June 2008 *Volume 2 Issue 1*

Trade Science Inc.

Nano Science and Nano Technology

An Indian Journal

Full Paper

NSNTAIJ, 2(1), 2008 [41-44]

Preparation and *in vitro* evaluation of glipizide loaded poly (D,L-lactide-co-glycolide) nanoparticles for effective management of type II diabetes

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Received: 27th January, 2008 ; Accepted: 2nd February, 2008

ABSTRACT

The aim of this study was to formulate glipizide loaded PLGA nanoparticles as a sustained release carrier with enhanced efficacy. PLGA nanoparticles (PLGA NPs) were prepared by oil-in-water (o/w) emulsion solvent extraction/evaporation technique with slight modification. The prepared Glipizide NPs demonstrate high drug loading and encapsulation efficiencies. Scanning electron microscope photograph showing spherical and porous morphology of the developed NPs. *In-vitro* drug release study revealed sustained release of glipizide from the delivery system following the Higuchi ($R^2 = 0.9732$) and Ritzer Peppas ($R^2 = 0.9825$) model explaining diffusion as well as erosion controlled release. The developed glipizide NPs are suitable for reduction in dosing frequency, decrease in side effects, and improved patient compliance.

KEYWORDS

PLGA NPs;
Glipizide;
Nanoparticles;
Sustained release.

INTRODUCTION

Glipizide, 1-cyclohexyl-3-[[4-[2-[[(5-methyl pyrazin-2-yl)carbonyl]amino]ethyl]-phenyl] sulphonyl] urea (figure 1) is a potential second generation oral hypoglycemic agent widely used for the treatment of noninsulin-dependent diabetes mellitus (NIDDM). Prior research work revealed that it has a good general tolerability, low incidence of hypoglycemia and low rate of secondary failure. In addition it has potential for slowing the progression of diabetic retinopathy. For these reasons, glipizide appears to be a drug of choice in long term sulfonylurea therapy for the control of NIDDM.

In general, rapid gastrointestinal (GI) absorption is required for oral hypoglycemic drugs, in order to prevent a sudden increase in blood glucose level after food intake in patients with diabetes mellitus. However the GI absorption rate of glipizide, in conventional dosage form (i.e. tablets), appears to be rather slow. Several studies using healthy volunteers or patients revealed that the time to reach peak serum glipizide concentration ranged from 1/2-1h following oral administration of the glipizide tablet. A slow absorption of drug usually originates from either poor permeability of the drug across the GI membrane. The dose of glipizide is 5mg tid, and hence there is always a need for the development of sustained release patient complaint formulation of glipizide, as it is short acting sulfonylurea[1].

Nanoparticles are one of the multiparticulate delivery systems and are prepared to obtain prolonged or

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controlled drug delivery and to improve bioavailability or stability. Nanoparticles can also offer advantages like limiting fluctuations within therapeutic range, reducing side effects, decreased dosing frequency, and improved patient compliance.

Poly (lactic-co-glycolic) acid (PLGA) is a biodegradable polymer and used for preparation of controlled and sustained release dosage forms. Release kinetics from PLGA and other biodegradable polymers are controlled by diffusion, erosion or a combination thereof, and are dependent on the polymer (Mw, copolymer ratio and crystallinity), drug properties, as well as the device characteristics (preparation conditions, particle size, morphology, porosity and drug loading) and the dissolution conditions[2].

The aim of this study was to formulate an optimized nanoparticulate delivery system containing glipizide to achieve a sustained release profile suitable for per oral administration with enhanced efficacy and which could overcome the drawbacks of glipizide delivery through conventional dosage forms.

MATERIALS AND METHODS

Materials

Poly (lactic-co-glycolic) acid (PLGA 50:50) (Mw =18000) was gifted by SPARK Laboratory. Tween-80®, methylene chloride and methanol were purchased from sigma-aldrich and Poly-vinyl alcohol (PVA) from SD Fine Chemicals. All other chemicals and solvents were of analytical grade. Triple distilled water filtered with 0.22 membrane has been used in the experimental work.

Methods

Preparation of nanoparticles

An oil-in-water(o/w) emulsion solvent extraction/ evaporation technique with slight modification was used for the formulation of glipizide nanoparticles. 200mg PLGA and 50mg of Glipizide were dissolved in 25ml methylene chloride using a vortex mixer for 30min. This organic phase was added slowly to a small amount of 0.5% (w/v) aqueous surfactant solution (50ml) and homogenized at 20,000 rpm for 30min. Further this emulsion was added to 250ml of a 0.5% (w/v) aqueous surfactant solution and stirred (3000 rpm on a magnetic stirrer) for 6h at 25°C. The resulting particles were filtered (Membrane Filter, 0.22μm), centrifuged and pellets obtained were washed three times with distilled water and freeze dried.

