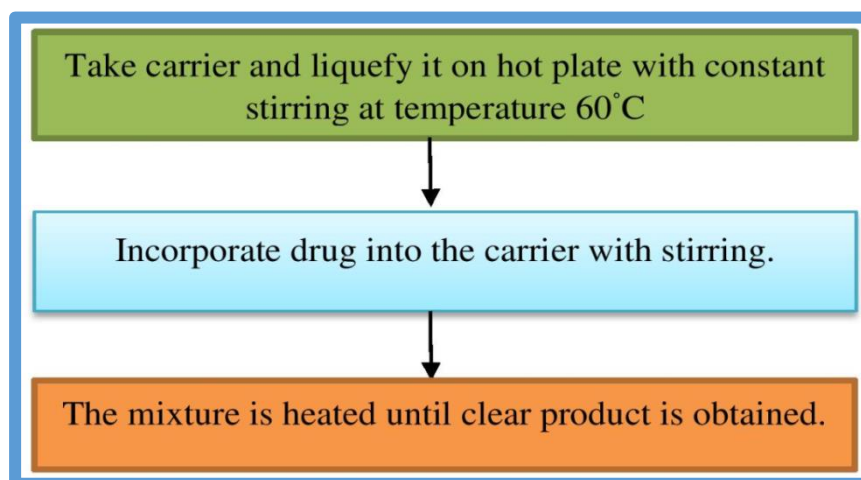
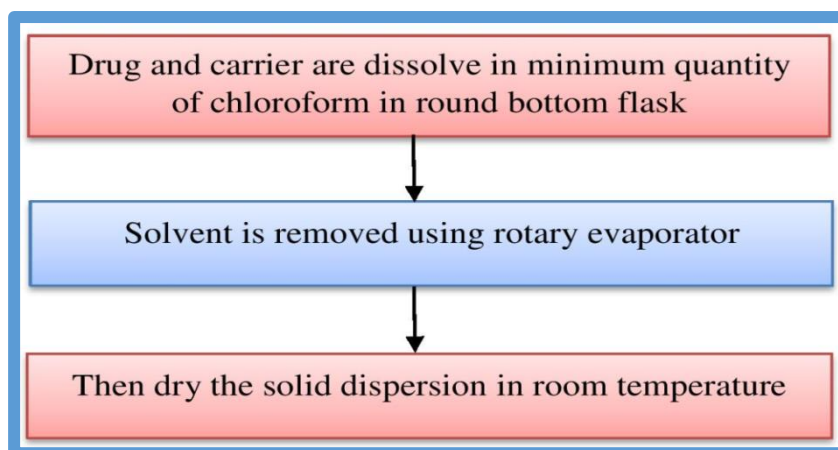
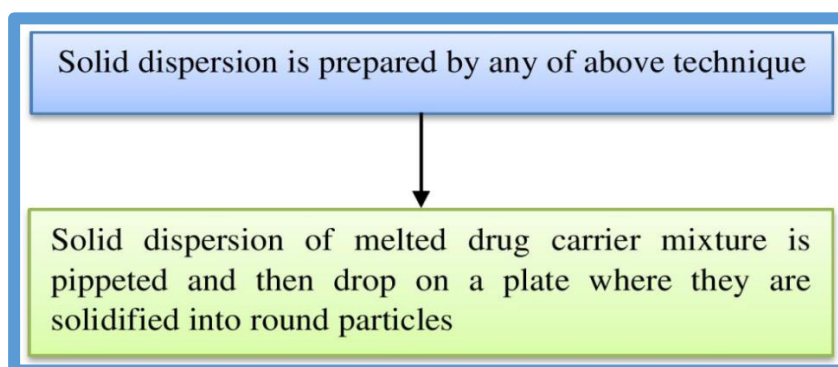


Fig.1.6 Steps of Fusion Melting Technique

1.6.5.2 Solvent method:**Fig.1.7** Steps of the solvent method**1.6.5.3 Dropping method:****Fig.1.8** Steps of Dropping Method**1.6.6 pH adjustment**

Drugs which are poorly water soluble can be dissolved by changing the pH. The solubility of the poorly soluble drug can be increased by protonating and deprotonating the molecule. The solubility of any compound depends on the pH and pKa value. By this method hydrophobic drugs can be dissolved by protonating and deprotonating the molecule, and for a weakly acidic drug with low pKa value and for weakly basic drugs with high pKa value. This is the simplest and easiest technique which is used to increase the solubility of poorly soluble drugs. (Patil et al., 2013)

1.6.7 Complexation

Complexation may be defined as the process of interaction between two or more molecules to form a non-bonded compound with defined stoichiometry.

Complexation involves weak forces like London forces, hydrogen bonding, and hydrophobic interactions. (Vimalson, 2016)

Table 1.5

Examples of complexing agents are as follows

| Sr.no. | Types | Examples |
|--------|----------------------------|--------------------------------|
| 1 | Inorganic complexing agent | I_B^- |
| 2 | Chelates | EGTA,EDTA |
| 3 | Metal olefin | Ferrocene |
| 4 | Molecular complexes | Polymers |
| 5 | Inclusion | Choleic acid,cyclodextrin |
| 6 | Coordination | Hexamine cobalt (III) chloride |

Complexation is the most widely used technique to enhance the solubility and stability of hydrophobic drugs.

There are two types of complexation:-

1.6.7.1 Staching complexation

Stacking complexes are formed by the association of nonpolar area of drug and complexing agent which results in the exclusion of water molecule from the nonpolar area. This causes a reduction in total energy of the system. These complexes can be homogenous/mixed. Examples of compounds that forms stacking complexes are Nicotinamide, pyrene, anthracene, salicylic acid, and benzoic acid.

1.6.7.2 Inclusion complexation

Inclusion complexes are an association of nonpolar region of one molecule into the cavity of another molecule. It is non-bonded entity does not include forces so called as non-bonded complexes. Cyclodextrin and their derivatives are widely used in complexation. Cyclodextrin forms complex with drug and enhance its solubility and bioavailability. Most common derivatives of cyclodextrin used in pharmaceutical formulations are: R- cyclodextrin, and hydroxyl propyl-R-cyclodextrin HP-R-CD (Kadam et al., 2013)

1.6.7.3 Techniques which are used in complexation

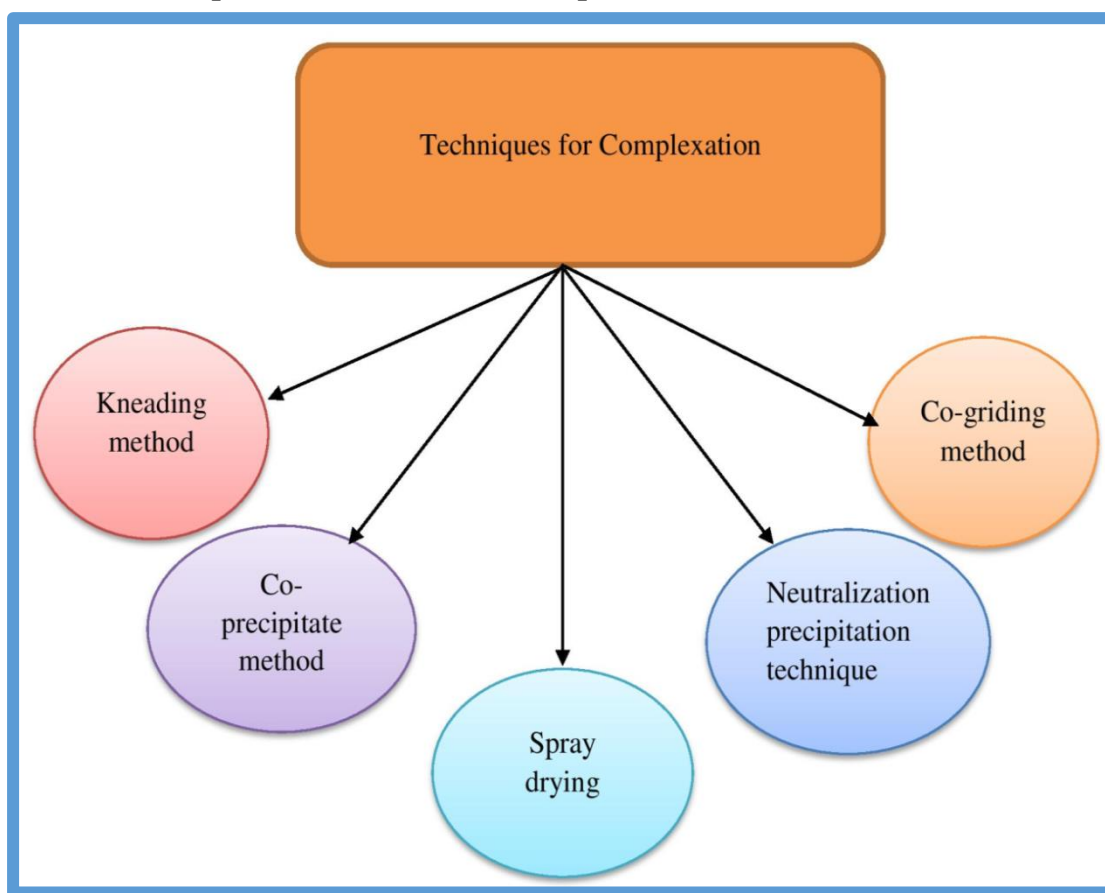


Fig.1.9 Complexation Techniques

1.6.7.3.1 Kneading method: (Kumar and Singh, 2016)

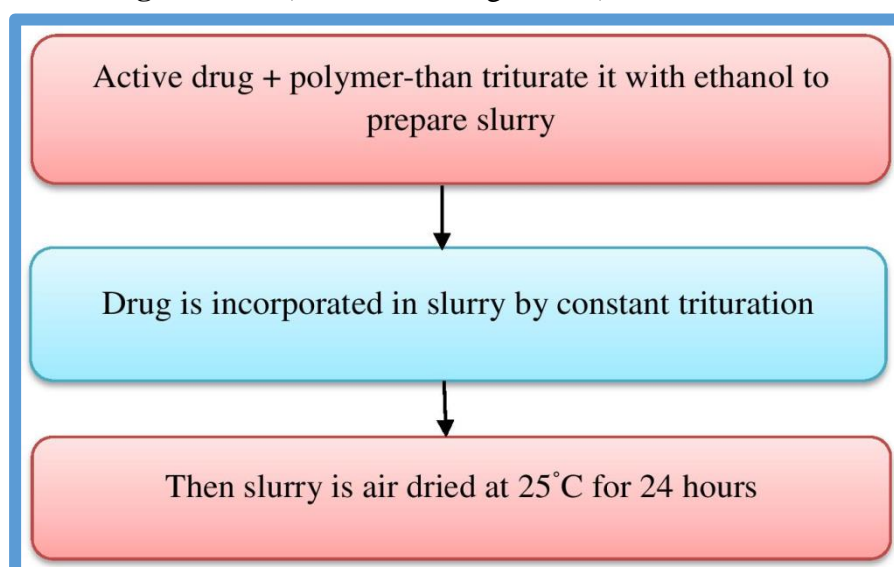
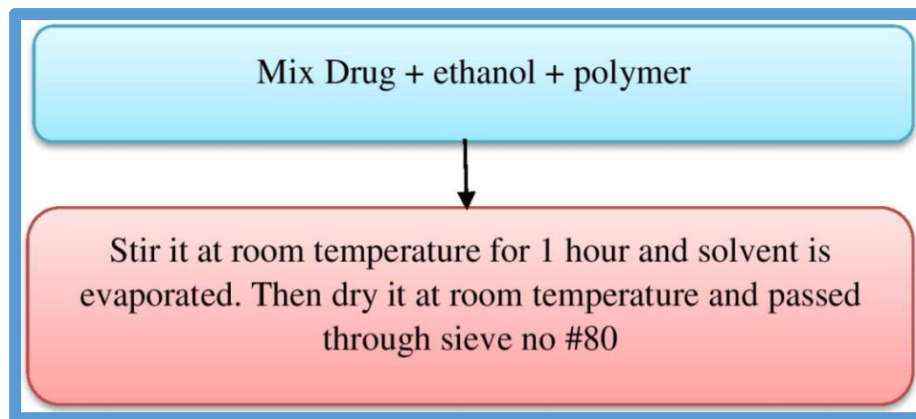
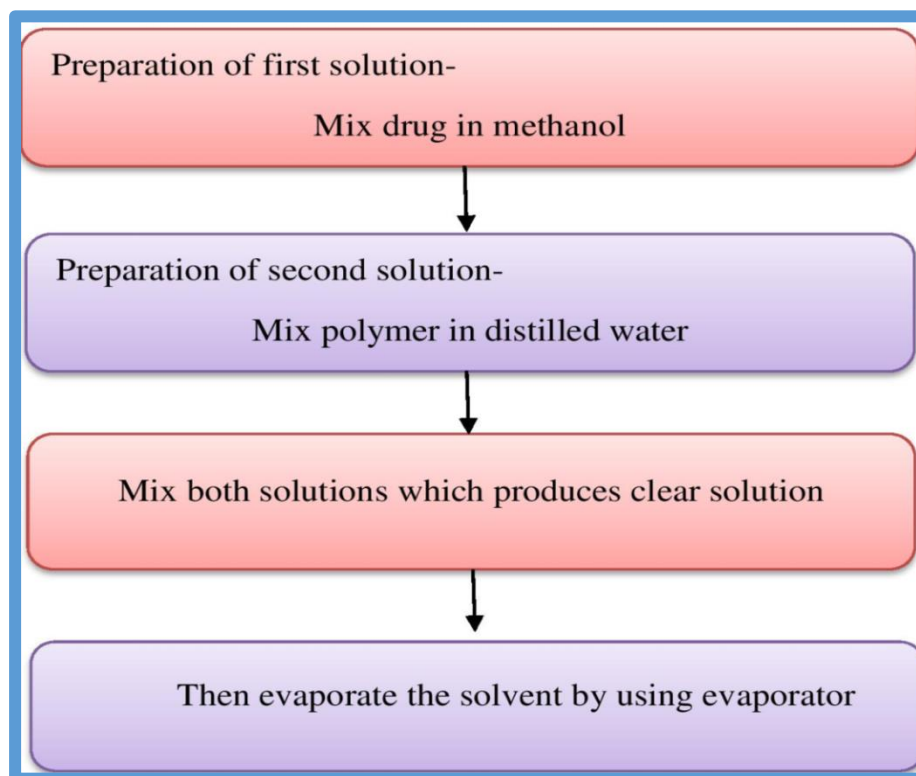


Fig.1.10 Kneading method

1.6.7.3.2 Co-precipitate method:**Fig.1.11** Co-precipitate method**1.6.7.3.3 Spray drying:****Fig.1.12** Steps of Spray drying

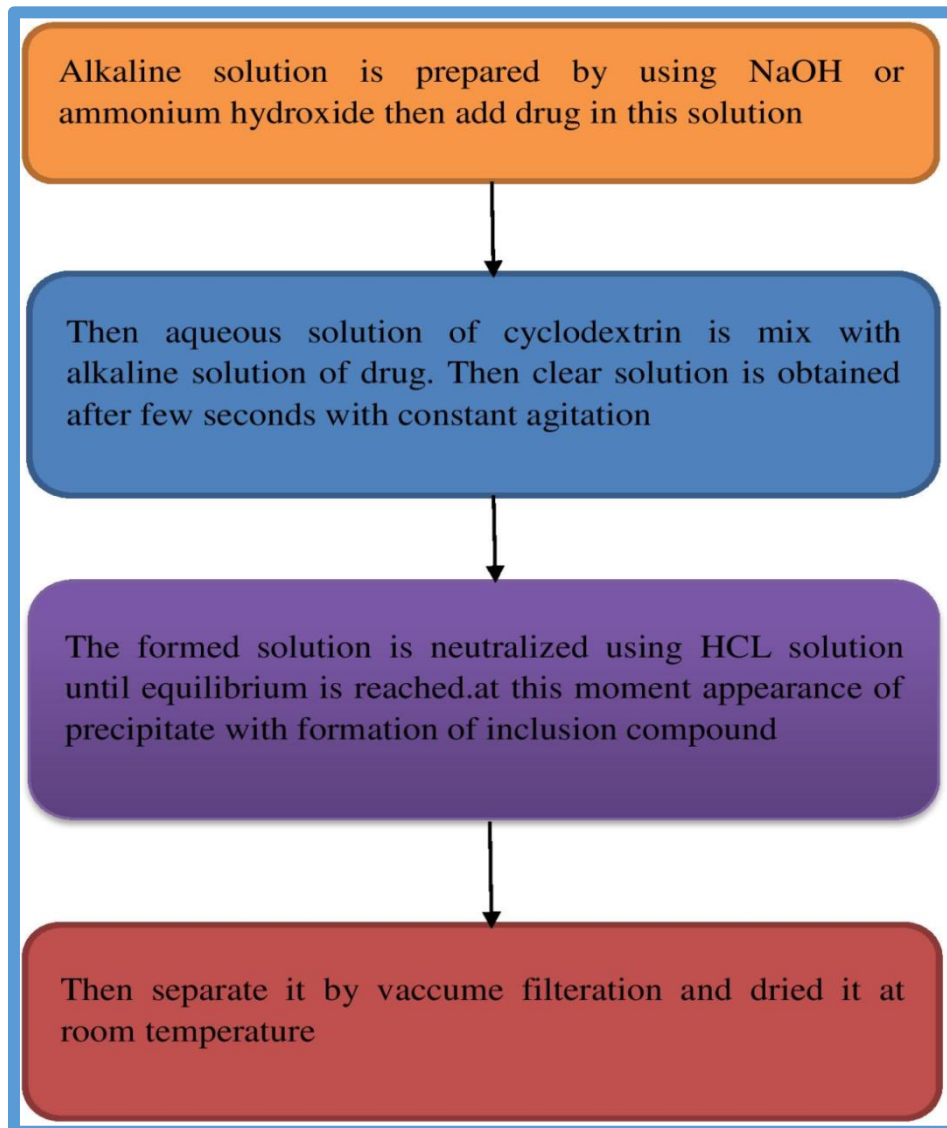
1.6.7.3.4 Neutralization precipitation technique

Fig.1.13 Neutralization precipitation technique (Patil et al., 2013)

