

Preparation and Characterization of Biodegradable Glimepiride Loaded PLA Nanoparticles by o/w Solvent Evaporation Method Using High Pressure Homogenizer: A Factorial Design Approach

Mokale VJ, Naik JB*, Verma U, Patil JS and Yadava SK

Department of Pharmaceutical Technology, University Institute of Chemical Technology, North Maharashtra University, Maharashtra, India

***Corresponding author:** Naik JB, Department of Pharmaceutical Technology, University Institute of Chemical Technology, North Maharashtra University, Jalgaon, 425 001, Maharashtra, India, Fax: +91257 2258403, Tel: +91257 2258441, E-mail: jitunaik@gmail.com

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ABSTRACT

Glimepiride is used for the treatment of type 2 diabetes mellitus, one of the third generation sulfonylurea drugs having poor aqueous solubility, slow dissolution rate and low elimination half life. The Aim of the present study was to prepare Glimepiride loaded poly-D, L-lactide (PLA) Nanoparticles by o/w solvent evaporation method using high speed homogenizer. Prepared Nanoparticles were characterized for the drug content, encapsulation efficiency. Physical state of the drug, polymer and Nanoparticles were determined by Particle size distribution, differential scanning calorimetry (DSC), X- ray diffraction (X-RD), Fourier transform infrared spectroscopy (FTIR). Particle morphology was observed by FE-SEM. The results of this study showed the potential of the O/W emulsion for drug delivery application. Drug content and Encapsulation efficiency were found to be 40.27 and 80.55% respectively and particle size of the Nanoparticles was 442 nm (dnm). DSC and XRD study showed partial interaction between the drug and polymer.

Drug release of optimized batch showed 73.72% to 78.12% drug released up to 12 hrs and optimized formulation followed first order kinetics. From this study we can conclude that the Glimepiride loaded PLA nanoparticles were successfully prepared and these nanoparticles seem to be promising for sustained drug release application leading to improved patient compliance.

Introduction

According to the biopharmaceutical classification system, approximately 40% of drugs in the industry pipeline belongs to the BCS class II having low aqueous solubility and high permeability and class IV having low solubility and low permeability. Drugs belong to these classes have low bioavailability [1-3]. Therefore, these drugs possess formulations and delivery problems [4]. For the treatment of diabetes, type 2, the use of oral anti-diabetic drugs increases rapidly [5]. Glimepiride is used for the treatment of type 2 diabetes, one of the third generation sulfonylurea drug having a poor aqueous solubility, slow dissolution rate and low elimination half-life (2-3 hrs) [6]. Glimepiride have a number of advantages over other members of sulfonylurea, currently in the market such as lower dosages, fast onset of action and lower C-peptide level of insulin, this is because of less secretion of insulin and more pronounced extra pancreatic effects [7]. Glimepiride acts by binding to the specific site on pancreatic β -cells and block the ATP-Dependent potassium channels to stimulate the insulin release. Due to the short elimination half-life frequent dosing is required, which leads to the adverse effects such as headache and gastrointestinal disorders [8].

Due to its short elimination half-life repeated doses are required which may cause different side effects to avoid the repeated dosing and enhance the bioavailability of glimepiride sustained release nanoparticles were developed. Nanotechnology is promising application in drug delivery system that accounts for the main part of nanomedicine [9]. There are number of biocompatible polymers are available in the market such as ethyl cellulose, PLA, poly (lactic-co-glycolic acid) (PLGA), poly-ε-caprolactone (PCL) and poly glycolic acid (PGA) for the formulation of sustained as well as controlled release nanoparticles [10]. Of these, PLA is more suitable polymer for sustaining the drug release and is used for the encapsulation of many therapeutic agents due to their biocompatibility, biodegradability, low toxicity, high mechanical strength and slow drug release [11].

Factorial analysis (FA) has been widely adopted for optimization of various parameters in drug formulation and development. This is a collection of statistical and mathematical techniques useful for developing, improving, and optimizing processes in which a response of interest is influenced by multiple variables and the objective is to optimize this response. Optimization of drug encapsulation efficiency by utilizing FA may allow more comprehensive analysis of the interactions between the experimental variables than a single-factor or two factor experimental designs [12,13]. Consequently, this could lead to a better understanding. Apart from that, it also reduces the number of experimental runs required to generate statistically-validated results [14,15].

In recent years, several approaches have been investigated for the development of nano range drug delivery system. Method of preparation of nanoparticle can be classified into the top-down and bottom up processes [3]. Top-down process involves size reduction of the larger particle into the micrometre or nano range. In this process size reduction of the particle can be achieved by milling, high pressure homogeniser or micro fluidization [16] where as in bottom up process, the drug nanoparticles are formed from molecules in a solution or in an emulsion. Emulsion evaporation, solvent displacement, solvent diffusion and rapid freezing have been widely used to prepare drug nanoparticles. Nanoparticle produced by top down process are generally crystalline in nature and require high energy and high pressure for the development of nano size particles, there is a chance of contamination if a milling medium used. Whereas bottom up process involves dissolution followed by precipitation or drying [17-19].

In the present study Glimepiride loaded PLA Nanoparticles were prepared by an o/w solvent evaporation method using a high pressure homogenizer to overcome the dosing frequency and side effects.

