IJRAR.ORG E-ISSN: 2348-1269, P-ISSN: 2349-5138

INTERNATIONAL JOURNAL OF RESEARCH AND ANALYTICAL REVIEWS (IJRAR) | IJRAR.ORG

An International Open Access, Peer-reviewed, Refereed Journal

DEVELOPMENT AND CHARACTERIZATION OF GLIMEPIRIDE LOADED MUCOADHESIVE NANOPARTICLES

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ABSTRACT

A metabolic condition known as type 2 diabetes mellitus (T2DM) causes hyperglycemia, polyuria, glycosuria, polyphasia, polydipsia, and weight loss. Because the body gets tolerant to insulin in T2DM and does not utilise blood glucose for energy generation, the blood glucose level rises and the body's sensitivity to insulin is diminished, even with normal pancreatic beta cell secretion. If left untreated, this illness might have major side effects such cardiopathy, neuropathy, nephropathy, and retinopathy.

and retinopathy.

The development of efficient medication delivery systems for the treatment of type 2 diabetes is now the focus of many researchers. Sulfonylureas are a family of medications that are frequently used to treat type 2 diabetes mellitus and regulate the patient's blood glucose levels. In general, third generation sulfonylurea conventional dosage forms like glimepiride have many disadvantages including short biological half life, low gastric residence time (high transit rate), and low aqueous solubility (BCS Class: II).

These drawbacks lead to frequent dosing, fluctuation in drug plasma concentration, low bioavailability, and patient noncompliance. It was necessary to create a new controlled release formulation that may provide additional important advantages in order to get rid of these drawbacks. Therefore, mucoadhesive nanoparticles containing glimepiride were produced in the current study for successful oral delivery.

The mucoadhesive property of the prepared mucoadhesive drug delivery system may make it superior to polymeric nanoparticles and microspheres. This mucoadhesive property ultimately lengthens the gastric residence time, which improves site-specific drug absorption from the stomach over a prolonged period of time and further increases bioavailability. Due to their large surface area and tiny particle size, mucoadhesive nanoparticles have an advantage over mucoadhesive microspheres in terms of medication solubility and absorption. Additionally, the suggested technique eliminates adverse effects and plasma medication volatility while enhancing patient compliance.

KEYWORDS: Hyperglycemia, Polyuria, Glycosuria, Polyphasia, Polydipsia

INTRODUCTION

1.1 DIABETES MELLITUS

The term diabetes mellitus describes a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia accompanied by disturbances in carbohydrate, lipid, and protein metabolism caused by defects in insulin secretion, insulin action, or both. The consequences of diabetes include long-term damage, dysfunction, and failure of various organs. Diabetes can present characteristic symptoms such as thirst, polyuria, blurred vision, and weight loss.In the most severe form, ketoacidosis or a non-ketotic hyperosmolar state can develop without effective treatment and lead to lethargy, coma, and death.

Often symptoms are not severe or may not be present, so hyperglycemia sufficient to causes pathological and functional changes may appear long before diagnosis. Long-term effects of diabetes include retinopathy that can lead to blindness, kidney disease that can lead to kidney failure, and the progressive development of specific complications of neuropathy with risk of foot ulcers, amputations, Charcot joints, and autonomic dysfunction, including sexual disfunction. Patients with diabetes are at increased risk of cardiovascular, peripheral, and cerebrovascular disease (Alberti et al. 2001).

1.2 TYPES OF DIABETES MELLITUS

1.2.1 Type 1 (beta-cell destruction, usually leading to absolute insulin deficiency)

This kind of diabetes, sometimes referred to as insulin-dependent diabetes or juvenile-onset diabetes, is brought on by an autoimmune reaction that kills the pancreatic beta cells. The pace of annihilation varies greatly amongst people, being fast in some and sluggish in others. The fast advancing variant can also affect adults, although it is typically seen in youngsters. The slowly progressing variant, which often affects adults, is also known as latent autoimmune diabetes in adults (LADA).Ketoacidosis may be the initial sign of the illness in some people, especially children and teenagers. Others have mild fasting hyperglycemia that, in the context of an illness or other stressors, can quickly progress to severe hyperglycemia and ketoacidosis.People with this kind of Type 1 diabetes frequently become dependent on insulin for survival and run the danger of developing ketoacidosis (WHO 1999).

1.2.2 Type 2 (predominantly insulin resistance with relative insulin deficiency)

This kind of diabetes mellitus is often referred to as adult-onset diabetes or non-insulin-dependent diabetes.It is a label used to those who have a relative (as opposed to an absolute) insulin insufficiency. The action of insulin is commonly resistant in those with this kind of diabetes.These people do not require insulin therapy to live, at least initially and frequently during their lifespan.Because the hyperglycemia is usually not severe enough to generate observable signs of diabetes, this kind of diabetes commonly goes untreated for many years.Typically, such individuals are more likely to experience macrovascular and microvascular problems (Kaul et al. 2014).

The majority of patients with this form of diabetes are obese, and obesity itself causes or aggravates insulin resistance. Although individuals with this kind of diabetes may have insulin levels that look normal or increased, if their beta-cell activity were normal, it would be predicted that the high blood glucose levels in these diabetic patients would cause even higher insulin values.. Women with a history of GDM as well as people with hypertension or dyslipidemia are more likely to experience it. Its prevalence varies among racial and ethnic categories.It is frequently linked to a significant familial propensity that is probably hereditary. The genetics of this kind of diabetes, however, are complicated and little understood (WHO 1999).

1.2.3 Gestational diabetes mellitus (GDM)

GDM is a disorder where pregnant women who have never been diagnosed with diabetes have excessive blood glucose (blood sugar) levels (especially during their third trimester).Insufficient insulin receptor activity leads to gestational diabetes. Pregnancy-related variables, such as the presence of human placental lactogen, which interacts with insulin receptors that are sensitive, are probably to blame for this. In turn, this results in unjustifiably increased blood sugar levels (Brody et al. 2003).

Table 1: Comparison between type 1 and type 2 diabetes

Feature	**Type 1 diabetes**	**Type 2 diabetes**
Onset	Sudden	Gradual
Age at onset	Mostly in children	Mostly in adult
Body habit	Thin or normal	Often obsesses
Keto-acidosis	Common	Rare
Auto antibodies	Usually present	Absent
Endogenous insulin	Low or absent	Normal, decreased or increased
Prevalence	**~10%**	**~90%**

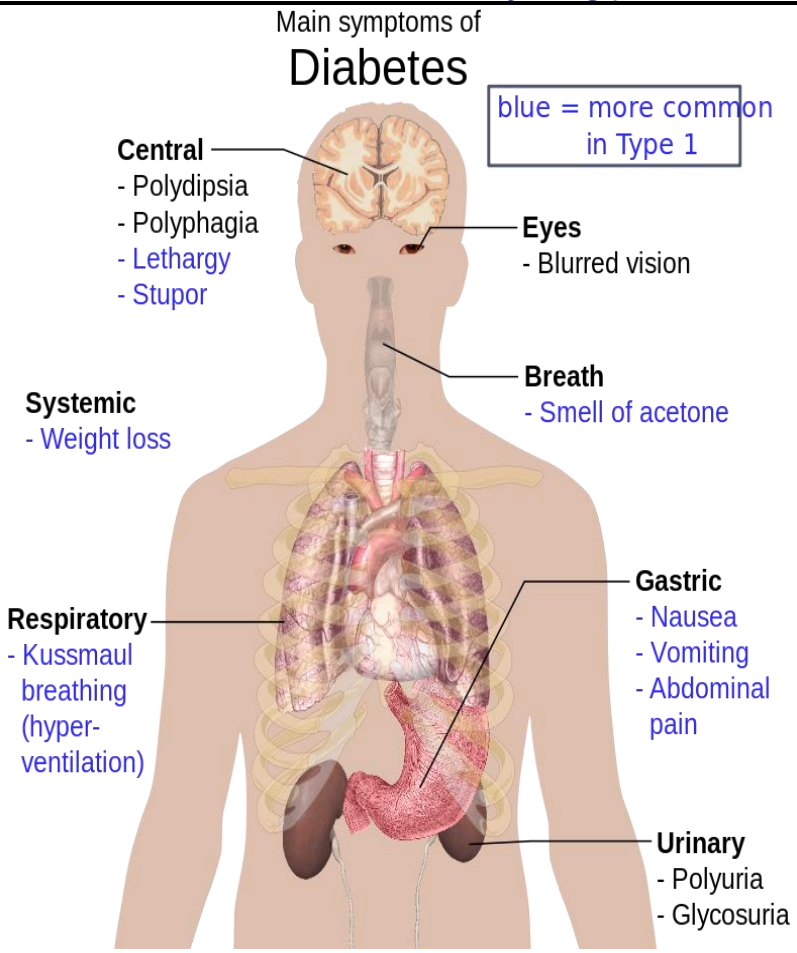


Figure 1: Symptoms of diabetes

1.4.1 Genetic Susceptibility

Type 2 diabetes susceptibility is greatly influenced by genes. A person's chance for acquiring the illness may be increased or decreased by having particular genes or gene combinations.The high incidence of type 2 diabetes in families, identical twins, and the significant differences in diabetes prevalence by ethnicity point to the potential influence of genes.Variants of the TCF7L2 gene have been linked to an increased risk of type 2 diabetes, according to studies.The risk of getting type 2 diabetes is around 80% higher for those who inherit two copies of the variations than for those who do not.

1.4.2 Obesity and Physical Inactivity:

Obesity and physical inactivity are highly correlated with the emergence of type 2 diabetes.When these risk factors are present, those who are genetically predisposed to type 2 diabetes are more vulnerable.

1.4.3 Insulin Resistance:

People who are overweight or obese, have considerable abdominal fat, and are not physically active frequently develop insulin resistance.Cells in the muscles, fat, and liver stop reacting to insulin as they should, causing the pancreas to overproduce insulin as a means of restitution.

1.4.4 MEDICATION-INDUCED DIABETES:

Numerous medicinal drugs may worsen glucose control if given to people who already have diabetes because they may predispose to or trigger the disease, especially when preexisting risk factors are present.They might do this via raising insulin resistance, interfering with insulin secretion, or both.These medicines can be classified into two categories for ease of use: those used often that are weakly diabetogenic, and those used for specific purposes that are more severely diabetogenic.These medicines can be classified into two categories for ease of use: those used often that are weakly diabetogenic, and those used for specific purposes that are more severely diabetogenic.

1.5 DIABETES COMPLICATIONS

1.5.1 Macrovascular disorder:A 2 to 4 times increased risk of stroke (cerebral), coronary heart disease (CHD), and peripheral vascular disease, which can result in ulceration, gangrene, and lower extremity amputations, may be caused by macrovascular problems that damage the big arteries of the circulatory system.These macrovascular consequences include the migration of leukocytes to the site of arterial damage and are basically accelerated types of atherosclerosis (Klien R. 1998).

1.5.2 Microvascular disorder In addition to contributing to diabetic neuropathy (nerve damage), nephropathy (kidney disease), retinopathy (eye disease), and foot difficulties (sores and blisters on the feet), microvascular complications also cause damage to the tiny blood vessels (Klien R. 1998).

1.6 PATHOPHYSIOLOGY OF TYPE 2 DIABETES

1.6.1 Insulin resistance: Food is broken down into its simple elements during digestion. Simple sugars, principally glucose, are formed when carbohydrates are broken down. For the body's cells, glucose is a vital source of energy. Glucose must go from the blood and enter the cells in order to provide them energy.The pancreas produces the hormone insulin, which tells cells to take up glucose as it travels through the blood.The pancreas generates more insulin when blood glucose levels increase (for as following a meal).

Figure 2: Showing Insulin Resistance

When the body's cells do not respond to insulin effectively, type 2 diabetes develops.Insulin resistance is what causes this. The amount of glucose that the cells take in from the blood is less than it should be. The actions of insulin are resisted by the cells.As a result, blood glucose levels start to rise. The pancreas "sees" the blood glucose level growing in persons with insulin resistance.In response, the pancreas produces more insulin to keep blood sugar levels normal (Stumvoll et al. 2005).

1.7 DIAGNOSIS

Blood sugar levels are tested to determine diabetes. After fasting all night, blood is checked in the morning.Even after a fast, the body typically maintains blood sugar levels between 70 and 110 milligrammes per deciliter (mg/dL). Diabetes is identified if a fasting blood sugar level exceeds 125 mg/dL.

Hemoglobin A1C (HbA1c) test: The A1C test, commonly known as the haemoglobin A1C test or glycohemoglobin test, is a blood test that gauges the typical blood glucose level during the previous three months.

- **Lipid profile:** evaluates triglyceride, total, HDL, and LDL cholesterol levels. By doing so, the risk of atherosclerosis is assessed.

1.8 MANAGEMENT OF TYPE 2 DIABETES

The four major groups of anti-diabetic agents are:

a) Biguanides, such as metformin, may inhibit liver gluconeogenesis Insulin secretagogues, such as sulfonylureas, are medications that encourage the pancreas to produce insulin (Glipizide, glibenclamide and glimepiride),

b) Thiazolidinediones are insulin sensitizers that increase the sensitivity of peripheral tissues to insulin (Rosiglitazone and pioglitazone).Insulin analogues (Aspro, lispro and glargin insulin) can provide recombinant insulin, an external source of insulin. In extreme situations, these medications are combined to improve blood glucose regulation (Kaul et al. 2014).

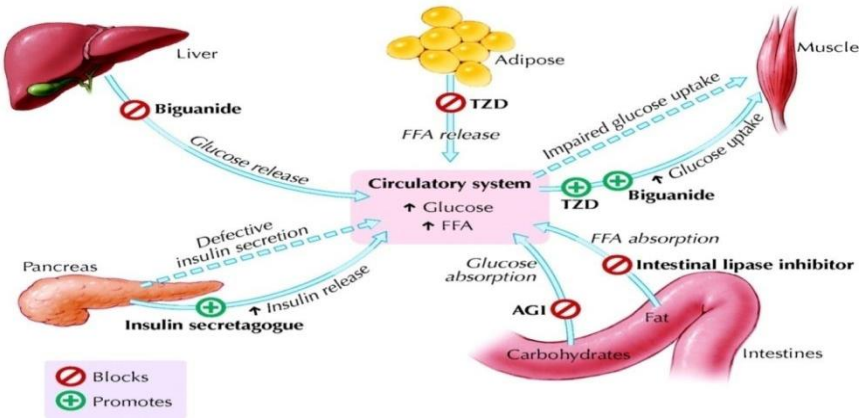


Figure 3: Different hypoglycemic agent with their mechanism of action (FFA- Free fatty acid, TZD-Thiazolidinedione, AGI- Alfa glucose inhibitor).

1.9 FUTURE OF DRUGS AND THERAPIES FOR TYPE 2 DIABETES

Exenatide, Liraglutide, and Albiglutide, which are injectable glucagon-like peptide 1 (GLP 1) agonists, and oral dipeptidyl peptidase 4 (DPP 4) inhibitors are some of the novel anti-diabetic medications that function on the incretin system (Alogliptin, Linagliptin, Saxagliptin, Sitagliptin, Vildagliptin).Potentially stimulating insulin secretion are GLP-1 agonists. The GLP 1 incretin hormone is elevated as a result of the DPP 4 inhibitors' inhibition of the DPP 4 enzyme.Additionally, drugs like Dapagliflozin, Remogliflozin, and Sergliflozin that limit sodium glucose transport protein 2 (SGLT 2) absorption through the SGLT 2 transporter are being created (Kaul et al. 2014).

