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Formulation and evaluation of poly (L-lactide-co- -caprolactone) loaded gliclazide biodegradable nanoparticles as a control release carrier

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Abstract

A biodegradable nanoparticle has been used frequently as drug delivery carrier due to its better encapsulation capacity, sustained/ control release property and less toxicity. Gliclazide (GLZ) is a second generation of hypoglycemic sulfonylurea and acts selectively on pancreatic ß cell to control diabetes mellitus. The objective of this study was to produce controlled release nanoparticles of Gliclazide using poly (L-lactide-co- -caprolactone) (PLCL). The method was optimized using design of experiments by employing a 3-factor, 3-level Design Expert (version 8.0.7.1) Statistical Design Software and was subjected to various characterization studies including Field Emission Scanning Electron Microscopy (FE-SEM), X-ray diffraction (XRD), Encapsulation efficiency (%EE), Particle Size Distribution (PSD), etc. Formulated nanoparticles were also subjected to Fourier Transform Infrared Spectroscopy (FT-IR), Differential Scanning Calorimetry (DSC) for studying interaction between drug and polymer and the effect of lyophilization (Freeze Drying) on developed nanoparticles. The release profiles and encapsulation efficiencies are depended on the concentration of PLCL. These data demonstrated the efficacy of the biodegradable polymeric nanoparticles in controlling the gliclazide drug release profile as novel drug delivery system.

Keywords: Poly (L-lactide-co- -caprolactone), Gliclazide, Biodegradable Nanoparticles, Lyophilization, High Pressure Homogenization

Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by high blood glucose concentrations (hyperglycemia) caused by insulin deficiency and it is often combined with insulin resistance[1]. Non-insulin-dependent diabetes mellitus (NIDDM) is a heterogeneous disorder, comprising milder forms of diabetes that occur predominately in adults. Most diabetic patients have NIDDM[2]. Gliclazide 1-(1-azabicyclo- [3,3,0]-oct-3-yl)-3-(*p*-tolylsulfonyl urea) is a potential second generation oral hypoglycemic agent widely used in the treatment of NIDDM. A full dose of GLZ is required before each meal; hence, therapy may become inconvenient[3].

Nanoparticles (including nanospheres and nanocapsules of size 10-200 nm) are in the solid state and are either amorphous or crystalline. They are able to absorb and/or encapsulate a drug, thus protecting it against chemical and enzymatic degradation. Nanocapsules are vesicular systems in which the drug is confined to a cavity surrounded by a unique polymer membrane, while nanospheres are matrix systems in which the drug is physically and uniformly dispersed. Nanoparticles as drug carriers can be formed from both biodegradable as well as non-biodegradable polymers. In recent years, biodegradable polymeric nanoparticles have attracted considerable attention as potential drug delivery devices in view of their applications in the controlled release of drugs, in targeting particular organs / tissues, as carriers of DNA in gene therapy, and in their ability to deliver proteins, peptides and genes through the peroral route [4]. For biodegradable polymers, researchers found that the degradation rate of these nanoparticles is nearly constant for a given set of experimental conditions, ideal for the controllable release of drugs. It is helpful to note that the degradation of these nanoparticles in dispersion is different from that of a bulk material [5]. Biodegradable polymers offer a novel approach for developing controlled/sustained release drug delivery systems that are simple and convenient to patient[6]. Controlled drug delivery take place when a polymer, whether natural or synthetic, is sensibly combined with a drug or other active agent in such a way that the active agent is released from the material in a predesigned manner [7]. Biodegradable polymers such as poly (glycolic acid), poly (lactic acid) (PLA) and their copolymers, poly (p-dioxanone), PLCL and copolymers of trimethylene carbonate and glycolide have been used in a number of clinical applications [8]. Solvent evaporation method to formulate micro-spheres of poly (lactic acid) (PLA), and its co-polymer poly (lactic-co-glycolic acid) (PLGA) has been studied extensively due to the biocompatibility of these polymers. The method of microencapsulation by solvent

evaporation is widely applied in pharmaceutical industries to obtain the controlled release of drug[9-12]. The formulation of biodegradable nanoparticles offers many advantages over conventional oral dosage forms. Drugs can be delivered in a sustained and continuous manner, encapsulated drugs are protected in the polymer network from gastric and enzymatic degradation and daily administration may not be required[13]. Controlled release systems (microparticles, nanoparticles, and liposome etc.) are prepared to obtain prolonged or controlled drug delivery, to improve bioavailability and to target drug to specific sites. These systems can also to protect drugs from degradation and reduce the toxicity or side effects [14-15]. Thus, the aim of the present study was to design a novel delivery system to maintain peak plasma levels of GLZ for the long-term management of diabetes mellitus.