Experimental

Materials

Glimepiride was obtained as a gift sample from Wockhardt Research Centre, Aurangabad, Maharashtra, India. Poly-D, L-lactide (PLA) was purchased from Merck specialties Pvt. Ltd. Dichloromethane (DCM) and Acetone were purchased from Merck specialties Pvt. Ltd. and RFCL limited, India respectively. Poly vinyl alcohol was purchased from S.D fine-chem. Ltd, India. All other chemicals and reagents were of analytical grade and used as provided.

Methods

Factorial design: A number of preliminary experimentations were conducted to determine the formulation parameters and conditions at which the process to yielded. To optimize the formulation a 3^2 full factorial design was applied for the preparation of inclusion complex using Design-Expert® Software (Version-8.0.7.1, Stat-Ease Inc., Minneapolis,) which allows evaluation by nine experiments in order to limit the number of experiments. Response surface methodology (RSM) was used for the analysis of results. The amount of PLA used as release retarding polymer (X_1 ,mg) and PVA as a surfactant (X_2 , %w/v) with respect to drug was selected as independent variables. Such statistical models were used to evaluate the effect of independent variables on the dependent variables like encapsulation efficiency (Y_1 , %), practical drug loading (Y_2 , %). The actual and coded values of independent variables are shown in Table 1 along with their low (-1), medium (0) and high levels (+1). The significance of the model was determined by the comparisons of statistical parameters, and the best model (suggested) was decided based on reasonable agreement between adjusted R^2 and predicted R^2 ; higher values of adjusted R^2 and predicted R^2 ; model p value (should be less than 0.05). Two-dimensional (2D) contour plots and three-dimensional (3D) response plots resulting from the equations were constructed using Design-Expert® software [19,20].

Preparation of nanoparticles: Nanoparticles containing anti-diabetic drug as a core material were prepared by O/W solvent evaporation method [21,22]. Accurately weighed quantity of drug (50mg) was dissolved in DCM & acetone (5ml each) and polymer in DCM (10 ml) separately and added into the aqueous phase (100 mL distilled water) containing surfactant 0.45% PVA using a high speed homogenizer (OMNI International TH) at 20000 rpm for 20 min and 35000 rpm for 3 min at 4 °C, by adding organic phase into aqueous phase. Then emulsion was passed through the high pressure homogenizer at a pressure of 500 bar for one cycle. The emulsion was kept on lab stirrer (Remi, Mumbai) at 1000 rpm for 3 hrs at room temperature for the evaporation of organic solvent, after that the nanoparticles were collected by centrifugation for 5 minutes at 10000 rpm (Remi, Mumbai). PVA is a water soluble surfactant, during centrifugation it was removed along with decant. Trace amount of PVA present in nanoparticles were removed by washing with distilled water. After washing nanoparticles were lyophilized for 48 hrs. To minimize the potential risk in patients treated with sustained release glimepiride the lowest effective dose should be used for the shortest possible duration [19].

Independent variables	Level used (actual, coded)			
	Low actual	High actual	Low coded	High coded
X_1	50	150	-1.00	1.00
X_2	0.15	0.45	-1.00	1.00

Table 1: Variables in a 3^2 full factorial design

Characterization of Nanoparticles

Drug loading (DL %) and encapsulation efficiency (E.E. %)

The amount of Glimepiride encapsulated into nanoparticle was determined by UV spectrophotometer (HITACHI U-2900, Tokyo, Japan). An accurately weighed 10 mg of nanoparticles were stirred with dichloromethane (5 ml) to dissolve the polymeric coat and extracted in phosphate buffer solution (pH 6.8). Stirring continued for 30 min at 30-40 °C to facilitate an evaporation of organic solvent. The drug content was determined in the filtrate after appropriate dilution with phosphate buffer solution at 226 nm using UV-spectrophotometer. The drug content and encapsulation efficiency of Glimepiride were calculated by using the following equations:

$$\% \text{ Drug Loading} = [\text{drug determined} / \text{actual drug added}] \times 100 \quad - \text{ (I)}$$

$$\% \text{ Encapsulation efficiency} = [\text{actual quantity} / \text{theoretical quantity}] \times 100 \quad - \text{ (II)}$$

Differential scanning calorimetry (DSC)

Thermal behavior of the sample was determined by Differential Scanning Calorimetry (DSC-60, Shimadzu & 821, Mettler Toledo). Accurately weighed samples (5-10 mg) were sealed in an aluminium pan and scanned at a temperature range of 30 °C to 400 °C at the rate of 10 °C/min under dry nitrogen atmosphere purge of 50mL/min.

X-ray diffraction (XRD)

X-ray diffraction analysis of sample was carried out to characterize the physical form i.e. amorphous or crystalline nature of glimepiride in sample of optimized batch in an X-ray diffractometer (D8 Advance, Bruker) with Cu K α radiation ($\lambda=1.54060$ Å). The scanning rate 10°/min and diffraction angle 2θ was 10-80°.

Particle morphology by Field Emission Scanning electron microscopic (FE-SEM)

The morphology of the samples was carried out using scanning electron microscopy (FE-SEM, Type-II, Model-S-4800, Hitachi, Japan). The surface morphology was analyzed at a working distance of 8.7-8.8 mm and 1.0 KV accelerating voltage.

Particle size distribution and Zeta Potential

The particle size distribution and zeta potential was determined in water as a dispersion medium by laser diffraction size analyzer, Malvern Zetasizer (Model: ZS 200).

