Research Article

Preparation, Physicochemical Characterization, Optimization and I*n vitro Comparative* Evaluation of Gliclazide-Eudragit Nanoparticles and Gliclazide-Poly (lactide-co-glycolide) Nanoparticles With Marketed Products for the Treatment of Diabetes mellitus

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ABSTRACT

Gliclazide is used in the treatment of diabetes mellitus, but it is having limitations such as low solubility leading to lower oral bioavailability. Gliclazide conventional formulation has to be given frequently to maintain blood glucose levels, which can be overcome by sustained approach. So the major objective was to formulate polymeric nanoparticles, which can increase solubility and oral bioavailability along with sustained release of the drug. In the present study, it was proposed to develop nanotechnology based systems, for selected poorly water soluble drug Gliclazide using PLGA (GL-PLGA) and Eudragit (GL-EU) as polymers (with different drug: polymer ratios) which was expected to improve dissolution properties by comparing with marketed immediate and sustained release forms. The polymeric nanoparticles were subjected to particle size evaluation, SEM study, drug content, entrapment efficiency and *in vitro* release studies. GL-PLGA in drug: polymer ratio of 1:3 has shown a particle size, drug loading and entrapment efficiency of 130±2.1nm, 61.28% and 18.15 % respectively. GL-EU in drug: polymer ratio of 1:1 has shown a particle size, drug loading and entrapment efficiency of 131± 2.5nm, 81.82% and 39.96 % respectively. *In vitro* drug release for GL-EU nanoparticles has shown sustained drug release of 86.39±0.19 % within 72 hours with a initial burst release of 42±0.12% within 7.5 hours where as Gliclazide-PLGA nanoparticles has shown lesser drug release of 59.85±1.89%. GL-EU nanoparticles shows similar release profile comparable to the marketed sustained release forms, which concludes its usage as alternative for the already available marketed product.

Keywords: Diabetes mellitus, Encapsulation efficiency, Gliclazide, Polymeric nanoparticles, Sustained.

INTRODUCTION

Diabetes mellitus is a metabolic disorder, which is characterized by their insulin dependency that is insulin dependent diabetes mellitus (Type I) or non-insulin dependent diabetes mellitus (Type II) and its inability to transport glucose from blood to the cells.[1]

Gliclazide belongs to the second-generation sulfonylureas that can be used as oral hypoglycemic agent in the treatment of non-insulin-dependent diabetes mellitus (NIDDM). Sulphonyl ureas act by binding to sulphonylurea receptors on pancreatic β-cells, leading to increased secretion of insulin. The rationale behind selecting Gliclazide as active pharmaceutical ingredient is because of its low incidence of hypoglycemia and good tolerability. More over researches have confirmed that Gliclazide is having a low rate of secondary failure along with its potential that helps in delaying the progress of diabetic retinopathy.[2-4]

Most of the newly developed molecules for treatment of diabetes are suffering from variability in absorption that limits their therapeutic efficacy, which can be attributed to their change in physicochemical properties. The medication for diabetes II needs to be given frequently which can be attributed to the low solubility of Gliclazide, which belongs to biopharmaceutical classification system II (BCS Class II). BCS class II indicates low solubility and high permeability for the drug molecules. As the drug is having low solubility, it dissolute very poorly so it delays the absorption which indicates that the rate of dissolution is the controlling step for absorption.[5-6]

Gliclazide is available in the market and the conventional form is given via oral route with the dose ranging from 40-320 mg per day.[7-8] Gliclazide is having a molecular weight of 323.412 g/mol and shows good permeability with log P of 2.6. It also exhibits p^H dependent solubility and 94% plasma protein binding. Previous researches have indicated that Gliclazide dissolution rate depends on gastric emptying time and dissolution rate in the small intestine where the drug is soluble.[9-10]

So Gliclazide despite having good permeability, its therapeutic effect was limited mainly due to its poor aqueous solubility and poor drug dissolution, which leads to improper absorption and decreased oral bioavailability. So these limitations have to be addressed in order to achieve intended therapeutic effect, which can be done by preferring nanotechnology approach over others.