Materials and Methods

Materials

Gliclazide was obtained as gift a sample from Wockhardt Research Centre, Aurangabad (M.S.) India. Poly (L-lactide-co- -caprolactone) (PLCL) polymer, Dichloromethane (DCM), hydrophilic surfactant polyvinyl alcohol (PVA) and n-Hexane was obtained from Merk Pvt. Ltd. All other chemicals and materials were of analytical grade and were used as procured.

Preparation of Nanoparticles

Preparation Poly (L-lactide-co- -caprolactone) (PLCL) loaded Gliclazide Nanoparticles by solvent evaporation - high pressure homogenization method (HPH)

Polymer and drug was dissolved in Dichloromethane (DCM), mixed properly till to obtained homogeneous solution, this organic solution was then added into polyvinyl alcohol (dissolved in water) with constant stirring at 1200 rpm at room temperature, oil -in-water (o/w) type emulsion was formed. The formed emulsion were stirred for 4 hr using lab mechanical stirrer (Remi) at 500 rpm and kept overnight for removal of residual organic (DCM) solvent[16].
Formulated microparticles were collected and then subjected to high pressure homogenization. Homogenization was carried out at 400 bars for three cycles. The processed emulsion was evaporated overnight to remove the residual DCM. The final emulsion was centrifuged at about 10000 rpm for 15 min, then kept for Lyophilization (freeze-drying) for 48 hrs [17]. The obtained free flowing nanoparticles were stored in desiccator for further analysis.

Physiochemical Characterization of NPs

Particle size and zeta potential

The average particle size and zeta potential of the Poly (L-lactide-co- -caprolactone) (PLCL) loaded Gliclazide nanoparticles were determined by Particle Size Analyzer (Zetasizer Ver System; Serial Number: MAL 1051945; Malvern Instruments Ltd, Malvern, UK) at temperature -25 ^{0}C, Count Rate (kcps): 557.2, duration used: 40s, cell Description: Disposable sizing cuvette at Attenuator:11. The result of average particle size is shown in Figure 1.

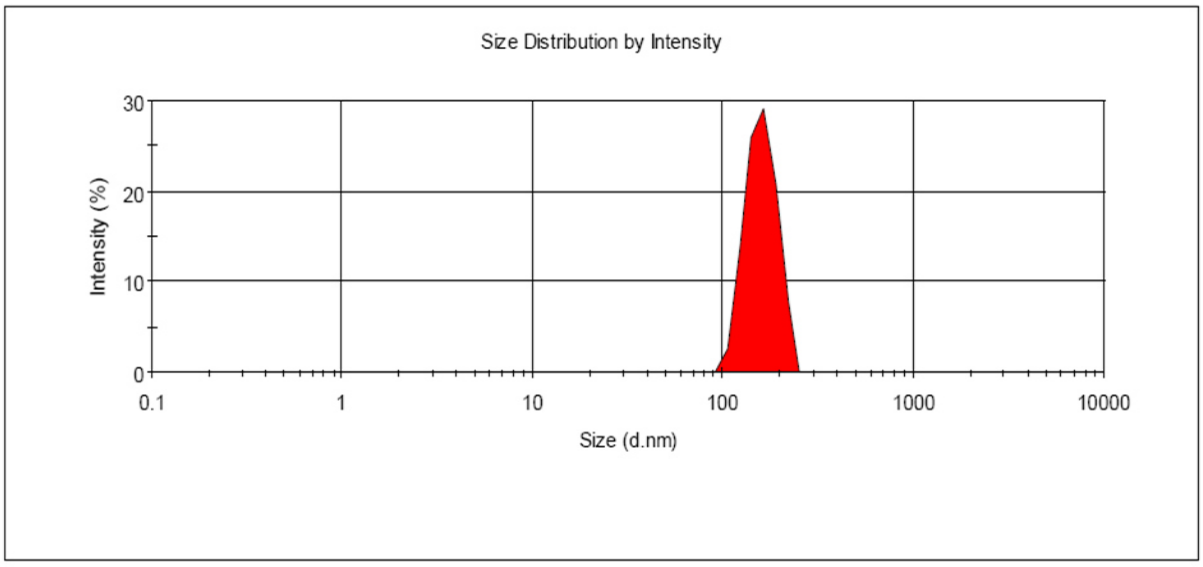

Fig 1: Average particle size

Encapsulation Efficiency

Table 1: Encapsulation Efficiency of Gliclazide nanoparticles embedded in the PLCL matrix