1.10 INTRODUCTION OF MUCOADHESIVE NANOPARTICLES

Solid, submicron-sized (10–1000 m), potentially biodegradable drug carriers are referred to as nanoparticles (Couvreur et al. 1995). Both nanospheres and nanocapsules are referred to as nanoparticles together. Nanospheres have a structure like a matrix.Drugs may be contained within the particle or absorbed onto the surface of the sphere.Drugs are contained within vesicular structures called nanocapsules, which have a cavity made of

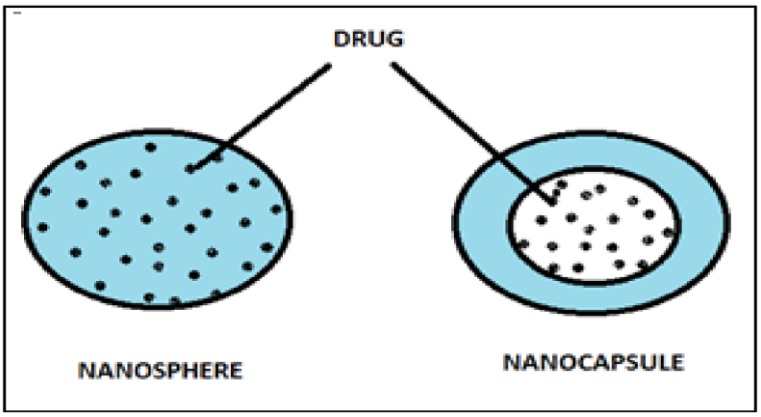


Figure 4: Difference between the nanosphere and nanocapsule

In nanocapsule inner liquid core surrounded by a polymeric membrane. Nanoparticles of a submicron size provide a variety of benefits over microparticles, including a considerably greater intracellular absorption rate.In terms of intestinal uptake, in addition to their particle size, the nature of the nanoparticles and their charge characteristics appear to have an impact on the uptake by intestinal epithelia.More hydrophilic particles may be quickly removed because uptake of nanoparticles made from hydrophobic polymers appears to be greater than that of particles with more hydrophilic surfaces.As opposed to this, positively charged, hydrophilic nanoparticles exhibit a considerable increase in bioadhesive qualities and are absorbed by both M cells and absorptive enterocytes.The gastrointestinal uptake appears to be positively impacted by the interaction of enhanced hydrophilicity of the matrix material and nanoparticle surface charges (Nagavarma et al. 2013).

Mucoadhesive nanoparticles (MNPs) are tiny particles comprised of polymers that attach to mucosal tissue and release drugs locally as well as systemically over an extended period of time (Jangey et al. 2014).

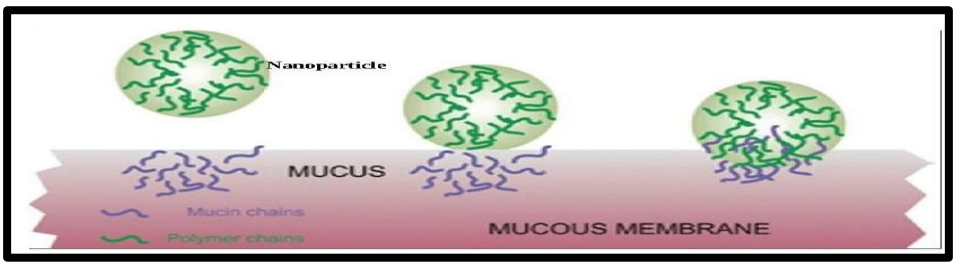


Figure 5: Showing mucoadhesion of nanoparticles

By keeping the drug concentration between the effective and harmful levels, preventing the drug from diluting into bodily fluids, and enabling the targeting and localization of a medication at a specific region, mucoadhesive controlled release devices can increase the efficacy of a medicine.Additionally, mucoadhesion lengthens and improves the closeness of contact between a drug-containing polymer and a mucosal surface. Because of the direct drug absorption and the reduced excretion rate brought on by the longer residence period, the medication's bioavailability can be improved while requiring a lower dosage and less frequent administration (Vyas et al. 2002Any drug delivery system's major objective is to obtain a target drug concentration in blood or tissue that is therapeutically efficacious and non-toxic for an extended length of time.The two fundamental components of medication delivery—spatial placement and temporal delivery—are where the target is pointing.Targeting a medicine to a particular organ or tissue is known as spatial placement, whereas regulating the pace of drug delivery to that organ or tissue is known as temporal delivery.**(Patil et al. 2011).**Due to its simplicity of administration, patient compliance, and adaptability in formulation, oral medication delivery is by far the preferred method of drug delivery (Dhole et al. 2011).

Over time, oral medication delivery methods have advanced from instantaneous release to site-specific delivery.Every patient aspires to the perfect medication delivery system, which must have two essential characteristics: a dosage form that releases the active ingredient directly at the site of action, and a single dose or less frequent dosing during the course of therapy. The most popular and frequent route for medication administration is through the gastrointestinal tract (GIT).The comparatively high volume of fluid present, the peristaltic motions of the stomach and intestines, the substantial blood flow through the mesenteric circulation, the vast mucosal region across which absorption can occur, and these physiological characteristics of the GIT all encourage absorption. Drugs with short half-lives and easy GIT absorption are removed from the systemic circulation swiftly. These medications must be dosed often to get the desired therapeutic effect.The development of oral sustained controlled release formulations is an effort to bypass this restriction by slowly releasing the medication into the GIT and maintaining an effective drug concentration in the systemic circulation for an extended period of time. After oral administration, such a drug delivery would be retained

in the stomach and release the drug in a controlled manner, so that the drug could be supplied continuously to its absorption sites in the GIT **(Jangey et al. 2014).**

1.10.1 Classification of mucoadhesive polymer

On the basis of formulation there are mainly two types, natural and synthetic type polymer.

Table 2: Classification of mucoadhesive polymer

Natural	Synthetic
Alginate, chitosan, dextran, collagen, gelatin, albumin, cyclodextrin, hyaluronic acid	Poly lactic acid, polyglycolic acid, poly hydroxybutyrate, poly(lactide-co-glycolide), poly(e-caprolactic acid), poly dioxane, poly amide, polyhydrides

1.10.2 Mechanisms of drug release from MNPs (Ghosh PK. 2000)

Any one of the three common physico-chemical methods is used by the polymeric drug carriers to deliver the drug to the tissue location.

1. through hydration-induced swelling of the polymer nanoparticles, followed by release by diffusion.

2. By an enzymatic process that causes the polymer at the site of delivery to break down or cleave, releasing the medication from the inner core that had been imprisoned there.

3. Drug de-adsorption/release from the swollen nanoparticles and dissociation of the drug from the polymer.

1.11 Types of nanoparticles

1.11.1 Inorganic nanoparticles

In the field of Modern material science Inorganic nanoparticle has been developed the role based upon their unique physical properties and particularly in biotechnology. Based upon these two factors of inorganic nanoparticles they have certain physical properties that mainly include size-dependent optical, magnetic, electronic, and catalytic properties. Bio related application are involved for the preparation of these interesting nanoparticles like iron oxides, gold, silver, silica, quantum dots etc (Ladj*etal.*, 2013). Novel physical properties mainlyrelated because of their size approaches nanometer scale dimension (Mark Asta*etal.*, 2007).

1.11.2 Polymeric nanoparticles

Polymeric nanoparticles are also a type of nanoparticle. In recent year polymeric nanoparticles have had a tremendous development in the field of research. The dispersion of preformed polymers and the polymerization of monomers are two strong strategies mainly involved for preparation (Prasad Rao and Kurt Geckeler, 2011). 10 to 1000nm is the range of size involved with solid particles.

1.11.3 Solid lipid nanoparticles

For controlling the drug delivery in the 1990s Solid lipid nanoparticles played a dominant role. There are certain alternate carrier systems to emulsions, liposomes and polymeric nanoparticles as a colloidal Carrier system (Abhilash, 2010).

1.11.4 Liposomes

Liposomes are one of the methods based upon the different types of nanoparticles. Structure of liposomes consists of one or more phospholipid bilayers and they are sphere-shaped vesicles to carry compound of interest. Today liposomes have been useful in the field of reagent and tool in various scientific disciplines. Since many features involved in liposome they made their own way in the market. Cosmetic andpharmaceutical industries numerous molecules act as a carrier, and in the field of Food and farming industries liposomes involved in encapsulation to grow delivery system that can entrap unstable compounds (AbolfazlAkbarzadeh*et al.*, 2013).

1.11.5 Nanocrystal

A nanocrystal is a type based upon material particle having at least one dimension smaller than 100 nanometres and mainly composed of atoms in either a single or poly-crystalline arrangement (Jens-Uwe *etal.*, 2008). Nanocrystals are aggregates ofaround hundreds or thousands of molecules that combine in a crystalline form, composed of pure drug with only a thin coating comprised of surfactant or combination of surfactants.

1.11.6 Nanotube

A nanotube is a nanometer scale tube likestructure. Nanotubes are members of the fullerene structural family. Their name is derived from their long, hollow structure with the walls formed by one-atom-thick sheets of carbon called graphene. These sheets are rolled at specific and discrete ("chiral") angles and the combination of the rolling angle and radius decides the nanotube properties; for example, whether the individual nanotube shell is a metal or semiconductor. Nanotubes are categorized as single-walled nanotubes (SWNTs) and multi-walled nanotubes.

1.11.7 Dendrimers

Dendrimers arise from two Greek words: Dendron meaning tree and Meros meaning part.Structure of dendrimers has a well-defined size, shape and defined molecular weight and also Dendrimers are hyper-branched, globular, monodisperse, three dimensional nanoscales synthetic Polymers. Molecular chemistry and

polymer chemistry both exhibit well-defined characteristics features of Dendrites (Anirudha Malik *et al.*, 2012).

1.12 Strategies used to synthesize nanoparticles

Traditionally nanoparticles were produced only by physical and chemical methods. Some of the commonly used physical and chemical methods are ion sputtering, solvothermal synthesis, and sol gel technique. Basically there are two approaches for nanoparticle synthesis namely the Bottom up approach and the Top down approach.

In the Top down approach, scientists try to formulate nanoparticles using larger ones to direct their assembly. The Bottom up approach is a process that builds towards larger and more complex systems by startingat the molecular level and maintaining precise control ofMolecular structure (Prathna*et al.*, 2010).

In Top down process bulk material is converted to fine particle in Bottom up process atom is processed to nuclei and finally to nanoparticles these are the process employed for the synthesis of nanoparticles.

1.13 Nanoparticle synthesis- Physical and chemical methods

1.13.1 Sol-gel technique: In Sol-gel techniquediscrete particles are integrated network precursor involved in chemical solution that mainly used for the fabrication of metal oxides hence it is a chemical technique. The precursor sol can be either deposited on the substrate to form a film or used to synthesize powders.

1.13.2 Solvothermal synthesis: In Solovothermalsynthesis process the polar solvents are involved in different condition like at temperatures above their boiling points and in the condition of under pressure at versatile low temperature. Hence the reaction does not involve in lower temperature because the solubility of reaction get significantly increases in Solovothermal condition.

1.13.3 Chemical reduction: Sodium borohydride,hydrazine hydrate and sodium citrate are some of the commonly used reducing agents in which the ionic salts get involved in reduction process by an appropriate medium in the presence of surfactant were involved using reducing agents are used.

1.13.4 Laser ablation: The laser ablation laserbeam is a technique that used for removing materials from a solid surface. Absorbed laser energy and evaporates mainly involves when the material is heated at low laser flux.The material is converted to plasma in case of higher flux. For example Carbon nanotubes can be produced by this method.

Inert gas condensation: In inert gas condensation there is an ultra-high vacuum chamber filled with helium or argon gas at typical pressure of few 100 Pascal s where different metals are evaporated in separate crucibles inside. As a result of inter atomic collisions with gas atoms in chamber, the evaporated metal atoms lose their kinetic energy and condense in the form of small crystals which accumulate on liquid nitrogenfilled cold finger. Example gold nanoparticles have been synthesized from gold wires. Synthesis using bio organisms is

compatible with the green chemistry principles. Environmental friendly, non-toxic and safe reagents are mainly involved in green synthesis of nanoparticle.

1.14 METHOD FOR MAKING MUCOADHESIVE NANOPARTICLS (Nagavarma et al. 2012)

1.14.1Dispersion of premade polymer nanoparticle preparation techniques

a) Solvent evaporation

b) Nanoprecipitation

c) Emulsification/solvent diffusion

d) Salting out

e) Dialysis

f) Supercritical fluid technology (SCF)

1.14.2 Methods for preparation of nanoparticles from polymerization of monomers

a) Emulsion

b) Mini emulsion

c) Micro emulsion

d) Interfacial polymerization

e) Controlled/Living radical polymerization(C/LRP)

1.14.3 Ionic gelation method

The use of biodegradable hydrophilic polymers like gelatin, sodium alginate, and chitosan is one of the well-known methods for creating polymeric nanoparticles.Ionic gelation is a technique that Calvo and colleagues used to create hydrophilic chitosan nanoparticles. **Dexamethasone Sodium Phosphate-loaded chitosan nanoparticles were reported to have been made using the ionic gelation process by Dustgani et al. in 2008.**In this method, two aqueous phases are combined, one of which is the polymer chitosan, which is a di-block co-polymer of ethylene oxide or propylene oxide (PEO-PPO), and the other of which is a poly anion sodium tripolyphosphate.In this method, the positively charged amino group of chitosan interacts with the negatively charged tripolyphosphate poly anion to generate coacervates with a nanometer size range.Coacervates are created when two aqueous phases contact electrostatically, but ionic gelation includes a substance changing from a liquid to a gel under circumstances of ionic interaction that occur at ambient temperature (Nagavarma et al. 2012).