In the last few decades, polymeric nanoparticles (PNPs) have gained considerable attention for drug delivery systems as they can deliver the drug in controlled manner to the site of action along with its release in time dependent manner. This approach helps us in increasing the therapeutic efficacy, minimizing the un-required side effects and reducing the dose to be administered.[11] Polymers mainly used for formulation of polymeric

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nanoparticles can be both biodegradable and non-biodegradable. Of these two, biodegradable polymers have captured attention mainly for developing controlled/sustained release and can be compliant to patient.[12] The main rationale behind opting for the biodegradable nanoparticles is that they provide constant rate of degradation, which is ideal for sustained/controlled drug delivery systems.[13]

Out of the all, the most biocompatible and proven are Poly (lactide-co-glycolide) (PLGA) and Eudragit RL-100 (EU), which allows for the control of their size and morphology during the formulation of polymeric nanoparticles as particle size has influence on the solubility as decrease in the particle size increases the effective surface area which can increase the solubility. Eudragit RL-100, a polymer also known as Eudragit Retard L is a copolymer of poly (Ethylacrylate, Methyl-methacrylate, and Chloro-trimethyl-ammonioethyl methacrylate) containing an amount of quaternary ammonium groups between 8.8% and 12%. The reason behind opting for this EU is its capability to swell to a limited extent, which attributes to its proven dispersion of drugs slowly.[14-15] Poly (l -lactic-acid) (PLA) and its copolymers with glycolic acid (PLGA) have been extensively used for controlled drug delivery systems.[16-18] Glycolide polymers chains are cleaved by hydrolysis into natural metabolites (lactic and glycolic acids), which are eliminated from the body by the citric acid cycle. PLGA provides a wide range of degradation rates, from months to years, depending on its composition and molecular weight, which makes this a suitable polymer.[19-21]

The problem with most of the recently formulated nanoparticles is that they are practically proven for the activity, but are not proven whether they are capable of replicating the activity or enhancing the activity when compared with the marketed products. Marketed products are the one, which were already approved by various regulatory agencies like NDA (New drug application), ANDA (Abbreviated New drug application), FDA (Food and Drug administration) prior to their release. So by comparing with the marketed product, we can come to a conclusion that the formulated product can be somewhere near by its standards. Previous researches have been conducted on these polymeric nanoparticles, but none was compared to the marketed formulations (immediate release and sustained release) of the API available in the market, which can give the valuable information regarding the commercial viability of the nanoparticles in the treatment of diabetes mellitus. So the ultimate goal of the formulator is to develop a nanoparticles, which can be viable in the society and can benefit the patient or acting as an alternative to the current marketed form, this comparative *in vitro* drug release approach was preferred.

This gave an impetus for us to develop Gliclazide polymeric nanoparticles by two different polymers separately i.e; Eudragit L-100 (EU) and PLGA. Focus was to develop a polymeric nanoparticles, which have to sustain the drug release so as to retard the progress of the disease and there by reducing dose frequency that justifies the usage of formulated polymeric nanoparticles over conventional form. So the polymeric nanoparticles were developed by solvent evaporation method using two polymers and the better of the two was optimized that can enhance the poor solubility and poor oral bioavailability of the drug along with sustained release to achieve intended therapeutic effect.

MATERIALS AND METHODS

Materials

Gliclazide was obtained from Gen Pharma Pvt. Ltd., Bhosari, Pune, which was used as API. Eudragit RL-100 polymer was obtained as a gift sample from Evonik Degussa India Pvt. Ltd., Mumbai, India that was used as polymer. PLGA was obtained from Sigma Aldrich, Mumbai that was used as polymer. Dichloromethane (DCM) was obtained from Ranbaxy fine chemicals, New Delhi. Pluronic F68 was used as surfactant and Acetonitrile was obtained from Sigma Aldrich, Mumbai. Hydrophilic surfactant polyvinyl alcohol (PVA) was obtained from Merck Pvt Ltd. Water was doubly distilled. All other chemicals and materials were of analytical grade and were used as procured.