Fourier transforms infrared spectroscopy (FTIR)

Drug polymer interactions were studied by using an FTIR spectrophotometer (Shimadzu, FTIR-8400). FTIR analysis of the pure drug, polymer and nanoparticles of glimepiride was carried out by KBr pellet method. The sample was mixed with KBr and compressed into a disc in a manual press. The spectrum was scanned from 4000 to 400 cm^{-1} .

In-Vitro drug release studies

Drug release studies of the nanoparticles were performed, in a Tablet Dissolution Test Apparatus, Type-II (Paddle method, Electrolab, TDT 06) at 37 ± 0.5 °C and at a paddle speed of 75 rpm. The release studies were carried out in a 900 mL dissolution medium of pH 1.2 for the first 2hrs, and continued in phosphate buffer pH 6.8 up to 12 hrs. Change in pH was made by the addition of 0.2M tribasic sodium phosphate. All Dissolution media was contained 0.2% Sodium lauryl sulfate. 5 mL sample was withdrawn from the dissolution apparatus at different time intervals and filtered through membrane filter. The drug content was determined at 226 nm by Double beam ultraviolet spectrophotometer. The withdrawn sample was replenished with 5mL of fresh media.

Kinetic analysis of drug release profiles

To study the drug release mechanism from sustained release nanoparticles the release data were fitted to zero-order kinetics, first-order kinetics [19], Higuchi model and Korsmeyer-Peppas kinetics models [23]. The kinetic model with the highest value of the coefficient of correlation (r^2) was considered to be a best fit model for describing the release of glimepiride from the nanoparticles [24].

Results

Statistical Analysis of Data

Using 3^2 factorial design, a total of nine runs was carried out for the preparation of inclusion complex and investigate the effects of two independent variables on the dependent variables (responses) using factorial design. The quadratic mathematical model (suggested) generated by 3^2 factorial design was used to evaluate the responses.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_2 + \beta_4 X_1^2 + \beta_5 X_2^2 \quad (3)$$

Where, β_0 is the intercept; β_1 to β_5 are the estimated coefficient obtained from the observed experimental values of Y; X_1 and X_2 are the coded levels of the factor. The coefficient corresponding interaction ($X_1 X_2$) and the quadratic effects (X_1^2 and X_2^2) were determined from the results of the experiments. Results of all the nine experiments carried out are summarized in Table 2. A study showed that the formulation parameters had an influence on the encapsulation efficiency and solubility of FMT in complex. The polynomial model describing the correlation between the formulation variables and the response can be represented by the following equation.

$$Y_1 = 73.22 - 0.083967X_1 + 39.3X_2 - 0.8575X_1 X_2 + 3.2066X_1^2 + 1.0266X_2^2 \quad (4)$$

$$Y_2 = 55.87 - 0.43397X_1 - 8.9166X_2 - 0.171X_1 X_2 + 0.0012X_1^2 + 102.66X_2^2 \quad (5)$$

Experimental run order	Variables				Theo. DL(%)
	Independent variables		Independent variables		
	X ₁	X ₂	Y ₁	Y ₂	
1	50	0.15%	65.22%	32.50%	50%
2	50	0.30%	70.37%	35.38%	50%
3	50	0.45%	80.55%	40.27%	50%
4	100	0.15%	61.72%	20.505	33.30%
5	100	0.30%	63.95%	21.20%	33.30%
6	100	0.45%	66.40%	22.27%	33.30%
7	150	0.15%	52.96%	13.24%	25%
8	150	0.30%	56.28%	14.07%	25%
9	150	0.45%	61.11%	15.37%	25.00%

Note: X_1 = PLA (mg) and X_2 = PVA (%), Y_1 = Encapsulation Efficiency, Y_2 = Practical DL
Theo. DL= Theoretical drug loading

Table 2: Drug Loading (DL) and Encapsulation Efficiency (E.E.) of Glimepiride Loaded PLA Nanoparticles

The equation represents the quantitative effects of factor (X_1 and X_2) upon the responses (Y_1 and Y_2). The sign of the coefficient shows how the factor influences the response. If the coefficient is positive, the response is increased (synergistic effect) as the factor moves from low level (-1) to high level (+1); the contrary is obtained (inverse relationship/antagonist effect) if the coefficient is negative. Linear, cross-product contribution (2FI), quadratic and cubic models were generated for the responses by the software. The quadratic model showed a best fit for the responses. Table 3 showed the model summary statistics of responses. Data in Table 4 showed the coefficient estimate and p values of each factor for the measured responses. Significant values indicated in bold faces. Significant factors affecting the response Y_1 were X_1 (amount of PLA as a polymer p value 0.0009), X_2 (amount of surfactant, p value 0.0058), and quadratic term X_2^2 (p value, 0.050). Significant factors affecting the response Y_2 were X_1 (p value, <0.0001), X_2 (p value, 0.0066), and quadratic term X_2^2 (p value, 0.0097). To validate the model, all the points were selected and observed their experimental and predicted value for the responses. Therefore, it can be concluded that the model is best suitable because of the difference between experimental and predicted value is very low.

Drug loading and encapsulation efficiency (% E.E.)