Std.	Run	Drug (mg)	Factor 1 A:Polymer (mg)	Factor 2 B:Surfactant (mg)	Response: Encapsulation efficiency (%)	Drug loading (%)
1	3	100	100	150	70.30	35.15
2	2	100	200	150	73.54	36.70
3	1	100	300	150	81.37	40.68
4	8	100	100	300	68.13	22.68
5	7	100	200	300	71.84	23.91
6	5	100	300	300	78.86	26.25
7	6	100	100	450	60.00	15.00
8	4	100	200	450	66.28	16.57
9	9	100	300	450	68.42	17.10

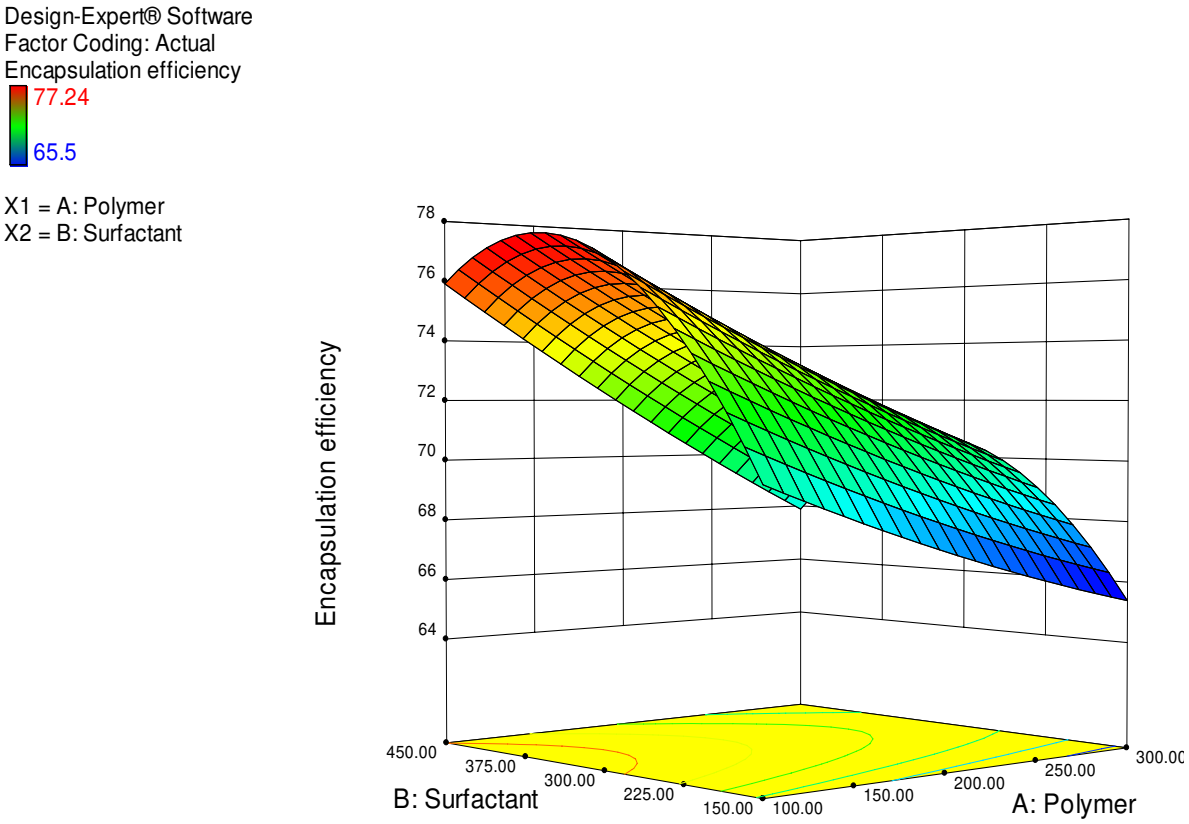

Figure 2: 3-D graph (Map) of Encapsulation Efficiency of fresh O/W emulsions processed by HPH between 100 and 400 MPa (single-stage, single-pass homogenization; T_{in} =24 ^{0}C) for Poly (L-lactide-co- -caprolactone) (PLCL) loaded Gliclazide Nanoparticles