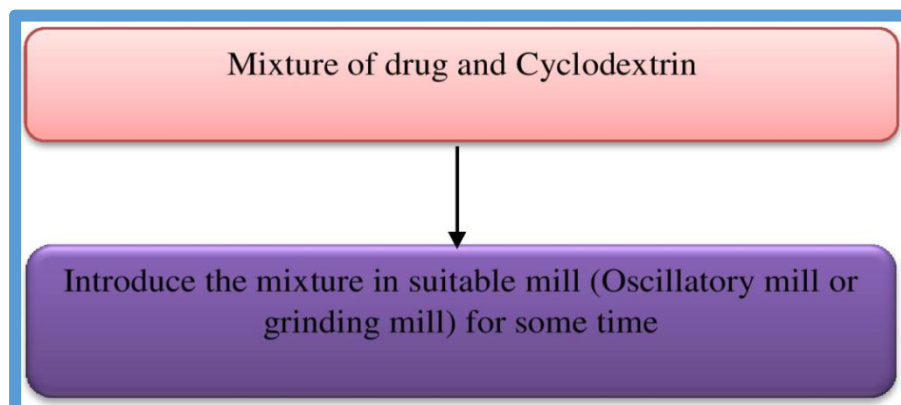
1.6.7.3.5 Co-grinding method

Fig.1.14 Steps of Co-grinding Method

1.6.8 Cryogenic technique

Cryogenic techniques produce Nano range amorphous drug particles with high porosity at very low temperature thus enhances the dissolution rate of the drug. Factors which affect the cryogenic inventions are type of injection device (capillary, rotatory, pneumatic and ultrasonic nozzle), location f nozzle (above or under the liquid solvent), and the composition of cryogenic liquid (hydro fluoroalkanes, nitrogen, argon, oxygen and organic solvent).After the completion of the process, drying can be done by various techniques: spray freeze drying, atmosphere freeze drying, vacuum freeze drying and lyophilization. (Savjani et al., 2012)

1.6.9 Salt formation

Almost 75% of all drugs are weak base, 20% are weak acids, and remaining 5% are non-ionic, amphoteric, and alcohols in nature. Salt formation is the simplest and most efficient method to increase the solubility of the acidic and basic drugs. Improvement in solubility of these drugs can be achieved by selection of suitable salt. Weak acid and weak base can be used to form a salt of these drugs. (Vimalson, 2016)

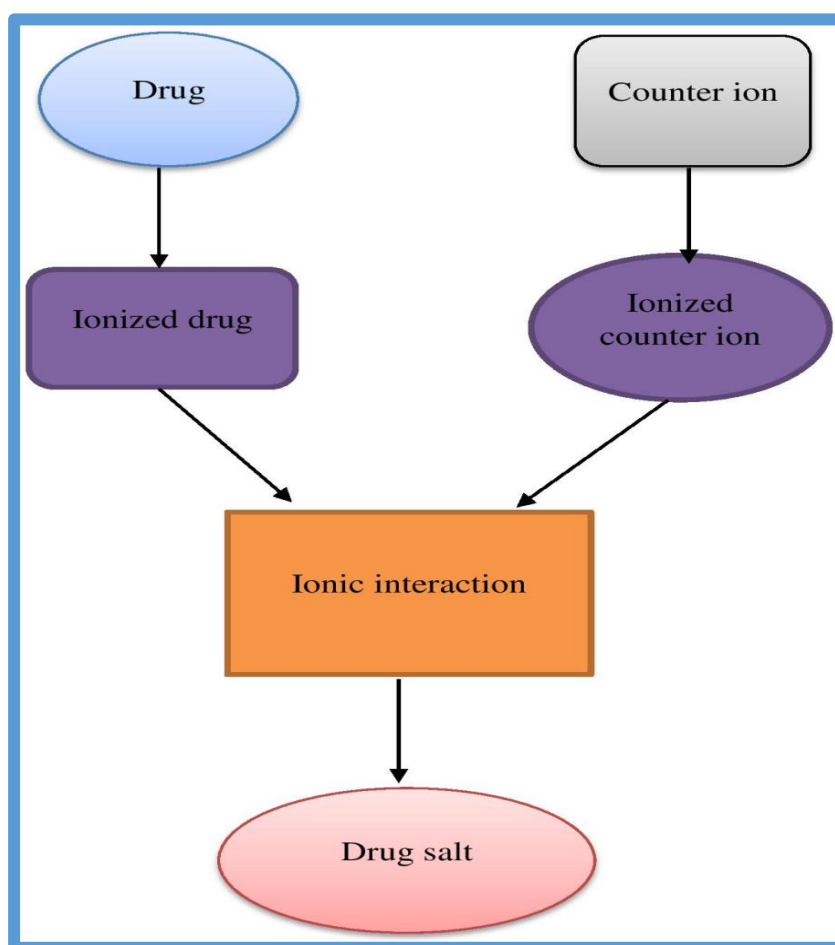


Fig.1.15 Steps of salt formation

1.6.10 Polymorphism

Polymorphism is the phenomenon of those compounds which are existing in more than one crystalline form. The compounds which are showing this kind of phenomenon is known as polymorph. Polymorphs are the compounds which are having different physicochemical properties and biological activities like solubility, melting point, shelf life etc. Polymorphs exist in three forms:- (Khar.K.R et al. ,2013)

- Stable
- Metastable
- Amorphous

1.6.10.1 Stable polymorphs

Stable polymorphs are polymorphs having low solubility, lower energy state, highest melting point, higher stability. This form having good stability but due to its low solubility, this form will not be recommended for solubility enhancement approach.

1.6.10.2 Metastable polymorphs

Metastable polymorph is the polymorph having high solubility, high energy, and low melting point but having stability greater than stable form and less than amorphous form.

1.6.10.3 Amorphous polymorph

Amorphous polymorph is the polymorph having highest energy state and high solubility but less stability. So due to its low stability it is not recommended in solubility enhancement approach. We will prefer metastable form in solubility enhancement approach.

We can increase the solubility of poorly soluble drugs by using any of above approaches but by these methods having both advantages and disadvantages so these approaches having limited utility in solubility enhancement. And other techniques which can be used for solubility enhancement like, emulsions, microemulsion, microspheres, liposomes etc. Shows success in solubility enhancement but not that much because these techniques are not suitable for all drugs which are not soluble in both aqueous and organic solvents but, Nanosuspension has the potential to solve the problem related to the solubility of the drug in aqueous as well as organic solvents.

1.7 Nanosuspension

Nanosuspension are the biphasic system which consists of a submicron colloidal dispersion of pure drug which is stabilized by using surfactants, polymers, and a

mixture of both surfactants and polymers which are dispersed in an aqueous solvent in which the diameter of suspended particle is less than 1 μm in size. In nanosuspension technology, the drug is maintained in crystalline form having reduced particle size which leads to increase in solubility and dissolution of drug and therefore having improved bioavailability. (Geetha et al., 2014)

1.7.1 Techniques to Prepare Nanosuspension

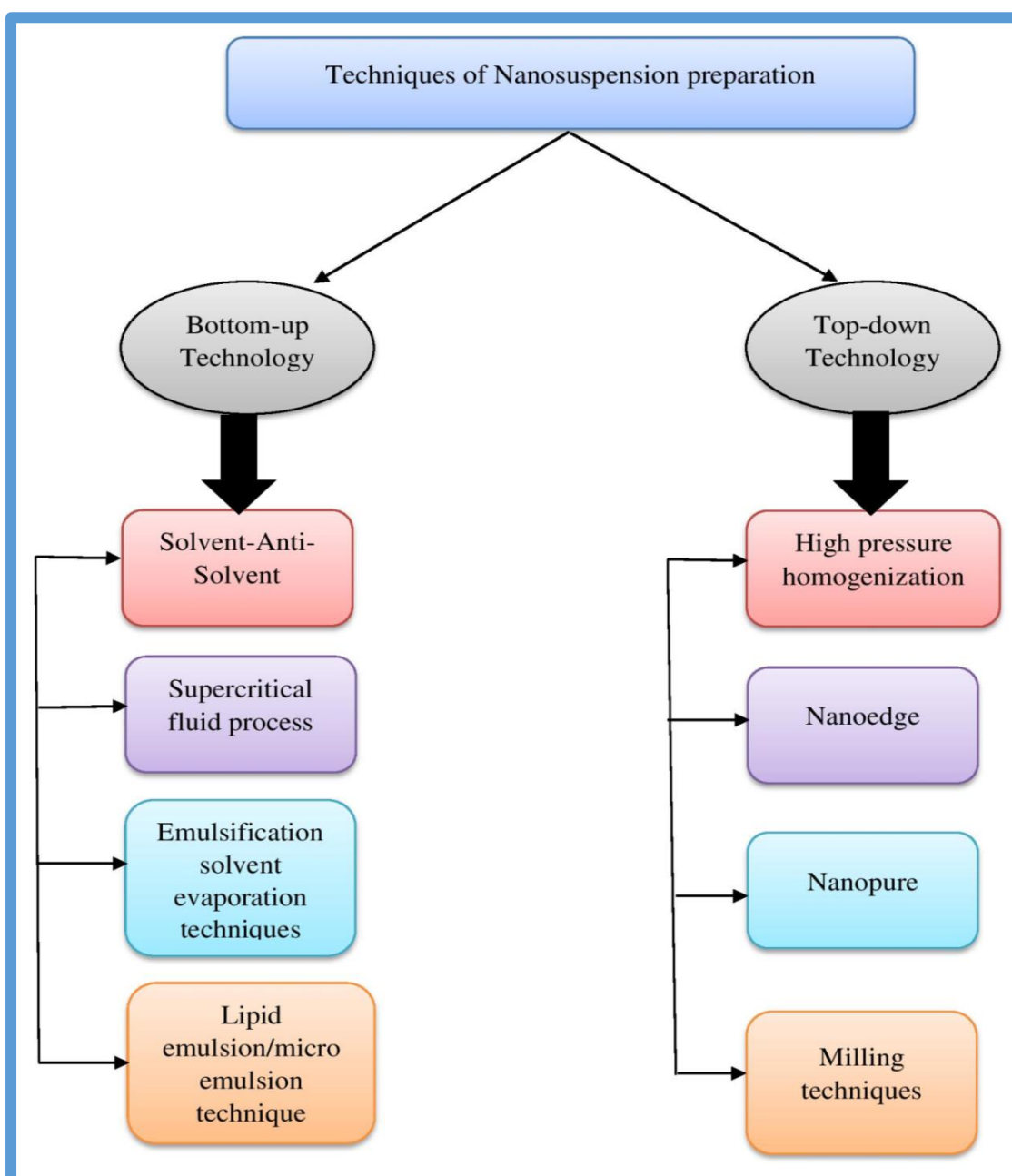


Fig.1.16 Techniques which are used to prepare nanosuspension

1.7.1.1 Bottom-up technology

In bottom-up technology the drug is dissolved in the solvent and dissolve that drug solvent mixture in non-solvent due to which precipitation of crystal takes place. The bottom-up process starts from molecular level to the formation of solid.(Sinha et al., 2013)

There are different techniques which are used in bottom-up technology:-

1.7.1.1.1 Solvent-Antisolvent method

In this method, the drug is dissolved in the solvent than this drug solution is mixed with a miscible anti-solvent in presence of surfactant.Rapid addition of drug solution in antisolvent leads to sudden supersaturation and formation of crystalline and amorphous drug. (Mansour Mansouri,1, Hamid Reza Pouretedal2, 2011)

1.7.1.1.2 Supercritical fluid process

In this process, there is the use of supercritical fluid and supercritical fluid is non-condensable , the dense fluid having pressure and temperature greater than critical pressure and critical temperature.SCF process allows micronization of drug particle to submicron level. (Shid et al., 2013)

1.7.1.1.3 Emulsification-solvent evaporation technique

In this process, the drug solution is prepared by emulsification process then add this solution in that solvent which is nonsolvent for the drug then evaporate the solvent.When the solvent is evaporated there is the formation of precipitates/crystals of the drug.growth of the crystal can be controlled by using high shear forces using high-speed stirrer. (Arunkumar et al., 2011)

1.7.1.1.4 Lipid emulsion method

In this method dissolve the drug in organic solvent then emulsify it in aqueous solution using a suitable surfactant.Then evaporate the organic solvent under reduced pressure,and evaporation of the organic solvent leads to the formation of crystals/precipitates in the aqueous phase forming an aqueous suspension.Then dilute the aqueous suspension to get nanosuspension.(Geetha et al., 2014)

1.7.1.2 Top-down technology

This technique is opposite to bottom-up technology and follows disintegration approach i.e.conversion of large size particle to nanosized particles. (Shid et al., 2013)

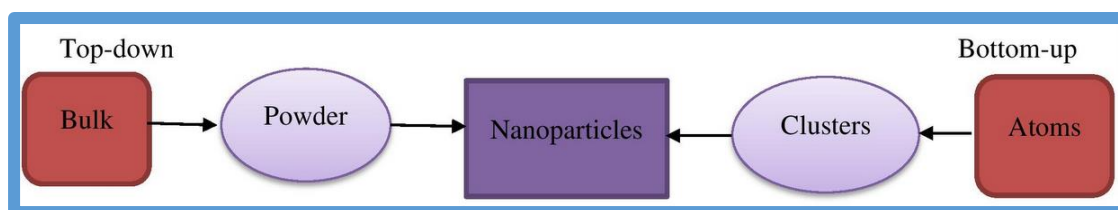


Fig.1.17 Top-down and Bottom-up Technique

1.7.1.2.1 High-pressure homogenization

This method is most widely used in pharmacy for the preparation of nanosuspension of poorly soluble drugs. This process involves three steps which are as follows:-

Step-1

Drug powder + stabilizer solution \longrightarrow formation of presuspension.

Step-2

Then, homogenize the presuspension in a high-pressure homogenizer at low pressure for the purpose of pre-milling.

Step-3

At last, homogenize the above mixture at high pressure for 10-25 cycles until desired size nanosuspension is prepared.

The other methods like nanopure and nano edge which are under top-down technique are based on the same principle of high-pressure homogenization method. (Patravale et al., 2004)

1.7.1.2.2 Media Milling Techniques

In this method, nanosuspension is prepared by the high-pressure mill. milling chamber is filled with drug, milling media (milling media composed of zirconium oxide+glass+highly crosslinked polystyrene resin), water, and the stabilizer solution and rotate the chamber at a very high shear rate under controlled temperature for 2-7 days. (George and Ghosh, 2013)

CHAPTER-2**LITERATURE REVIEW**

A Literature review is to establish the theoretical framework for the topic. With the help of literature review, the research scholar can follow the chronological method to describe the topic.

2.1 Work Done on Nanosuspension:

Geetha.G,et al.,2014 The solubility of poorly soluble drugs especially drugs which belong to BCS class II has increased by using Nanotechnology. In nanotechnology, Nanosuspension technique is used. Formation of Nanosuspension is applicable to all drugs which are water insoluble. Nanosuspension are prepared by using different techniques like high-pressure homogenization, solvent evaporation, melt emulsification and supercritical fluid process. Nanosuspension can be delivered by oral, parenteral, ocular and pulmonary routes.

Tejkrishana.M et al., 2013 Mucoadhesive microbeads are formulated by using ionic gelation method with HPMC and NaCMC as a coating polymer to make sustained release formulation of glimepiride to enhance GIT residence time. Microbeads are evaluated for GI residence time, size distribution, tapped density, entrapment efficiency, wall thickness, scanning electron microscopy, drug release studies and influence of the concentration of polymers on the rate of drug release etc. The rate of drug release was found to be decreased by increasing the concentration of coat polymers. The formulation in which NaCMC is used having decreased drug release than compare to HPMC. Microbead prepared with HPMC and Glimepiride in ratio of 1:9 shows a prolonged drug release upto 12 hours.

Godse.Z.S et al., 2013 In this article there is description of Different techniques which are used to increase the solubility of hydrophobic drugs like physical modification, chemical modification of the drug. And other miscellaneous methods are used to increase the solubility of the drug. Most of the drugs are lipophilic in nature. so different techniques are used to increase the solubility of lipophilic drugs.

Bastaki.S,2005 Diabetes is an endocrine disorder which is due to the defect in insulin secretion, insulin action and both. deficiency of insulin leads to chronic hyperglycemia. diabetes is divided into two types i.e IDDM and NIDDM. To treat IDDM diabetes insulin therapy is used. And for treating type-2 diabetes mellitus

oral hypoglycemic agents are used. Hypoglycemic agents include Sulphonylureas, Biguanides, Thiazolidinediones, Alpha-glucosidase inhibitors etc.

T. Ethiraj et al., 2013 Nanosuspension of Glimpiride is developed and evaluated for the improvement of solubility and bioavailability of poorly soluble drugs. Nanosuspension of glimepiride is prepared by using a different ratio of urea and PVP combination by nanoprecipitation technique. FTIR spectroscopy is used to confirm the interaction between drug and carriers. Four formulations like F1, F2, F3, and F4 are prepared by using a different ratio of urea and the PVP. All formulation is prepared by using poloxamer as a stabilizer, acetone as the organic phase, a mixture of drug and distilled water containing carrier as the aqueous phase. Then physicochemical properties and in vitro release was evaluated.

Patel. L.Z et al., 2012 Solubility is a very important framework to increase the oral bioavailability of poorly soluble drugs which directly affect the in vivo absorption. Poor solubility directly affects the bioavailability of the drug. So different techniques are used to increase the solubility of poorly soluble drugs like physical method, chemical method, co-crystallization, co-solvency, molecular encapsulation with cyclodextrin and nanotechnology approach etc.

Kannan et al., 2011 Drug utilization pattern of the oral hypoglycemic agent is done on type-2 diabetic patients. Study is executed on 202 patients for 9 months in the hospital. Patients who are taking oral hypoglycemic drugs are applicable for this study. Then disease data, demographic data and utilization of different classes of oral hypoglycemic agents as well as the individual drug is analyzed. From study it is found that 51.98% patients are male. Large no. of patients are in age group of 51-60 years. Conclusion is that the combination of glimepiride and metformin are most commonly used.

Patil. J.S et al., 2010 Complexation technique is apply to increase the solubility and bioavailability of the poorly soluble drugs. Different techniques like solid dispersion, solvent deposition, micronization are approaches which are used to solubility enhancement, but all of these approaches have some limitations but complexation technique is mostly used to increase the dissolution, solubility, and bioavailability of the drug which are poorly soluble. cyclodextrin is used as a complexing agent which form inclusion complex with the insoluble drug.

Kotak.U et al.,2015 There are so many techniques which are used to increase the solubility of the drug but co-crystallization has an advantage over salt formation technique.co-crystallization is a technique in which formation of hydrogen bond between drug and co-former.Interaction which are responsible for the formation of cocrystal includes π -stacking,hydrogen bonding, vander walls forces.

Kadam.S.V et al.,2013 40% Oral administered drugs will suffer from formulationdifficultieslikewatersolubility.Bioavailability,dissolution,absorption,distribution and excretion of drug mainly depend on its solubility.On the base of solubility of drugs are classified into four classes of BCS classification.Class II and Class IV having low solubility so we have to increase the solubility of drugs belongs to class II and class IV by using different solubility enhancement techniques.

Reddy.K.B et al.,2011 The BCS classification system is the result of mathematical analysis for the elucidation of kinetics and dynamics of drug process in gastrointestinal tract (GIT),New drug application (NDA), Abbreviated New Drug Application (ANDA), and biowaivers. And this step reduces the time duration of new drug development process and also reduces the drug exposure in healthy volunteers and also increase the impact of bioequivalence studies within in vitro dissolution tests.

Kalra.S et al.,2015 There is a description about the clinical pharmacology of anti-diabetic drug's class sulphonylureas.They will give the various advantages,disadvantages and compare the uses of sulphonylureas in different clinical situations.They give complete guidance for safe and effective use of sulphonylureas class drug and compare the use of various sulphonylureas in different clinical situations.

Patravale V.B et al.,2004 Nanosuspension is the best approach for the effective delivery of hydrophobic drugs.Nanosuspension are used in different dosage form like mucoadhesive hydrogels.Rapid steps are taken for delivery of nanosuspension by different routes like parenteral, ocular, peroral and pulmonary routes.Now efforts are made to increase their use in site-specific drug delivery system.