Drug loading and encapsulation efficiency of different experimental runs of Glimepiride nanoparticles are given in Table 2. Glimepiride loaded PLA nanoparticles showed practical drug loading 40.27% and 80.55% encapsulation efficiency respectively which is shown in Figure 1. From the study it was observed that the increase in PVA concentration increases the %E.E and %DL but decreases with increase in polymer concentration. This may be due to the decrease in interfacial tension with increase in PVA concentration and also increases the surface area of PLA nanoparticles, which leads to the more diffusion of the drug from the nanoparticles and it may decrease the %E.E., but in our findings it increased due to the free drug available on the surface of nanoparticles in place of entrapment on the nanoparticles. While %E.E. decreases with an increase in the amount of polymer concentration, this may be due to the more time taken for the precipitation of polymer which was in higher amount [25]. Among the nine formulations, Run R_3 and R_9 are the optimized formulations in terms of %E.E. and % DL.

Response	Model	Standard deviation	R ²	Adjusted R ²	Predicted R ²	Significance
Y ₁	Linear	1.86	0.9613	0.8970	0.5296	
	2FI	1.73	0.9316	0.8906	0.7209	-
	Quadratic	1.68	0.9043	0.8725	0.7602	Suggested
	Cubic	0.11	0.9999	0.9995	0.9896	-
Y ₂	Linear	2.13	0.9635	0.9514	0.9139	-
	2FI	2.21	0.9675	0.948	0.8333	-
	Quadratic	0.76	0.9976	0.9937	0.9727	Suggested
	Cubic	0.47	0.9997	0.9976	0.9460	-

Table 3: Model summary statistics of responses Y1 and Y2

Response	Y ₁		Y ₂	
	Coefficient of estimate	p value	Coefficient of estimate	p value
X ₁	-4.98	0.0009	- 10.88	<0.0001
X ₂	3.93	0.0058	1.5	0.0066
X ₂ ²	3.93	0.0500	1.5	0.0097

Table 4: Coefficient estimate and p values of each factor for the measured responses Y₁ and Y₂

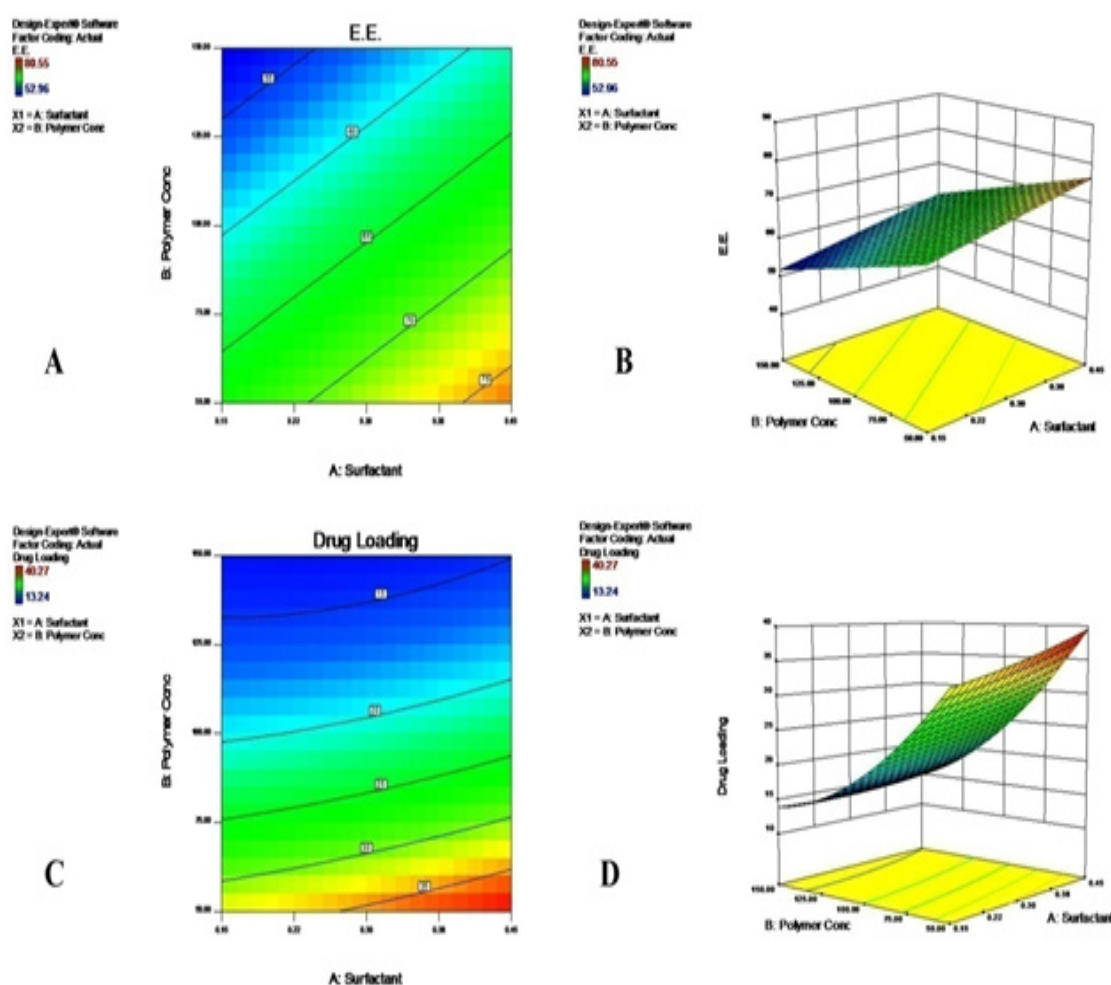


Figure 1: A) Counter graph of Entrapment Efficiency, B) 3D graph of Entrapment Efficiency, C) Counter graph of Drug Loading D) 3D graph of Drug Loading of Glimepiride loaded PLA nanoparticles respectively.