2.1 REVIEW OF LITERATURE

Author's Name	Brief about work done	Year
Parasuram RR *et. al.,*	formulated a nanoemulsions based emulgel with flurbiprofen and concentrations were optimized after the evaluation of the in vitro studies. They are in-vivo studies of emulgel are compared to the marketed gel, which were revealed into a significant increase in the skin penetration of profile and the anti-inflammatory effect.	**W J Pharma & Pharm Sci (2016)**
Harsha P *et. al.,*	Reported using the Büchi Nano Spray Dryer B-90 to create mucoadhesive nanospheres, and due to their advantageous narrow particle-size distribution, these nanoparticles were deemed appropriate for oral administration. Aminated gelatin was responsible for the improved mucoadhesion outcomes. The formed nanospheres may be administered orally, which would minimise frequent dosage intervals and avoid variations of the therapeutic substance. This is suggested by the fact that the nanospheres were maintained to a greater extent both in vitro and in vivo.	*Int J Pharm Sci a & Res* *2015*
Dwivedi *et. al.,*	**F**ocused on the preparation of nanoemulsions loaded with the arteether for its improved efficacy against malaria parasite. Arteether nanoemulsions was prepared by using high pressure homogenization technique with the aim of improving its solubility and bioavailability .The pharmacokinetic study was showed significantly enhanced bioavailability and high antimalarial efficacy. It can be a promising oral delivery system for the arteether.	**Int J Tech Sci & Res (2015**)

Emani *et. al.*,	described the development of glipizide controlled release alginate-chitosan nanoparticles, their characterisation, and their optimization. Ionotropic controlled gelation was used to create the nanoparticles. They developed a novel controlled-release, bioadhesive, nontoxic, biocompatible, and biodegradable particle technology	*Ino Amr.J Pharm res 2014*
gaur S *et. al.*,	reported nanoemulsions served as the better contrivance to provide better traversing of the drug through skin layer due to a very small size the incorporating even hydrophobic drug and the conversion into gel provides sustained effect to overcome the shorter half-life of the drug.	Indo Glo J og Sci & Res **(2014)**
Mokale *et. al.*,	reported employing a high pressure homogenizer to evaporatively prepare and characterise biodegradable glimepiride-loaded PLA nanoparticles. An o/w solvent evaporation technique was used to successfully create glimepiride-loaded PLA nanoparticles. Drug and polymer interaction was partially demonstrated by DSC and FT-IR research.	*J Pharm & Pharmcol 2014*
Jangdey *et. al.*,	reviewed of the sustained drug delivery method using gastro-retentive mucoadhesive nanoparticles. To boost bioavailability by preventing drug denaturation in the gastro intestinal lumen, they were created as mucoadhesive gastro-retentive nanoparticles.	*Drug Deliv 2014*
Jaiswal *et. al.*,	developed nanoemulsions to control the major problems regarding conventional drug delivery system. They gave a detailed idea about nanoemulsions system which is formed improving were in the delivery of active pharmaceutical ingredient having nanosized particles. The authors focused to give a general thought about its formulation, method of the preparation, characterization techniques parameters and the various applications of nanoemulsions. Nanoemulsions formulation may be the considered as safe and with increase bioavailability.	*Ino Amr .J Pharm res* **(2014)**

Sharma *et. al.*,	carried out studies on preparation and optimization of the nanoemulsions for targeted drug delivery by the presenting some method of preparation such as phase inversion method, high pressure homogenizer, micro fluidization, high amplitude ultrasound where as optimization by held with phase behaviour studies, variation of parameters, experimental designs of the evaluation of nanoemulsions.	J Pharma Sci & Bio **(2013)**
Suria *et. al.*,	developed to a comparative to the vitro release for the diclofenac sodium gel from many different marketed formulation was studied, diclofenac for a NSAIDS frequently prescribed for a long term treatment of rheumatoid arthritis, osteoarthritis and inclosing sodalities in the drug when the substantial first pass effect and are the 50% drug is available systemically. The study were to compare the in – vitro release in the diclofenac sodium from many marketed products are 4 different marketed products 1, 2, 3, and 4 are studied, in their permeation are dialysis membrane.	Indo Glo J of Sci & Res **(2012)**
Alam *et. al.*,	reported nanoemulsions was prepared by aqueous phase titration method and prepared nanoemulsions was subjected to different thermodynamic stability tests and characterized for droplet size, viscosity and to refractive index.	Int J Pharm App Sci & Res **(2012)**
Inderbir singh *et. al.*,	reported the emulsion gelation method by the preparation of domperidone loaded mineral oil entrapped gel buoyant beads of alginate polymer offers flexible, easily to controllable and consistent process for achieving the homogeneity and uniformity of beads formation.	J Pharma Sci & Bio **(2011)**

Chouksey *et. al.*,	Reviewed and summarized to the formation, characterization, properties & applications of the nanoemulsions. They are thermodynamically stable isotropic system in which two immiscible liquids was mixed to a form in a single phase by means of an appropriate surfactant. They show great promising future cosmetics, diagnostic, drug therapies and biotechnologies. They are also used for controlled drug delivery and targeting.	*J Drug Deliv & Therapeutics* **(2011)**
Kharia *et. al.*,	studied the development and assessment of a mucoadhesive nanoparticle formulation of acyclovir for gastro-retentive medication administration	*J Drug Deliv & Therapeutics . 2013*
Bhosal *et. al.*,	reported on the creation and improvement of an acyclovir mucoadhesive nanodrug system. In order to increase mucoadhesiveness, PLGA nanoparticles were produced using a solvent disposal approach and coated with polyacrylic acid.	*Int J Pharm Sci Res 2011*
Makala *et. al.*,	explained how to make gliclazide microspheres that are mucoadhesive using natural gum. They adjusted the drug polymer ratios for better results and employed the ionic orifice gelation and emulsion ionic gelation procedures to create mucoadhesive microparticles.	*Int J Drug Deve & RES 2011*
Gaba *et. al.*,	Gliclazide mucoadhesive microspheres' formulation and evaluation were described. They employed two distinct polymers, HPMC K4M and CMC, and various volumes of glutaraldehyde as a cross-linking agent to apply a straightforward emulsification phase separation technique.	*Int J pharm bio,pharmcy 2011*

Gaba *et. al.*,	For the treatment of type 2 diabetes mellitus, galactomannan-coated mucoadhesive microspheres of glipizide were examined. The produced formulation was evaluated both in vitro and in vivo**.**	*Int J pharm bio,pharmcy 2011*
Dora *et. al.*,	reported on the creation and characterisation of glibenclamide nanoparticles using the solvent displacement approach. To produce a superior release profile suited for oral administration with increased effectiveness, they employed Eudragit L100.	*J Pharm innov 2010*
Shah *et. al.*,	reviewed nanoemulsions as the sub micron sized emulsions for under high investigation for the drug carrier better delivery to the therapeutic agents. Nanoemulsions are thermodynamically stable having droplet size of 20-200nm. In this review, the author focused to provide introduction regarding nanoemulsions formulation aspect,& method of the preparation, characterization techniques to the special emphasis on the various application of nanoemulsions in the different areas that such as in cancer treatment, in the drug targeting, to a vehicle of form to the transdermal drug delivery etc.	Indo Glo J og Sci & Res **(2010)**
Baboota *et. al.*,	reported the nanoemulsion formulation of celecoxib containing 2% (m/m) of CXB, 10%(m/m) for the oil phase (sefsol 218 and Triacetin), 50% (m/m) to the surfactant mixture (Tween 80 & Transcutol-p) and 40% (m/m) for the above of distilled water has been optimized and concluded for the developed nanoemulsion toward include a great potential for transdermal drug delivery	Int J Pharm App Sci & Res **(2007)**

Stagni *et. al.,*	prepared a 0.3% HPMC gel of meloxicam the effect of four different combinations of co-solvents (ethanol, PEG-400, Propylene glycol and water) on MLX permeability were determined in vitro-through isopylene microstate saturated cellulose.	*J Pharm & Pharmcol* **(2007)**
Andes *et. al.,*	proposed the study of pharmacokinetics involves understanding the interaction of drug are the host including measurements of mainly absorption distribution, metabolism & excretion whereas study of pharmacodynamics provide insight into the link between drug pharmacokinetic, in vitro susceptibility and treatment efficacy. This provide an understanding of the relationship between drug dosing and treatment efficacy.	*J Pharm & Pharmcol* **(2006)**
Patel *et. al.,*	research was done on the creation and assessment of mucoadhesive glipizide microspheres. By adopting a straightforward emulsification phase separation approach using glutaraldehyde as a cross-linking agent, they created glipizide-loaded chitosan microspheres. The mucoadhesive microspheres of glipizide showed considerable hypoglycemic efficacy in the in-vivo investigation.	*J Drug Deliv & Therapeutics . 2005*
Kyu *et. al.,*	designing and developed by retinoic gels intended for enhanced transdermal delivery. They studied the release characteristics of drug from carbopol gel are according to temperature, receptors medium and drug concentration. The carbopol gel of retinoic containing and the enhancer could be developed for the enhanced transdermal deliver of drug.	Int J Pharm App Sci & Res **(2005)**

Solan *et. al.*,	reviewed & summarized to the formation properties and also the application of nanoemulsion. Nanoemulsions are also can be formed by the dispersion or high energy emulsification method or to low energy in the emulsification method. Due to their small droplet size nanoemulsion possesses stability against sedimentation or creaming. Nanoemulsion droplets act as nonreactors. Nanoemulsion formulation can be used for controlled drug delivery and targeting.	*Int J Pharm Sci a & Res* **(2005)**
Chowdary *et. al.*,	explored the development of mucoadhesive microcapsules of glipizide for oral controlled release and their in vitro and in vivo evaluation. Alginate and a mucoadhesive polymer were used in the orifice ionic gelation process, and the researchers reported on the effects of various mucoadhesive polymers on drug release and shape, size, and microencapsulation effectiveness.	*J Pharm & Pharmcol* **(2003)**

3.1 RESEARCH ENVISAGED

A metabolic condition known as type 2 diabetes mellitus (T2DM) causes hyperglycemia, polyuria, glycosuria, polyphasia, polydipsia, and weight loss. Because the body gets tolerant to insulin in T2DM and does not utilise blood glucose for energy generation, the blood glucose level rises and the body's sensitivity to insulin is diminished, even with normal pancreatic beta cell secretion. If left untreated, this illness might have major side effects such cardiopathy, neuropathy, nephropathy, and retinopathy.

The development of efficient medication delivery systems for the treatment of type 2 diabetes is now the focus of many researchers. Sulfonylureas are a family of medications that are frequently used to treat type 2 diabetes mellitus and regulate the patient's blood glucose levels. In general, third generation sulfonylurea conventional dosage forms like glimepiride have many disadvantages including short biological half life, low gastric residence time (high transit rate), and low aqueous solubility (BCS Class: II).

These drawbacks lead to frequent dosing, fluctuation in drug plasma concentration, low bioavailability, and patient noncompliance. It was necessary to create a new controlled release formulation that may provide additional important advantages in order to get rid of these drawbacks. Therefore, mucoadhesive nanoparticles containing glimepiride were produced in the current study for successful oral delivery. The mucoadhesive property of the prepared mucoadhesive drug delivery system may make it superior to polymeric nanoparticles and microspheres. This mucoadhesive property ultimately lengthens the gastric residence time, which improves site-specific drug absorption from the stomach over a prolonged period of time and further increases

bioavailability. Due to their large surface area and tiny particle size, mucoadhesive nanoparticles have an advantage over mucoadhesive microspheres in terms of medication solubility and absorption. Additionally, the suggested technique eliminates adverse effects and plasma medication volatility while enhancing patient compliance.

3.2 AIM OF PRESENT DEVELOPMENT

- The main objective was an attempt to design a formulation to improve the oral therapeutic efficacy with optimal control of plasma drug level which contains combination of three antidiabetic drugs containing Glimepiride as a meucoadhesive nano drug delivery, Antidiabetic as the immediate release.
- The rapid introduction of nanotech drug delivery therapy with consist of oral anti-diabetics helps in targeting the effect and also reduced adverse effects.
- Yet another objective of the development is to reduce the dose of active ingredient by extending the drug release over period of time.
- The present development discloses stable pharmaceutical composition of three active ingredients, formulated in a single dosage form providing different release profiles comprising dual release drug absorption system that is DURADAS technology. The composition involved use of a disintegrant which helps in the mucoadhesive nano drug delivery of drug comprising Antidiabetic drug.

3.3 OBJECTIVES OF STUDY

- To formulate the mucoadhesive nano drug deliverycontaining antidiabetic as therapeutic action.
- To study the effect of varying concentration of excipients on formulation characteristic.
- To study the effect of composition of various excipients on mucoadhesive drug delivery.
- To study the effect of temperature and relative humidity on mucoadhesive drug delivery.
- To compare or match the *in-vitro* release mucoadhesive drug deliveryof the formulation with the competitor formulation.
- To provide once a day dosage form for the treatment of diabetes. This implies the formulation of an mucoadhesive release dosage form. Pharmacokinetic profile of glimepiride suggested that it could be formulated as ananotech release part because of its shorter half-life, low volume of distribution and rapid clearance.
- To evaluate the initial dissolution of mucoadhesive polymer and then diffusion of the dissolved
- To evaluate such polymers or combination of polymers in the optimized concentration that would provide maximum release in the small intestine and also within the specified compendial limits;
- To obtain immediate release of glimepiride from the dosage form. The amount of glimepiride released from the dosage form in 1 hour should not be less than 80%. Therefore, the objective was to select such ingredient and diluents which would provide maximum release within 1 hour.

- The process involves reduced manufacturing steps and manufacturing time and finally makes a cost-effective formulation.
- To formulate a physically and chemically stable dosage form.
- Tablets to have satisfactory properties.
- Product to meet with competitor formulation.

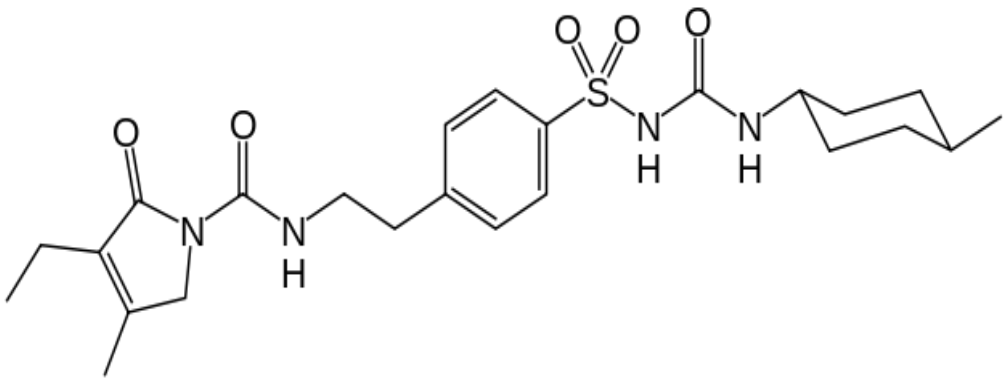
MATERIAL AND METHOD

4.1 DRUG PROFILE

4.1.1 Name: Glimepiride

4.1.2 Synonyms: Amaryl, Glista OD

4.1.3Structure:



4.1.4 IUPAC Name:3-ethyl-4-methyl-*N*-(4-[*N*-((1*r*,4*r*)-4-methylcyclohexylcarbamoyl) sulfamoyl] phenethyl)-2-oxo-2,5-dihydro-1*H*-pyrrole-1- carboxamide

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

4.1.6 Molecular weight: 490.62 gm/mol

4.1.7 Appearance: Powder that ranges in colour from white to yellow.

4.1.8 Melting point:207 °C

4.1.9 BCS Classification: Class 2: High Permeability and Low Solubility

4.1.10 Solubility: Insoluble in water, very soluble in DMSO, and soluble in ethanol.

4.1.11 Pharmacodynamics: In healthy adults, a modest reduction in blood glucose levels first emerged after single oral dosages as low as 0.5–0.6 mg.

It took around two to three hours to attain the minimal blood glucose level and maximal impact.

4.1.12 Mechanism of action: Glimepiride's ability to reduce blood sugar levels appears to depend on its ability to stimulate the production of insulin from active pancreatic beta cells and boost the sensitivity of peripheral tissues to insulin. The pancreatic cell surface has ATP-sensitive potassium channel receptors (SUR-1), which glimepiride is expected to bind to. This decreases potassium conductance and depolarizes the membrane.