Preparation of Gliclazide nanoparticles

Solvent evaporation method was used for the preparation of polymeric nanoparticles using different drug and polymer ratios. The polymers used were PLGA and Eudragit L-100. In this method, polymer was dissolved in respective organic solvents i.e., acetonitrile for PLGA and DMSO for Eudragit. Gliclazide was dissolved separately in methanol. Then oil in water emulsion was prepared by emulsifying a mixture of polymer and drug solution (25ml) into an aqueous solution containing pluronic F-68 as surfactant along with polyvinyl acetic acid (1%) (PVA) for stabilization under constant stirring at 500 rpm. Then emulsion was subjected to sonication for 30 seconds, which was followed by homogenization. Removal of organic solvent was done by evaporation and there after centrifuged for 30 minutes at 30,000 rpm. The supernatant was kept aside for determination of encapsulation efficiency. Excess of PVA was removed by washing the settled nanoparticles. Method was done in triplicate in order to maintain reproducibility and also for desired amount of product needed for further analysis. Different ratios of drug: polymer was prepared by keeping drug as constant and optimizing the polymer effect on the formulation. Both polymers PLGA and EU were taken in drug: polymer ratios of 1:1, 1:2, 1:3, 1:4 for the optimization of the best formulation by comparing both the polymers that can provide the intended outcomes of this study.

Preformulation Studies

The drug solution was diluted prior to scanning by ultraviolet Spectrophotometric method for estimation of Gliclazide. Scanning was done within the wavelength range of 400-200nm. Maximum wavelength at which absorption has taken place was selected and that was used for further quantitative analysis. Further FTIR, DSC, XRD studies were carried out to confirm drug purity in accordance with Pharmacopoeial standards.[22]

Development of calibration curve

10 mg of Gliclazide was accurately weighed, dissolved in 10 ml of phosphate buffer pH 7.4 (5 mg SLS is dissolved in 100 ml of buffer) and make up the volume with pH 7.4 buffer to give a stock solution of 1 mg/ml (1000µg/ml) concentration. From this, 1ml of the solution was taken and diluted to 100 ml with phosphate buffer pH 7.4 to get a concentration (100 µg/ml). Aliquots of series of 10ml volumetric flask were added with 0.5ml, 1ml, 1.5ml, 2ml, 2.5ml from the secondary stock solution and the volume was made up to 10ml using phosphate buffer pH 7.4 (5mg SLS dissolved in 100 ml of buffer) to a get a concentration range of 5 µg/ml to 25 µg/ml. The absorbance of these solutions was measured at 225.5 nm using phosphate buffer pH 7.4 as blank in UV-Spectrophotometer.

Differential scanning calorimetry (DSC)

DSC analysis was performed using DSC Q200, TA instruments, Mumbai, India. The samples were heated in a sealed aluminium pans at a rate of 10° C per/min in a 30 to 300° C temperature under nitrogen flow of 40 mL/min. DSC analysis was performed for PLGA, Eudragit L100, Gliclazide, physical mixture of polymer and drug and finally for GL-PLGA, GL-EU polymeric nanoparticles.

X-ray diffraction studies (XRD)

Molecular arrangements of drug Gliclazide in nanoparticulate formulations were performed on an x-ray diffractometer (PANalytical X'pert, Almelo, Netherlands) using CuKα radiation. The data were collected over an angular range from 3 degrees to 50 degrees 2θ in continuous mode using a step size of 0.02 degree 2θ and step time of 5 seconds.[23]

Particle size and zeta potential

The average particle size and zeta potential of the PLGA - Gliclazide and EU-Gliclazide nanoparticles were determined by Particle Size Analyzer (Zetasizer Ver System; Malvern Instruments Ltd, Malvern, UK). To analyze particle size, nanosuspension was diluted with filtered (0.22µm) ultra pure water.[24]