Differential scanning calorimetry (DSC)

DSC thermo gram of pure drug, polymer and an optimized batch of nanoparticle are shown in Figure 2. Thermogram of pure drug showed a sharp endothermic peak at 234.13 °C which corresponds to melting point of the drug, which is same as reported in literature. A result of DSC analysis confirms the identity and purity of the drug. Glass transition temperature of the pure PLA polymer ranges from 150 to 160 °C. Pure PLA polymer showed T_g at 141.63 °C and Glimepiride loaded PLA nanoparticle show an endothermic peak at 262.48 °C during the DSC analysis. There was an increase in endothermic peak in nanoparticle as compared to pure drug, suggesting that the interaction between the drug and polymer occurs which is due to changes in physical form of crystalline to amorphous state [26].

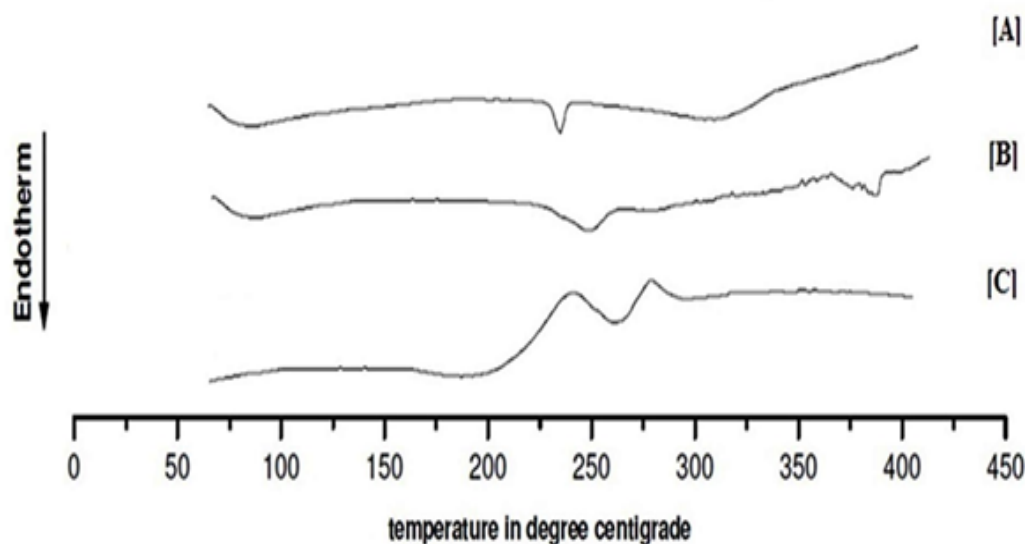


Figure 2: A) DSC of pure Glimepiride, B) DSC of pure PLA polymer and C) DSC of Glimepiride nanoparticles embedded in the PLA matrix.

X-ray diffraction (XRD)

X-RD patterns of pure drug, polymer and nanoparticles were shown in Figure 3. The diffraction spectra of pure drug showed sharp and intense peaks of crystallinity. On the other hand X-RD pattern of nanoparticles prepared by the o/w solvent evaporation method showed reduction in number and intensity of the peaks in comparison to the pure drug indicates that the decrease in crystallinity or slightly change in amorphous nature of the drug [27].