Table 2: Model Summary Statistics

Standard		Adjusted		Predicted			
Source	Dev.	R-Squared	R-Squared	R-Squared	PRESS		p-value
Linear	2.485071167	0.697117899	0.596157199	0.34557724	80.05965		0.0278
2FI	2.585515315	0.726782237	0.562851579	0.051056453	116.0902		0.4943
Quadratic	0.578009836	0.991807123	0.978152329	0.900421091	12.18211	Suggested	0.0052
Cubic	0.088333333	0.999936219	0.999489749	0.988375843	1.422056	Aliased	0.0882

Table 3: Analysis of variance

Sum of			Mean		p-value
Source	Squares	Df	Square	F-Value	
Model	121.3340028	5	24.26680056	72.63435147	0.0025
A-Polymer	56.24281667	1	56.24281667	168.3435978	0.0010
B-Surfactant	29.04	1	29.04	86.92128828	0.0026
AB	3.629025	1	3.629025	10.86224271	0.0459
A^2	0.154938889	1	0.154938889	0.463756468	0.5447
B^2	32.26722222	1	32.26722222	96.58087206	0.0022
Residual	1.002286111	3	0.33409537		
Cor Total	122.3362889	8			

Response: Encapsulation efficiency
ANOVA for Response Surface Quadratic Model

Analytical Techniques.

Morphological examinations of NP were performed using a FESEM (Hitachi, S-4800[Type II] High Technologies Corporation, Japan).

Surface/Internal morphology

The size and morphology of the Gliclazide nanoparticles embedded into a PLCL polymer matrix were determined using a field emission scanning electron microscope (FE-SEM) (Hitachi, S-4800[Type II] High Technologies Corporation, Japan). The powders were previously fixed on a brass stub using double-sided adhesive tape and then were made electrically conductive by coating, in a vacuum, with a thin layer of platinum for 100 s and at 30 W. Photographs were taken at an excitation voltage of 5.0 kV. The particle size and surface morphology of Gliclazide embedded into poly (L-lactide-co- -caprolactone) (PLCL) latex determined by FESEM analysis shown in (Fig. 4).

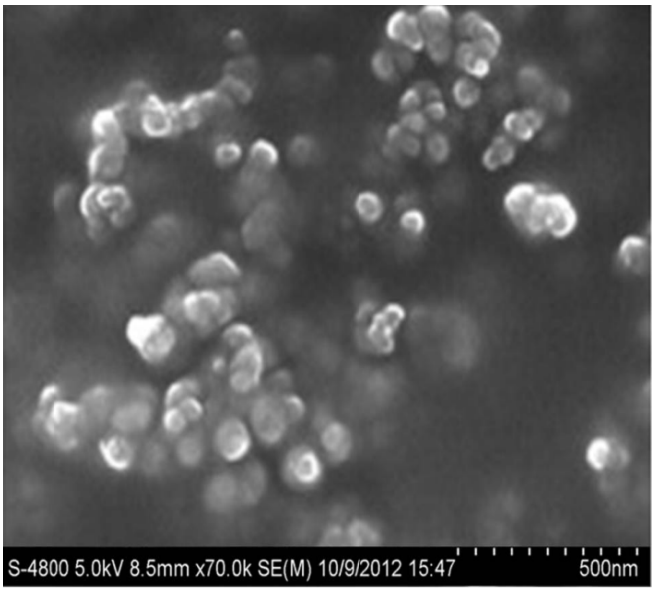

Figure 4: FE-SEM image of Gliclazide nanoparticles embedded in to PLCL matrix.

Differential Scanning calorimetry

Thermo gravimetric analysis was carried out using a DSC, Model-821, Make- Mettler Toledo from 25 to 300 C at a heating rate of 2 C/min under a nitrogen atmosphere (Fig. 5).