Drug content and entrapment efficiency

Nanoparticles (20mg) after freeze-drying were added to their specific solvent (10 ml) to facilitate the coat of the nanoparticles to get dissolved. The resultant suspension was subjected to evaporation for further removal of the solvent prior to filtration. Then the residue was washed and diluted appropriately with phosphate buffer of pH 7.2 to determine drug content and entrapment efficiency. Samples were measured at an absorbance of 225.5 nm in Double beam U.V Spectrophotometer. Drug content loading and entrapment efficiency of Gliclazide in nanoparticles were determined by the following equations: [25]

$$\text{Drug loading content (\% w/w)} = \frac{\text{Weight of drug in nanoparticle}}{\text{Weight of nanoparticle recovered}} \times 100$$

$$\text{Entrapment efficiency (\%)} = \frac{\text{Weight of the drug in nanoparticle}}{\text{Weight of the drug fed initially}} \times 100$$

Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) was used to verify uniformity of particle shape and size. Freeze-dried nanoparticles were re-suspended in distilled water and were later dropped onto a silicon grid and dried under room temperature. The nanoparticle suspension was vacuum coated with gold for 3min. The surface morphology of the samples was observed under a scanning electron microscope (JEOL–JAPAN) operated at 15-keV pulse at different resolutions.[22]

In Vitro release studies

In vitro release of Gliclazide from the polymeric nanoparticles was evaluated by performing *in vitro* studies using USP type II (TDT 08T, Electro-lab, Mumbai, Maharashtra, India) dissolution test apparatus. Dissolution test was conducted in phosphate buffer (PB) p^H 7.4, which was maintained at 37.5^0C, and paddle rotation speed was maintained at 100 rpm. The main rationale behind selecting phosphate buffer of p^H 7.4 as Gliclazide is a hydrophobic drug it was found to be appreciably more soluble (2-4 times) in phosphate buffer of p^H 7.4 as solvent. Nanoparticles were suspended in 900 ml of PB with continuous stirring at 100 rpm. Samples were withdrawn from the dissolution medium at particular time intervals and replenished with fresh buffer after each sampling. The sample solutions were filtered and diluted up to 10 ml and the absorbance was measured at 225.5 nm using Double Beam UV/VIS Spectrophotometer. The study was done in triplicate which suggest each data point in the *in vitro* release graph represents an average of three measurements.[24]

In vitro drug release studies were conducted to the marketed Gliclazide immediate and sustained release forms in order to compare the viability of the optimized nanoparticles with the marketed forms which ensures better applicability to this study. This *in vitro* release graph was plotted by using Graph pad Prism software (V 5.01) in Microsoft windows 7 workstation.

RESULTS AND DISCUSSION

Development of calibration curve

Calibration studies were performed by U.V Spectrophotometric method at 227.5 to conclude the

linearity and it was found ranging from 5.0µg/ml to 30 µg/ml, which can be seen in the Figure 1. Drug was dissolved in methanol and then the standard plot was prepared in phosphate buffer of pH 7.4.
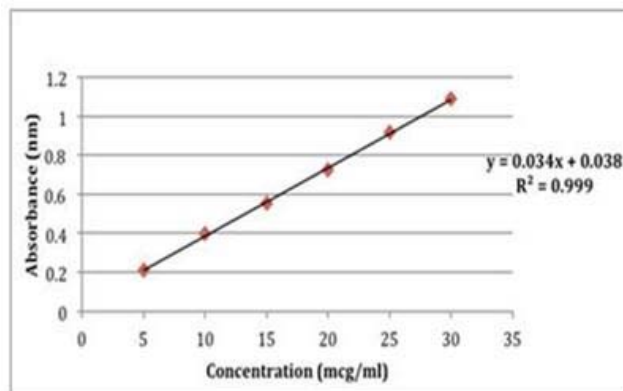