Through voltage-sensitive calcium channels, membrane depolarization promotes calcium ion influx. Insulin is secreted as a result of this rise in intracellular calcium ion concentration.Sulfonylureas may potentially lower blood glucagon levels and enhance the effects of insulin on extrapancreatic organs.

4.1.13 Indication and Usage: Glimeperide is recommended as a supplement to diet and exercise for persons with type 2 diabetes mellitus in order to enhance glycemic control.

4.1.14Absorption: Glimepiride is absorbed from the GI tract 90–95 percent after oral dosing.

4.1.15 Metabolism: Following an IV or oral dosage, glimepiride is entirely metabolised via oxidative biotransformation. The cyclohexyl hydroxy methyl derivative (M1) and the carboxyl derivative are the main metabolites (M2). It has been demonstrated that the biotransformation of glimepiride into M1 involves cytochrome P450 2C9. By means of one or more cytosolic enzymes, M1 is further converted to M1.11. not M2, but M1**.15 Volume of distribution:** 8.8 litre (113 ml/kg) The majority (65%) of glimepiride and its metabolites are eliminated in the urine and faeces.

4.1.17 Total body clearance: 47.8 ml/min

4.1.18 Protein binding: 99.5%

4.1.19 Half life: 5 hours

4.1.20 Route of administration: Oral and intravenous.

4.1.21 Toxicity: Glimepiride frequently causes nausea, vomiting, headaches, and dizziness as adverse effects. In addition, allergic reactions and rashes might happen. Sulfonylureas, such as AMARYL, can cause hypoglycemia when used in excess. Without unconsciousness or neurologic signs, mild hypoglycemia symptoms should be actively addressed with oral glucose as well as medication dose and meal timing modifications.

4.1.22 Dosage: For the treatment of diabetes mellitus with AMARYL or any other hypoglycemic medication, there is no set dosing schedule.

4.1.23 Drug interaction: When used with certain drugs, glimepiride's effectiveness to control blood sugar may be diminished. Furosemide, prednisone, and methyl prednisone are corticosteroids in this group, along with phenytoin and somatotropin, which are diuretics. This might lessen the impact of glimepiride and lead to greater blood sugar levels.

Table 3: Optimized formulation ratio

Ingredients	F1	F2	F3	F4	F5	F6
Drug (Glimepride)	20mg	20mg	20mg	20mg	20mg	20mg
PEG 6000	40 mg	40 mg	50 mg	50 mg	60 mg	60 mg
Guar Gum	110mg	100mg	100mg	90 mg	90mg	100mg
Sodium alginate	100 mg	110 mg	100 mg	110 mg	100 mg	90 mg
Water	80 ml	80 ml	80 ml	80 ml	80 ml	80 ml
0.5N NaoH	20 ml	20 ml	20 ml	20 ml	20 ml	20 ml
0.05M $Cacl_2$	50 ml	50 ml	50 ml	50 ml	50 ml	50 ml

4.3 FORMULATION

Pharmaceutical formulation is the process by which several chemicals, including the active ingredient in the medicine, are mixed to create the finished pharmaceutical product. Particle size, polymorphism, pH, and solubility are a few examples of variables that may affect a drug's bioavailability and therefore its action. The medication must be mixed with inert fillers in a way that guarantees a constant level of medication is present in each dosing unit.

Making formulas is a crucial component of producing medications because they guarantee that the drug's active ingredient is delivered to the proper area of the body at the right concentration and pace (not too fast and not too slowly). Drug delivery methods that take use of supersaturation are an excellent example. Additionally, they must have a pleasant flavour (in the case of pills, tablets, or syrups), be stable enough physically and chemically during transportation from the point of manufacturing to the final user, and survive long enough in storage to be safe and effective when used (Simler et al. 2008).

In the study that was presented, the formulation of glimepiride-loaded mucoadhesive nanoparticles for stomach administration to treat type 2 diabetes was the main objective.

4.3.1 Preparation Of mucoadhesive nanoparticles by ionic gelation method

By using a slightly modified version of the ionic gelation approach described by Emami et al. in 2014, glimepiride-loaded mucoadhesive nanoparticles were created. Accurate amounts of PEG 6000 (50 mg), guar gum (100 mg), and sodium alginate (100 mg) were added to a beaker containing 80 ml of distilled water, and the mixture was agitated on a magnetic stirrer at 50–600°C until the polymers were completely dissolved. 20 ml of 0.1 N NaOHsolution were used to dissolve the 20 milligrammes of glimepiride.

The drug-containing solution was then combined with the polymeric solution, and the combination was agitated using a magnetic stirrer until it was homogenous. Using a 23 gauze syringe and a mechanical stirrer, this mixture was then injected dropwise into 50 ml of 0.05 M CaCl2 solution. It was continuously swirled at the same rate for six hours. Microadhesive nanoparticles were extracted from the formulation by mini-ultracentrifugation (SPINWIN) at 10,000 rpm for 45 minutes after it had been sonicated for 30 seconds using a probe sonicator. By using ethanol to wash and centrifuge the formulation three times, it was cleaned. The mucoadhesive nanoparticles that had been collected at the bottom of the centrifuge tube were then transferred to a round-bottom flask and dried by lyophilization.

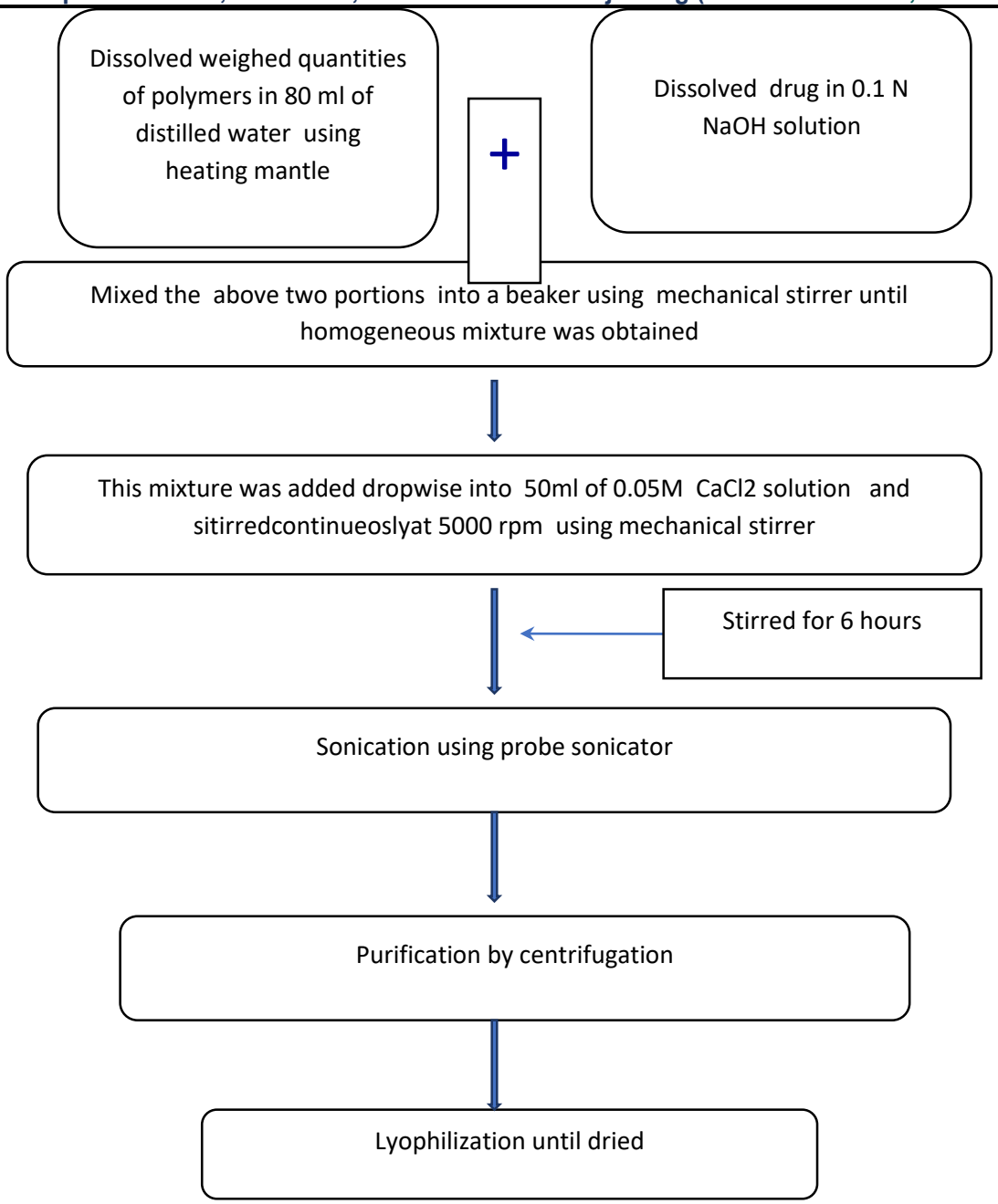


Figure 6: Flow chart for preparation of mucoadhesive nanoparticles

4.4 OPTIMIZATION OF FORMULATION AND PROCESS VARIABLES

Optimization of formulation and process variables for mucoadhesive nanoparticles include:

A. Optimization of sodium alginate and guar gum concentration
B. Optimization of PEG 6000 concentration
C. Optimization of drug concentration
D. Optimization of calcium chloride solution concentration
E. Optimization of stirring speed
F. Optimization of stirring time
G. Optimization of sonication Time
H. Optimizations of formulations were done on the basis of several parameters like Particle size and % Entrapment efficiency

5.1 RESULT

The process of optimising a medicine by the identification or specification of those physical and chemical features thought to be crucial in the creation of a stable, efficient, and secure dosage form is known as preformulation. In order to provide the ideal circumstances for creating a viable drug delivery system, preformulation studies are thus carried out. Preformulation refers to the stage of development that determines the formulation conditions relating to process variation, component selection, and amount. Studies on the preformulation that came after were conducted.

5.1.1 Melting Point

The temperature ranges at which the sample melts were detected after the heating process had begun.

Table 4: Physical Properties of Drug

Physical Property	Reported	Characteristics
Color	White to yellowish white	White
State	Crystalline	Crystalline powder
Odor	Odorless	Odorless
Hygroscopicity	Hygroscopic	Hygroscopic
Melting Point	207^0C	208– 210 ^{0}C

5.1.2 IR Spectroscopic Analysis

IR spectrophotometer was used to get the IR spectra of glimepiride (Bruker, TENSOR- 47).

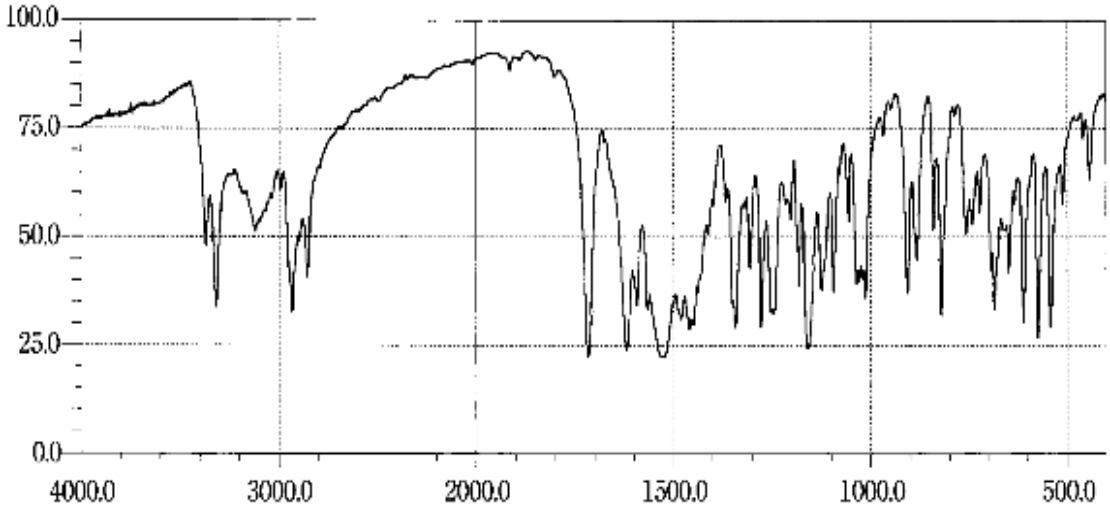


Figure 7: IR spectra of Glimepiride standard

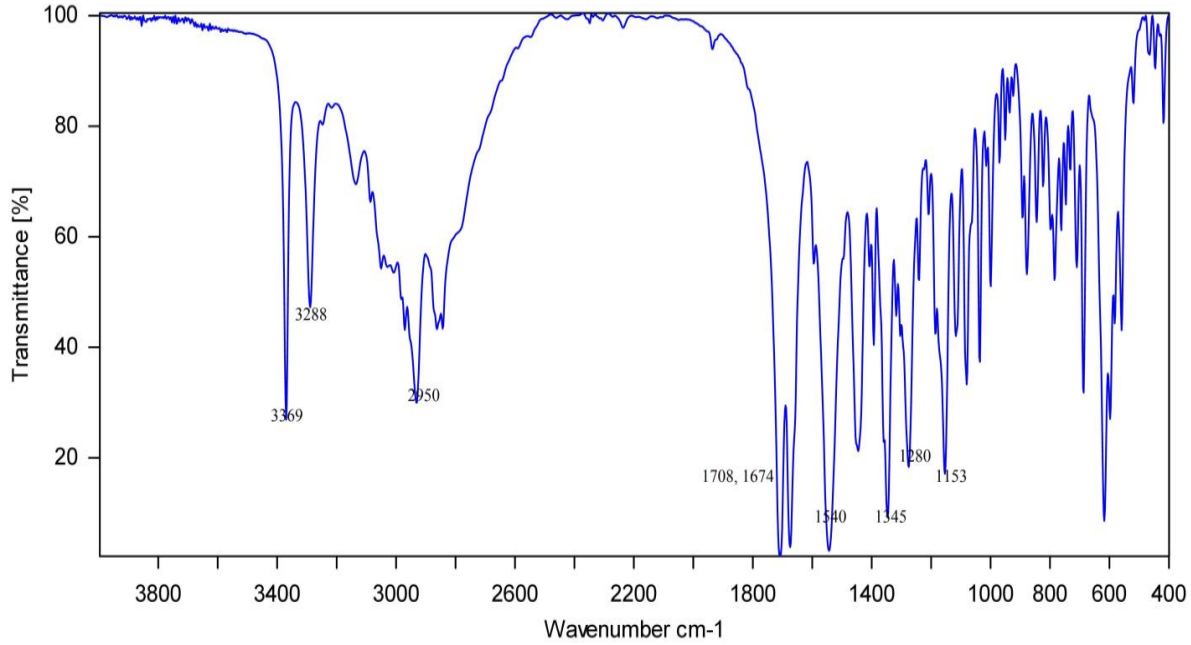


Figure 8: IR spectrum of Glimepiride sample

Table 5: Interpretation of FTIR spectrum

Wave number (cm^{-1})	**Functional group**
3369, 3288	*Amide N-H Stretch*
2950	*Aliphatic C-H Stretch*
1708, 1674	*Amide C=O Stretch*
1673	*Aromatic N-H bend*
1540	*Aromatic C=C stretch*
1345,1153	*S=O Stretch*
1280	*C-N stretch*

5.1.3 Determination of Absorption Maxima

The maximum absorption wavelength, represented by the symbol max, is the range across which more general quantitative findings may be achieved. The 10 g/ml samples of glimepiride in PBS (7.4 pH), ethanol:water, and 0.1N HCl (in 40% ethanol) were each collected in a 1 cm2 standard cuvette and scanned using a spectrophotometer between 200 and 400 nm (UV Mini 1240, Shimadzu). In Figures 3.3 to 3.5, the wavelengths for maximum absorption (max) are identified and displayed, accordingly.