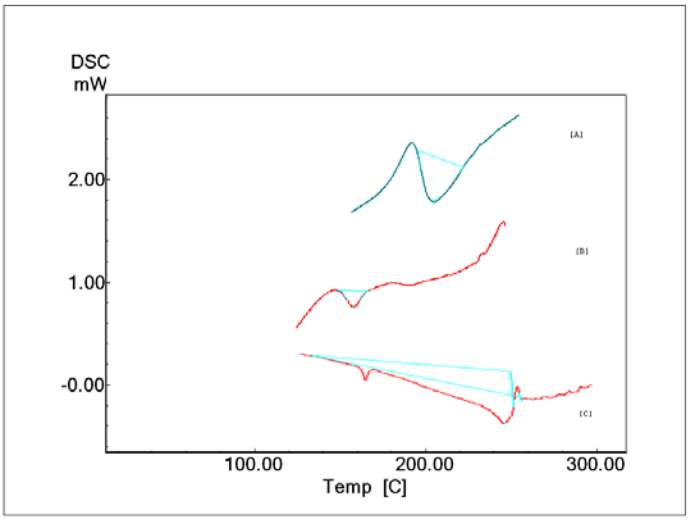

Figure 5: A] DSC of pure Gliclazide, B] DSC of pure PLCL polymer and C] DSC of Gliclazide nanoparticles embedded in the PLCL matrix.

Interpretation of FTIR spectra

The principle peaks of gliclazide were obtained at wave numbers 3271.38, 3115.14, 1708.99 1595.18, 1437.02, 1350.22. (3271.38 cm^{-1}) for N-H stretching, (3115.14 cm^{-1}) for =C-H stretching, (1708.99 cm^{-1}) for O=C stretching, (1595.18 cm^{-1}) for aromatic nucleus, (1437.02 cm^{-1}) for C-H deformation, and (1350.22 cm^{-1}) for SO_2-NH stretching.

X-ray diffraction analysis (XRD)

The state of gliclazide, its physical mixtures and solid dispersions were evaluated with X-ray powder diffraction. Diffraction patterns were obtained using D8 ADVANCE diffractometer with Vario1 Johansson focusing monochromator features high-flux K-alpha-1 radiation with DAVINCI design (Bruker AXS) with a radius of 240 mm. The Cu K radiation (K 1.54060 Å) was Ni filtered. A system of diverging and receiving slits of 1 and 0.1 mm, respectively, was used. The pattern was collected with 40 kV of tube voltage and 40 mA of tube current and scanned over the 2θ range of 5–60 (Fig. 7).