Figure 1: Calibration curve of Gliclazide

Differential Scanning Calorimetry (DSC)

Thermogram of Gliclazide, PLGA, EU and corresponding drug polymer systems were illustrated in Figure 2. The DSC curve of Gliclazide exhibit corresponding exothermic peak at peak temperature of 162.8 ^{0}C corresponding to its melting point. Polymers, PLGA and Eudragit shown peaks at a temperature of 65.12 and 231.2 ^{0}C respectively. Gliclazide-PLGA polymeric nanoparticles have shown a minor peak at 65^{0}C and peak position at Gliclazide was found to be vanished, which can be attributed to the entrapment of drug in the nanoparticles. Gliclazide-EU nanoparticles thermogram has shown peak at 230.1 ^{0}C and a minor peak at the area where Gliclazide presents. These studies further strengthen the evidence that there is compatibility between the drug and the polymers and also the chance of entrapment of drug inside the polymer in the polymeric nanoparticles. The DSC studies support our rationale, as stability is the primary concern, which can effect the formulation in many ways. To achieve stability, compatibility between the drug and the polymer must be ensured which can be confirmed by the DSC and further by XRD studies.
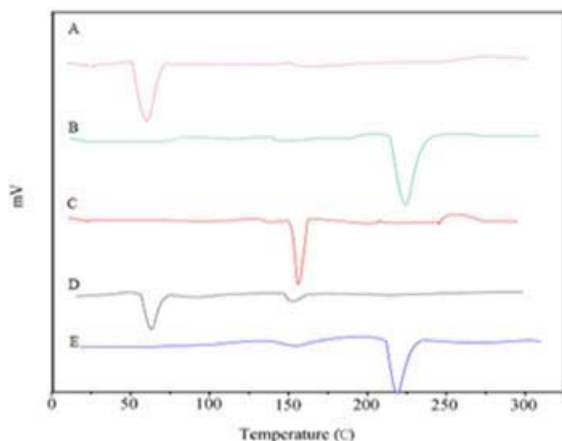

Figure 2: DSC Thermograms of (A) PLGA (B) Eudragit L 100 (C) Gliclazide (D) GL-PLGA (E) GL-EU

XRD studies

An XRD peak mainly depends on the crystal size as they indicate the crystalline nature at particular value at 2θ range. In this study, pure drug Gliclazide had shown a sharp single peak and the highest one at 2θ equals 11.5 ^{0} that indicates its crystalline nature. Polymers PLGA and EU diffractograms had shown peaks at 23^0 and 14^0 respectively which can be seen in Figure 3. The diffractograms of pure polymer was found to be different from the drug loaded polymeric nanoparticles, as we noticed a little decrease in the intensity of the peak which can be attributed to the lower level of detection of the encapsulated drug as it was dispersed in molecular level and moreover the slight disappearance of the Gliclazide peak indicates the entrapment of drug inside the polymers. The XRD peaks of GL-PLGA and GL-EU were shown in Figure 4.
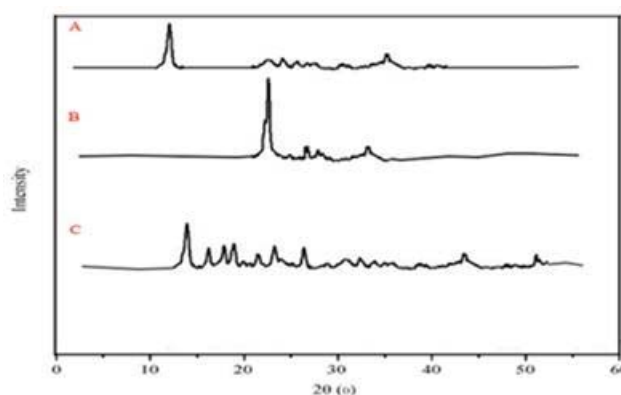