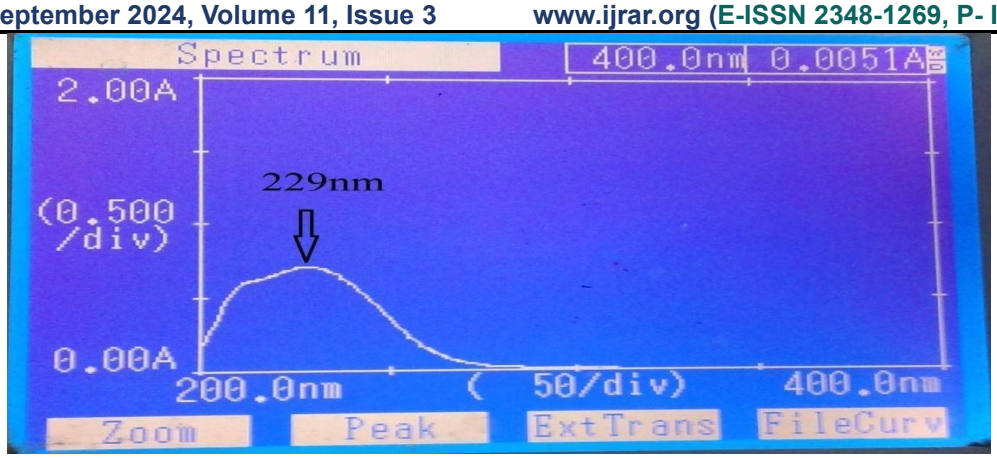


Figure 9: Absorption of Glimepiride maxima in PBS(7.4 pH)

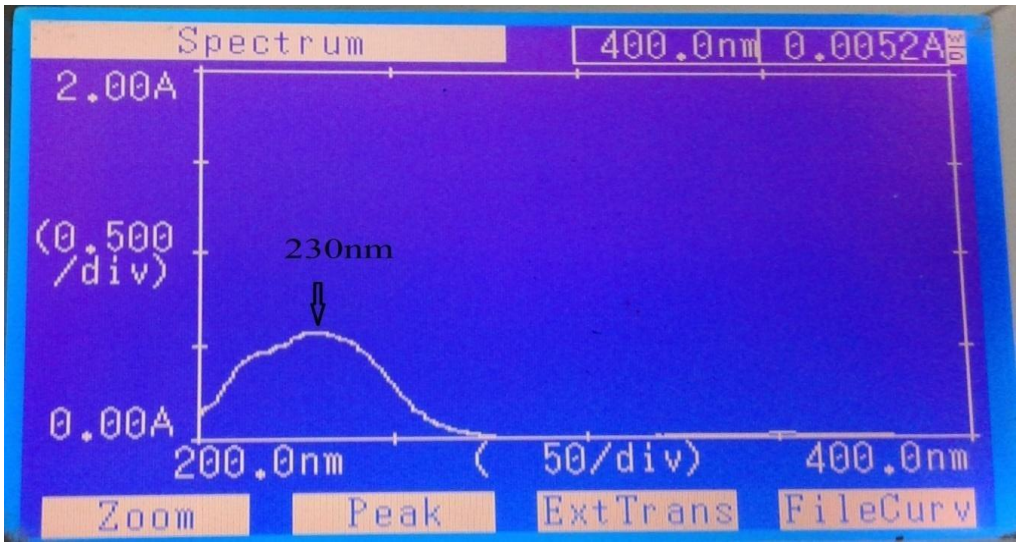


Figure 10: Absorption maxima ofGlimepiridein ethanol:water (1:1)

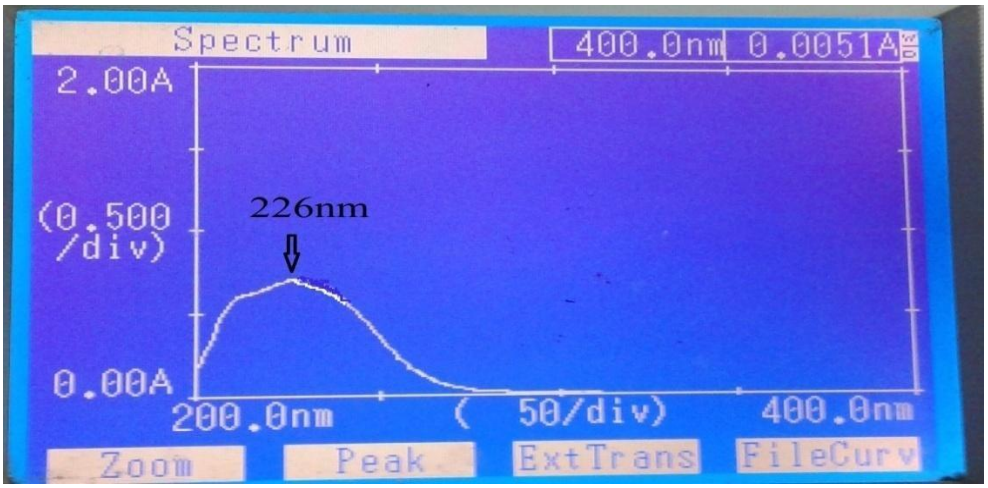


Figure 11: Absorption maxima ofGlimepiride in 0.1N HCl (in 40% ethanol)

5.1.4 Preparation of Standard Curve of Glimepiride in PBS (pH 7.4) at λmax 229nm

A precise weight of 10 mg of glimepiride was used to dissolve it in 30 ml of 0.1N NaOH. Then, PBS was used to dilute the volume to 100 ml, yielding a stock solution containing 100 g/ml. Then, various aliquots of 2, 4, 6, 8, 10, 12, 14 and 16 g/ml were made and placed into volumetric flasks with a 10 ml capacity. PBS (pH 7.4) was used as a blank for measuring the absorbance at 229 nm.

Table 6: Standard curve of Glimepiride in PBS (pH 7.4)

Concentration (µg/ml)	Absorbance	Statistical Parameter
2	0.12	y = 0.093x + 0.020 R^2 = 0.997
4	0.2091	
6	0.2935	
8	0.4025	
10	0.4839	
12	0.5713	
14	0.6562	
16	0.7901	

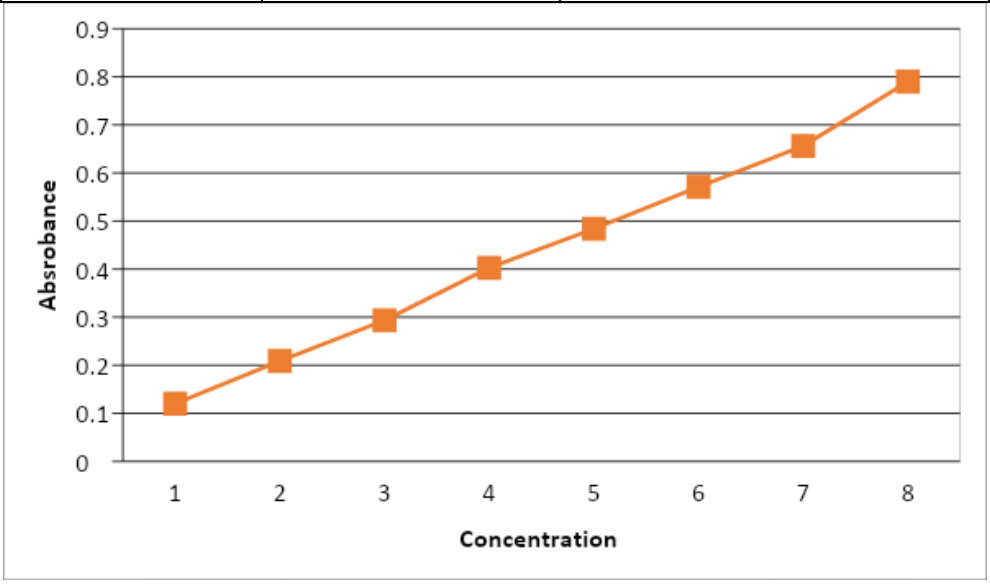


Figure 12: Calibration curve of Glimepiride in PBS (pH 7.4)

5.1.5Preparation of Standard Curve of Glimepiride in ethanol:water(1:1) at λmax 229 nm

A precise weight of 10 mg of glimepiride was used to dissolve it in 30 ml of 0.1N NaOH. Then, a stock solution containing 100 g/ml of ethanol:water was diluted to a volume of 100 ml. Then, various aliquots of 2, 4, 6, 8, 10, 12, 14 and 16 g/ml were made and placed into volumetric flasks with a 10 ml capacity. At 230 nm, the absorbance was measured with ethanol:water used as a blank.

Table 7: Standard curve of Glimepiride in ethanol:water (1:1)

Concentration (µg/ml)	**Absorbance**	**Statistical Parameter**
2	0.12	y = 0.080x + 0.045 R^2 = 0.998
4	0.201	
6	0.2987	
8	0.3773	
10	0.449	
12	0.5195	
14	0.5975	
16	0.6958	

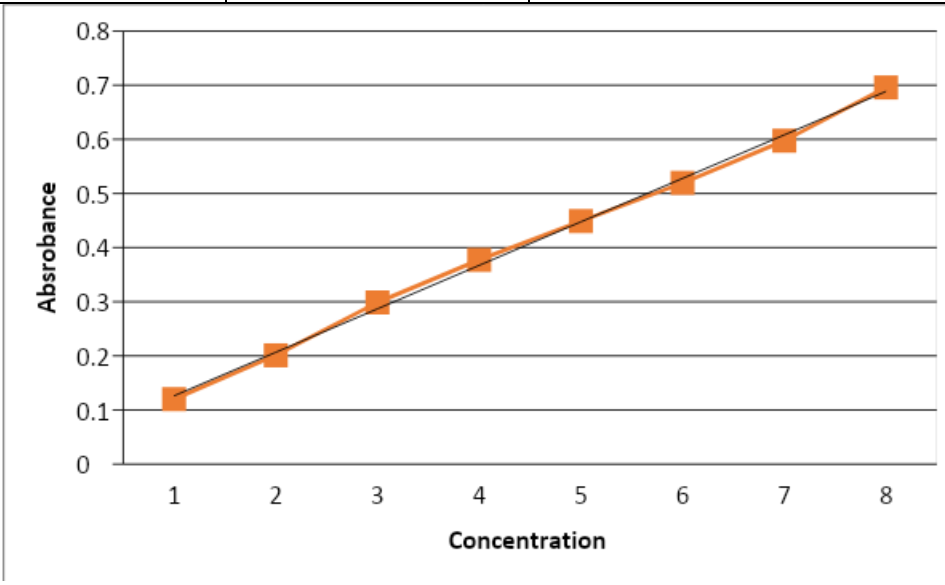


Figure 13: Calibration curve of Glimepiride in ethanol:water (1:1)

5.1.6 Preparation of Standard Curve of Glimepiride in in 0.1N HCl (in 40% ethanol) at λmax 226 nm

A precise weight of 10 mg of glimepiride was used to dissolve it in 30 ml of 0.1N NaOH. Then, 0.1N HCl (in 40% ethanol) was used to dilute the volume to 100 ml, yielding a stock solution containing 100 g/ml. Then, various aliquots of 2, 4, 6, 8, 10, 12, 14 and 16 g/ml were made and placed into volumetric flasks with a 10 ml capacity. A blank solution of ethanol:water was used to test the absorbance at 226 nm.

Table 8: Standard curve of Glimepiride in 0.1N HCl (in 40% ethanol)

Concentration (µg/ml)	**Absorbance**	**Statistical Parameter**
2	0.0152	**y = 0.062x - 0.044** **R^2 = 0.998**
4	0.0806	
6	0.1423	
8	0.2145	
10	0.2717	
12	0.328	
14	0.4031	
16	0.45	

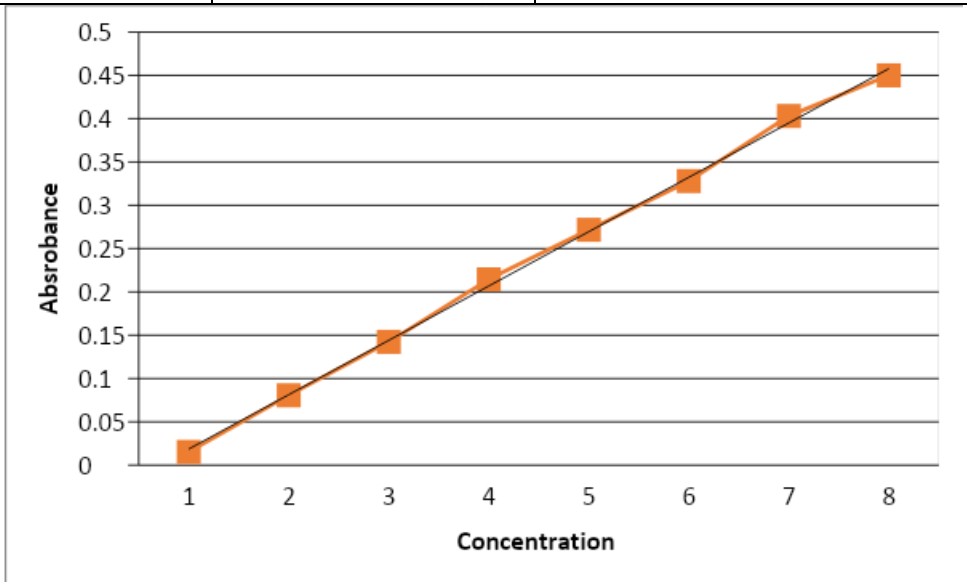


Figure 14: Calibration curve of Glimepiride in 0.1N HCl (in 40% ethanol)

5.2 Solubility Study

5.2.1 Determination of Solubility of Glimipiride

Both quantitative and qualitative investigations on solubility were carried out. To achieve saturation for the quantitative investigation, excess quantities of glimepiride were weighed, suspended in 5 ml of a separate solvent at room temperature in sealed test tubes, and intermittently shaken for 24 hours. Samples were withdrawn for UV analysis at their respective max once saturation was confirmed. Then, as shown in Table 3.6, concentration and the quantity of medication soluble in each solvent were calculated and compared.