Krishna Bala K et al.,2011 In this article, there is a description of the characterization,preparation methods like Homogenization,milling,precipitation techniques etc and application of nanosuspension and by which route we can give the nanosuspension like oral, topical parenteral,ocular and pulmonary

routes. Nanosuspension can be stabilized by using various surfactants and polymers. characterization of nanosuspension is done by particle size analysis, zeta potential, X-ray diffraction and dissolution studies.

Paun J.S et al.,2012 The effectiveness of the drug is greatly influenced by its solubility problem which is a major problem in dosage form design, but nanotechnology is one way to improve this problem. By making nanosuspension solubility problem can be eliminated. Nanosuspension can solve the problem of solubility and bioavailability as well as increases the safety and efficacy by altering the pharmacokinetic profile. In this article, there is a description of preparation, properties, advantages and applications of nanosuspension.

Nagajyothi N. et al.,2014 This article provides the complete information about the formulation and characterization of nanosuspension of pitavastatin by precipitation method to improve the dissolution characteristics. Evaluation of nanosuspension was done by different studies like zeta potential analysis, FTIR, SEM, solubility and in-vitro drug release studies. As result by different evaluation test it was found that the solubility and dissolution of pitavastatin drug was increased.

Masilamani.K et al.,2012 This articles reveals the information about the preparation of nanosuspension of aceclofenac drug by oil in water emulsion method by using eudrajit L100 as polymer and tween 80 as surfactant by using their different concentration with different sonication time and agitation speed. evaluation was done by drug content and entrapment efficiency. Result was found that formulation and process variables having significant effect on drug content and entrapment efficiency.

Aher.J.S. et al.,2014 This articles explain about the preparation and characterization of zaltoprofen nanosuspension and the aim of this work to increase the in-vitro dissolution and oral bioavailability of drug zaltoprofen. Zaltoprofen nanosuspension was prepared by emulsification solvent diffusion method in which different concentration of drug polymer and stabilizer was used. The formulation with ratio 1:1:0.5 shows reduce particle size, increased dissolution and more drug entrapment.

Detroja .C et al.,2011 The objective of this article to increase the bioavailability of candesartan cilexetil by preparing nanosuspension. Media milling technique was used to prepare nanosuspension. Evaluation of nanosuspension was done by zeta potential, crystal study, surface morphology, differential scanning calorimetry and dissolution

behavior. The result was found that there is significant increase in antihypertensive activity of candesartan when formulated as nanosuspension.

Langguth.P et al.,2005 This article gives the complete information about the preparation,advantages and properties of spironolactone nanosuspension. Evaluation of nanosuspension was done by drug content and entrapment efficiency.The result was found that there is increase in the solubility and bioavailabilty of spironolactone.

Zhang.X et al.,2006 This article reveals the information about the nanosuspension all trans retioic acid which is prepared by modified precipitation method.Mean particle size of retinoic acid is reduced from 337nm to 155nm.the morphology of retinoic nanoparticle vary with different concentration of retinoic acid solution in acetone.As a result it is found that nanosuspension prepared by modified precipitation method is stable and controllable to a certain extent.

Zhang.D et al.,2007 In this article there is a description about the nanosuspension of azithromycin which is prepared by high-pressure homogenization,by lyophilization technique nanosuspension becomes more stable.analysis of azithromycin nanosuspension was done by differential scanning calorimetry and X-ray diffraction and size of nanoparticle found to be 400nm which is analysed by transmission electron microscopy.In-vitro studies show that the dissolution of nanosuspension is increased.

Gao.L et al.,2007 In this article there is description about the preparation of oridonin nanosuspension which is prepared by high-pressure homogenization.The aim of this article is to get a stable nanosuspension with an increase in solubility and dissolution.characterization of nanosuspension is done by particle size,size distribution,zeta potential,DSC and X-ray diffraction etc.

Susan.D et al.,2014 This articles gives the description about the drug release from nano formulation of drug by different methods like, continue flow method,dialysis memberane method and sample and separate method.in this article gives the description about the each methods and there advantages and disadvantages and from study it comes to know that SS method is very simple to find out the drug release with simple setup but sampling is difficult in this case.and in case of continue flow method sampling and drug release is good but set up is time consuming.In dialysis memberane method sampling and setup is easier than both methods but this method is

not suitable for that drugs which binds to memberane. And novel method having possibilities of measurement of drug release but it may be restricted for some drugs. Atlast they conclude that efforts are made to focus on developing mathematical model that can describe the mechanism of drug release.

Sambasivarao.A. et al., 2016 This article gives the description about the formulation and evaluation of immediate release tablets of glimepiride by solid dispersion technique. In this study solid dispersion of glimepiride was prepared by melting method by using different carrier in different ratios. It is found that rate dissolution of glimepiride is increased with increase in carrier ratio.

Sharma.M. et al.,2015 The objective of this article is to formulate and evaluate microspheres of glimepiride.the microsphere of glimepiride was prepared by emulsification solventdiffusion method by using different polymers in different ratios.the evaluation of microspheres was done by percentage yeild ,percentage encapsulatio efficiency and in-vitro drug release.

Venkatesh.B. et al.,2014 This article gives the detailed information about the formulation and evaluation of glimepiride oral capsule.the rationale of study is to increase the solubility and dissolution of drug glimepiride.The cubosomes of glimepiride was prepared by top down method by using lipid and stabelizer.characterization of prepared formulation was done by encapsulation efficiency,particle size ,zetapotential ,FTIR, SEM and in-vitro drug release.

Kumar.A.N. et al.,2010 The objective of study to preapare nanosuspension of poorly soluble drug by high pressure homogenization technique to enhance the solubility and dissolution of drug.characterization of prepared formulation was done by thermogravimetric analysis ,differential scanning calorimetry,X-ray diffraction and in-vitro drug release.

Kumar.G.Y. et al.,2015 This article reveals the information about design and development of sustained released matrix tablets of vidagliptin by wet granulation method by using synthetic and natural polymers in different ratios and evaluation of prepared granules of vidagliptin was done by bulk density,tapped density and

compressibility index and the characterization was done by weight variation, thickness, hardness, friability, drug content and in-vitro drug release.

Singh Baliar.O.P. et al.,2009 The objective of this article is to investigate the physicochemical properties of glimepiride in solid dispersion along with PEG 20000. The evaluation of solid dispersion was done by FTIR, X-ray diffraction and dissolution studies. From this it comes to know that dissolution of glimepiride increases by increasing the amount of PEG 20000.

Chaudhari. M.D. et al.,2012 This article reveals the information about the enhancement of solubility and dissolution of poorly water soluble glimepiride by preparing it as solid dispersion which was prepared by solvent evaporation technique and the tablet of solid dispersion was prepared by direct compression technique. The evaluation of solid dispersion was done by FTIR, XRD, SEM and in-vitro dissolution stability studies of solid dispersion was done by FTIR studies.

Cho Young.H. et al.,2016 This article gives the information about the preparation and evaluation of solid self-emulsifying drug delivery system containing paclitaxel for lymphatic drug delivery which is prepared by spray drying method. The evaluation of formulation was done by mean droplet size, zeta potential and encapsulation efficiency.

Wagh V. T. et al.,2012 This article gives the detailed information about the formulation and evaluation of glimepiride solid dispersion tablets by kneading technique and the characterization of solid dispersion by in-vitro solubility study, drug content, FTIR and in-vitro drug dissolution study. The tablet of solid dispersion was prepared by direct compression technique and evaluated for various pharmaceutical characteristics like hardness, friability, weight variation, in-vitro dissolution profile.

Mansouri.M. et al.,2011 This gives the detailed information about the preparation and characterization of ibuprofen nanoparticles by using solvent/antisolvent precipitation method. The characterization of nanoparticles was done by in-vitro dissolution. At last they conclude that anti-solvent precipitation is an effective approach to produce nanoparticles of poorly soluble drug.

Kumar.A. et al.,2009 This articles gives the information about the nanosuspension technology and its application in drug delivery for drugs which are insoluble in water and this approach can be used to improve the stability of as well as bioavailability of poorely soluble drugs .in this there is description about the various methods of preparation of nanosuspensions like high pressure homogenization, wet milling techniques,emulsification solvent evaporation and supercritical fluid process.

Mahesh.V.K. et al.,2014 This articles gives the detailed information about the preparation of glipizide nanosuspension by top down and bottom up technique for comparative study.in this work nanosuspensions were prepared by two techniques,antisolvent precipitation and media milling by using bead mill.they concluded that ratio of polymer and drug, milling time, milling speed plays a important role in controlling zetapotential and particle size of nanosuspension prepared by media milling technique and in case of antisolvent evaporation method increase in particle size was observed under accelerated conditions.

Bipul.N. et al.,2016 The main objective of this study was to find the interaction between glimepiride and excipients for the preparation of extended release tablets which were prepared by direct compression method, and compatibility study was done by differential scanning calorimetry and FTIR. And evaluation of extended release tablets was done by kinectics study,physical properties and stability studies.from DSC and FTIR it comes to know that there wasno interaction between drug glimepiride and excipients.

Papdiwal.A. et al.,2014 The purpose of this study was to improve the solubility and dissolution of drug nateglinide by preparation of nanosuspension by nanoprecipitation technique.the evaluation of prepared nanosuspension was done by particle size, in-vitro dissolution studies and characterization was done by DSC and optical microscopy.

Hyma.P. et al.,2014 In this work the purpose of preparing self microemulsifying drug delivery system is to increase the solubility and bioavailability of poorly soluble drug.these drug delivery system was the isotropic mixture of surfactant ,co-surfactant and oil incorporated with drug.pseudoternary titerations were done by using

surfactant ,co-surfactant and oil in different ratios against water.and FTIR was done for investigating drug excipients compatibility.

Sandhya.P. et al.,2014 The aim of this article is preparation of bilayer tablets of glimepiride and metformin Hcl.the tablets of metformin hydrochloride was prepared as sustained release by wet granulation method and glimepiride as immediate release matrix tablets as a dosage form by direct compression method by using different polymers.evaluation of these tablets was done by FTIR for detection of any type of interaction between drug and polymers.

Basu.S.K. et al.,2008 The objective of this work was to prepare nitrendipne-loaded eudrajit RL 100 microspheres to acheive sustained release formulation.these microspheres was prepared by emulsion solvent evaporation method.the evaluation of these microspheres was done by particle size, in-vitro dissolution and drug loading,and to determine the physical state of microspheres SEM, DSC , X-ray diffraction and FTIR was done.

Ahmed.S. et al.,2015 This article gives the detailed information about the validation of UV spectrophotometric method for the assay of tolfenamic acid in organic solvents.validation was done according to ICH guidelines and parameters like linearity, accuracy, precision, sensitivity and robustness have been studied.the method was found to be efficient for determination of TA in all solvents,on the basis of statistical data 1-octnol is better than ethanol and methanol.

Kocbek.P. et al.,2006 The main objective of this article was preparation and evaluation of nanosuspension by melt emulsification method for enhancing the solubility and dissolution of poorly soluble drugs and this method is evaluated against solvent diffusion method. The advantage of melt emulsification method against sovent diffusion is avoidance of organic solvent during production.and due to use of various stabelizers the nanosuspension of average particle size was produced.

Sriram.N. et al.,2013 The aim of present study was to formulate and evaluate glimepiride microspheres bu emulsification solvent evaporation method.evaluation of glimepiride microspheres was done by SEM, in-vitro drug release. They concluded that there was increase in drug release with increase in concentration of polymers.

Kumar.S.C. et al.,2016 This article gives the detailed information about the formulation and evaluation of nanosuspension of aceclofenac to improve the solubility of aceclofenac.in this work nanosuspension was prepared by sonoprecipitation method and evaluated for particle size analysis,zeta potential,SEM , in-vitro dissolution study ,drug content,DSC ,XRD,

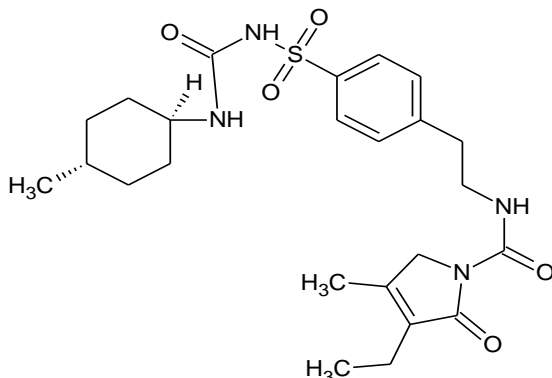
Aly . A. M.et al.,2011 The objective of this work was to prepare glimepiride rapidly disintegrated tablets by direct compression method and evaluate new excipient i.e. pharmaburst which is newly introduced excipient for this type of tablets and another goal to evaluate the stability of the formulation as well as in-vivo effect of the formulation.atlast it is found that pharmaburst was found to have a faster onset of action and have a good stability in accelerated stability conditions.

Rout.K.P.et al.,2009 The present investigation describes about the preparation of microspheres of losartan by solvent evaporation and w/o emulsion solvent evaporation methods along with in-vitro characterization of microspheres to evaluate the effect of method of preparation on physical properties and drug release profile of microspheres.atlast they concluded that formulation which was prepared by solvent evaporation method has potential to deliver losartan potassium in controlled manner.

2.2 Drug profile (IP-2010)

Name of drug: - Glimepiride

Molecular structure: -



Molecular formula: - C₂₄H₃₄N₄O₅S

Molecular weight: - 490.6

IUPAC Name: - 1-[[4-[2-(3-ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido)ethyl]phenyl]sulphonyl]-3-trans-4-methylcyclohexyl)urea.

Colour: - White

Odor: - odorless

Solubility: - Practically insoluble in water, slightly soluble in dichloromethane and methanol. It is soluble DMSO (dimethylsulfoxide) (Sharma et al., 2015)

Melting point: - 207°C

Mechanism of action: - Glimepiride increases the insulin secretion by acting on β-cells of the pancreas. It binds to sulphonylurea (SUR) receptors which are present on β-cell on the plasma membrane, which closes the ATP-sensitive potassium channel leading to depolarization of cell membrane, by which there is the opening of voltage-gated calcium

channel due to which there is an influx of calcium ions causes secretion of the preformed insulin molecule.

Pharmacokinetics:

Plasma half-life:- 1 to 4 hours

Duration of action: - up to 24 hours (Mk.L.Thomas, foy's principles of medicinal chemistry)

Protein binding: - >99.5%

Volume of distribution: - 0.18 L/kg

pK_a value: - 5.0

Log p: - 2.9

Elimination: - <0.5% unchanged drug in urine

37% to 52% in urine as metabolites (Beale.M.J, et al., 2010)

Maximum daily dose: - 1-8 mg/day

BCS class: - II

Food effect: - No

2.3 Excipients profile

2.3.1 Surfactants

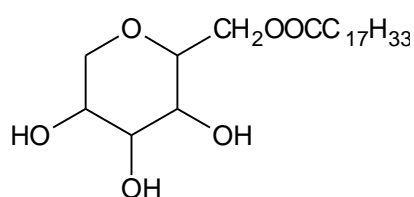
Surfactants or surface active agents are the pharmaceutical chemical compounds which help in reducing the surface tension/interfacial tension between two immiscible liquids.

2.3.1.1 Span-80

Span-80/polysorbate-80: - It is type of nonionic surfactant

Colour: - yellow (viscous)

Molecular structure: -



Span 80

Molecular formula: - C₂₄H₄₄O₆

Molecular weight: - 428.61

Solubility: - Span 80 is soluble in ethanol , isopropyl alcohol,mineral oil and vegetable oil etc.

Mechanism of action:

- Span-80 increases the solubility of poorly water-soluble drugs by the formation of micelles.formation of micelles takes place in different steps:
- Firstly dissolve surfactant in water phase in a very small concentration then fraction of surfactant will be adsorbed at air-water interface whereas remaining will be in bulk.
- When more of surfactant will be added then air-water interface becomes saturated and the surfactant is imposed into the bulk of the liquid.At high concentration the molecule or ion of the surfactant aggregate to form particles of a colloidal size which are known as micelles.The concentration at which micelles formation occurs is known as critical micelles concentration (CMC).

HLB value: - 4.3

Other names: - Sorbitan Oleate, Emulsifier S 80

Uses: -

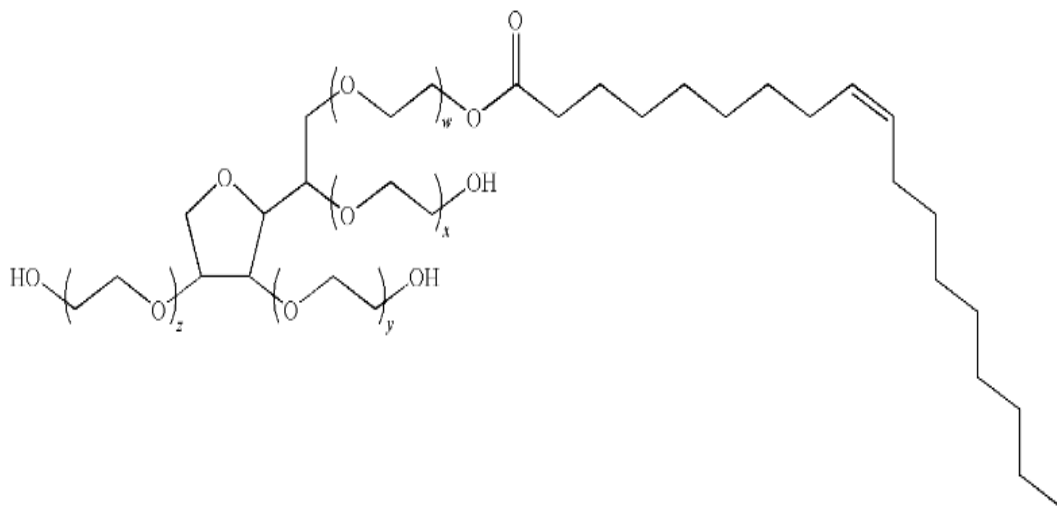
Span 80 is used as solubilizer, emulsifier, stabilizer.

2.3.1.2 Tween-80

It's a type of nonionic surfactant

Colour: - yellow (viscous)

Molecular structure:



Molecular formula: - $C_{64}H_{124}O_{26}$

Molar mass: - 1,310 g/mol

Solubility: - It is easily soluble in water, soluble in ethanol, vegetable oil and insoluble in mineral oil

HLB value: - 15.0

Other names: - Emulsifier T 80

Uses: - Used as emulsifier, wetting agent, penetrating agent

2.3.2 Polymers

2.3.2.1 Polyethyleneglycol(PEG)-400

Synonyms: Carbowax, Macrogels

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

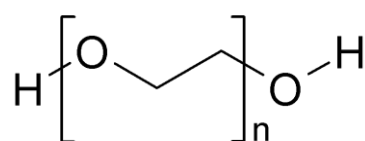
Colour: Colourless

Odor: Odorless

Molecular formula: $C_{2n}H_{4n+2}O_{n+1}$, The value of n lies between 8.2 to 9.1

Molecular weight: 380-420

Molecular structure:



Solubility:

- PEG 400 is soluble in acetone ,alcohol,benzene,glycerine and water.
- And slightly soluble in aliphatic hydrocarbons.