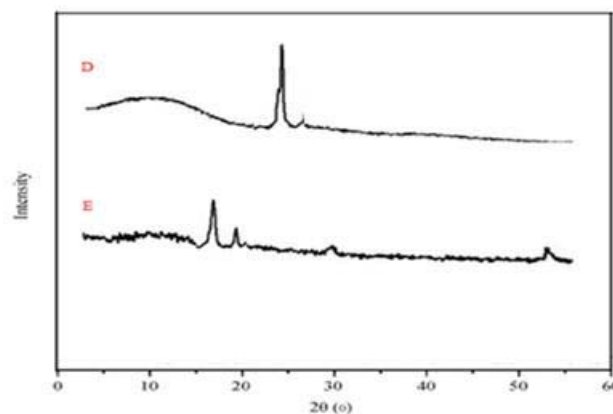**Figure 3:** XRD patterns of (A) Gliclazide (B) PLGA (C) Eudragit

Figure 4: XRD patterns of (D) GL-PLGA (E) GL-EU

Particle size and zeta potential analysis

The mean particle size of GL-PLGA and GL-EU was found to be smaller than 139 nm. So the nanoparticles size range was found to be satisfactory and was according to the specifications. This was performed in replicate of three times (n=3) in order to ensure reproducibility to minimize the error. PDI values were found to be lesser than 0.2, which indicates that the system has a relatively narrow distribution. Zeta potential was found to be in the limit and it further proves the stability of the prepared

polymeric nanoparticles, which justifies the rationale of preparing stable nanoparticles, as stable nanoparticles can be easily dispersed which enhances its solubility. The results evaluated were shown for different ratios of drug: lipid in Table 1. The particle size intensity for GL-PLGA (1:3) and GL-EU (1:1) were 130±2.1nm and 131± 2.5nm respectively which can be seen in Figure 5. Both these were found to be satisfactory among the all by evaluating the entrapment efficiency which was confirmed later.

Table 1: Particle Size, Entrapment efficiencies and Drug loading (%) of Gliclazide-PLGA and Gliclazide-Eudragit nanoparticles

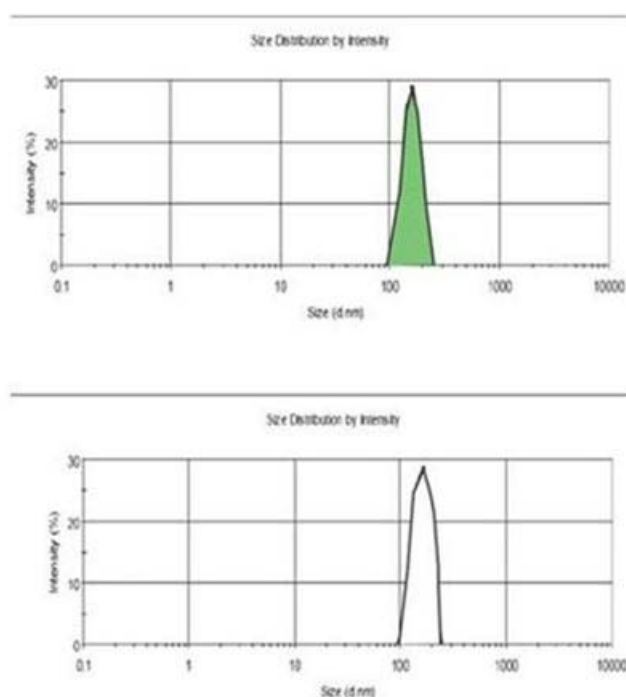| Drug: Polymer ratio	Particle size (nm)	Entrapment efficiency (%)	Drug loading (%)
1:1 (PLGA)	142± 3.8	44.93	15.25
1:2 (PLGA)	138±0.29	56.18	16.82
1:3 (PLGA)	130±2.1	61.28	18.15
1:4 (PLGA)	131±0.15	54.84	14.29
1:1 (EU)	131± 2.5	81.82	39.96
1:2 (EU)	129±1.12	64.58	21.24
1:3 (EU)	130±1.05	60.81	19.21
1:4 (EU)	138±2.91	58.12	18.93

Figure 5: Average particle sizes of GL-PLGA (1:3) and GL-EU (1:1) nanoparticles respectively.