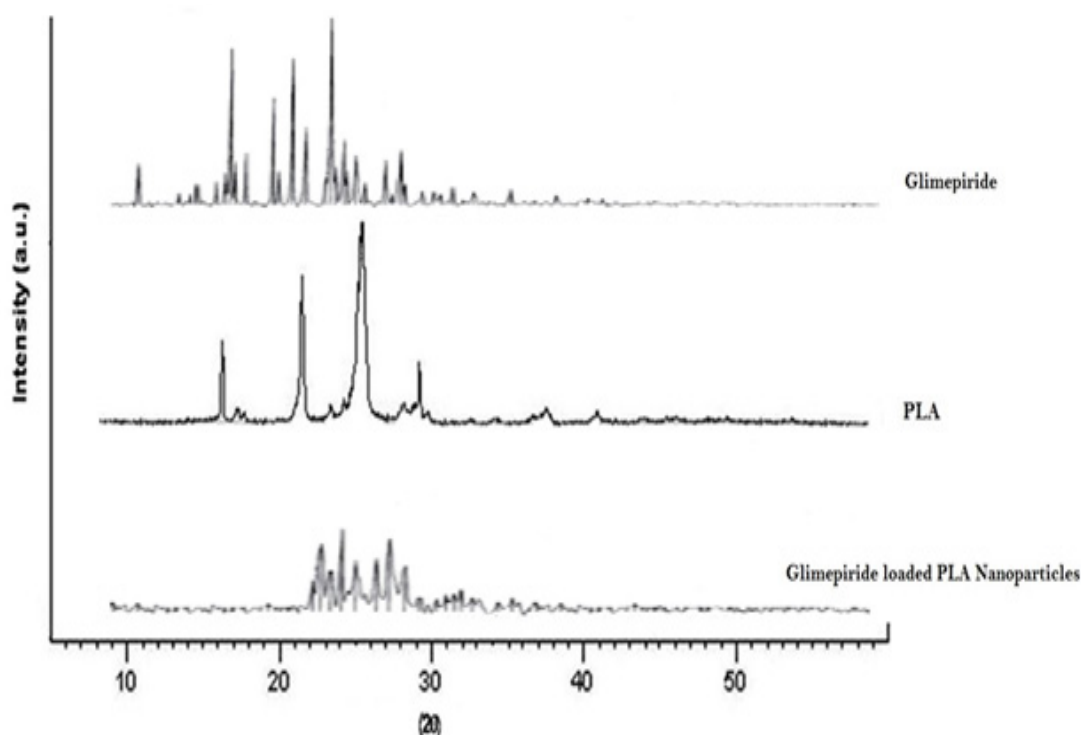


Figure 3: XRD pattern for pure Glimepiride, pure PLA polymer, and Glimepiride Loaded PLA Nanoparticles

Particle morphology by Field Emission Scanning electron microscopic (FE-SEM)

The field emission scanning electron micrographs (FESEM) of the nanoparticle prepared by the o/w solvent evaporation method and were found to be spherical in shape Figure 4.

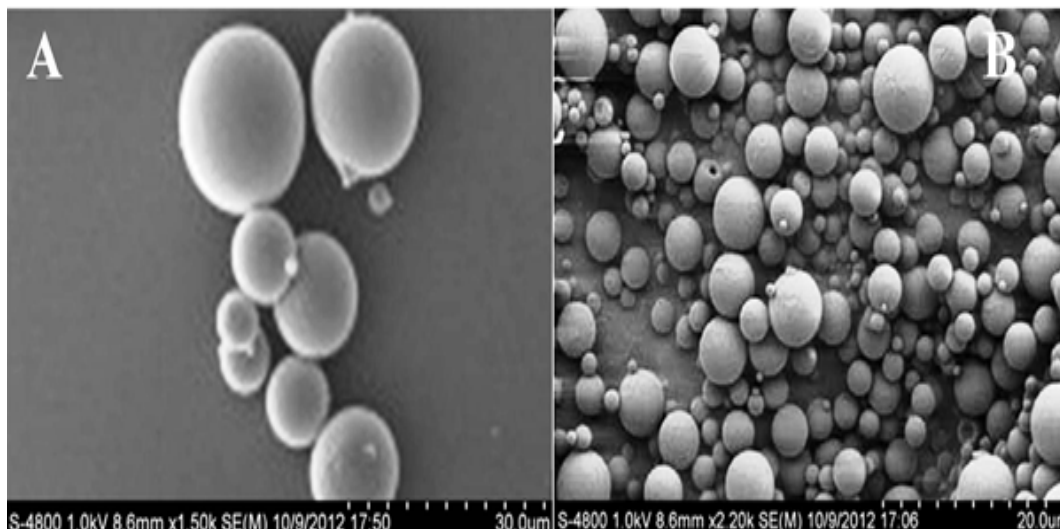


Figure 4: A) and B) FE-SEM Image of Glimepiride loaded PLA-NP

Particle size distribution and Zeta potential

Particle size distribution and zeta potential of Glimepiride loaded PLA Nanoparticles formulation was found to be 442.2(d. nm.) and Zeta potential -31.2mV. Zeta potential value was found to be in the range of ± 30 to ± 40 in optimised formulation (R_3 and R_9) signifying an increase in stability of glimepiride loaded PLA nanoparticles. Result of particle size and Zeta potential distribution was shown in Figure 5a and 5b.