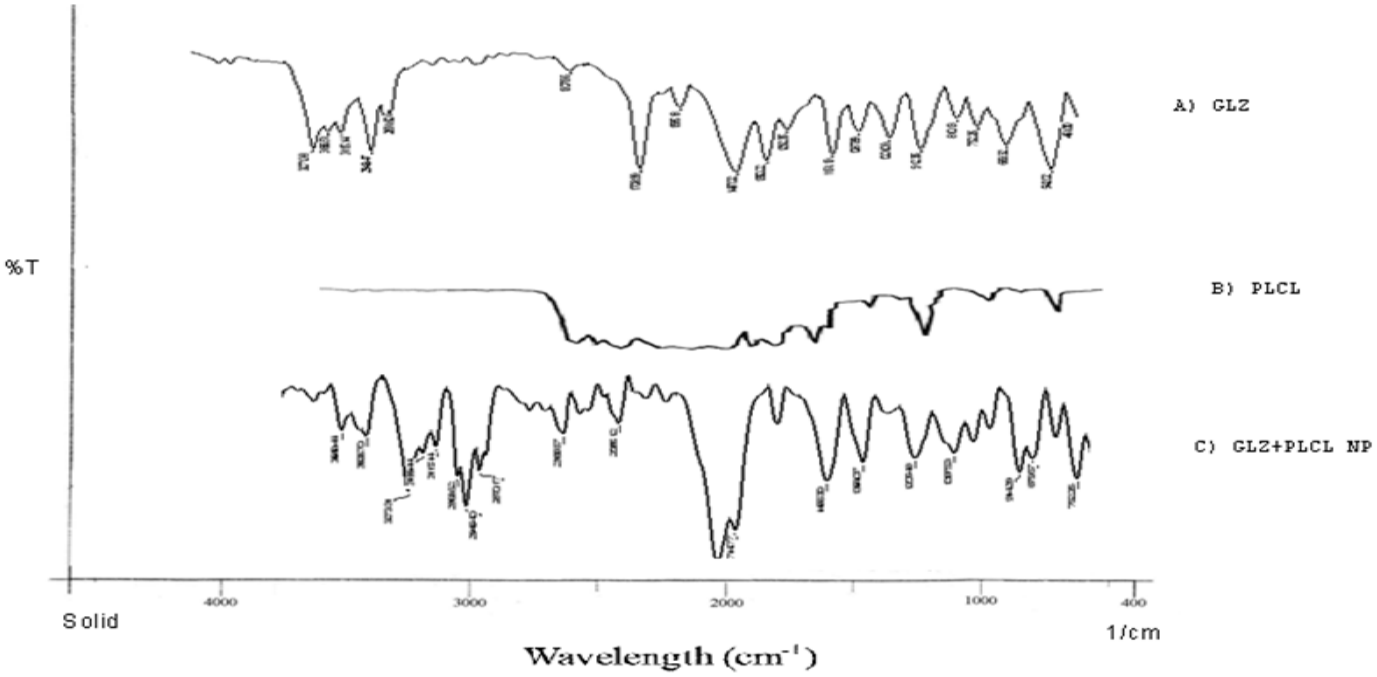

Figure 6: FT-IR spectra of A] pure Gliclazide, B] pure PLCL polymer and C] Gliclazide nanoparticles embedded in the PLCL matrix.

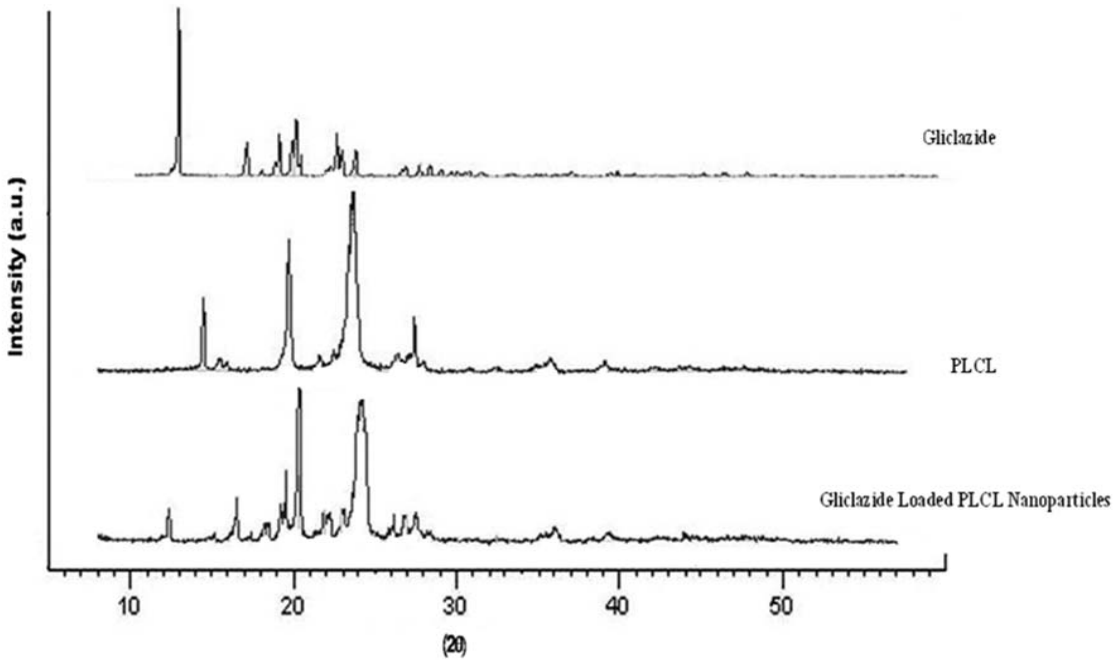

Figure 7: XRD pattern for pure Gliclazide, pure PLCL polymer, and Gliclazide Loaded PLCL Nanoparticles

Encapsulation Efficiency

The amount of gliclazide entrapped within NP was determined using following equation:

% E.E. = [wt. of drug determined (mg) / wt. of dug added (mg)] x 100 (1)

For estimation of drug loading, the freeze-dried nanoparticles containing around 5 mg of gliclazide was dissolved in 5 ml of methanol and 5 ml of pH 7.4 Phosphate buffer solution, after complete evaporation of methanol, the drug content was analyzed using a UV-Spectrophotometer (U-2900; Hitachi, UV/VIS spectrophotometer 200V) at 225.5 nm.