Drug Loading and Entrapment efficiency studies

Drug loading has a very important influence in the polymeric nanoparticles preference over others such as solid lipid nanoparticles. The reason behind giving importance for the entrapment of the drug inside the polymeric nanoparticles, as improper entrapment leads to the initial burst release of the drug, which hinders its

sustained release property. More over its essential for the formulator to make sure that intended therapeutic dose have been retained within the formulated polymeric nanoparticles to achieve intended therapeutic effect to the patient. Entrapment efficiency of all the polymer nanoparticles made of different polymers was shown in Table 1.

Based on the evaluation parameters of entrapment efficiency and drug content, Gliclazide-PLGA (GL-PLGA) polymeric nanoparticles of ratio 1:3 (131± 2.5nm) and Gliclazide-Eudragit polymeric nanoparticles (GL-EU) of 1:1 (131± 2.5nm) were optimized. Some past researches support the increase in the encapsulation efficiency with increase in the polymer, which was true to some extent in case of Gliclazide-PLGA nanoparticles as entrapment efficiency increased up to 61.28% till 1:3 ratio of drug: polymer.[26] But contrary to that gliclazide-EU nanoparticles had shown decrease in the entrapment efficiency with increase in the polymer ratio, which can be attributed to the more compact polymer coat, which limits its entrapment, and also to the hydrophobicity nature of the drug. GL-EU nanoparticles has shown an entrapment efficiency of 81.82% and that decreased to 58.12% for the drug: polymer ratio of 1:4. The optimized ratio of 1:1 of GL-EU and 1:3 ratio of GL-PLGA were given primary importance for further studies. Also the particle size of the optimized ratios was found satisfactory which enables us to select the both GL-PLGA and GL-EU for *in vitro* release studies.

Scanning electron microscopy

Scanning electron microscopy was performed for GL-PLGA and GL-EU to obtain more information on the particle size and morphology. The photos of polymeric nanoparticles had shown that the formulated Gliclazide nanoparticles of different polymers were of spherical shape with size range from 95 to 139 nm, which were shown in Figure 6. More over the nanoparticles were observed with smooth surface, which may contribute to its release of the drug in a sustained manner when compared to the nanoparticles having rough surface. This property was evaluated thoroughly in the upcoming *in vitro* drug release studies.
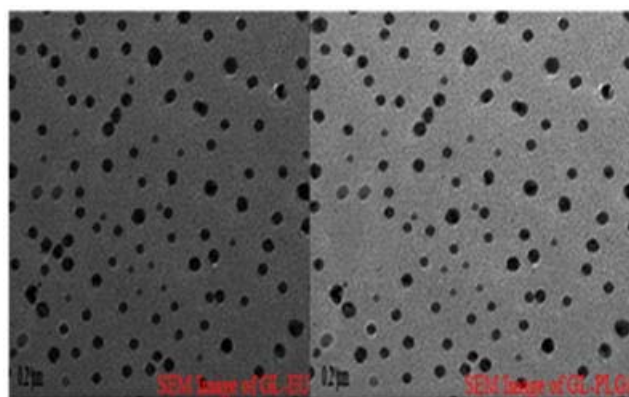