Table 9: Solubility study data

Solvents	Solubility
Distilled water	---
PBS (7.4 pH)	+ (2.15 mg/ml)
0.1N NaOH	+++
0.1N HCl (in 40% ethanol)	+ (1.94 mg/ml)
Ethanol	++
Ethanol:water (1:1)	+

+++Soluble = 10-30 parts of solvent

++ Sparingly soluble = 30-100 parts of solvent

+ Slightly soluble = 100-1000 parts of solvent

--- insoluble = >10000 part of solvent

5.2.3 Partition Coefficient(P)

The ratio of a compound's concentrations in a mixture of two immiscible phases at equilibrium is known as the partition coefficient. This ratio consequently represents the variation in the compound's solubility between these two phases. The log P represents the partition coefficient's log value.

$P_{o/w} = [C_o/C_{w.}]$ equilibrium

Where,$P_{O/W}$=Partition coefficient of drug

C_O = Concentration of drug in octanol

C_w= Concentration of drug in water

Accurately weighing five (05 mg) mg of glimepiride, it was then added to a 25 ml separating funnel that contained 10 ml of each of two immiscible phases, n-octanol and aqueous phase (PBS pH 7.4). For 24 hours, the mixture was shaken on a wrist action shaker. After an appropriate dilution, the aqueous phase was divided into the two using a separating funnel, and the quantity of medication in it was then determined (UV-mini 1240shimadzu). It was decided what the concentration was in the aqueous phase.

Table 10: Log partition coefficient (log P)

Solvent system	Reported (log P)	Observed
n-Octanol : PBS (pH 7.4)	2.38	2.41

The identification of Glimepiride was established by all the aforementioned findings.

5.3 CHARACTERIZATION OF OPTIMIZED MUCOADHESIVE NANOPARTICLES

5.3.1 Particle shape and surface morphology

Utilizing a transmission electron microscope, the surface morphology of the produced mucoadhesive nanoparticles was evaluated (TECHNAI G-2S T30). On a glass slide, a drop of mucoadhesive nanoparticles dispersion (in deionized water) was applied. A copper grid was then put over the sample, and the copper grid was kept in place for one minute to allow the MNPs to attach. The surplus solution was then drained off using filter paper after the grid was left on a drop of 1 percent phosphotungstic acid for more than 5 seconds. After allowing the grid to dry naturally, the sample was investigated by a TEM, and pictures were obtained at an appropriate magnification

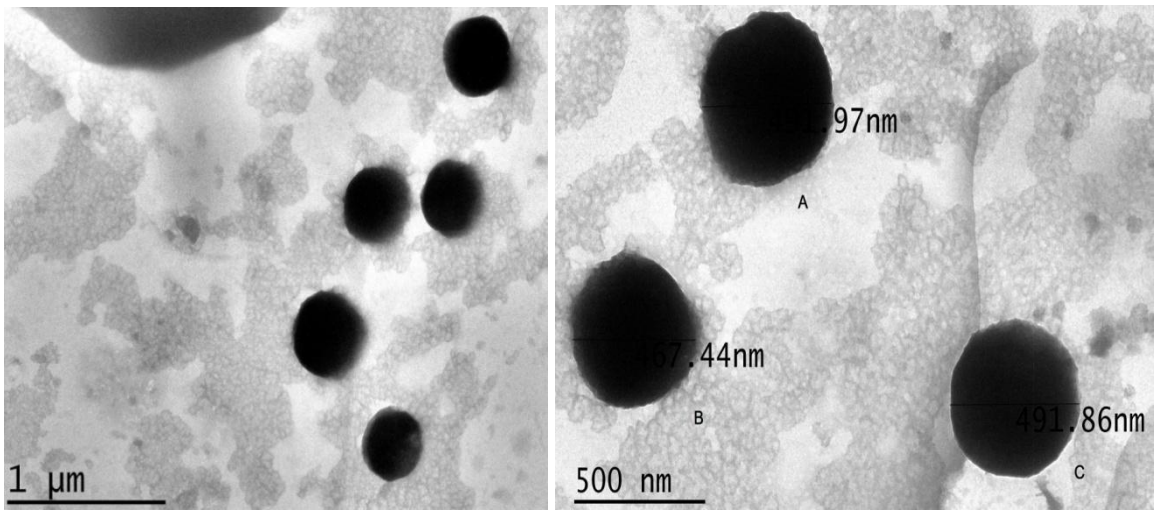


Figure 15: TEM images of mucoadhesive nanoparticles (size of particle A- 481.97nm, B- 467.44nm, and C- 491.86nm)

5.3.2 Particle size, size distribution and zeta potential

The zeta potential, polydispersity index (PI), and z-average diameter of the drug-laced MNPs were measured using the dynamic light scattering technique (Malvern Instruments Ltd). 2 mg of mucoadhesive nanoparticles were dissolved in 10 ml of deionized water in order to evaluate the particle size. The material was poured into the cuvette, and the nanoparticles' sizes were assessed. All measurements were made in triplet at a temperature of 25 °C and were recoded in Table 4.9.

Table 11: Particle Size, PDI, Zeta potential of formulations

Formulation Code	Avg. particle Size (nm)	Polydispersity Index (PDI)	Zeta potential (mV)
$AG_2P_2D_2C_2R_2T_2S_2$	**480.48±3.25**	0.230	-24.8

Mean± SD (n=3)

Size Distribution Report by Intensity

v2.1

Malvern

Sample Details

Sample Name: F-7 S Size 1
SOP Name: mansettings.nano
General Notes:

File Name: F-7 S Size.dts
Record Number: 1
Material RI: 1.59
Material Absorbtion: 0.010
Dispersant Name: Water
Dispersant RI: 1.330
Viscosity (cP): 0.8872
Measurement Date and Time: Saturday, May 08, 2021 3:09:4...

System

Temperature (°C): 25.0
Count Rate (kcps): 140.3
Cell Description: Disposable sizing cuvette
Duration Used (s): 60
Measurement Position (mm): 4.65
Attenuator: 7

Results

		Size (d.nm):	% Intensity	Width (d.nm):
Z-Average (d.nm): 482.5	Peak 1:	482.5	100	22.26
PdI: 0.230	Peak 2:	0.000	0.0	0.000
Intercept: 0.898	Peak 3:	0.000	0.0	0.000

Result quality : Good

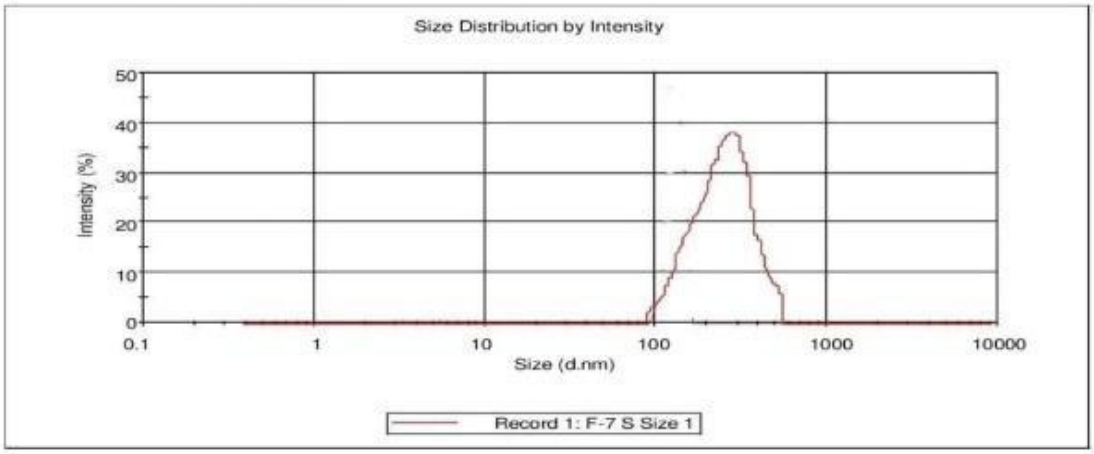


Figure 16: Particle size distribution of MNPs

Zeta Potential Report

v2.2

Malvern

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Sample Details

Sample Name: F-7 S Zeta 1
SOP Name: mansettings.nano
General Notes:

File Name: F-7 S Zeta.dts
Record Number: 1
Date and Time: Friday, May 07, 2021 2:11:5...
Dispersant Name: Water
Dispersant RI: 1.330
Viscosity (cP): 0.8872
Dispersant Dielectric Constant: 78.5

System

Temperature (°C): 25.0
Count Rate (kcps): 337.8
Cell Description: Clear disposable zeta cell
Zeta Runs: 12
Measurement Position (mm): 2.00
Attenuator: 7

Results

		Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -24.8	**Peak 1:**	-24.8	100.0	3.70
Zeta Deviation (mV): 3.70	**Peak 2:**	0.00	0.0	0.00
Conductivity (mS/cm): 0.393	**Peak 3:**	0.00	0.0	0.00

Result quality : Good

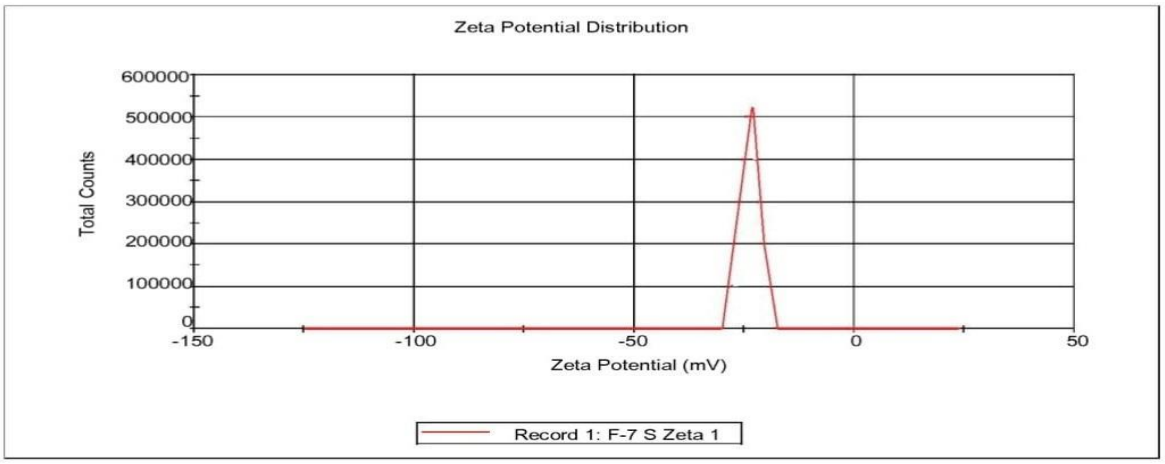

Figure 17: Zeta potential distribution of MNPs

5.4 In-Vitro Drug Release Study

Drug development experts now identify in-vitro dissolution as a crucial component. It is possible to utilise it as a stand-in for the evaluation of bioequivalence under specific circumstances. Dissolution of drugs from immediate-release and modified-release dose forms is described by a number of theories and kinetics models. There are a number of models that may be used to depict the drug dissolution profiles, where f is a function of time that is linked to the amount of medication that has been dissolved from the pharmaceutical dosing system. The use of a general equation that mathematically translates the dissolution curve in function of various factors linked to the pharmaceutical dosage forms facilitates the quantitative interpretation of the data acquired in the dissolution experiment. In some circumstances, such as in zero order kinetics, that equation may be determined by a theoretical understanding of the process. In the majority of circumstances, whether dealing with tablets, capsules, coated forms, or delayed release forms, a more suitable empirical equation is employed instead of a theoretical foundation. The release kinetics of a medication can be affected by its type,

polymorphic form, crystallinity, particle size, solubility, and dose in a pharmaceutical dosage form (Coastaet al., 2001).

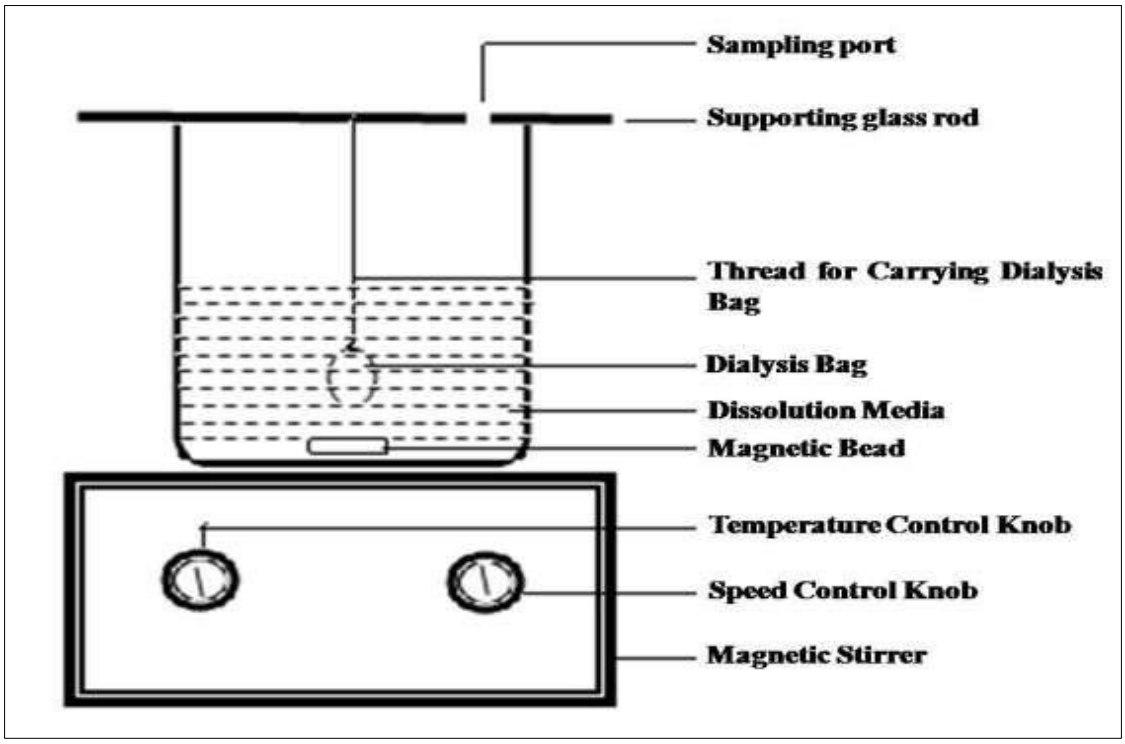

Figure 18: Schematic illustration of the assembly for in-vitro drug release study

To analyse the drug release profile, an in vitro release study in 0.1N HCl (in 40% ethanol) solution was conducted. A dialysis bag diffusion research was carried out to examine in vitro medication release. Before being used for the experiment, the dialysis membrane (molecular weight cut off 12 KD) was soaked in double-distilled water for 12 hours. Drug-loaded MNPs containing 10 mg of glimepiride were suspended in a 500 ml beaker containing 400 ml of newly manufactured 0.1N HCl in 40% ethanol at a temperature of 370.5°C. The dissolving media comprised 400 ml of freshly prepared 0.1N HCl in 40% ethanol. with constant 75 rpm magnetic stirring. To maintain consistent volume, samples were taken out at predetermined intervals and the same volume of new medium was added right away. The sink condition was kept throughout the release studies. The UV-mini 1240 Shimadzu spectrophotometer was used to analyse the samples at 226 nm. The drug release profile from the commercial formulation (a powdered tablet containing 10 mg of glimepiride) was examined using the same methodology. These two formulations' release profiles were contrasted and displayed .