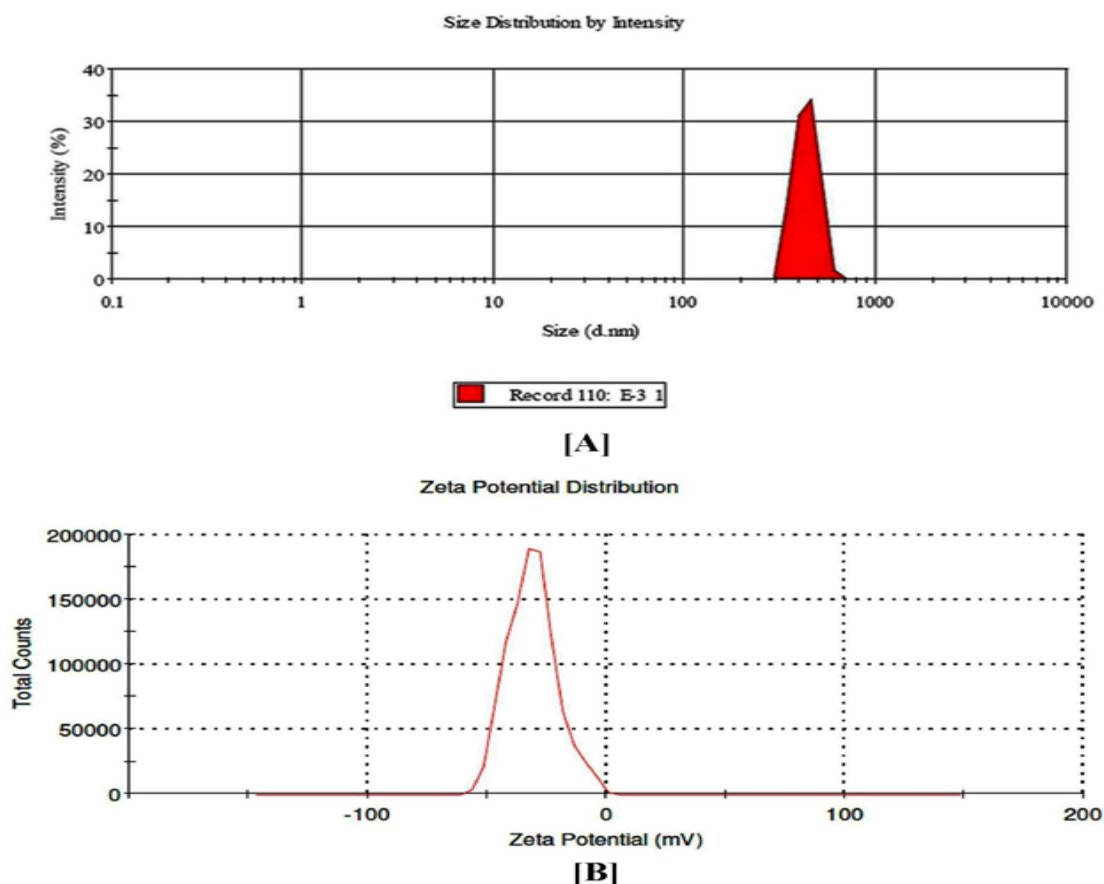


Figure 5: Graph [A] For average particle size (d.nm) determination of Glimepiride-loaded PLA polymeric nanoparticles and [B] Zeta Potential Distribution of PLA nanoparticle

Fourier transforms infrared spectroscopy (FTIR)

FTIR spectra of pure drug, polymer, and Glimepiride loaded PLA nanoparticles of optimized formulation are shown in Figure 6. FTIR spectra of pure drug showed characteristic peaks at 3369 cm^{-1} and 3288 cm^{-1} corresponding to N-H stretching, 1707 cm^{-1} and 1674 cm^{-1} due to carbonyl stretching, 1345 cm^{-1} showing C-N stretching, 1153 cm^{-1} showing S=O stretching vibration. FTIR spectrum of different batches of glimepiride shows decrease in the intensity of N-H and S=O stretching vibration of glimepiride.

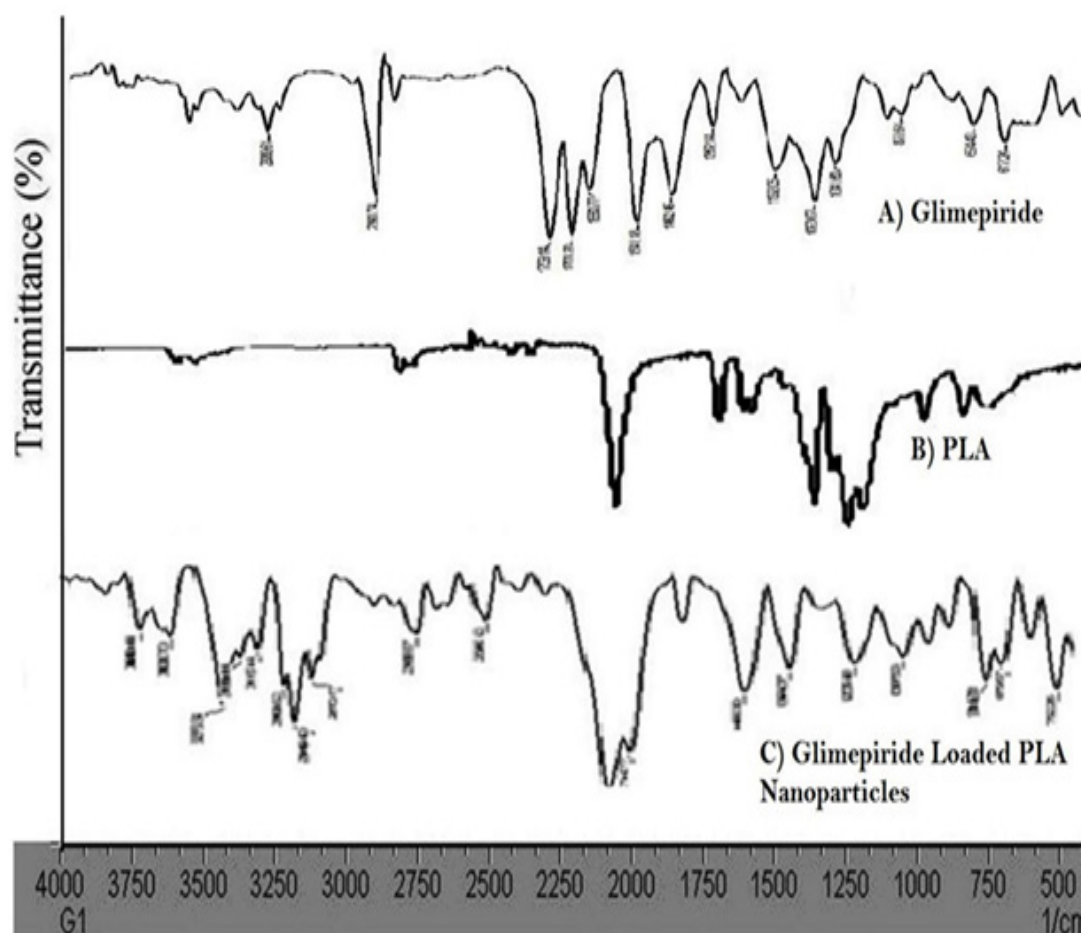
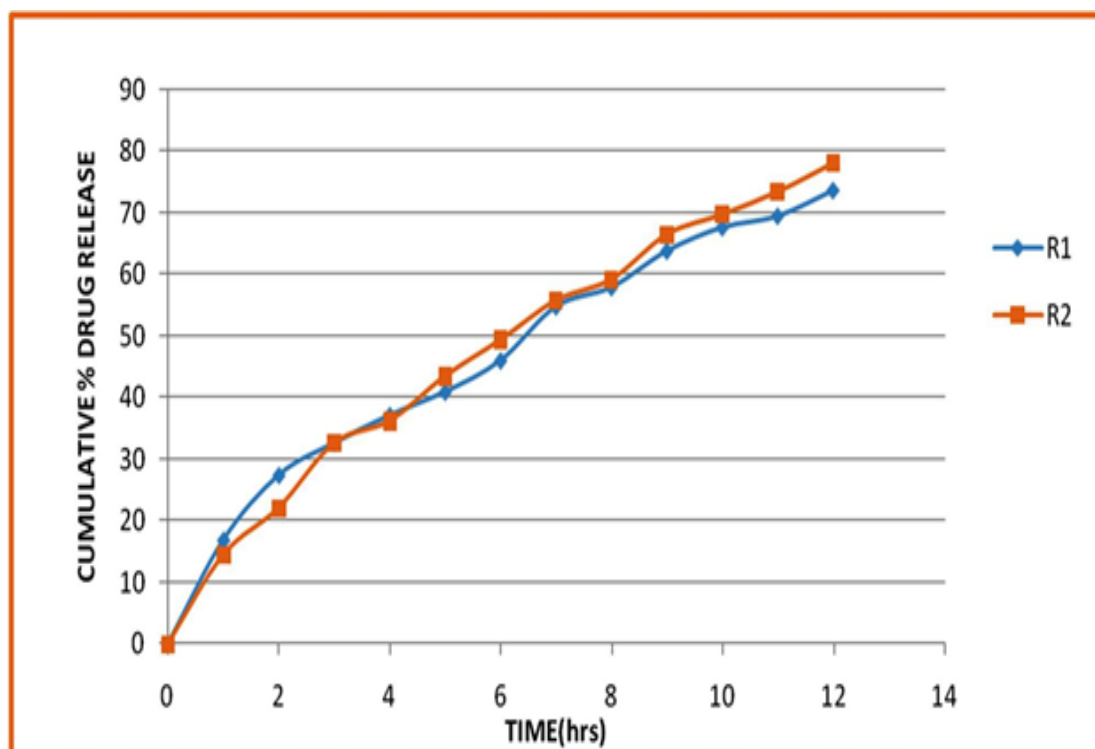