Figure 6: SEM image of GL-PLGA nanoparticles and GL-EU nanoparticles

In Vitro Drug Release

In vitro release of Gliclazide from two polymeric nanoparticles- GL-PLGA (1:3) and GL-EU (1:1) were shown in Figure 7. Cumulative drug release was observed for the optimized polymeric nanoparticles till 72 hours. Along with the nanoparticles, pure drug solution of Gliclazide was also taken for *in vitro* studies in order to differentiate the sustained release of the polymeric nanoparticles. Out of the two optimized batches, GL-EU (1:1) showed better drug release when compared to the GL-PLGA (1:3). GL-EU nanoparticles showed an initial burst release up to 42±0.12% in 7 .5 hours and there after the drug release was sustained by releasing 86.39±0.19 % of the drug with in 72 hrs. GL-PLGA nanoparticles shown drug release of 59.85±1.89% only which indicates to its less drug release, which may be due to the compact nature of the polymer coat around the drug minimizing its release. Initial burst release in GL-EU (1:1) nanoparticles may be attributed to the partial un-encapsulated drug which may be present outside the polymer and there after sustained release was achieved which can be due to the release of the drug from the polymer. The pure drug solution of Gliclazide (GL-SOL) had shown drug release of 68.25±0.09% within 4 hours, which had elevated the importance of the GL-EU (1:1) polymeric nanoparticles.

In vitro drug release has justified the rationale as increase in the cumulative drug release indicates the increased solubility of the Gliclazide using polymeric nanoparticles as it helps in dispersing the drug in the buffer used in the dissolution medium where as in Gliclazide pure solution initially showed drug release but hindered after some time because of its low solubility. Also the drug release over 72 hours has proven the sustained release of the nanoparticles, which will benefit diabetic patients than oral conventional form. As the solubility has been increased in polymeric nanoparticles it may increase the oral bioavailability of Gliclazide.

Comparative *in vitro* drug release evaluation was done by comparing optimized batches of the polymeric nanoparticles with marketed immediate release (IR) and sustained release (SR) forms in order to know the viability of the nanoparticles in the treatment. GL-EU (1:1) has shown a similar release profile to that of the marketed sustained release form. IR dosage form has shown 100% drug release with in 2 hrs, which elevates the importance of sustained release and also shows the drawback of the IR in providing prolonged treatment. SR dosage form has shown that drug release was more in the first six hours (52±0.19%) and there after sustained the drug release till 72 hours (82.18±0.23%) which may be attributed to the fact that drug may have reached the plateau phase. GL-EU (1:1) has shown better drug release albeit slightly when compared to the SR, which can prove the fact that it can be used as an alternative to the already available marketed SR in the treatment of diabetes mellitus.
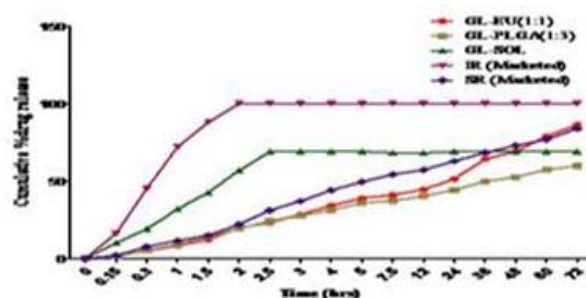

Figure 7: *In vitro* drug release of nanoparticles in comparison with GL-SOL, Marketed IR and SR forms

CONCLUSION

It can be evident from the study that gliclazide was successfully encapsulated in both PLGA and Eudragit polymer by solvent evaporation method. Eudragit PNP's (drug: polymer ratio of 1:1) yielded more entrapment efficiency, drug content and cumulative drug release when compared to the PLGA polymeric nanoparticles. *In vitro* release concluded that GL-EU PNP's exhibited biphasic pattern with initial burst release, which was followed by sustained release that fulfilled our objective. We can conclude that the formulated nanoparticles improved drug release contributes to increased oral absorption of the drug, which can enhance its oral bioavailability. Also the polymeric nanoparticles has shown better drug release and also similar sustained pattern in comparison with marketed IR and SR dosage forms which concludes the viability of the drug in the market and concludes that it can be also used as an alternative for the already existing marketed sustained form.

These changes benefit the patient in decreasing the dosing frequency and dose that can be administered. So we can conclude that nanoparticles prepared by this method using the same polymer with the optimized ratio can represent as potential drug delivery approach for effective delivery of the active pharmaceutical ingredient.

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