Table 12: In-vitro release study of MNPs and marketed formulation in 0.1N HCl (in 40% ethanol)

S.no	Time (hrs)	% drug release from MNPs	% drug release from marketed glimepiride tablet formulation(MF)
1	0.5	4.43±2.31	25.54±2.45
2	1	11.76±2.28	46.67±3.24
3	2	18.54±2.62	69.21±3.63
4	3	26.34±2.19	91.34±2.79
5	4	31.21±1.92	92.63±2.64
6	5	37.41±2.24	-
7	6	43.13±3.31	-
8	7	52.31±3.82	-
9	8	61.58±3.52	-
10	24	91.67±2.34	-

Mean± SD (n=3)

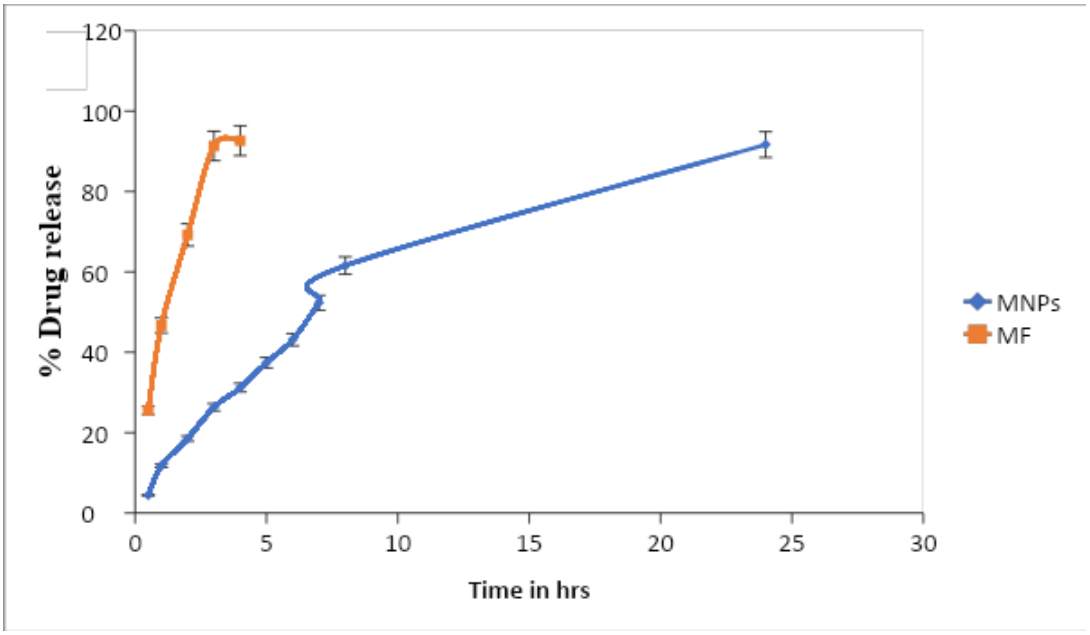

Figure 19: In-Vitro release profile of Glimepiride loaded MNPs(blue line) and marketed tablet formulation (red line) in 0.1N HCl (in 40% ethanol)

Mucoadhesive nanoparticles were created by slightly altering the ionic gelation process described by Emami et al. (2014), employing different amounts of sodium alginate, guar gum, PEG 6000, and calcium chloride.

To produce the best-sized nanoparticles with the highest drug entrapment efficiency, prepared MNPs were optimised based on process variables such as stirring speed, stirring time, and sonicatin time as well as formulation variables such as sodium alginate, guar gum, PEG 6000, drug, and CaCl2 concentrations.

In order to produce mucoadhesive nanoparticles, sodium alginate and guar gum were first subjected to optimization on the basis of average particle size by varying the ratio of the two substances. These formulation codes, AG1, AG2, AG3, and AG4, had sodium alginate and guar gum ratios of 10:05, 10:10, 10:15, and 10:20, respectively.

It was discovered that the average particle size was 380.633.59, 498.553.68, 646.434.21, and 865.523.23nm, respectively. From these data, it can be seen that the particle size of nanoparticles progressively rises as sodium alginate concentrations decline. This can be because calcium ions interact with alginate ions more often when there is a low concentration of alginate ions present. Therefore, the 10:10 ratio with an average particle size of 498.55 3.68 nm was chosen as the optimal formulation for future research. Different formulations were created and coded as AG2P1, AG2P2, AG2P3, and AG2P4 with 25, 50, 75, and 100 mg of PEG 6000 concentrations, respectively, for the purpose of optimising PEG 6000 concentration. It was discovered that the average particle size was 651.25 nm, 495.83 nm, 389.32 nm, and 318.27 nm, respectively. According to these findings, the particle size of nanoparticles gradually reduces as PEG 6000 concentrations rise. This may be because PEG 6000 at high concentrations makes polymers more soluble in water, which causes the particle size to shrink. The amount of PEG 6000 in the formulation that comprised 50 mg was determined to be ideal.

In this study, drug concentration was optimised by changing the drug concentrations while maintaining the other parameters constant. The formulations, coded as AG2P2D1, AG2P2D2, AG2P2D3, and AG2P2D4, had drug concentrations of 10, 20, and 40 mg, respectively. It was discovered that these formulations had average particle sizes of 478.26 nm, 482.35 nm, 515.82 nm, and 568.28 nm, as well as. In this study, drug concentration was optimised by changing the drug concentrations while maintaining the other parameters constant. The formulations, coded as AG2P2D1, AG2P2D2, AG2P2D3, and AG2P2D4, had drug concentrations of 10, 20, and 40 mg, respectively. It was discovered that these formulations had average particle sizes of 478.26 nm, 482.35 nm, 515.82 nm, and 568.28 nm, as well as

Further, the CaCl2 molar concentration was optimised by changing the CaCl2 concentration while maintaining the other parameter constant, and formulations were coded as AG2P2D 2C1, AG2P2D 2C1, AG2P2D 2C1, and AG2P2D2C1 having 0.01, 0.05, 0.1, and 0.15M CaCl2 concentration respectively. It was discovered that these formulations had average particle sizes of 281.43 nm, 493.64 From these findings, it can be seen that the particle size of nanoparticles gradually increases with increasing CaCl2concentrations. This might be because more calcium ions were available for the ionic interaction with a fixed amount of alginate ions, leading to an increase in particle size, and because after the optimum concentration, no significant effect on percent

entrapment efficiency was seen. The best CaCl2 concentration among the ones tested was determined to be 0.05M.

Then, the stirring speed of the process was optimised in relation to the average particle size (nm) and the percentage of entrapment efficiency. The average particle size and entrapment efficiency of the formulations with the codes AG2P2D2C2R1, AG2P2D2C2R2, AG2P2D 2C2R3, and AG2P2D 2C2R1 were determined to be 710.314.23, 493.223.47, 382.633.62, and 320.344.36 nm, 81.212.34, and 80.182.16, respectively, when they were operated. These findings show that when stirring speed is increased, nanoparticle particle size and percent entrapment effectiveness steadily decline. This may be because the stress of high swirling speed causes bigger nanoparticles to break into smaller ones. It was determined that the best outcome came from AG2P2D 2C2R2 with a 5000 rpm stirring speed.

The formulations were coded as AG2P2D2C2R2T1,AG2P2D2C2R2T2, AG2P2D2C2R2T3, and AG2P2D2C2R2T4 respectively, and the optimization of process variables also included stirring time (hrs), which was 5, 6, 7, and 8 hours. It was discovered that these formulations had average particle sizes of 780.14, 508.43, 451.62 and 378.31 nm, From these data, it can be seen that nanoparticle particle size and percent entrapment effectiveness steadily decline as stirring duration increases. This may be because nanoparticles break down or rupture when stirred at a steady speed over an extended period of time. The best outcome came from AG2P2D2C2R2T2, which had a 6 hour stirring period.

The final stage of the optimization process took into account the average particle size and the percentage of entrapment efficiency before sonication time (sec) was optimised. Formulations were identified by the codes AG2P2D2C2R2T2S1, AG2P2D2C2R2T2S2, AG2P2D2C2R2T2S3, and AG2P2D2C2R2T2S4. having 30, 60, 90, and 120 seconds of sonication time were discovered to have, respectively, average particle sizes of 715.29 nm, 480.48 nm, 321.67 nm, and 278.36 nm, and entrapment efficiencies of 80.68 nm, 80.12 nm, 75.24 nm, and 70.13 nm. These findings show that when sonication time is increased, nanoparticle particle size and percent entrapment effectiveness steadily decline. This may be because bigger nanoparticles break down into smaller ones under the prolonged high sonication stress. It was determined that the optimal outcome from AG2P2D2C2R2T2S2had a 60-second sonication time.

In the end, the formulation code AG2P2D2C2R2T2S2 was identified as an optimal formula with an entrapment efficiency of 80.122.42 percent and an average particle size of 480.483.25 nm.

By using a transmission electron microscope, the surface morphology of the MNPs formulation AG2P2D2C2R2T2S2 was examined. MNPs were shown as having a spherical form in the TEM images in Figure 4.8.

The AG2P2D2C2R2T2S2 formulation's PDI was discovered to be 0.230. This outcome demonstrated that the AG2P2D2C2R2T2S2 formulation included monodispersed nanoparticles.

It was discovered that formulation AG2P2D2C2R2T2S2 had a zeta potential of -24.8 mV. The absence of aggregates in the formulation and the presence of monodispersed nanoparticles were indicated by the negative sign.

For in vitro drug release, the improved formulation AG2P2D2C2R2T2S was investigated. The dialysis bag method was used to determine the formulation's in vitro drug release characteristics.

The percent drug release profile of MNPs was found to be 91.672.34 percent up to 24 hours in 0.1N HCl (in 40% ethanol), while the conventional dosage form that is commercially available showed 91.342.79 percent up to 4 hours when compared under the same circumstances with an equivalent amount of glimepiride (Table4.10; Fig. 4.13). thus that MNPs have a 24-hour controlled release pharmacological profile.

5.5 STABILITY STUDIES

The ability of the formulation to stay within specified boundaries for a certain amount of time is typically used to characterise the stability of any pharmaceutical product (shelf life of the product). The ability of a certain formulation in a specific container to continue to meet the physical, chemical, microbiological, therapeutic, and toxicological standards is referred to as a product's durability.

Stability testing is done to show how a formulation's quality changes over time and is impacted by many environmental conditions, including temperature, humidity, and light under tropical circumstances with greater ambient temperatures and humidity, degradation is more prone to happen. The drug stability studies can be used to determine the amount of drug inactivation caused by different environmental unfavourable circumstances. As a rate process, it is stated. These studies aid in predicting a product's shelf life and expiration dates. The period needed for the reactant concentration to drop to 90% of its starting concentration is referred to as the shelf life. Time/conc unit representation of shelf life is t90. A key quality-attribute for all pharmacological dosage forms is the expiration date. Preferably, the expiration date should be accompanied by information on the particular storage conditions, which are specified in the pharmacopoeia for this reason (preservation, packaging, storage and labelling).

The stability assessment of any new medication or drug product is a crucial component in determining or predicting the shelf life of the formulation, according to ICH guidance line QA1. Under approved storage conditions, the product's potency shouldn't drop below 95%, and it should still have the same appearance and functionality as when it was initially created.

Any drug product will degrade since it has a certain activation energy (Ea). It is obtainable from the Arrhenius plot's slop. Assuming a low amount, such as 10Kcalmol-1, is wise since it will speed up the process. Drug degradation can range from 10 to 100 kcalmol-1, although it often occurs between 15 and 60 kcalmol-1, with a mean of 19.8 kcalmol-1 (Aulton, 2002).

Table 13: Relationship between λmax and Associated Energy of Various form of light (Aulton, 2002)

Types Of Radiation	**Wavelength (nm)**	**Energy(Kcalmol^{-1})**
UV	50-400	287-72
VISIBLE	400-750	72-36
IR	750-10000	36-01

The capacity of a prepared product to remain stable on the shelf plays a crucial role in the effective creation of a dosage form. As a result, creating a stable testing strategy is crucial for creating mucoadhesive nanoparticles (MNPs). The durability of the improved MNPs formulations was evaluated by keeping them at three different temperatures: room temperature (25°C), accelerated temperature (40°C), and refrigerator temperature (4°C; 65–5 percent relative humidity). The REMI Stability Chamber was used to store the improved MNPs formulations in screw-capped glass bottles under the required storage conditions. After 7, 14, 21, and 28 days, samples were examined for particle size and residual drug content.

5.5.1 Effect of Storage Conditions on Particle Size

Zetasizer (Malvern Instrument Ltd.) was used to measure the effects of different storage conditions, such as 4°C in the refrigerator, 25°C at room temperature, and 40°C at accelerated temperature, on the particle size of formulations after a specific period of time of 7, 14, 21, and 28 days. The results are shown in Table 5.2 and Fig. 5.1.

Table 14: Effect of Storage Conditions on the Particle Size of mucoadhesive nanoparticles

Duration in days	**Influence of storage condition on the Particle Size (nm) of mucoadhesive nanoparticles**		
	Storage condition		
	4±2˚C	25±2˚C (65±5%RH)	40±2˚C (75±5%RH)
Initial	480.48±3.25	480.48±3.25	480.48±3.25
7	481.23±2.65	483.43±2.51	487.65±3.86
14	483.53±2.45	487.78±3.25	499.34±2.95
21	486.27±2.14	491.21±4.32	512.87±2.73
28	489.34±3.34	496.46±3.45	519.43±2.86