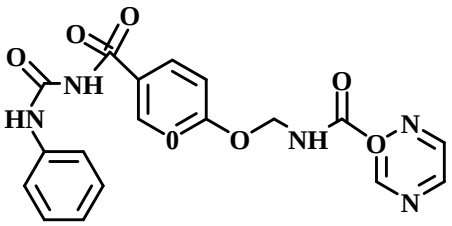

Figure 1: Structure of Glipizide

Evaluation of nanoparticles

Particle size distribution

Average particle size was determined using Particle Size Analyzer (Malvern S4700 PCS System, Malvern Instruments Ltd, Malvern, UK). The analysis was performed at a scattering angle of 90^0C and at a temperature of 25^0C using samples appropriately diluted with filtered water (0.2μ filter). For each sample, the mean diameter ± standard deviations of six determinations were calculated applying multimodal analysis. Values reported are the mean diameter ± standard deviation for two replicate samples.

Zeta potential

The zeta potential of the particles was determined by Laser Doppler Anemometry (Malvern Zetasizer IV, Malvern Instrments Ltd, Malvern, UK). All analyses were performed on samples appropriately diluted with 1mM HEPES buffer (adjusted to pH 7.4 with 1M HCl) in order to maintain a constant ionic strength. For each sample the mean value ± standard deviation of four determinations were established. Values reported are the mean value ± standard deviation for two replicate samples.

Particle morphology

Morphological evaluation of the nanoparticles was performed using scanning electron microscope (S-3000N, magnification=5000).

Encapsulation efficiency and drug loading

The amount of glipizide entrapped within NP was

determined by measuring the amount of nonentrapped drug in the supernatant recovered after centrifugation and washing of the NP by a UV method[3].

%encapsulation efficiency=Mass of drug added during NP preparation-Mass of free drug in supernatant/Mass of drug added during NP preparation×100

For estimation of drug loading, 10mg of the freeze dried nanoparticles were dissolved in 10 ml of methanol and after suitable dilution analyzed by the UV spectrophotometer (Shimadzu, Pharmaspec-1700) for glipizide contents. (Prior studies reveals that no absorbance interference from PLGA polymer under the same conditions). Encapsulation efficiency was determined as reported. All the measurements were conducted in triplicate and the mean values and standard deviations are reported[4].

% Drug Loading= Mass of drug in nanoparticles/Mass of nanoparticlesrecovered×100

In vitro drug release studies

The *in vitro* drug release studies were performed with some modification[5,6]. Briefly, glipizide-loaded PLGA NPs (equivalent to 10mg of glipizide) were suspended in 100mL of pH 7.4 phosphate buffer (USP-XIV) in a 250mL glass bottle. The glass bottle was placed in a stirring bath (50rpm), with temperature adjusted to 37°C. At selected time intervals, 5.0mL of the sample was withdrawn and subjected for centrifugation; pellets were resuspended in 5ml of fresh phosphate buffer and added to glass bottle to maintain the sink condition. The sample was then filtered through 0.22μ membrane and analyzed by UV-spectrophotometry at λmax of 271.5nm.

RESULTS AND DISCUSSION

Particle size distribution and zeta potential

In this study the selected drug polymer ratio was 1:4, homogenization speed 20000 rpm, surfactant 0.5% tween 80, and the stirring speed 3000 rpm. The average particle size of PLGA nanoparticles loaded with glipizide was obtained 505 ± 12.13nm. Nanosizing can improve the bioavailability of class II drugs having high permeability but low aqueous solubility either by increasing the rate of dissolution or through bypassing the prerequisite of dissolution before a drug can be absorbed.