Figure 6: FT-IR spectra of A) pure Glimepiride, B) pure PLA polymer and C) Glimepiride nanoparticles embedded in the PLA matrix.

In vitro drug release studies

In vitro drug release studies of glimepiride loaded PLA nanoparticles were performed to determine the percentage of drug released from the nanoparticles in gastric fluid and intestinal fluid. The *in vitro* drug release study of optimized formulation (R_3 and R_9) with same concentration surfactants was performed for the first two hours at pH 1.2 and at phosphate buffer pH 6.8 for next 12 hrs. The release patterns of glimepiride from nanoparticles are shown in Figure 7. The drug released from the nanoparticles was found to be in the range of 14.46 to 16.89 % in first 2 hrs at pH 1.2 and 73.72 to 78.12% up to 12 hrs at pH 6.8. From the drug release behavior it was observed that if the polymer concentration increase drug release decreases due to the high viscosity of PLA which on contact with the dissolution medium, surface of nanoparticles become wet and forms viscous gel layers. As the concentration of PLA increases viscosity of the gel layers increases while the diffusion coefficient of drug decreases [28,29].

To study the release behavior of glimepiride loaded PLA nanoparticles the release data were fitted to the various kinetic models such as a zero - order, first-order, Higuchi and Korsmeyer-Peppas kinetics models. In this present study *in-vitro* drug release profile of the optimized batch (R_2) followed first-order kinetics and plot shows highest regression coefficient (r^2 0.9915). The slope (n) value obtained from Korsmeyer-Peppus model was found to be less than 0.89 indicates drug releases by diffusion mechanism [30].



Notes: R1 – cumulative % drug release drug- polymer ratio (1:1). R2 – cumulative % drug release drug-polymer ratio (1:3)

Figure 7: % cumulative drug release from sustained release Glimepiride-Loaded PLA Nanoparticles

Conclusion

Glimepiride loaded PLA nanoparticles were successfully prepared by an o/w solvent evaporation method using a high pressure homogenizer with encapsulation efficiency of 80.55% and drug loading 40.27%. DSC and FT-IR study showed the partial interaction with drug and polymer due to the hydrogen bonding between N-H group and carbonyl group. A slow drug released from the glimepiride loaded PLA nanoparticles was observed and particle size 442.2 nm (denim) can make the suitable candidate for the further development of nano medicines.

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