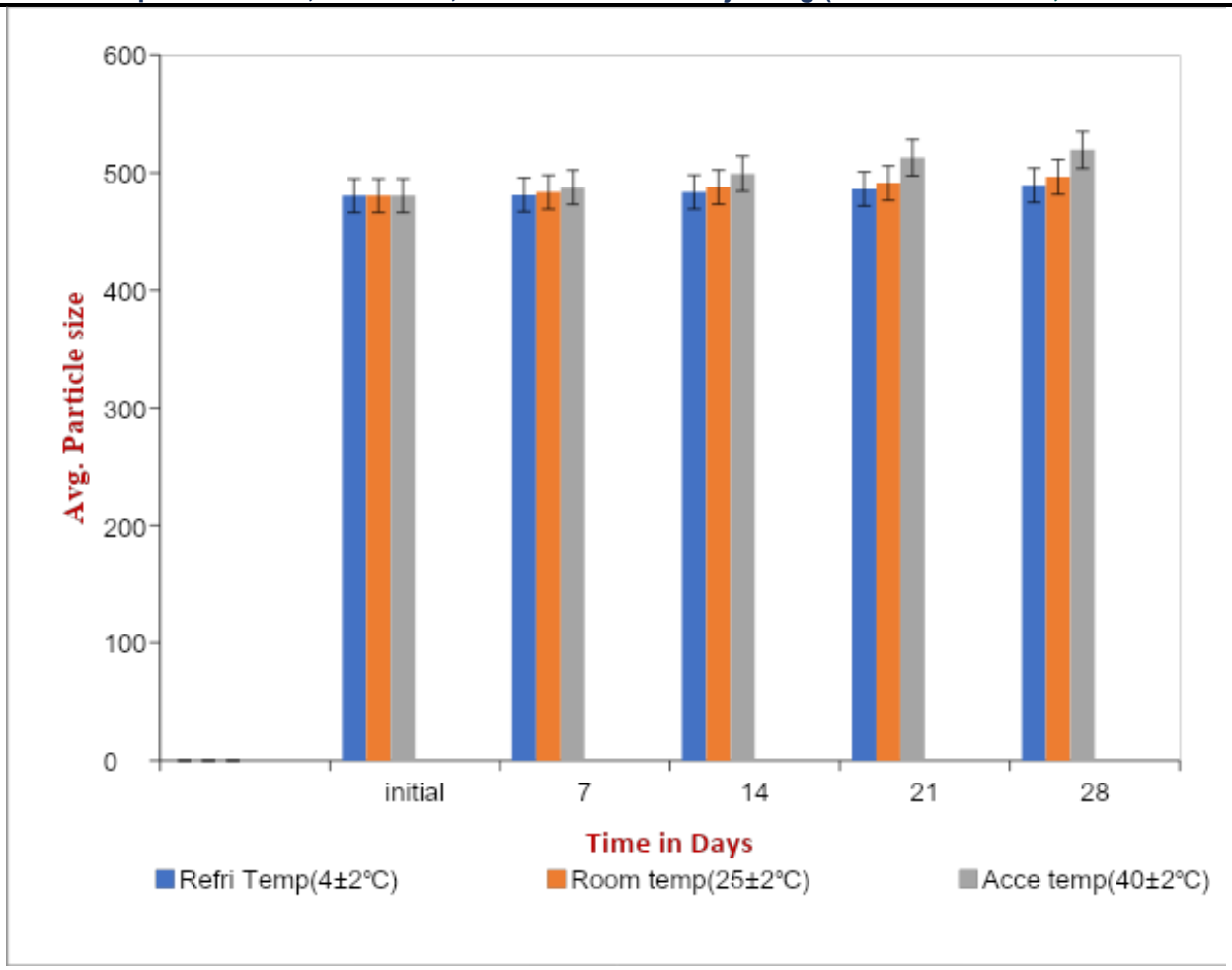

Figure 20: Histogram showing effect of storage conditions on Particle Size of mucoadhesive nanoparticles

5.5.2 Effect of Storage conditions on % Residual Drug Content

By using a UV spectrophotometer, the formulations' residual drug content was assessed after being stored for the predetermined amounts of 7, 14, 21 and 28 days. It was completed by using a pestle and mortar to triturate known amounts of materials in PBS (pH 7.4) and then setting it aside for 24 hours for extraction. After that, samples were filtered using Whatman filter paper, and a UV spectrophotometer was used to measure the absorbance of the filtrates (UV-mini 1240 shimadzu). In Table 5.3 and Fig. 5.2, the percent drug residual is computed and displayed.

Table 15: Effect of Storage Condition on % Residual Drug Content of mucoadhesive nanoparticles

Duration in days	Influence of storage condition on % Residual Drug Content size of mucoadhesive nanoparticles		
	Storage condition		
	4±2˚C	25±2˚C (65±5%RH)	40±2˚C (75±5%RH)
Initial	100.00±0.00	100.00±0.00	100.00±0.00
7	98.86±1.78	98.23±2.31	94.43±3.24
14	97.21±2.36	96.43±2.35	88.34±2.68
21	96.53±3.23	94.31±3.362	81.26±3.23
28	95.12±2.54	91.22±3.24	78.23±2.56

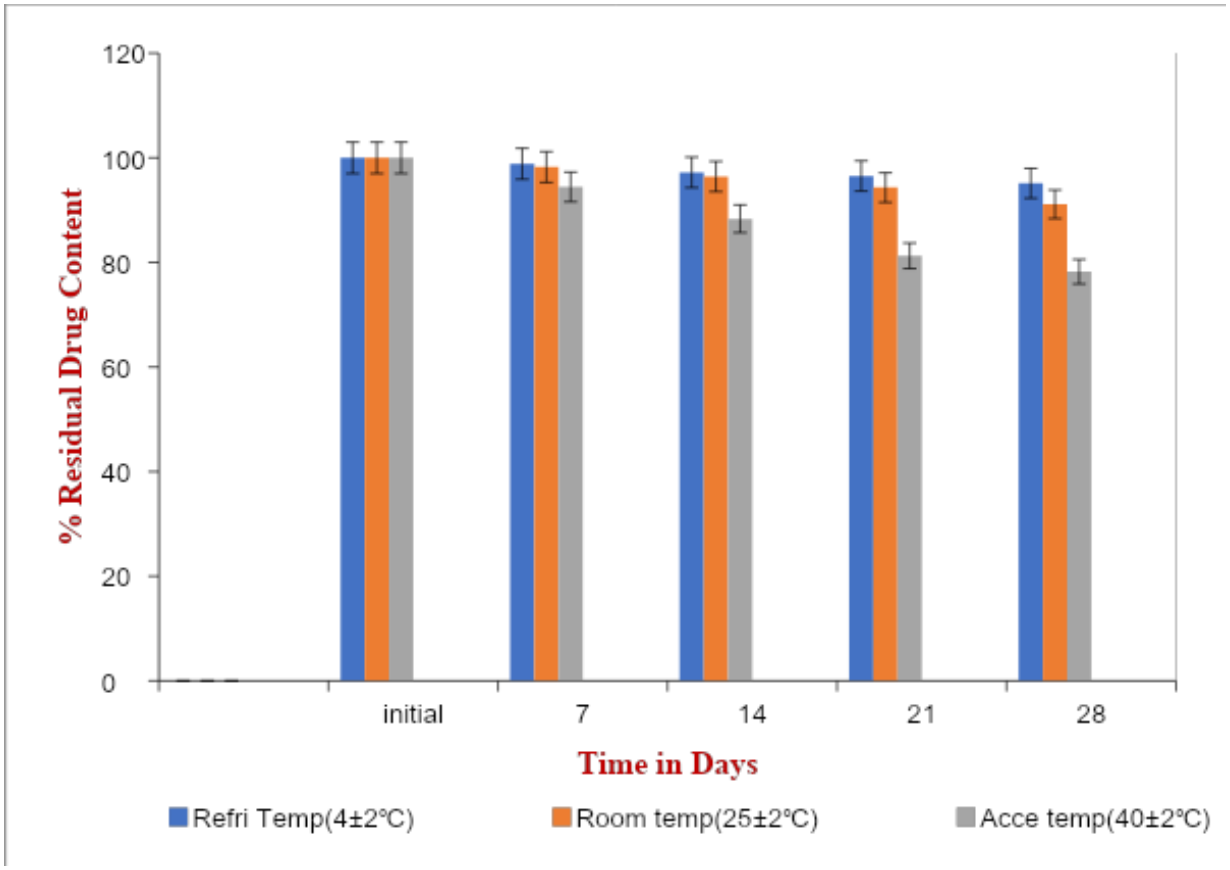

Figure 21: Histogram showing effect of Storage conditions on % Residual Drug Content of mucoadhesive nanoparticles

Using the REMI Stability Chamber, the stability of mucoadhesive nanoparticles was studied throughout time periods of 7, 14, 21, and 28 days at various temperatures and relative humidity levels, including ambient temperature (27°OC), accelerated (40°OC), and refrigerated temperature (4°OC). The stability of the produced formulations was assessed based on changes in particle size and the percentage of residual drug content. On storage, mucoadhesive nanoparticles' average particle size was shown to increase, which may be the result of particle aggregation.

This impact was minimised in the formulation held at 42OC, indicating that aggregation may be controlled by adjusting storage temperature. As a result, 42OC was determined to be the best storage temperature for mucoadhesive nanoparticles. (Fig. 5.1, Table 5.2). After 28 days, it was discovered that the mucoadhesive nanoparticles' percent residual drug content was 95.122.54, 91.223.24 and 78.232.56 percent, respectively, when kept at 42OC, 252OC (655 percent RH), and 402OC (755 percent RH).

The formulations that were kept at 4°C had substantial levels of drug residue (Table 5.3, Fig.5.2) means least degradation. The results of the stability tests showed that mucoadhesive nanoparticles held at 4°C were more stable than those stored at 27°C and 40°C at 75% and 75% relative humidity, respectively. For best stability, this formulation must be kept in the refrigerator. The prepared mucoadhesive nanoparticles, however, must be stored at refrigerator temperature (42OC), though they can also be kept at room temperature. Never, however, should they be kept at higher temperature due to the significant drug degradation that occurs at higher temperature, according to the analysis of Table (5.2 and 5.3) and Fig. (5.1 and 5.2).

6.1 DISCUSSION

According to the drug preformulation investigations, the medication obtained from Ravian Life Science, SIDCUL, Haridwar, complies with the requirements.

The substance was discovered to be an odourless, white, crystalline powder. Glimepiride's melting point was discovered to be between 208 and 2100°C, which was close to the previously reported figure of 207°C.

The FT-IR spectrum of the drug showed two amide N-H stretches at 3288 and 3369cm^{-1}, aliphatic C-H stretch at 2950cm^{-1} , two amide C=O stretches at 1674 and 1708cm^{-1} and aromatic C=C stretch at 1540 cm^{-1}, whichconfirmed the presence of different functional groups, which were identical to the spectra of reference drug given in literature.

UV spectrophotometer measurements revealed that the absorption maxima of glimepiride in PBS (pH 7.4), ethanol:water, and 0.1N HCl (in 40% ethanol) were 229 nm, 230 nm, and 226 nm, respectively.

The calibration curves were created in 0.1N HCl, PBS (pH 7.4), and ethanol:water (in 40 percent ethanol). The standard curve data were linearly regressed, and correlation coefficients and equations for standard curves with straight lines were found. The standard curve correlation coefficient was discovered to be quite close to one, indicating a strong co-linear relationship between concentration and absorbance. As a result, the medication obeyed Beer-Law Lambert's between 2.0 and 16.0 g/ml. The glimepiride was discovered to be insoluble in distilled water but soluble in 0.1 N sodium hydroxide solution, ethanol, and PBS (pH 7.4).

In n-octanol/PBS with a pH of 7.4, the log partition coefficient of glimepiride was calculated and found to be 2.41. Studies on the drug's solubility and partition coefficient revealed that it was hydrophobic by nature.

A metabolic condition known as type 2 diabetes mellitus (T2DM) causes hyperglycemia, polyuria, glycosuria, polyphasia, polydipsia, and weight loss. Because the body gets tolerant to insulin and does not utilise blood glucose for energy generation, which results in T2DM, the body's sensitivity to insulin is diminished, even with normal pancreatic beta cell secretion. If left untreated, this illness might have major side effects such cardiopathy, neuropathy, nephropathy, and retinopathy. In order to manage blood glucose levels for a long time with a single dose, an efficient medication delivery mechanism must be created. Glimipiride is an oral hypoglycemic agent that belongs to the third generation of sulfonylureas and is used to treat type 2 diabetes. It is typically formulated as a conventional dosage form, which has several drawbacks including a short biological half-life, low solubility, frequent dosing, erratic drug plasma concentrations, low bioavailability, and patient noncompliance. To get over these drawbacks, it is necessary to create a more modern medication delivery method. Therefore, it is suggested to create mucoadhesive nanoparticles that can solve all of the aforementioned issues with traditional dosage forms. The mucoadhesive nanoparticles have the capacity to stick to the stomach mucosa, prolong gastric residence duration, and release the medication in a controlled way at a particular spot from where absorption takes place for a prolonged length of time.

To determine the identification and purity of the medication, a preformulation research was conducted prior to the production of glimepiride-loaded mucoadhesive nanoparticles. The substance was discovered to be an odourless, white, crystalline powder. Glimepiride's melting point was discovered to be between 208 and 2100°C, which was close to the previously reported figure of 2070°C.

The FT-IR spectrum of the drug showed two amide N-H stretches at 3288 and 3369cm^{-1}, aliphatic C-H stretch at 2950 cm^{-1} , two amide C=O stretches at 1674 and 1708cm^{-1} and aromatic C=C stretch at 1540 cm^{-1}, which confirmed the presence of different functional groups, which were identical to the spectra of reference drug given in literature.

The absorption maximums of glimepiride in PBS (pH 7.4), in ethanol:water and in 0.1N HCl (in 40% ethanol) were determined by UV spectrophotometer (UV mini 1240 Shimadzu) and were found to be **229 nm, 230 nm and 226 nm,** respectively.

The calibration curves were created in 0.1N HCl, PBS (pH 7.4), and ethanol:water (in 40 percent ethanol). The standard curve data were linearly regressed, and correlation coefficients and equations for standard curves with straight lines were found. The standard curve correlation coefficient was discovered to be quite close to one, indicating a strong co-linear relationship between concentration and absorbance. As a result, the medication obeyed Beer-Law Lambert's between 2.0 and 16.0 g/ml. Insoluble in pure water, the glimepiride was found to be soluble in 0.1 N sodium hydroxide solution, sparingly soluble in ethanol, and mildly soluble in PBS (pH 7.4).

In n-octanol/PBS with a pH of 7.4, the log partition coefficient of glimepiride was calculated and found to be 2.41. The drug's solubility and partition coefficient results demonstrated its hydrophobic nature. The identification of Glimepiride was established by all the aforementioned findings.

Ionic gelation was used to create mucoadhesive nanoparticles. To produce the best-sized nanoparticles with the highest drug entrapment efficiency, prepared MNPs were optimised based on process variables such as stirring speed, stirring time, and sonicatin time as well as formulation variables such as sodium alginate, guar gum, PEG 6000, drug, and CaCl2 concentrations. The formulation AG2P2D2C2R2T2S2 was discovered to be the best, with an optimal entrapment effectiveness of 80.122.42 percent and an average particle size of 480.483.25 nm.

When MNPs were examined using a transmission electron microscope, it was discovered that they had a spherical form. The zeta potential and PDI of the chosen formulation were determined to be -24.8 mV and 0.230, respectively, showing that the formulation included monodispersed nanoparticles without any aggregates.

The investigation of drug release from mucoadhesive nanoparticles serves as both a standard quality-control test to ensure homogeneity of the final product and a crucial step in the creation of novel formulations. MNPs' in-vitro release investigation revealed consistent release for up to 24 hours. As could be observed, 91.672.34 percent of the medicine that had been encapsulated inside the core of the MNPs was released, as opposed to 91.342.79 percent in the case of the traditional dosage form, which did so within 4 hours. As a result, MNPs displayed a delayed controlled drug release profile.

The results of the stability tests showed that mucoadhesive nanoparticles held at 4°C were more stable than those stored at 27°C and 40°C at 75% and 75% relative humidity, respectively. For best stability, this formulation must be kept in the refrigerator. The prepared mucoadhesive nanoparticles, however, must be stored at refrigerator temperature (42OC), though they can also be kept at room temperature. Never, however, should they be kept at higher temperature due to the significant drug degradation that occurs at higher temperature, according to the analysis of Table (5.2 and 5.3) and Fig. (5.1 and 5.2).

The "Glimeriride Loaded Mucoadhesive Nanoparticles" were successfully prepared, optimised, and evaluated and were found to be a promising candidate for treating type 2 diabetes mellitus. Additionally, the high mucoadhesive property improved its gastric residence time and led to controlled drug delivery, ultimately increasing therapeutic utility and reducing the side effects of single dosage formulation.

7.1 REFERNCES

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