Melting point: 4-8°C

Uses:

- Used as a plasticizer,solvent.
- Lubricant for tablets and capsules.

2.3.2.2 Polyethylene glycol 6000

Non-proprietary name: Macrogol 6000

Synonym: Macrogol, PEG, Pluriol E, Polyethylene glycol

Chemical Name: α -hydroxy- ω -hydroxypoly (oxy-1,2-ethanediyl)

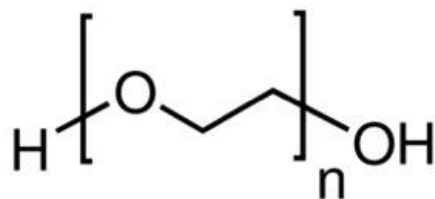
Empirical formula and Molecular formula:

$HOCH_2 (CH_2OCH_2)_m CH_2OH$, where m is equivalent to the no. of ethyl groups present.

Colour: White or off-White

Odor: sweet odor

Structural formula:

**Distinctive properties:****Melting point:** 55-63°C

Moisture content: hygroscopic in nature, where hygroscopicity decreases with increase in 'molecular weight.

Solubility profile:

In water: form gelly substance

In organic solvent: soluble

In oils: insoluble

Uses: lubricants, Drug carrier and stabilizing agent.

2.3.2.3 Polyvinyl pyrrolidone K30

Synonyms: PVP, Povidone, Copovidone, , crosopovidone, PVPP

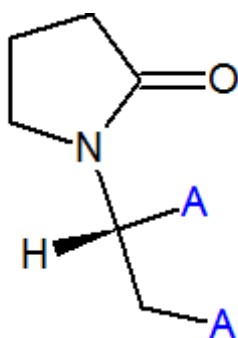
Chemical name: Poly[1-(2-oxo-1-pyrrolidiny)ethylene]

Colour: White or off white in colour

Odor: Odorless

Molecular formula: (-CH(NCH₂CH₂CH₂CO)CH₂-)_n

Molecular weight: 35000- 50000

Molecular structure:**Solubility:**

PVP K30 is soluble in water, methanol, ethanol, alcohol, chloroform and glycerol, acetic acid.

It is insoluble in dimethyl ether, acetone, toluene, ethyl acetate, xylene, and carbon tetrachloride

Melting point: 150- 180°C

Uses:

It is used as polymer in many pharmaceutical preparations as binder, stabilizing agent and emulsifier.

CHAPTER-3**RESEARCH ENVISAGED AND PLAN OF WORK****3.1 Rationale**

Glimepiride is an Anti-Diabetic drug belongs to class sulphonylurea which is mostly used for the treatment of type-2 DM. Glimepiride belongs to BCS (Biopharmaceutics classification system) Class-II, having low solubility and high permeability. (Gill.B et al., 2010). Solubility is a major problem of glimepiride, due to which it is having poor dissolution and bioavailability. Glimepiride in tablet form having some problems like poor solubility, incomplete dissolution, and low efficacy.

A formulation as nanosuspension is promising approach to solve these problems. The approach of nanosuspension formulation not solve only the problem of solubility and bioavailability but also can alter the pharmacokinetics and helps in improving the safety and efficacy of the drug. It also increases the Pharmacological action of glimepiride when given by parenteral route. Glimepiride in tablet dosage form only administered by oral route but in nanosuspension dosage form, it can be used by parenteral as well as oral route. (Agrawal and Patel, 2011)

3.2 Aim and Objective**3.2.1 Aim**

The Aim of presented work is “**Comparative In-vitro Evaluation of Glimepiride Nanosuspension prepared by different Techniques**”.

3.2.2 Objective

- To formulate nanosuspension of glimepiride by different techniques
- To compare the solubility of glimepiride nanosuspensions prepared by different techniques
- To compare the drug release profile of glimepiride nanosuspensions prepared by different techniques
- To find out the better technique meant for preparation of glimepiride nanosuspension for enhancement of drug solubility

3.3 Comprehensive plan of work

- Selection of Drug
- Selection of methods for preparation of nanosuspension
- Selection of excipients like surfactants and polymers

- Preformulation studies: compatibility studies, solubility analysis, melting point analysis, pre-screening studies
- To formulate nanosuspension of glimepiride by different techniques.
- Physical and chemical characterization of nanosuspension with respect to particle size analysis, in-vitro drug release studies
- To formulate nanosuspension of glimepiride by different techniques.
- Comparison of solubility of glimepiride nanosuspension which is prepared by different techniques
- From comparative study find out which technique is better for solubility enhancement

CHAPTER-4
MATERIAL AND METHOD

4.1 List of material used during the study:

Table 4.1

List of chemicals used in study

| Sr.no. | Chemical/Material | Batch no. | Source/Manufacturer |
|--------|--------------------------------|------------|-------------------------------------|
| 1 | Acetone | 1302500 | Loba Pvt. Ltd. Mumbai, India. |
| 2 | Dichloromethane | 9502500 | Loba Pvt. Ltd. Mumbai, India. |
| 6 | PEG-400 | 25900500 | Loba Pvt. Ltd. Mumbai, India. |
| 8 | Span-80 | 608800500 | Loba Pvt. Ltd. Mumbai, India. |
| 9 | Cellulose microcrystalline | 263000500 | Loba Pvt. Ltd. Mumbai, India. |
| 10 | PVP K30 | 531700100 | Loba Chemie Pvt. Ltd. Mumbai, India |
| 11 | PEG 6000 | 531200500 | Loba Chemie Pvt. Ltd. Mumbai, India |
| 12 | Tween 80 | 642000500 | Loba Chemie Pvt. Ltd. Mumbai, India |
| 13 | Potassium dihydrogen phosphate | 5357D00500 | Loba Chemie Pvt. Ltd. Mumbai, India |
| 14 | Sodium hydroxide | 589800500 | Loba Chemie Pvt. Ltd. Mumbai, India |
| 15 | Sodium dodecyl sulphate | | Loba Chemie Pvt. Ltd. Mumbai, India |

4.2 List of equipments used during the study

Table 4.2

| Sr.no. | Equipment | Model and Manufacture |
|--------|---|--|
| 1 | Bath sonicator | Athena technology,ATS-02 |
| 2 | Electronic weighing balance | Shimadzu Co.Ltd., Japan,CY360 |
| 3 | Eppendorf tubes | Tarsons Products Pvt. Ltd. Kolkata, India |
| 4 | FTIR spectrometer | Shimadzu Co.Ltd. Japan Spectrum 400 |
| 5 | Hot air oven | Cadmach Drying Oven, Cadmach Machinery Ltd.,Ahmadabad India |
| 6 | Heated/Magnetic stirrer | Remi, Pvt. Ltd. Mumbai, India Q-5247 |
| 7 | Particle size analyzer | Beckman coulter RQ-121 D |
| 8 | pH meter | Systronic, μ pH system, India |
| 9 | UV spectrophotometer | Shimadzu Co. Ltd. Japan 2M9F36500 |
| 10 | Transmission Electron Microscope (TEM) | Field Electron and Ion Co. TECNAI G F-20 |
| 11 | Trinocular microscope | Kyowa, Gentner, Japan 10390 |
| 12 | Hot plate | Popular, India |
| 13 | Dessicator | Tarsons Peoducts Pvt.Ltd.Kolkata India 5L |
| 14 | Borosilicate Type-1 glass | TarsonsProducts Pvt.Ltd.Kolkata,India |
| 15 | Syringe and needle | |
| 16 | Dissolution Apparatus | DS 8000 LABINDIA Thane West,Maharastra,India. |
| 17 | Centrifuge | Remi,Pvt.Ltd.Mumbai India CM- 12PLUS |
| 18 | Zeta analyzer | Beckman coulter |

CHAPTER-5**EXPERIMENTAL WORK****5.1 Physicochemical characterization and Identification of Glimepiride****5.1.1 Physical Appearance Test**

Glimepiride was characterized for various organoleptic properties such as colour, odour and appearance.

5.1.2 Melting point

Melting point of glimepiride was found out by capillary method.

Capillary method

In this method drug was filled into capillary tube which was sealed from one end at the height from 3mm from closed end. The capillary tube was introduced in digital melting point apparatus, then melting point was noted by recording the temperature at which drug starts melting. Temperature was noted when the total drug melted.

5.1.3 Fourier transform infrared spectral analysis

The FTIR spectrum of Glimepiride was observed by preparing potassium bromide disk (Brammer et al., 1991). The finely ground Glimepiride powder was mixed with powdered potassium bromide and was pressed with a specific hydraulic compression. The prepared KBr pellet was then observed under Fourier transform infrared spectrometer (FTIR) and the spectrum was recorded. The FTIR spectrum obtained was compared with the spectrum obtained with Glimepiride standard given.

5.2 Determination of Absorbance maxima (λ_{max})

10 mg of Glimepiride was accurately weighed from calibrated digital weighing balance.

Dissolve the 10 mg Glimepiride in small quantity of Methanol. Then, transfer the solution in 100 ml of volumetric flask, and make the volume up to 100ml with methanol to give stock solution of 1000 μ g/ml. 1ml of stock solution from 1000 μ g/ml was transferred to 10ml volumetric flask and make the volume up to 10 ml with methanol, the dilution with concentration 100 μ g/ml was prepared. Take 0.5 ml of stock solution from 1000 μ g/ml and add it in 10ml volumetric flask, and make the volume up to 10ml with methanol, dilution of 50 μ g/ml was prepared. Both the dilutions were scanned on double beam UV visible spectrometer.

The wavelength at which maximum absorbance was shown by both the dilutions, that was recorded as λ_{\max} Glimepiride.

5.3 Method validation for Glimepiride in Methanol

5.3.1 Calibration Plot for glimepiride in methanol

10 mg of glimepiride was accurately weighed on calibrated digital weighing balance and was dissolved in small quantity of methanol. The solution was then transferred to 10 ml of volumetric flask and make the volume up to 10 ml to give stock solution of 1mg/ml. Now from the stock solution, 2,4,6,8,10 ml of solution were transferred into 10 ml volumetric flasks and make the volume up to 10 ml to form concentrations 20,40,60,80 and 100 $\mu\text{g/ml}$ respectively. The absorbance was noted at λ_{\max} 228.50 nm. The analysis was carried out in triplicate.

5.3.2 Linearity and Range

Linearity is the ability of the method to obtain the results of test samples which were directly proportional to analyte concentration within a given range (ICH, Q2 (R1) guidelines, 2005). Range is the interval between the upper and lower levels of analytes which can be determined by accuracy, precision and linearity. The accepted criteria for linearity is that the correlation coefficient (R²) should not be less than 0.990. Different aliquots from stock solution were sufficiently diluted to get solution in concentration ranging 2-10 $\mu\text{g/ml}$ in triplicate. Calibration plots were obtained by plotting the graphs between absorbance versus concentration data and linear regression analysis was carried out for the same.

5.3.3 Accuracy

It represents the closeness of agreement between the values which is accepted either as a conventional true value or an accepted reference value. (ICH, Q2 (R1) guidelines, 2005). Accuracy was determined by performing recovery studies. It was performed by preparing different concentration levels (2, 3, and 4) $\mu\text{g/ml}$. The study was carried out in triplicate by preparing three sample solutions at each recovery level. Absorbance was analyzed on a U.V spectrophotometer. Percentage mean recovery along with percentage R.S.D were calculated.

5.3.4 Precision

The precision expresses the closeness of agreement (degree of scatter) between a series of measurement obtained from multiple sampling of the same homogeneous sample under the prescribed conditions (ICH, Q2 (R1) guidelines, 2005). The

precision of proposed method was determined for five concentrations (2,4,6,8 and 10 µg/ml) covering the entire linearity range by intraday (repeatability) and interday studies (intermediate precision). Intraday precision was determined by analyzing (2,4,6,8 and 10 µg/ml) at three different time points on the same day and interday precision was determined by analyzing the solutions at three different time points on different days. For analyzing the precision, percentage R.S.D was calculated for intraday and interday precision studies.

5.3.5 Robustness

It is the method in which there is measurement of the capacity of sample to remain unaffected by deliberate variation in the method parameters and gives an indication of its reliability during the normal usage (ICH, Q2 (R1) guidelines, 2005). The robustness of proposed method was estimated by evaluating by the interpersonally. The % R.S.D was determined. (Ahmed et al., 2015)

5.3.6 Limit of Detection (LOD) and Limit of Quantification (LOQ)

Limit of detection may be defined as the lowest amount of analyte in a sample which can be detected but can not quantified as an exact value (ICH, Q2 (R1) guidelines, 2005). The limit of quantification of an individual analytical procedure is the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy as shown in equation 5.1 and 5.2. Estimation of L.O.D and L.O.Q was based on the standard deviation of response and slope of the calibration curve. (Ahmed et al., 2015)

$$\text{L.O.D} = 3.3 \sigma / S \dots\dots\dots (\text{Equation 5.1})$$

(σ = Standard deviation of the intercept of linear regression equation)

(S= Slope of the regression equation)

$$\text{L.O.Q} = 10 \sigma / S \dots\dots\dots (\text{Equation 5.2})$$

(σ = Standard deviation of the intercept of linear regression equation)

(S= Slope of the regression equation)

5.4 Preformulation studies

5.4.1 Drug excipient compatibility

Compatibility study was carried out by using pure drug, excipients and drug: excipient mixture in ratio of 1:1. Then place above mixture in glass containers and stored at temperature 40°C (ICH, Q1 A guidelines, 2005). Observation of mixtures and

pure samples were made on 0th and 15th day physically for color change, appearance, state and lump formation.

5.4.1.1 Chemical characterization of drug excipients mixture

Chemical compatibility of drug excipients mixture was checked on 15th day by performing FTIR analysis of the drug with and without the excipients. The peaks of Glimepiride along with the excipients were observed. The effect of the excipients on the major peaks of glimepiride was observed to find the compatibility of the drug and excipients. The undisturbed peak of drug signifies compatibility of the drug with the excipients.

5.4.2 Solubility studies

The solubility study of Glimepiride was done by using various solvents and variable pH to understand the solubility profile of drug (IP, 2007). Standard plot of glimepiride was observed by using various solvents such as methanol, DCM (Dichloromethane), 6.8 Phosphate buffer and pH 7.8 Phosphate buffer. The samples of drug solution in different solvents were taken and diluted suitably to observe the absorbance of drug by using U.V spectrophotometer at λ_{max} 228.50 nm. The drug concentration in each solvent was calculated from the standard plot and the graph was plotted between the concentrations vs. absorbance.

5.4.3 Partition coefficient

The partition coefficient study was done by using octanol and water. 5 ml of both solvents were filled in glass container in which 100 mg of drug was added. Then mixture was allowed to shake for 24 hr at $37^{\circ}\text{C} \pm 2^{\circ}\text{C}$. The solution was then transferred to the separating funnel and was shaken intermittently for one hour. The separating funnel was kept undisturbed to separate the two layers. The aqueous and organic layer were collected separately and the concentration of drug was found using U.V spectrophotometer.

5.5 Screening studies

5.5.1 Screening of the method for the preparation of nanosuspension

Anti-solvent evaporation technique (Method 1): In this method nanosuspension was prepared by antisolvent evaporation followed by sonication technique. Appropriate amount of drug was taken and dissolved in an antisolvent which is immiscible with water. Then, 1ml solution of glimepiride was injected in to deionized water with or

without polymers or surfactants at 4°C with rapid stirring at 1200 rpm followed by intense sonication.

Nanoprecipitation technique (Method 2): Nanosuspension was prepared by nanoprecipitation technique. The drug Glimepiride was dissolved in methanol which was taken as organic phase. The polymer PVPK30, PEG 6000 and PEG400 were added to 40 ml of distilled water (antisolvent) which was taken as a aqueous phase. The organic phase was drop wise added to the aqueous phase with syringe which was kept at room temperature and stirred with a speed of 1000-1200rpm for 1hr using magnetic stirrers (Kumar and Baig, 2014)



Fig.5.1 Combination and nanoprecipitation method for the preparation of nanosuspension

5.5.2 Effect of preparation techniques on the nanosuspension formulation

Nanosuspension formulations prepared by using antisolvent precipitation followed by sonication technique. Glimepiride was weighed properly and 1ml solution of Glimepiride was injected into deionised water at 4°C temperature with continuous stirring at 1200 rpm and intense sonication. Various solvents were evaluated such as methanol, ethanol, isopropyl alcohol, and Di-chloromethane. Polymers and surfactants were chosen from PEG 400, PEG 6000, and PVP K30. Processing

condition were evaluated for their ability to prepare nanosuspension. (Y.Lu et al., 2013)

In case of second methods i.e nanoprecipitation method, the drug Glimpiride was dissolved in methanol which was taken as organic phase. The polymer PVPK30, PEG 6000 and PEG400 were added to 40 ml of distilled water (antisolvent) which was taken as a aqueous phase. The organic phase was slowly added to the aqueous phase in a drop wise manner with syringe and kept at room temperature and stirred with a speed of 1000-1200rpm for 1hr using Magnetic stirrers without sonication. Various solvents were evaluated such as methanol, ethanol, isopropyl alcohol, and Dichloromethane. Different types of Polymers and surfactants were chosen from PEG 400, PEG 6000, and PVP K30. Processing condition were evaluated for their ability to prepare nanosuspension.