Calibration curve of Gliclazide

The absorbance was measured at 225.5 nm using double beam UV/Visible spectrophotometer, (Figure. 8).

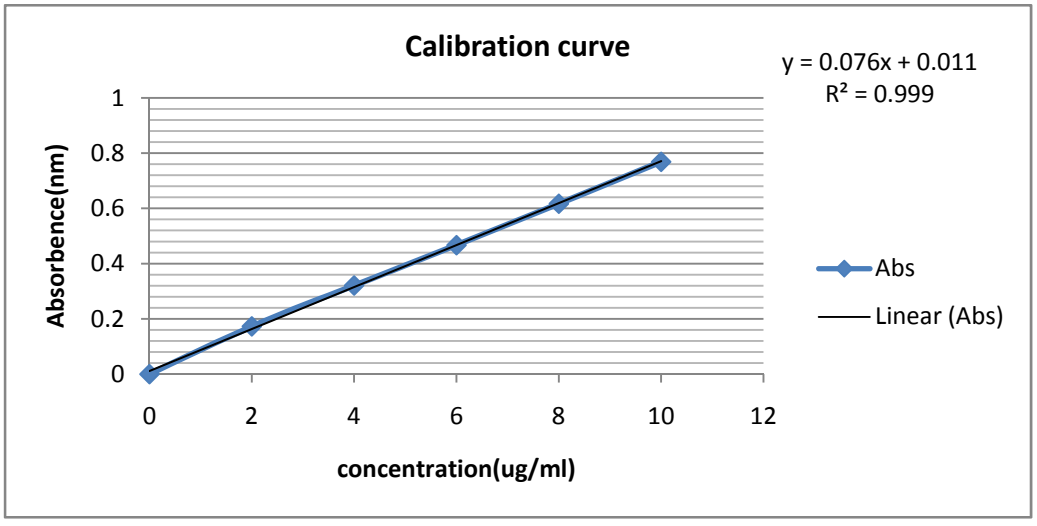

Figure 8:Calibration curve of Gliclazide

In vitro drug release studies-

In vitro dissolution studies of the nanoparticles were carried out using USP type II (TDT 08T, Electro-lab, Mumbai, Maharashtra, India) dissolution test apparatus. The dissolution test for all the formulations was carried out in standard phosphate buffer saline (pH 7.4) maintained at 37 ± 0.5^{0}C at the paddle rotation speed of 100 rpm. The conditions for *in vitro* were maintained to study the release behavior of a hydrophobic drug. Solubility of Gliclazide increases 3–6 times with phosphate buffer (pH 7.4) as solvent. Hence, the drug release study was carried out using phosphate buffer pH 7.4. Samples of nanoparticles containing Gliclazide were suspended in 900 ml of standard phosphate buffer saline, pH 7.4, and stirred at 100 rpm. Withdrawing 5 ml of samples at preselected time intervals up to 24 hours monitored progress of the dissolution. Same volume of dissolution medium was replenished 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.