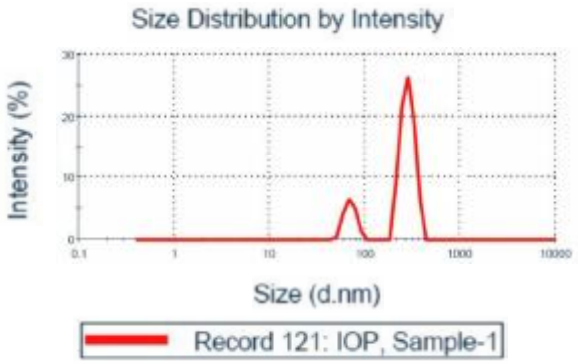

Figure 2: Particle size distribution of prepared Glipizide loaded PLGA nanopartiles

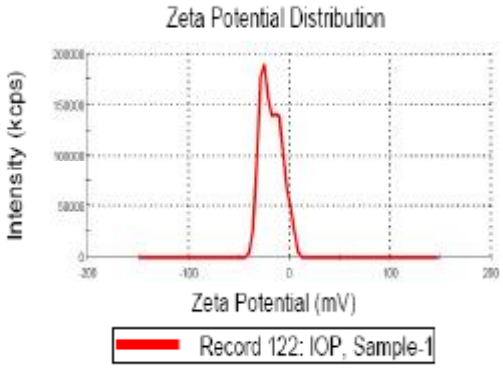

Figure 3: Zeta potential measurement of prepared Glipizide loaded PLGA nanopartiles

Since glipizide belongs to type II drug category (low soluble high permeable) the prepared delivery system will exert high bioavailability through bypassing the dissolution and increase in absorption of nanoparticles.

The zeta potential is an index of stability of nano particles. The higher the magnitude, irrespective of the charge type, the higher the stability is expected. Tween 80(pH 6.1) resulted in a negative zeta potential value of -18 .2mV, suggesting the stability of prepared nanoparticles.

Surface morphology

Figure 4 shows the SEM image of the particles as a function of polymer concentration. The particles were spherical and most likely porous bumpy. The particles from PLGA with the diameter less than 750nm were solid spheres, whereas the particles larger than that were collapsed.

Encapsulation efficiency and drug loading

The importance of enhanced drug incorporation

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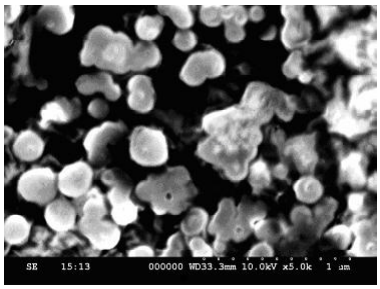

Figure 4: SEM image of Glipizide loaded PLGA nano particles

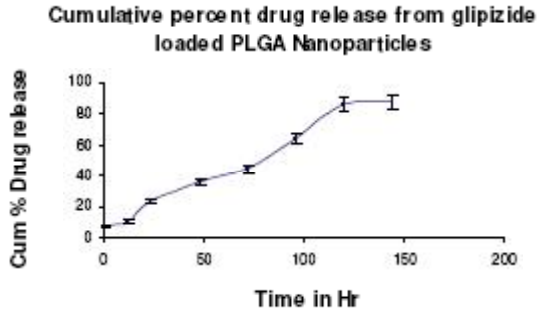

Figure 5: Cumulative percent drug release of glipizide loaded nanoparticles in pH 7.4 buffer

efficiency in nanoparticles has been emphasized earlier, since a high nanoparticles recovery is required for reducing manufacturing costs and since its size and morphology are important for quality control and biodistribution. The selection of optimal formulation in our study was, therefore, based on that which provided a combination of good morphology (in terms of particle size and surface properties) drug loading, and encapsulation efficiency.

In vitro drug release study

In-vitro drug release study of glipizide loaded of PLGA NPs in phosphate buffer (pH 7.4) showed a sustained release profile (R^2=0.9822). Drug release in "real-time" (37°C) typically shows a triphasic profile: (1) an initial burst release (7.78% in 1 Hr) of surface and pore associated drug, (2) a lag phase until sufficient polymer erosion has taken place (44.3% in 72hr) and (3) a secondary burst with approximately zero order release kinetics (64.29% in 96hr and finally 88.03% in 144hr). The initial burst release is controlled by diffusion, whereas the lag phase and secondary burst phase are dependent on polymer erosion as well as diffusion. This is probably because of the inherent encapsulation efficiency of glipizide with the tween 80.

The release data was well fitted to higuchi model revealing a diffusion controlled release for the PLGA NPs prepared by using tween 80 (R^2=0.9732). The release data was also fitted to Ritger Pappas model revealing that release is governed by diffusion and erosion of polymer matrix for the formulation of PLGA NPs prepared by using Tween 80 (R^2 = 0.9825).

CONCLUSION

Glipizide-loaded PLGA NPs were prepared by oil-in-water (o/w) emulsion solvent extraction/evaporation. *In vitro* release study revealed sustained release of glipizide from PLGA NPs. Sustained activity with decreased dose and enhanced bioavailability could reduce dose frequency, decrease side effects, and improve patient compliance.

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