Table 5.1

Screening the ratio of components for formulation prepared by combination technique

| Sr. No. | Batch code | Components | Ratio |
|---------|-----------------|---------------------|---------|
| 1 | G ₁ | GMP: PEG 6000 | 1:10 |
| 2 | G ₂ | GMP: PEG 6000 | 1:20 |
| 3 | G ₃ | GMP: PEG 6000 | 1:30 |
| 4 | G ₄ | GMP: PVP K30 | 1:10 |
| 5 | G ₅ | GMP: PVP K30 | 1:20 |
| 6 | G ₆ | GMP: PVP K30 | 1:30 |
| 7 | G ₇ | GMP: PEG 4000 | 1:10 |
| 8 | G ₈ | GMP: PEG 4000 | 1:20 |
| 9 | G ₉ | GMP: PEG 4000 | 1:30 |
| 10 | G ₁₀ | GMP:PEG6000:PVPK30 | 1:10:10 |
| 11 | G ₁₁ | GMP:PEG6000:PEG4000 | 1:10:10 |
| 12 | G ₁₂ | GMP:PEG4000: PVPK30 | 1:10:10 |

| Name of contents | G1 | G2 | G3 |
|---------------------------|------|------|------|
| Glimepiride (mg) | 1 | 1 | 1 |
| Polymer (PEG6000) (mg) | 10 | 20 | 30 |
| Water(ml) | 40 | 40 | 40 |
| Homogenization speed(rpm) | 1200 | 1200 | 1200 |




Fig.5.2 G1,G2 and G3 nanosuspension formulations prepared by method-1

| Name of contents | G4 | G5 | G6 |
|---------------------------|------|------|------|
| Glimepiride (mg) | 1 | 1 | 1 |
| Polymer (PVPK30) (mg) | 10 | 20 | 30 |
| Water (ml) | 40 | 40 | 40 |
| Homogenization speed(rpm) | 1200 | 1200 | 1200 |




Fig.5.3 G4,G5 and G6 nanosuspension formulation prepared by method-1

| Name of contents | G7 | G8 | G9 |
|---------------------------|------|------|------|
| Glimepiride (mg) | 1 | 1 | 1 |
| Polymer (PEG400) (mg) | 10 | 20 | 30 |
| Water(ml) | 40 | 40 | 40 |
| Homogenization speed(rpm) | 1200 | 1200 | 1200 |




Fig.5.4 G7,G8 and G9 nanosuspension prepared by method-1

| Name of contents | G10 | G11 | G12 |
|-----------------------------------|------|------|------|
| Glimepiride (mg) | 1 | 1 | 1 |
| Polymer (all three polymers) (mg) | 10 | 20 | 30 |
| Water(ml) | 40 | 40 | 40 |
| Homogenization speed(rpm) | 1200 | 1200 | 1200 |




Fig.5.5 G10,G11 and G12 nanosuspension prepared by method-1

Table 5.2

Screening the ratio of components for formulations prepared by nanoprecipitation technique

| Sr. No. | Batch code | Components | Ratio |
|---------|------------------|-----------------------|-------|
| 1 | G _i | Glimepiride: PEG 6000 | 1:10 |
| 2 | G _{ii} | Glimepiride: PEG 6000 | 1:20 |
| 3 | G _{iii} | Glimepiride: PVP K30 | 1:10 |
| 4 | G _{iv} | Glimepiride: PVP K30 | 1:20 |
| 5 | G _v | Glimepiride: PEG 400 | 1:10 |
| 6 | G _{vi} | Glimepiride: PEG 400 | 1:20 |

| Name of contents | Gi | Gii |
|---------------------------|-----------|-----------|
| Glimepiride (mg) | 1 | 1 |
| Polymer (PVPK30) (mg) | 10 | 20 |
| Water(ml) | 40 | 40 |
| Homogenization speed(rpm) | 1000-1200 | 1000-1200 |




Fig.5.6 Gi and Gii nanosuspension prepared by method-2

| Name of contents | Giii | Giv |
|---------------------------|-----------|-----------|
| Glimepiride (mg) | 1 | 1 |
| Polymer (PEG6000) (mg) | 10 | 20 |
| Water(ml) | 40 | 40 |
| Homogenization speed(rpm) | 1000-1200 | 1000-1200 |




Fig.5.7 Giii and Giv nanosuspension prepared by method-2

| Name of contents | Gv | Gvi |
|---------------------------|-----------|-----------|
| Glimepiride (mg) | 1 | 1 |
| Polymer (PEG400) (mg) | 10 | 20 |
| Water(ml) | 40 | 40 |
| Homogenization speed(rpm) | 1000-1200 | 1000-1200 |




Fig.5.8 Gv and Gvi nanosuspension prepared by method-2

5.6 Characterization and Evaluation of Nanosuspension

5.6.1 Optical Microscopy

Optical microscopy was done by optical microscope at 100 X using optical lens for viewing the abundance of nanoparticles and physical appearance. The morphological characteristics were studied for nanosuspension by optical microscopy.

5.6.2 Drug entrapment efficiency

Entrapment efficiency is defined as the percentage amount of drug which is entrapped by the nanosuspension. For the determination of entrapment efficiency, the un-entrapped drug was first separated by centrifugation at 20000 rpm for 30 minutes at 5° C by using cool ultra centrifuge. The amount of free drug was measured from

supernatant solution by taking the absorbance using UV visible spectrophotometer at 228 nm and 240 nm. (Ethiraj T*, Sujitha R and Dept., 2014)

The entrapment efficiency was calculated by given formula:

$$\% \text{Drug entrapment} = \frac{(\text{Total drug} - \text{Drug in supernatant liquid})}{\text{Total Drug}} \times 100$$

5.6.3 Particle size

The nanosuspension prepared by antisolvent precipitation supplemented by sonication were subjected to particle size analysis using “Beckman coulter” instrument.



Fig.5.9 Instruments of particles size and zeta potential analysis and transmission electron microscope

5.6.4 Zeta potential

Zeta potential determines the stability of nanosuspension. A minimum zeta potential of +30 mv or -30mV is required, where as in case of combined electrostatic or steric stabiliser, a zeta potential of 20 mV would be sufficient.(Kavitha et al., 2014).

5.6.5 Transmission electron microscopy (TEM)

A drop of a sample was placed on a carbon-coated grid and allowed it to dry. The grid in which the sample was introduced and observed under the transmission electron microscope with an accelerating voltage of 120 kV. The nanosuspension were observed by focusing the lens. The images were obtained after focusing the microscope with different magnifications of 19000-50000 X.

5.6.6 In-vitro drug release studies

Drug release profiles of nanosuspensions was done by using USP type II dissolution apparatus, at a rate of 50 rpm by using pH 6.8 phosphate buffer as dissolution medium. The temperature was maintained at 37° C Fourier transform infrared spectrometer. 5 ml of sample was withdrawn at 5,10,20,30,40,50 and 60 min. The same volume was replaced with fresh dissolution medium, required dilutions were made and samples were analysed at respective wavelength i.e 214 nm using UV-visible spectrophotometer.(Yadav et al., 2012)

CHAPTER 6

RESULTS AND DISCUSSION

6.1 Identification and characterization of Glimepiride

6.1.1 Physical description

The sample of glimepiride was identified and characterized as per requirements of COA (certificate of analysis) issued by the manufacturer and (USP 30 NF 25, 2007). Results are shown in table 6.1.

Table 6.1

Identification and characterization of glimepiride

| Parameters | Specifications as per COA | Observation |
|----------------|---------------------------|-------------|
| Physical state | Solid | Solid |
| Colour | White | White |
| Odor | Odorless | Odorless |
| Appearance | Crystalline | Crystalline |

6.1.2 Melting point analysis

The observed experimental melting point by capillary method complies with the reported melting point as shown in table 6.2 (Brammer *et al.*, 1991).

Table 6.2

Melting Point of glimepiride

| Parameter | Specification as per COA | Observation |
|---------------|--------------------------|----------------------|
| Melting range | 207 ^c | 203-205 ^c |

6.1.3 Identification of the drug glimepiride by FTIR spectra

An IR spectrum of sample was recorded to determine the presence of different functional groups. Fourier transforms infrared (FTIR) of pure glimepiride exhibited characteristic sharp peaks at 3369 cm⁻¹ and 3288 cm⁻¹ because of N-H stretching, 1707 cm⁻¹ and 1674 cm⁻¹ because of carbonyl group, 1345 cm⁻¹ due to C-N stretching vibration, 1153 cm⁻¹ due to S=O stretching vibration.

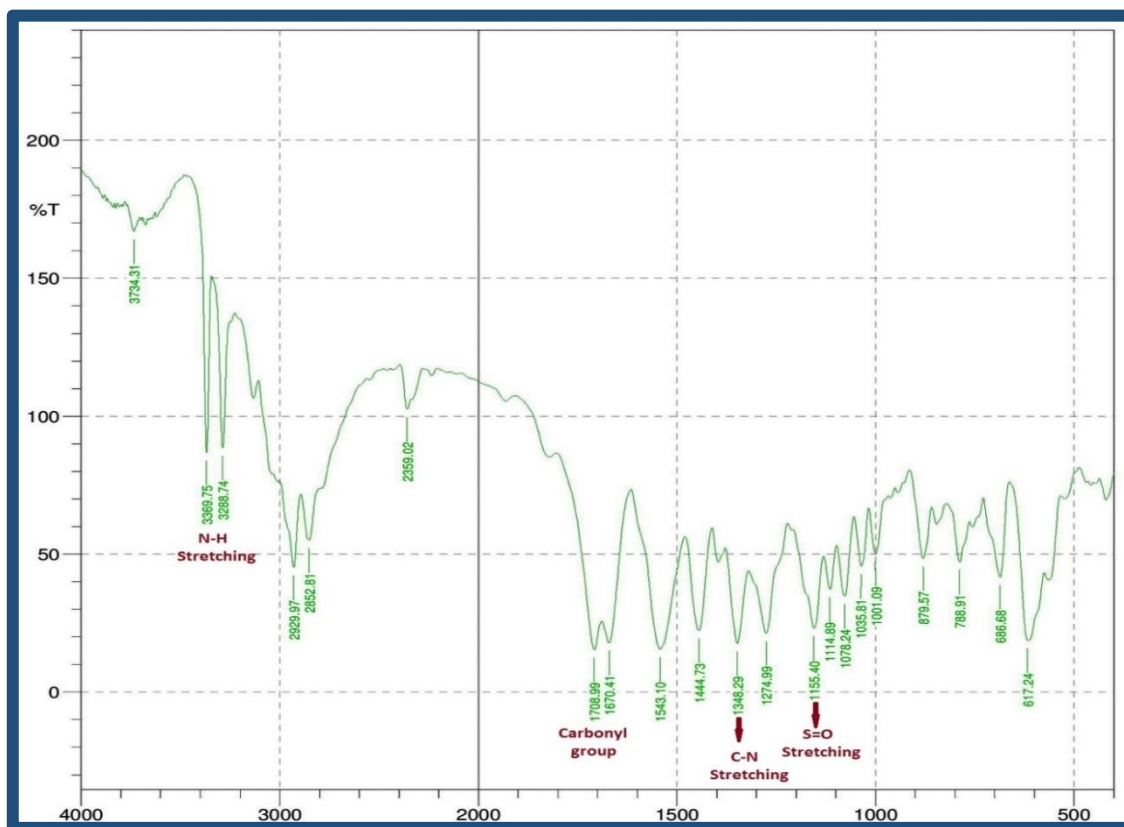


Fig.6.1. IR of glimepiride

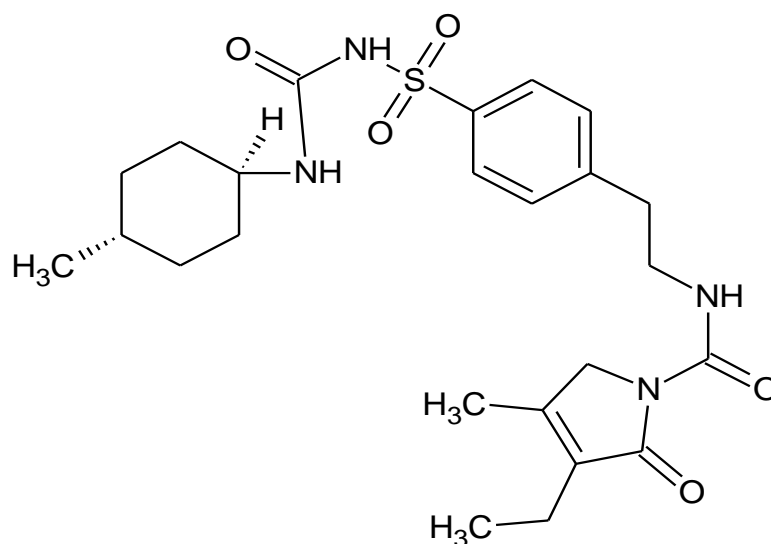


Fig.6.2. Structure of glimepiride

6.2 Determination of absorption maxima (λ_{\max}) of glimepiride

The λ_{\max} of glimepiride was found to be 228.50 nm in methanol. The scanning of the drug was done in the range (200-400 nm) as shown in the Fig.6.3.

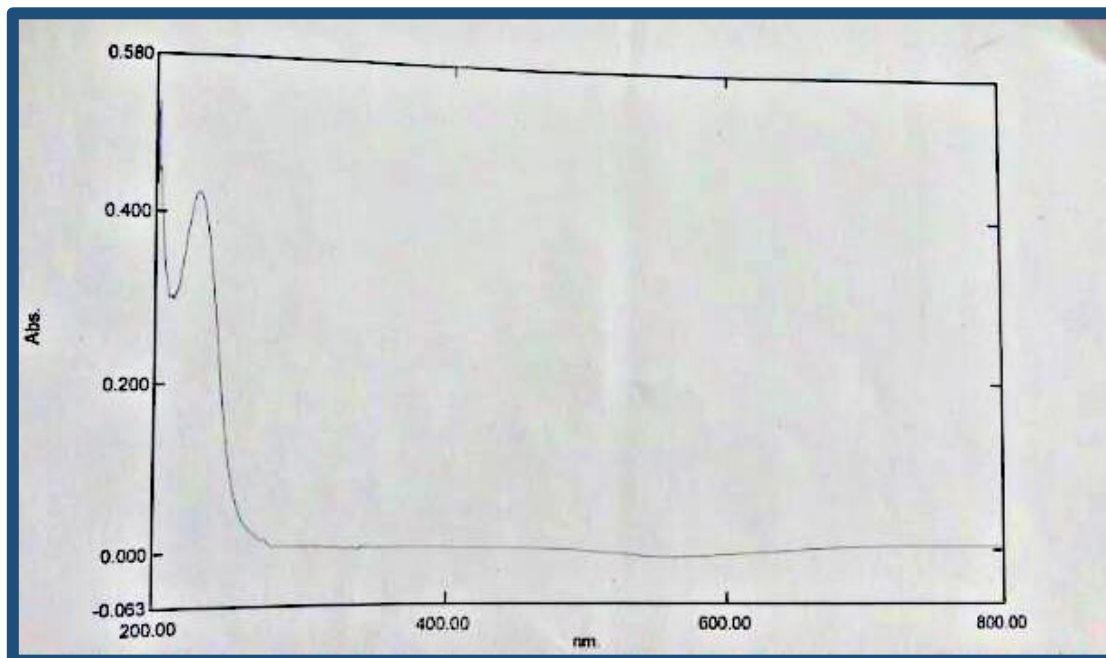


Fig. 6.3. Scan of glimepiride in methanol when scanned between 200- 400 nm

6.3 Analytical method validation of glimepiride methanol

The U.V spectroscopic method was validated to check the suitability for the purpose prescribed (ICH, Q2 (R1) guidelines, 2005). The process of validation depicts whether the method is good for its intended purpose or not. The selected method was validated according to ICH guidelines to determine the linearity, accuracy, precision, LOD, LOQ and robustness. The λ_{\max} selected was 228.50 nm and the linearity was established in the range of 2-10 $\mu\text{g/ml}$ with correlation coefficient, $R^2 = 0.9983$. The validity of the proposed method was further evaluated by recovery studies. The characteristic parameters are shown in table 6.8.

6.3.1 Calibration curve of glimepiride in methanol

The calibration plot of glimepiride was prepared by taking 2, 4, 6, 8, 10 $\mu\text{g/ml}$ (table 6.3) concentrations of glimepiride in methanol as shown in table 6.4. The experiments were performed in triplicate to find the standard deviation and percentage relative standard deviation. Absorbance range was found to be 0.129 - 0.543. The regression coefficient (R^2 value) was 0.9983 which showed linearity between 2-10 $\mu\text{g/ml}$

concentrations. The Lambert Beer law was obeyed within the linearity range. The standard regression equation was found to be $y = 0.0535x + 0.0068$.

Table 6.3

Absorbance of glimepiride in methanol at 228.50 nm

| Concentration ($\mu\text{g/ml}$) | Mean Absorbance \pm S.D (n= 3) | % Relative Standard deviation(RSD) |
|------------------------------------|-------------------------------------|---------------------------------------|
| 0 | 0 | 0 |
| 2 | 0.129 \pm 0.002 | 1.266 |
| 4 | 0.215 \pm 0.003 | 1.336 |
| 6 | 0.323 \pm 0.002 | 0.637 |
| 8 | 0.435 \pm 0.002 | 0.473 |
| 10 | 0.543 \pm 0.002 | 0.459 |
| Linear Regression | | (R^2) 0.9983 |

6.3.2 Linearity and Range

Table 6.3 shows concentration and absorbance at 228.50 nm. Linearity was observed in range of 2-10 $\mu\text{g/ml}$ at 228.50 nm with significant greater value of correlation coefficient, $r^2 = 0.998$ thus, follow Beer Lamberts law in this range as shown in Fig. 6.4.

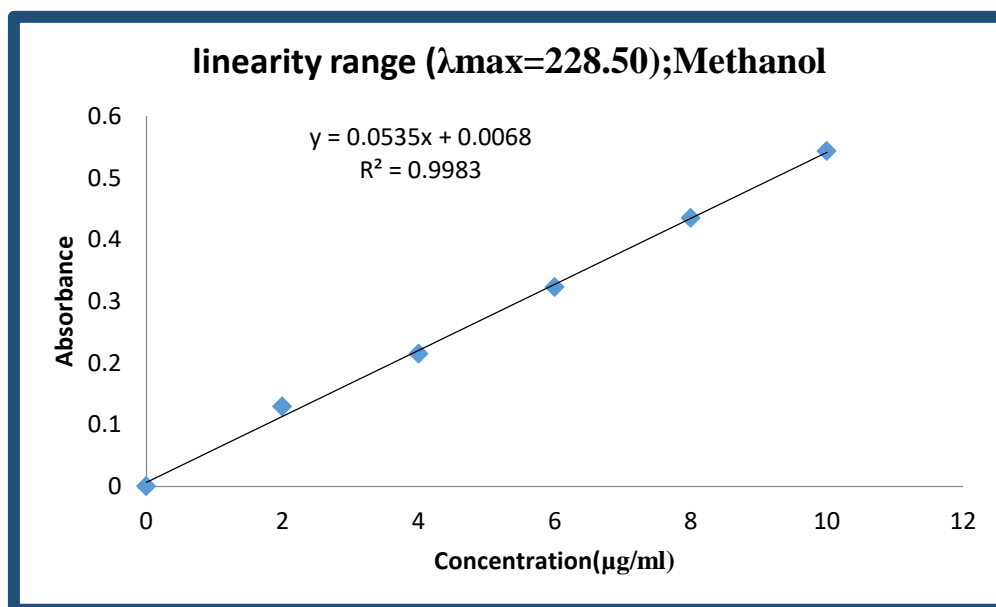


Fig. 6.4. Calibration curve of glimepiride in methanol at 228.50 nm

6.3.3 Accuracy

Accuracy results as shown in table 6.4 presents good reproducibility having RSD value less than 2. The method was found to be accurate as percentage recovery was

found to be within the range of 91.38 – 98.96. These results proved that the method is accurate.

Table 6.4

Result of accuracy of glimepiride in methanol

| Concentration ($\mu\text{g/ml}$) | Mean Absorbance \pm S.D (n= 3) | % Mean recovery | % Relative Standard deviation(RSD) |
|---------------------------------------|-------------------------------------|-----------------|---------------------------------------|
| 2 | 1.828 \pm 0.022 | 91.38 | 1.22 |
| 6 | 5.893 \pm 0.014 | 98.22 | 0.24 |
| 10 | 9.896 \pm 0.016 | 98.96 | 0.14 |

6.3.4 Precision

The results of intraday, interday repeatability and reproducibility have been summarized in table 6.5 and 6.6 respectively. The results were found to show good reproducibility with % RSD below 2. The results were very close to the true value. There was very less variation in intraday and interday precision. Percentage recovery of intraday precision was between 92.85-99.07 and interday precision was between 91.67-98.06.