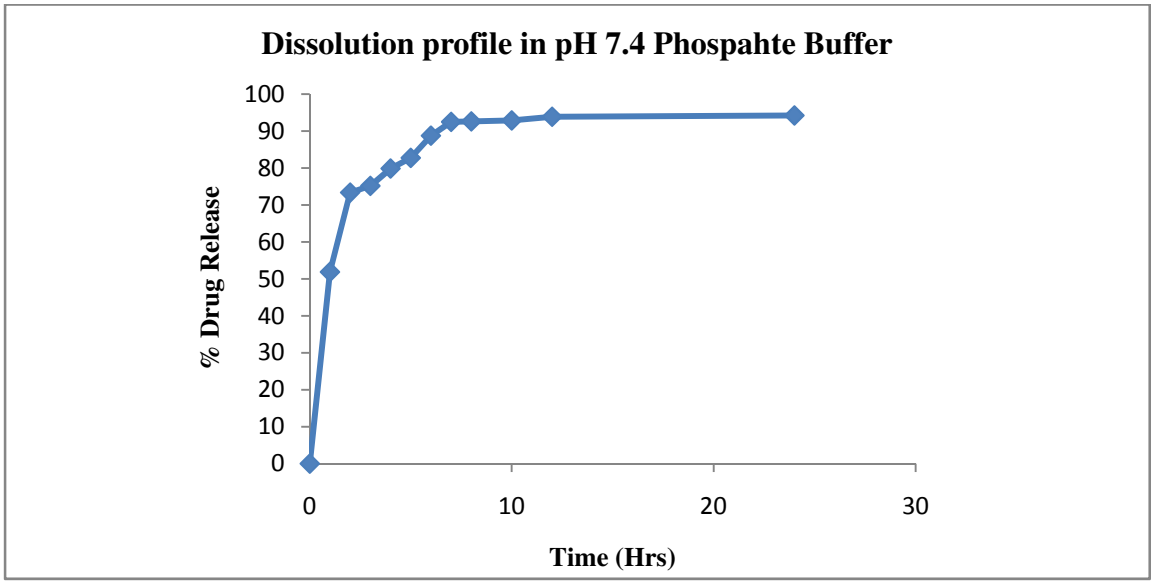

Figure 9: % cumulative drug release from controlled release nanoparticles

Result-

In our study, our attempt was to encapsulate gliclazide with the PLCL biodegradable polymer for sufficiently high entrapment efficiency by O/W single emulsion solvent evaporation method and then to obtained a nanosize under High Pressure Homogenizer. The importance of enhanced drug entrapment efficiency in nanoparticles has been emphasized earlier, since a nanoparticles recovery is required for reducing manufacturing costs and its size and morphology important for quality control and biodistribution, it was necessary to study the influence of processing parameters on nanoparticles preparation. Solvent evaporation method was used to prepare PLCL loaded nanoparticles. Different Gliclazide: PLCL ratios were tried. The effect of drug:polymer ratio on encapsulation efficiency is shown in table no.1, Initially various solvents like acetone, methanol and DCM were tried for preparation of nanoparticles. However, maximum solubility and encapsulation efficiency of gliclazide found only when DCM is used as an organic phase. Hence DCM used as an organic phase. All the optimized batches were further employed for particle size reduction using high pressure homogenizer (Panda Plus-200) which reduces the particle size up to 100 nm which is shown in the figure no. 4. PVA with concentration up to 0.3% shows excellent encapsulation efficiency with particle size reduction for Poly (L-lactide-co- -caprolactone) (PLCL) loaded Gliclazide biodegradable nanoparticle formulation.

The % cumulative release of Gliclazide from PLCL NPs was almost complete after 2 h, due to a single and rapid desorption process from the PLCL surface. The release of GLZ from PLCL NPs, it was observed a biphasic process with an early rapid release which took place within 2-5 h (up to 75% and 80%, respectively), the remaining drug being slowly liberated during the next 26 h as controlled release property of the nanoparticles (Fig. 9). GLZ release during the faster release phase may result polymer degradation which has been reported to be a very high solubility in the pH-7.4, suggests that the major fraction of GLZ was entrapped into the PLCL polymeric network rather than adsorbed onto the NP surface, which determined more complex drug diffusion through the polymeric shell.

Conclusions

From the results it can be concluded that the Gliclazide has successfully encapsulated in PLCL polymer employing O/W solvent evaporation and HPH method. Nanoparticles was successfully developed using response surface methodology by 3^2 factorial designs. PLCL polymeric matrix has yielded higher encapsulation efficiency and drug loading with potential carrier for efficient delivery of Gliclazide. *In vitro* drug release from microspheres followed a controlled drug release pattern. Nanoparticles prepared by such method may represent a promising approach for efficient delivery of Gliclazide.

Future perspective

In this study we have formulated successfully Gliclazide encapsulated PLCL biodegradable nanoparticles, however, in this novel drug delivery system including the field of nanomedicine is very beneficial for the patient who is and will be suffer from Diabetes a life threatening diseases. In future this kind of work should be carried out for further formulation or drug delivery systems to avoid the excess use of filler which is traditionally in the use of formulation for conventional dosage forms and we have to move towards nanotechnology for better patient compliance and to save them from this type of life threatening diseases.

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Authors contributions

Authors 1) and 2) have developed concept behind why we have to for design and development of PLCL loaded Gliclazide biodegradable nanoparticle formulation; authors 2) to 5) have been involved in preformulation studies and optimization of methodology to get the final required formulation and authors 6) and & 7) are involved in drafting the full manuscript suitable for publication in peer reviewed journal.

Conflict of Interest

All authors do not have any financial conflict of interest

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