Table 6.5

Result of intraday precision of glimepiride in methanol

| Concentration ($\mu\text{g/ml}$) | Mean Absorbance \pm S.D (n= 3) | % Mean recovery | % Relative Standard deviation(RSD) |
|---------------------------------------|-------------------------------------|-----------------|---------------------------------------|
| 2 | 1.857 \pm 0.013 | 92.85 | 0.69 |
| 6 | 5.917 \pm 0.076 | 98.61 | 1.28 |
| 10 | 9.907 \pm 0.135 | 99.07 | 1.36 |

Table 6.6

Result of interday precision of glimepiride in methanol

| Concentration ($\mu\text{g/ml}$) | Mean Absorbance \pm S.D (n= 3) | % Mean recovery | % Relative Standard deviation(RSD) |
|---------------------------------------|-------------------------------------|-----------------|---------------------------------------|
| 2 | 1.776 \pm 0.030 | 91.67 | 1.66 |
| 6 | 4.883 \pm 0.040 | 98.06 | 0.82 |
| 10 | 9.787 \pm 0.086 | 97.87 | 1.90 |

6.3.5 Robustness

Robustness results have been summarized in table 6.7 and were found to be good. All the samples prepared by interpersonal was showed % RSD below 2 and percentage mean recovery was also between 93.00-98.77 %. From the data it was found that slight changes by interpersonal do not affect the absorbance and method was validated.

Table 6.7

Result of robustness of glimepiride by interpersonal

| Concentration ($\mu\text{g/ml}$) | Mean Absorbance \pm S.D (n= 3) | % Mean recovery | % Relative Standard deviation (RSD) |
|---------------------------------------|-------------------------------------|-----------------|--|
| 2 | 1.860 \pm 0.024 | 93.00 | 1.32 |
| 6 | 5.787 \pm 0.037 | 96.44 | 0.64 |
| 10 | 9.877 \pm 0.029 | 98.77 | 0.29 |

6.3.6 Limit of Detection and Limit of Quantification

The LOD and LOQ were found to be 0.541 $\mu\text{g/ml}$ and 1.64 $\mu\text{g/ml}$ respectively as given in table 6.8. These results demonstrate that the method is sensitive and can detect the drug in the above mentioned concentration range.

Table 6.8

Characteristics for glimepiride in methanol

| Parameters | Values |
|---|-------------|
| λ_{max} (nm) | 228.50 |
| Linearity range ($\mu\text{g/ml}$) | 2-10 |
| Slope | 0.0068 |
| Intercept | 0.0535 |
| Correlation coefficient (R^2) | 0.9983 |
| Accuracy (Percentage mean recovery) | 91.38-98.96 |
| Intraday Precision (Percentage mean recovery) | 92.85-99.07 |
| Interday Precision (Percentage mean recovery) | 91.67-98.06 |
| Robustness (Percentage mean recovery) | 93.00-98.77 |
| LOD ($\mu\text{g/ml}$) | 0.541 |
| LOQ ($\mu\text{g/ml}$) | 1.64 |

6.4 Preformulation studies

6.4.1 Drug excipients compatibility

The results obtained from compatibility study of glimepiride with various excipients showed no physical and chemical incompatibility between glimepiride and excipients under stress conditions, and there was no change in color, appearance of glimepiride.

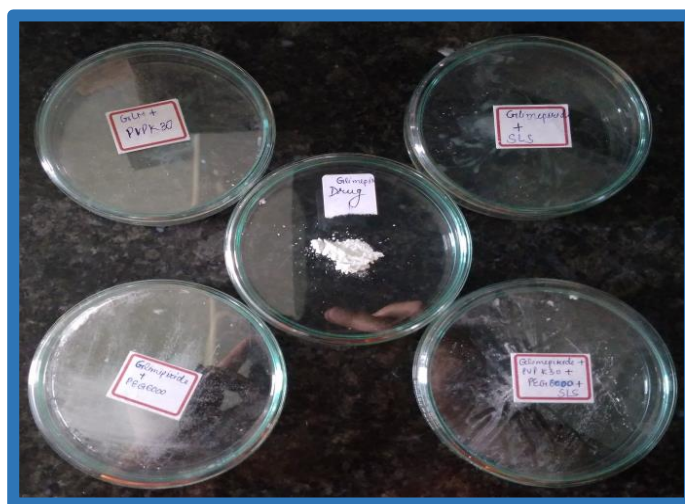


Fig.6.5 compatibility of drug glimepiride with different excipients

Table 6.9

Drug and excipients in 1:1 ratio for compatibility studies

| S.N o. | Ingredients | Color | Appearance | State | Lumps |
|-----------|--------------------------------|-------|-------------|-------|-------------|
| 1 | Glimepiride | White | Crystalline | Solid | Not present |
| 2 | Glimepiride:PVPK30 | White | Crystalline | Solid | Not present |
| 3 | Glimepiride:PEG 6000 | White | Crystalline | Solid | Not present |
| 4 | Glimepiride:SLS | White | Crystalline | Solid | Not present |
| 5 | Glimepiride:PEG6000:PV PK30 | White | Crystalline | Solid | Not present |
| 6 | Glimepiride:PEG6000:SLS | White | Crystalline | Solid | Not present |

| | | | | | |
|---|------------------------------------|-------|-------------|-------|-------------|
| | | | Crystalline | | |
| 7 | Glimepiride:SLS:PVPK30 | White | Crystalline | Solid | Not present |
| 8 | Glimepiride:SLS:PVPK30: PEG6000 | White | Crystalline | Solid | Not present |

Table 6.10

Drug and excipients in 1:1 ratio at different time intervals

| Ingredients | 1 st Day | 2 nd Day | 3 rd Day | 10 th Day | 15 th Day |
|----------------------------|------------------------|------------------------|------------------------|-------------------------|-------------------------|
| Glimepiride:PEG6000 | | | | | |
| Color | √ | √ | √ | √ | √ |
| Appearance | √ | √ | √ | √ | √ |
| State | √ | √ | √ | √ | √ |
| Lumps | √ | √ | √ | √ | √ |
| Glimepiride:PVPK30 | | | | | |
| Color | √ | √ | √ | √ | √ |
| Appearance | √ | √ | √ | √ | √ |
| State | √ | √ | √ | √ | √ |
| Lumps | √ | √ | √ | √ | √ |
| Glimepiride:SLS | | | | | |
| Color | √ | √ | √ | √ | √ |
| Appearance | √ | √ | √ | √ | √ |
| State | √ | √ | √ | √ | √ |
| Lumps | √ | √ | √ | √ | √ |
| Glimepiride:PEG6000:PVPK30 | | | | | |
| Color | √ | √ | √ | √ | √ |
| Appearance | √ | √ | √ | √ | √ |
| State | √ | √ | √ | √ | √ |
| Lumps | √ | √ | √ | √ | √ |
| Glimepiride:PEG6000:SLS | | | | | |
| Color | √ | √ | √ | √ | √ |
| Appearance | √ | √ | √ | √ | √ |
| State | √ | √ | √ | √ | √ |
| Lumps | √ | √ | √ | √ | √ |
| Glimepiride:PVPK30:SLS | | | | | |
| Color | √ | √ | √ | √ | √ |
| Appearance | √ | √ | √ | √ | √ |

| | | | | | |
|--------------------------------|---|---|---|---|---|
| State | √ | √ | √ | √ | √ |
| Lumps | √ | √ | √ | √ | √ |
| Glimepiride:SLS:PVPK30:PEG6000 | | | | | |
| Color | √ | √ | √ | √ | √ |
| Appearance | √ | √ | √ | √ | √ |
| State | √ | √ | √ | √ | √ |
| Lumps | √ | √ | √ | √ | √ |

6.4.2 Solubility analysis of glimepiride

Solubility of glimepiride was determined in methanol, DCM (Di-chloromethane), 0.1 N HCl, pH 6.8 Phosphate buffer and pH 7.8 Phosphate buffer. An excess amount of drug was added in 50 ml of conical flask and was kept under shaking for 72 hrs (Rotary shaker). Then absorbance of prepared solution was determined from that amount of drug solubilized drug was calculated as shown in table 6.11.

Table 6.11

Solubility of glimepiride in various organic solvents and buffers (IP, 2010)

| Sr.no. | Solvent | Solubility profile |
|--------|----------------------------------|--------------------|
| 1 | Water | Not Soluble |
| 2 | Di-chloromethane | Slightly soluble |
| 2 | Methanol | Slightly soluble |
| 3 | 0.1N HCl | Not Soluble |
| 4 | pH 6.8 Phosphate buffer | Not Soluble |
| 5 | pH 7.8 Phosphate buffer | Not Soluble |
| 6 | pH 6.8 Phosphate buffer+1%SLS | Very soluble |
| 7 | pH 7.8 Phosphate buffer+1%SLS | Very soluble |

6.4.3 Partition coefficient of glimepiride

The partition coefficient of glimepiride between octanol and water (log P) was determined (Florey, 2008). The study indicated that glimepiride has a log P value equals to 3.04.

6.4.4 Prescreening study for selection of ratio of components

Prescreening study was done to select the levels for design of experiment. For this, the formulations with suitable ratios of drug and polymers. (Abdul Hasan Sathali. A et al., 2013) Levels were decided on the basis of literature. The nanosuspension were prepared by the Antisolvent evaporation technique followed by sonication and nanoprecipitation method and were evaluated for various evaluation parameters. The ratio of the components were screened by optical microscopy as shown in Fig.6.8.

Table 6.12

Screening of ratio of components in Antisolvent evaporation followed by sonication technique

| Batch code | Drug: Polymer | Ratio | Nanoparticles |
|------------|-----------------------------------|---------|---------------|
| G1 | Glimepiride: PEG 6000 | 1:10 | Present |
| G2 | Glimepiride: PEG 6000 | 1:20 | Present |
| G3 | Glimepiride: PEG 6000 | 1:30 | Present |
| G4 | Glimepiride: PVP K30 | 1:10 | Present |
| G5 | Glimepiride: PVP K30 | 1:20 | Present |
| G6 | Glimepiride: PVP K30 | 1:30 | Present |
| G7 | Glimepiride:PEG 400 | 1:10 | Present |
| G8 | Glimepiride:PEG 400 | 1:20 | Present |
| G9 | Glimepiride:PEG 400 | 1:30 | Present |
| G10 | Glimepiride: PEG 6000: PVP K30 | 1:10:10 | Present |
| G11 | Glimepiride: PVP K30: PEG 400 | 1:10:10 | Present |
| G12 | Glimepiride: Peg 400: PEG 6000 | 1:10:10 | Present |

Table 6.13

Screening of ratio of components for preparation of nanosuspension by nanoprecipitation technique

| Batch code | Drug: polymer | Ratio | Nanoparticles |
|------------------|-----------------------|-------|---------------|
| G _i | Glimepiride: PEG 6000 | 1:10 | Present |
| G _{ii} | Glimepiride: PEG 6000 | 1:20 | Present |
| G _{iii} | Glimepiride: PVP K30 | 1:10 | Present |
| G _{iv} | Glimepiride: PVP K30 | 1:20 | Present |
| G _v | Glimepiride: PEG 400 | 1:10 | Present |
| G _{vi} | Glimepiride: PEG 400 | 1:20 | Present |

6.5 Formulation Development trials

6.5.1 Optimization of nanosuspension formulation prepared by combination technique

The design of optimization contained two independent variables (X1, X2) and one dependent variable (Y1). The X variables were drug (% w/w) and polymer (% w/w) respectively, whereas, the Y1 variable was percentage entrapment efficiency. According to the reference, 12 formulations were suggested and each of them were formulated and analysed for entrapment efficiency shown in table 6.14.

Table 6.14

Factor combination and responses for combination technique

| Run no. | Amount of drug (mg) (X1) | Amount of polymer (mg) (X2) | Homogenizati on speed (rpm) | Entrapment efficiency (%) |
|---------|--------------------------|-----------------------------|-----------------------------|---------------------------|
| G1 | 1mg Glimepiride | 10mg (PEG6000) | 1200 | 82.16 |
| G2 | 1mg Glimepiride | 20mg (PEG6000) | 1200 | 43.63 |
| G3 | 1mg Glimepiride | 30mg (PEG6000) | 1200 | 32.42 |
| G4 | 1mg | 10mg (PVPK30) | 1200 | 78.44 |

| | | | | |
|-----|-------------|-------------------|------|-------|
| | Glimepiride | | | |
| G5 | 1mg | 20mg (PVPK30) | 1200 | 74.02 |
| | Glimepiride | | | |
| G6 | 1mg | 30mg (PVPK30) | 1200 | 61.23 |
| | Glimepiride | | | |
| G7 | 1mg | 10mg (PEG400) | 1200 | 55.61 |
| | Glimepiride | | | |
| G8 | 1mg | 20mg (PEG400) | 1200 | 33.65 |
| | Glimepiride | | | |
| G9 | 1mg | 30mg (PEG400) | 1200 | 46.41 |
| | Glimepiride | | | |
| G10 | 1mg | 10mg | 1200 | 38.42 |
| | Glimepiride | (PEG6000:PVPK30) | | |
| G11 | 1mg | 20mg | 1200 | 72.42 |
| | Glimepiride | (PEG6000:PEG4000) | | |
| G12 | 1mg | 30mg(PEG4000: | 1200 | 77.24 |
| | Glimepiride | PVPK30) | | |

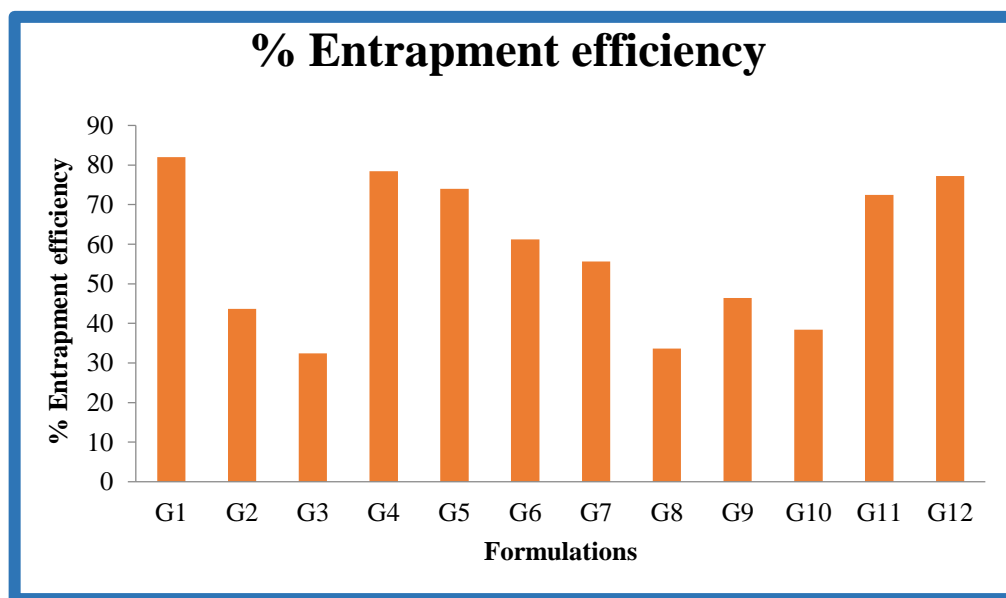


Fig 6.6 Percentage drug entrapment of different nanosuspension formulations prepared by combination technique

6.5.2 Optimization of nanosuspension formulation prepared by nanoprecipitation technique

The design of optimization contained two independent variables (X_i , X_{ii}) and one dependent variables (Y_i). The X variables were drug (% w/w) and polymer (% w/w) respectively, whereas, the Y_i variable was percentage entrapment efficiency. According to the literature, 6 formulations were suggested and each of them were formulated and analysed for entrapment efficiency shown in table 6.15

Table 6.15

Factor combination and responses for nanoprecipitation technique

| Run no. | Amount of drug (mg) (X_i) | Amount of polymer (mg) (X_{ii}) | Homogenization speed (rpm) | Entrapment efficiency (%) |
|-----------|-------------------------------|-------------------------------------|----------------------------|---------------------------|
| G_i | 1mg Glimepiride | 10mg PEG 6000 | 1000-1200 | 62.82 |
| G_{ii} | 1mg Glimepiride | 20mg PEG 6000 | 1000-1200 | 80.03 |
| G_{iii} | 1mg Glimepiride | 10mg PVP K30 | 1000-1200 | 49.24 |
| G_{iv} | 1mg Glimepiride | 20mg PVP K30 | 1000-1200 | 76.01 |
| G_v | 1mg Glimepiride | 10mg PEG 400 | 1000-1200 | 25.26 |
| G_{vi} | 1mg Glimepiride | 20mg PEG 400 | 1000-1200 | 63.64 |

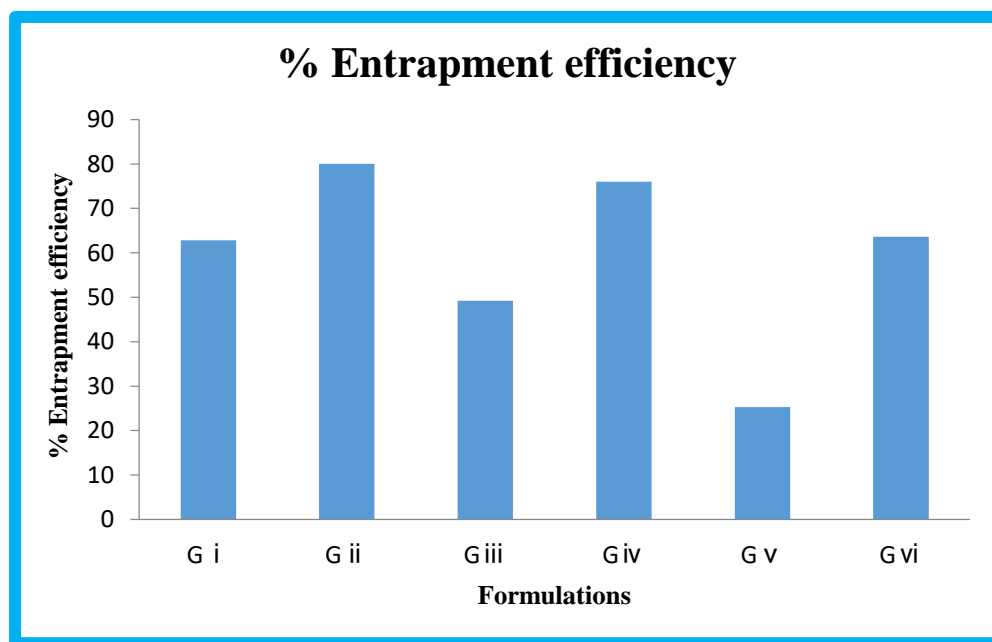
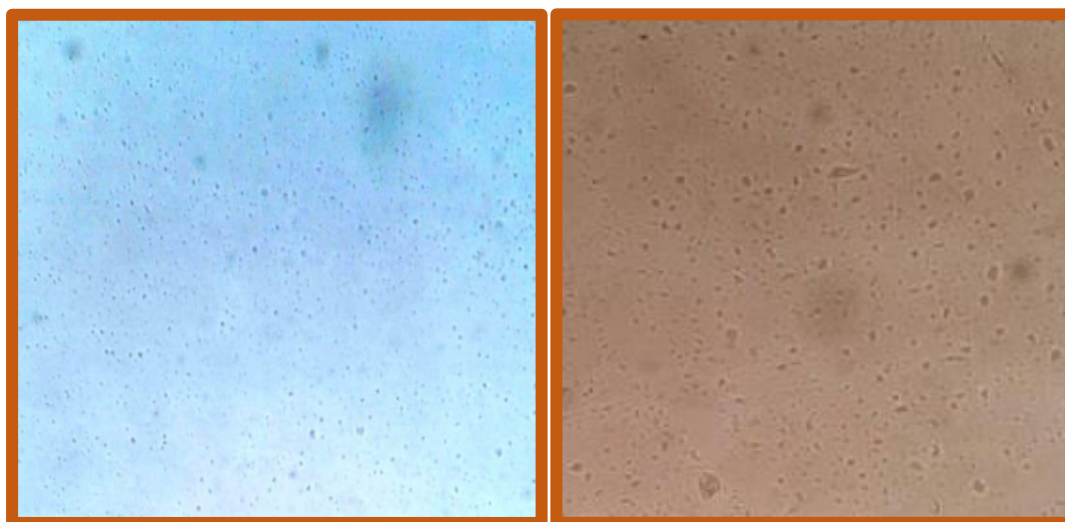


Fig.6.7 Percentage drug entrapment of different nanosuspension formulations prepared by nanoprecipitation method

6.6 Characterization and Evaluation of nanosuspension:

6.6.1 Optical microscopy

The prepared formulations were examined for optical microscopy as shown in Fig.6.8. Optical microscopy showed that the round particles were observed in formulations studied at 100 X. The micrographs of nanosuspension revealed the presence of round structure which were nano in appearance as shown in Fig.6.8



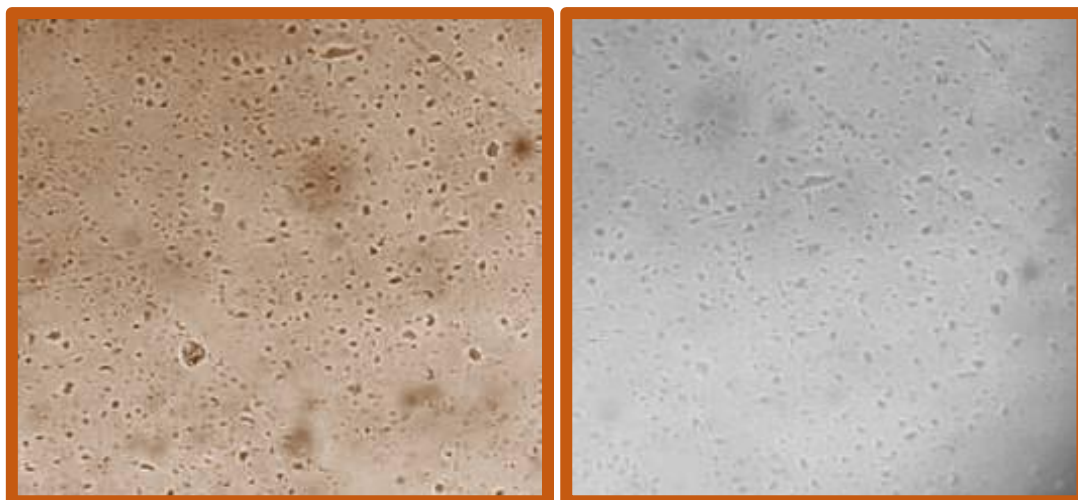


Fig. 6.8. Optical photomicrographs of some representative nanosuspension formulations

6.6.2 Analysis of particle size

The particle size and polydispersity Index (PI) values of nanosuspension are presented in table 6.16. The differences in the particle size of nanosuspension formulations prepared with variable ratios of polymer and drug were utilized to find the optimized formulation. The particle sizes were falling in the range of 129-180 nm with PI of 0.253 for G1 nanosuspension and 72- 383 nm with PI 0.358 for G2 nanosuspension as shown in Fig. 6.9. In general, nano and microcarriers with Polydispersity Index (PI) value higher than 0.5 shows large size distributions and have the tendency to aggregate. the value of polydispersity index varies from 0.0-1.0 and closer the value of PI to zero more will be the homogeneous nanosuspension formed. (Kotecha et al., 2013) The result shows that G1 formulation is best formulation which is prepared by combination technique having average particle size 180 nm and polydispersity index 0.253 which is shown in Fig. 6.9. From these values of particle size and polydispersity index it is come to know that optimized nanosuspension formulation is homogeneous and having uniform distribution.

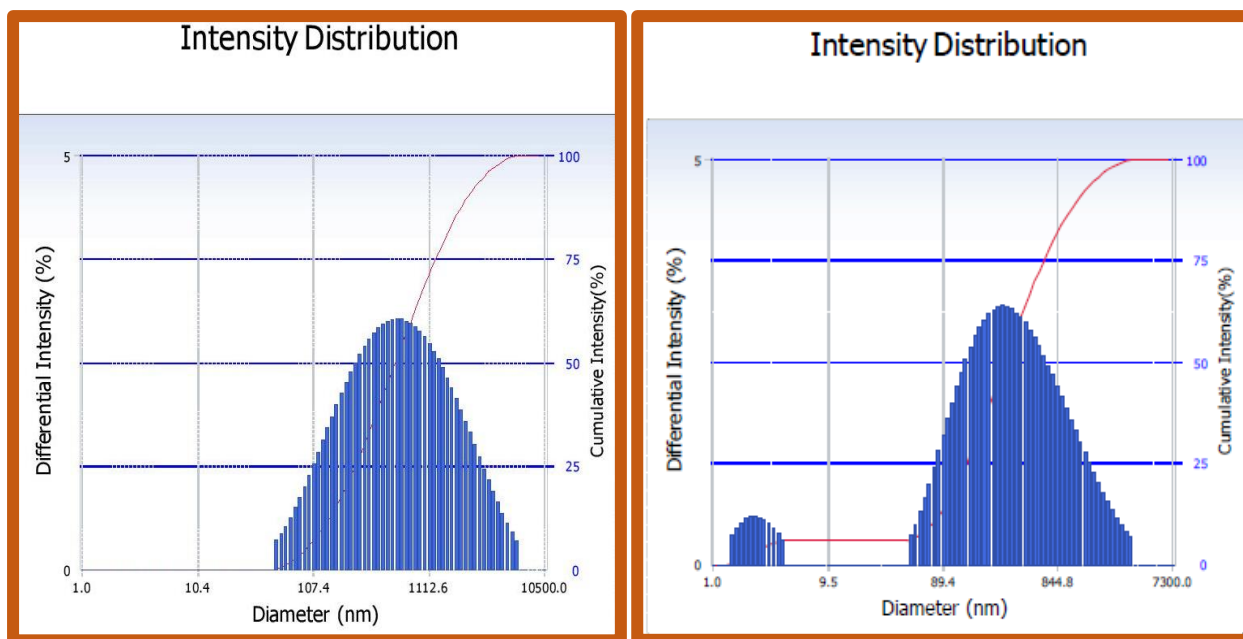


Fig. 6.9. Size distribution of optimized nanosuspension formulation G1 and Gii

Table 6.16

Results of particle size analysis

| Cumulants Results | | |
|----------------------------------|---------------------------------|---------------------------------|
| Parameters | G1 | Gii |
| Diameter (d) | 180.01 nm | 383nm |
| Polydispersity Index (PI) | 0.253 | 0.358 |
| Diffusion Const. (D) | 1.014e-008 cm ² /sec | 1.222e-008 cm ² /sec |
| Measurement Condition | | |
| Temperature | 25.1 °C | 25.0°C |
| Diluent Name | Water | Water |
| Refractive Index | 1.3328 | 1.3328 |
| Viscosity | 0.8878 (cP) | 0.8878 (cP) |
| Scattering Intensity | 6928 (cps) | 3244(cps) |

6.6.3 Zeta potential

Zeta potential will determine the stability of nanosuspension. A minimum zeta potential of +30 mv or -30mv is required where as in case of combined electrostatic or steric stabiliser, a zeta potential of 20 mv would be sufficient.(Kavitha et al., 2014).At 24.9° with greater zeta value toward positive side for formulation G1 was findout to be 30.16mV and for Gii it was -22.19 mV ,means G1 formulation having more stability as compare to Gii formulation.The high value of zeta potential shows electrostatic repulsion between particles, and zeta potential below ± 30 mV shows good stability.

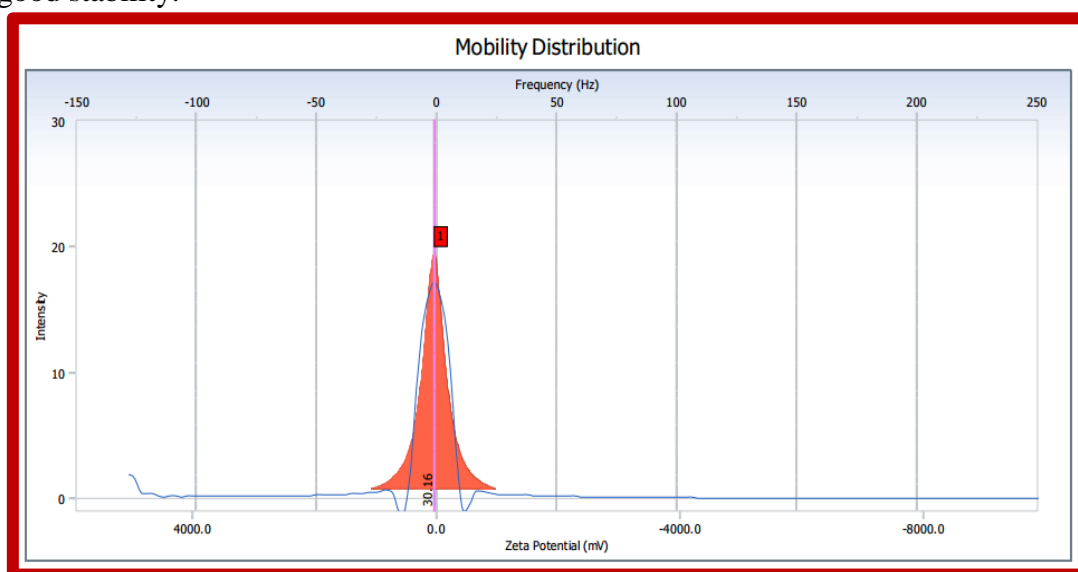


Fig 6.10 Zetapotential of nanosuspension G1

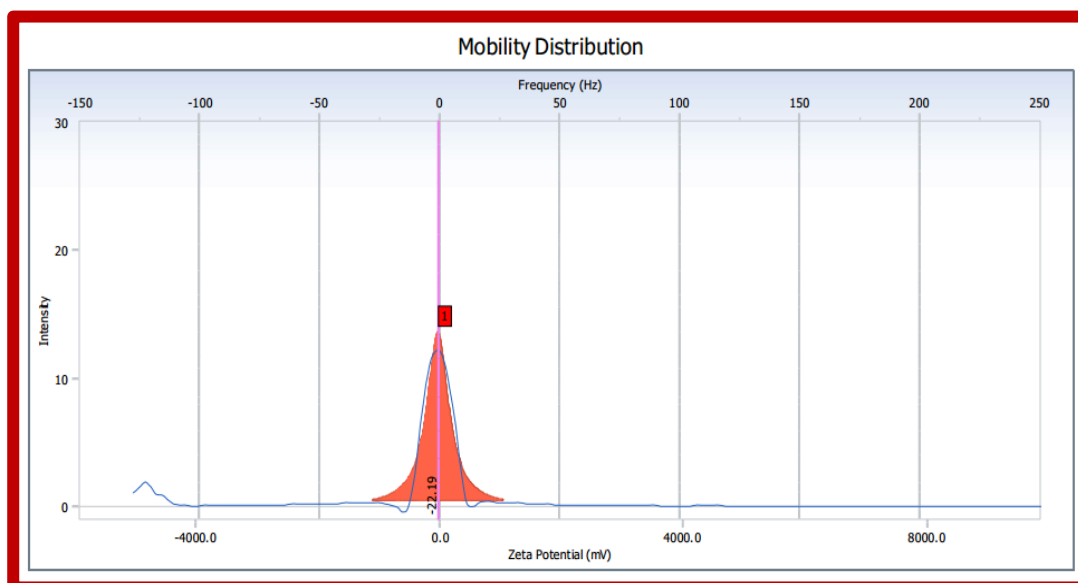


Fig 6.11 Zeta potential of nanosuspension Gii

6.6.4 In vitro drug release studies

Drug release profiles of Glimepiride nanosuspensions i.e G1 and Gii was done by using USP type II dissolution apparatus, at a rate of 50 rpm by using pH 6.8 phosphate buffer as dissolution medium. The temperature was maintained at $37\pm 0.5^\circ\text{C}$. withdrawn the samples at regular intervals of time and the same volume was replaced with fresh dissolution medium Required dilutions were made and samples were analysed at 214 nm using UV-visible spectrophotometer.(Yadav et al., 2012)

Calibration curve of Glimepiride in pH 6.8 phosphate buffer:

The calibration plot of glimepiride was prepared by taking 2, 4, 6, 8, 10,12,14,16,18 and 20 $\mu\text{g/ml}$ concentrations of glimepiride in pH 6.8 phosphate buffer. Absorbance range was found to be 0.121 to 0.589. The regression coefficient (R^2 value) was 0.9975 which showed linearity between 2 – 20 $\mu\text{g/ml}$ concentrations. The Lambert-Beer law was obeyed within the linearity range. The standard regression equation was found to be $y = 0.0254x + 0.0785$.

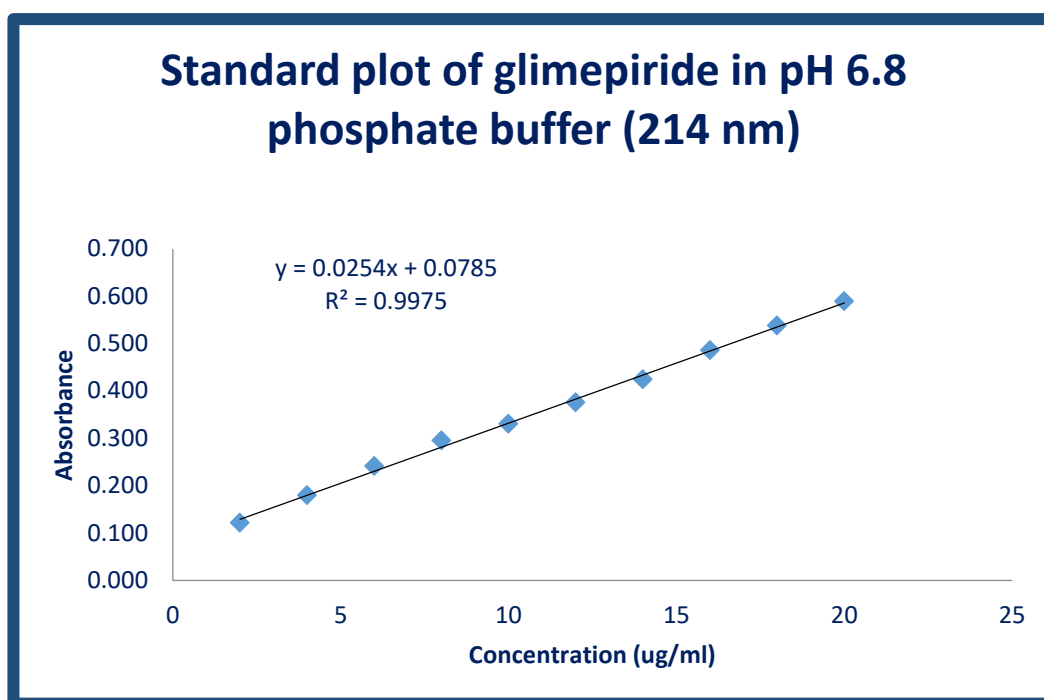


Fig.6.12 Calibration curve of glimepiride in pH 6.8 phosphate buffer at 214 nm

From the results of in-vitro dissolution study it is found that G1 formulation shows more drug release i.e 86.76% in comparison to Gii formulation which shows drug release 74.77% after 60 minutes in pH 6.8 phosphate buffer at wavelength 214 nm shown in table 6.17.

Table 6.17

Results of in-vitro dissolution of nanosuspensions G1 and Gii

| Time (G1 and GII) | %Drug release | |
|-------------------|---------------|-------|
| | G1 | Gii |
| 5 | 6.66 | 3.54 |
| 10 | 18.89 | 13.20 |
| 20 | 37.70 | 22.63 |
| 30 | 62.57 | 47.98 |
| 40 | 76.81 | 61.85 |
| 50 | 83.18 | 70.13 |
| 60 | 86.76 | 74.77 |

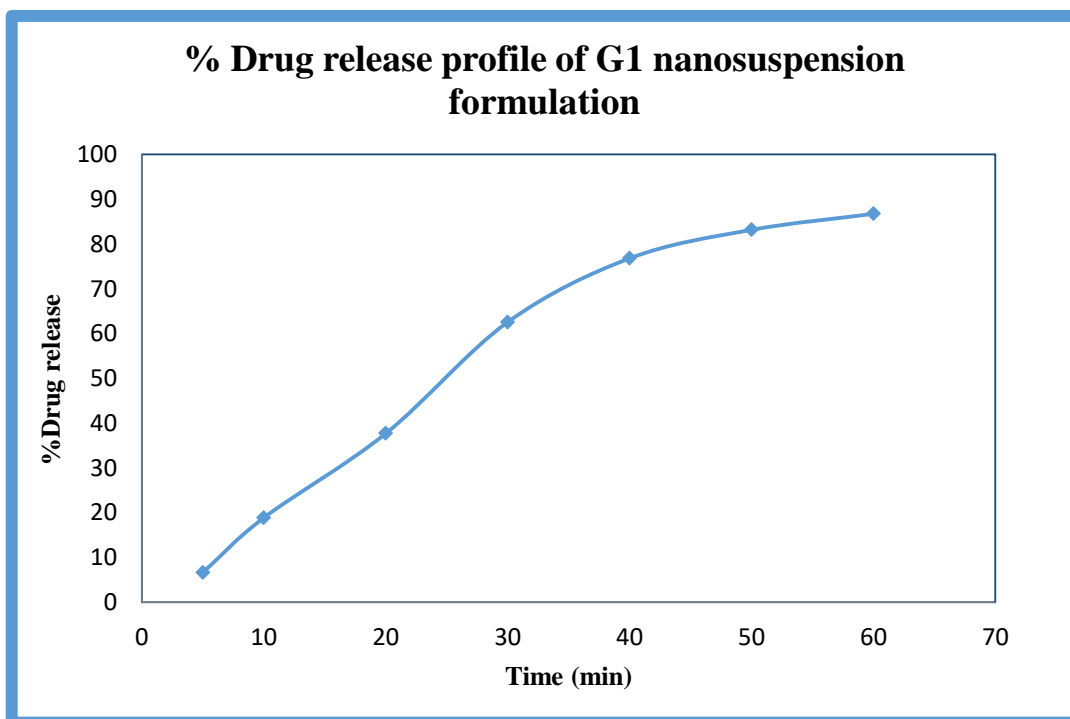


Fig 6.13 Graphical representation of drug release profile of nanosuspension formulation (G1)

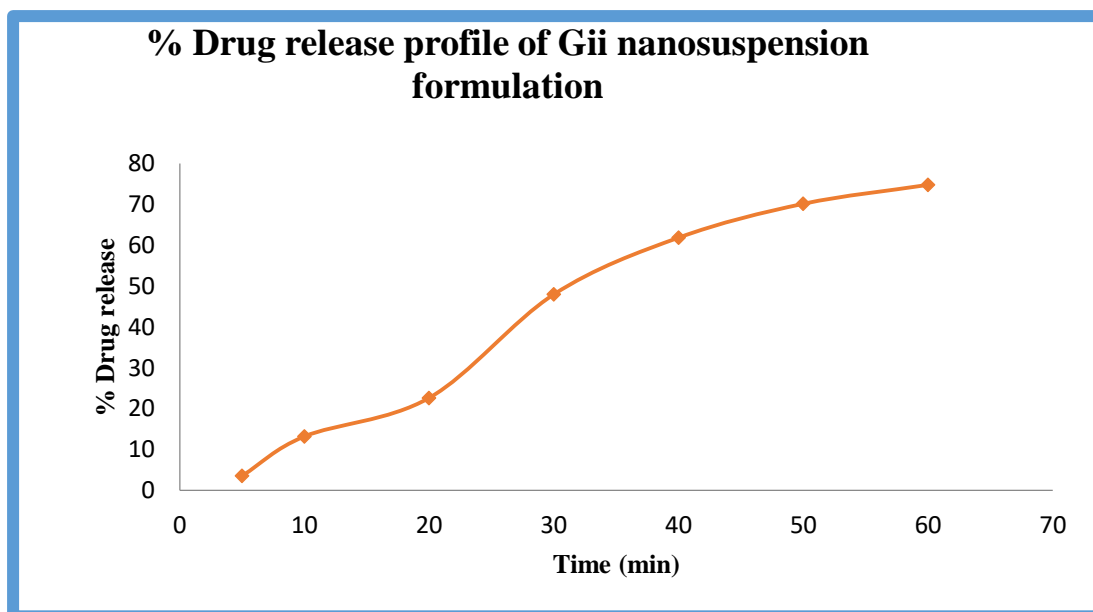


Fig 6.14 Graphical representation of drug release profile of nanosuspension formulation (Gii)

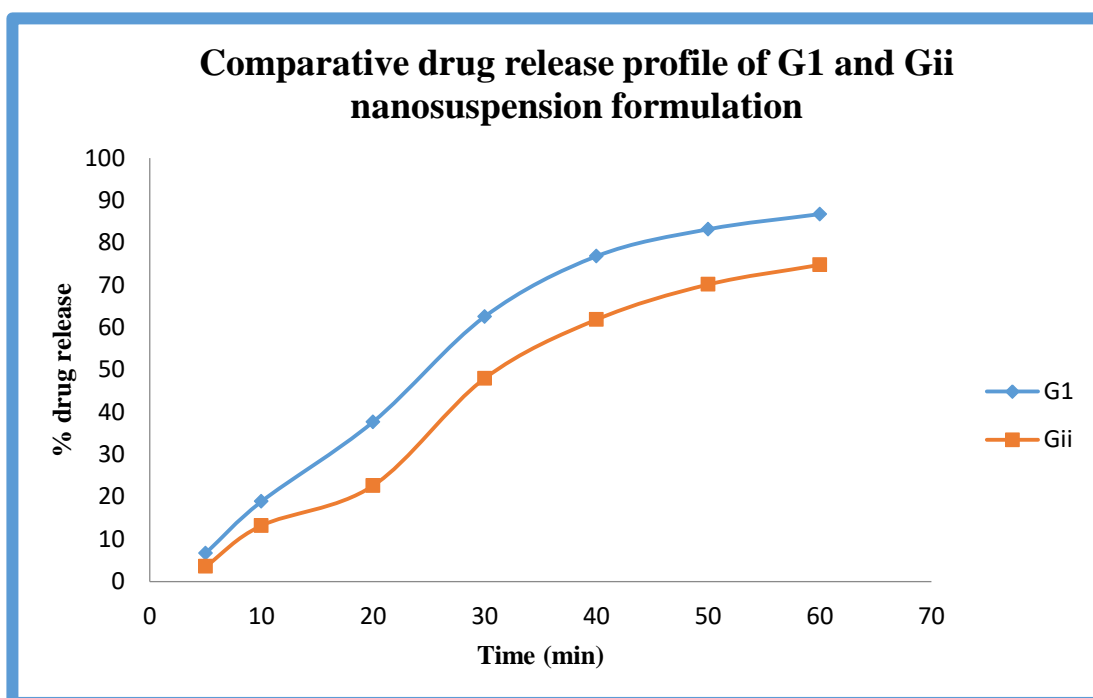


Fig. 6.15 Comparative drug release study of nanosuspension formulations G1 and Gii

6.6.5 Transmission electron microscopy (TEM)

TEM photomicrographs of some representable glimepiride nanosuspension are shown in Fig 6.16-6.17. The grid in which the sample was introduced and was observed under the transmission electron microscope with an accelerating voltage of 120 kV

with magnification between 19000 X – 50000 X. The diameter was found to be within the range of 20-100 nm.

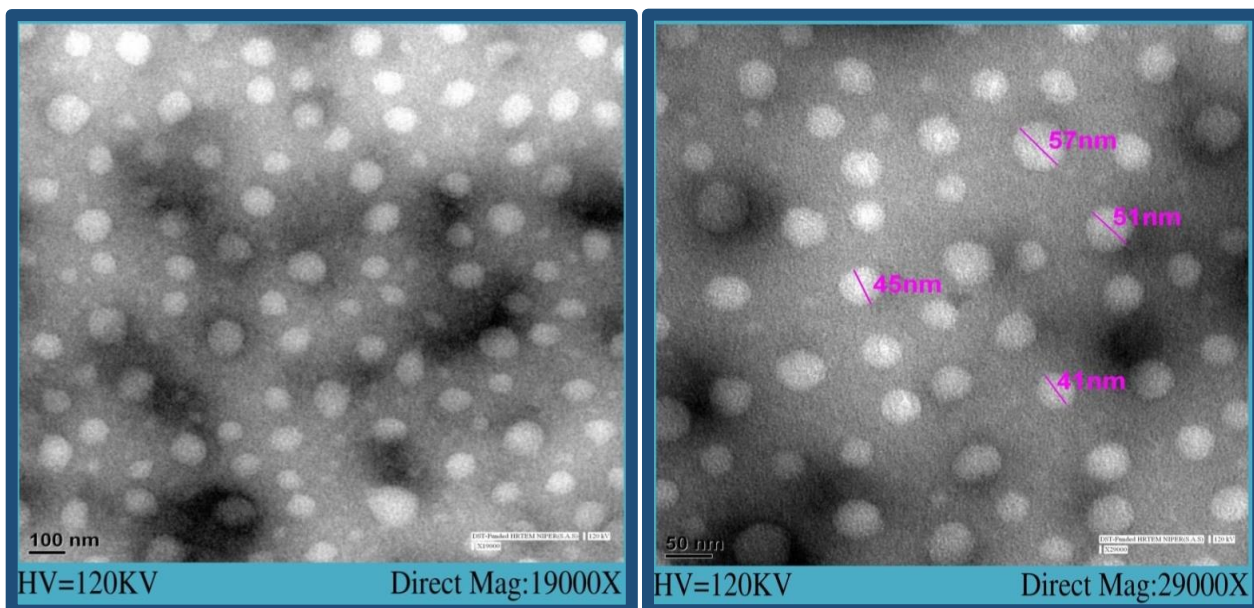


Fig.6.16. Transmission electron microscopy images of (G1) glimepiride nanosuspension with magnification of 19000X and 29000X

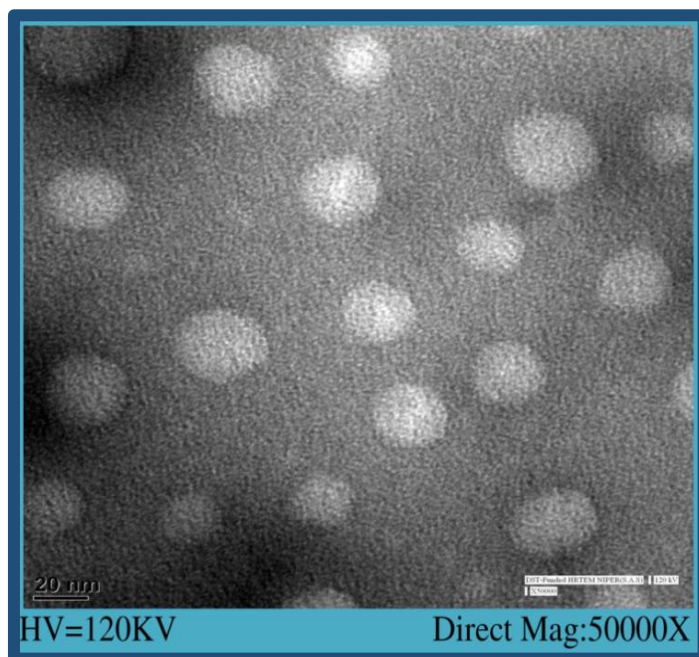


Fig.6.17. Transmission electron microscopy images of (G1) glimepiride nanosuspension with magnification 50000X

6.6.6 Stability study:

Stability studies were carried out by storing prepared nanosuspensions in high temperature in humidity control oven at $40\pm 2^\circ\text{C}/75\pm 5\%$ RH for 3 months according to ICH guidelines. After 3 months of storage, analysis of different parameters like zeta potential and entrapment efficiency was done by comparing with older results of zeta potential and entrapment efficiency of nanosuspensions which was taken as a control. (Yadav et al., 2012). After 3 months the entrapment efficiency values were reduced from 82.16% to 77.24% for G1 formulation and from 80.03% to 72.83% for Gii formulation and zeta values were also reduced to some extent from 30.16 to -25.09 mV for G1 formulation and from -22.19 to -16.14 mV for Gii formulation.

Table 6.18

Stability testing parameters of prepared formulations after 3 months of storage

| Evaluation parameters (after 3 months) | Previous results of parameters of fresh nanosuspensions | | After three months of storage | |
|---|---|--------|----------------------------------|--------|
| | G1 | Gii | G1 | Gii |
| Zeta potential | 30.16 | -22.19 | -25.09 | -16.14 |
| % Entrapment efficiency | 82.16% | 80.03% | 77.24% | 72.83% |

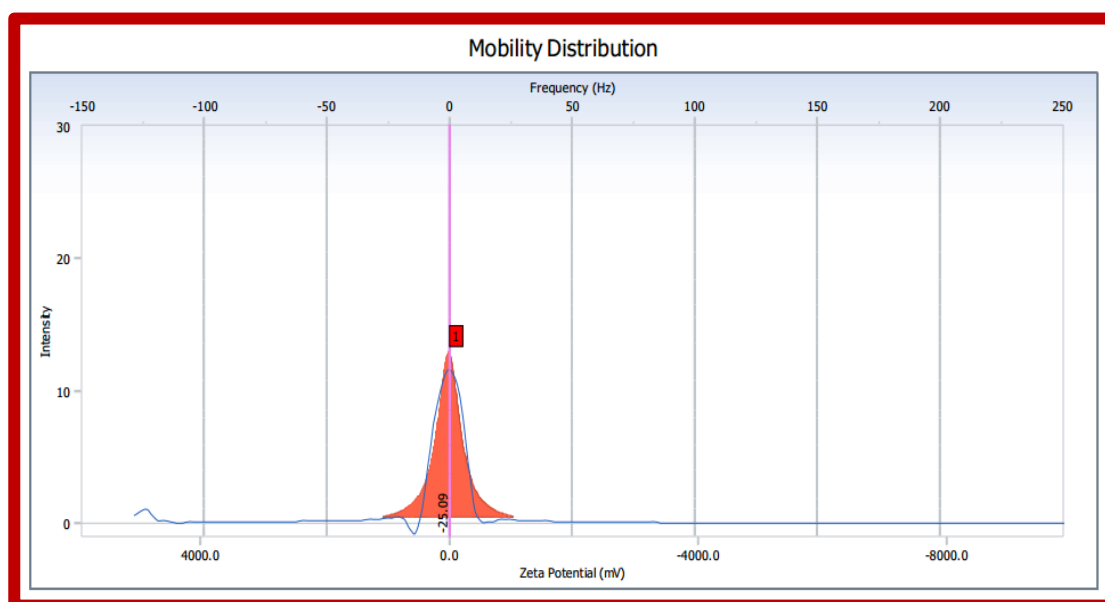


Fig.6.18 Zetapotential of nanosuspension G1 for stability study

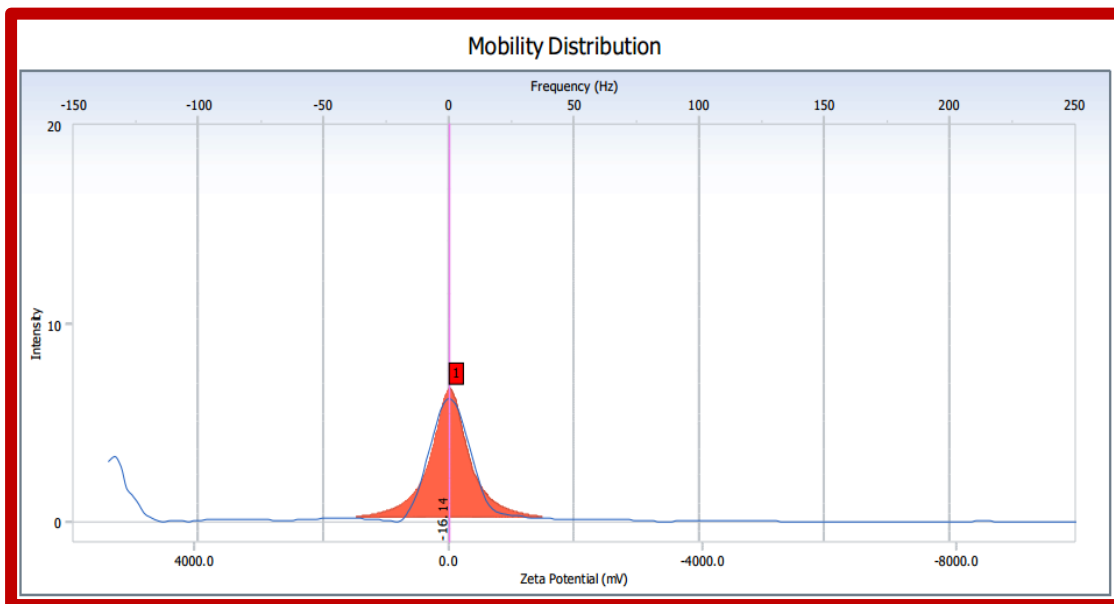


Fig.6.19 Zetapotential of nanosuspension Gii for stability study

CHAPTER-7**SUMMARY AND CONCLUSION**

Diabetes mellitus (DM) is chronic, life-long endocrine and metabolic disorder which occurs due to the defect in insulin secretion and insulin action. many peoples are suffering from this disease now a days. If diabetes is uncontrolled then it leads to severe diabetic complications like retinopathy, neuropathy, and various cardiovascular complications. diabetes is treated with various anti-diabetic drugs but most of the drugs having a problem related to poor solubility and dissolution so to avoid these problems new and effective formulation were developed to increase the solubility of the anti-diabetic drug by making it's as nanosuspension.

Glimepiride was selected as a model drug. The characterization of glimepiride was done by melting point analysis and FTIR. The solubility and partition coefficients analysis were done to determine the nature of the drug. Analytical method validation for glimepiride in methanol was carried out to establish a simple reproducible analytical method for estimation of glimepiride with the help of UV spectrophotometer. From Prescreening study the two techniques were selected, combination technique which includes antisolvent evaporation followed by sonication and nanoprecipitation technique by which nanosuspension was prepared. From literature review, the ratio of polymers and drug was selected to use in preparation of nanosuspension formulations which were prepared by both techniques. In combination technique 12 formulations and in nanoprecipitation method 6 formulations were prepared by varying the ratio of polymers like PEG6000, PEG 400 and PVPK30 in 1:10,1:20 and 1:30. The ratio of drug was taken similar in both cases.

The characterization and evaluation was done by optical microscopy, drug entrapment efficiency, particle size analysis ,zeta potential, in-vitro dissolution and TEM.

In optical microscopy round shape nanoparticles were seen at 100 X. Drug entrapment efficiency was done for 12 formulations(G1,G2,G3,G4,G5,G6,G7,G8G9, G10, G11 and G12) in which G1 shows highest entrapment efficiency i.e 82.16% for combination method and 6 formulations (G_i, G_{ii}, G_{iii}, G_{iv}, G_v and G_{vi}) in which G_{ii} shows highest entrapment efficiency i.e 80.03% for nanoprecipitation method. Then, particle size and zeta potential analysis were carried which presented the particle size average

range of 129-180 nm for G1 formulation having zeta potential value and PI values 30.16mV and 0.253. for Gii formulation particle size range was 72-383 nm having zeta and PI values -22.19 mV and 0.358. Then, G1 and Gii formulations were selected for in-vitro dissolution study. From the result of in-vitro dissolution study it is found that G1 formulation shows more drug release i.e 86.76% in comparison to Gii formulation which shows drug release 74.77% after 60 minutes in pH 6.8 phosphate buffer. From results of in-vitro dissolution G1 formulation was studied for morphology by TEM which ensured the formation of round nanoparticles of nanosuspension. Stability studies were carried out on G1 and Gii formulations for 3 months ,after 3 months the entrapment efficiency and zetapotential of G1 and Gii formulations were carried out and compare with previous results of entrapment efficiency and zeta potential. The entrapment efficiency values were reduced from 82.16% to 77.24% for G1 formulation and from 80.03% to 72.83% for Gii formulation and zeta values were also reduced to some extent from 30.16 to -25.09 mV for G1 formulation and from -22.19 to -16.14mV for Gii formulation.

From the different studies which were carried out on nanosuspensions G1 and Gii which were prepared by two different techniques, it is concluded that the formulation G1 prepared by combination technique shows good solubility, dissolution and drug release as compared to formulation Gii, prepared by nanoprecipitation method. So, the present study, concludes that the combination technique which include antisolvent evaporation followed by sonication is the better technique to prepare glimepiride nanosuspension.

FUTURE ASPECTS:

The present study has provided information regarding the preparation of nanosuspension of glimepiride to improve solubility and dissolution rate. The sincere efforts have been devoted to explore all the possible outcomes related to the development, validation and evaluation of the system. However, there is always a scope for a researcher to proceed further. The future aspects of the study involves: Ex vivo study of the nanosuspension to treat diabetes. With the advancement and researches carried, nanosuspension has emerged as a potential delivery system to increase the solubility and bioavailability of pharmaceutical dosage form.

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