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Research Article

Preparation and Evaluation of Glibenclamide-Loaded Biodegradable Nanoparticles

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Abstract

Purpose: *To formulate and evaluate glibenclamide (GB)-loaded poly(lactic-co-glycolic) acid (PLGA) nanoparticles (NPs) for controlled release.*
Methods: *GB-loaded PLGA NPs were prepared by solvent evaporation technique using methanol/dichloromethane (2:1) and characterized by transmission electron microscopy (TEM), and differential scanning calorimetry (DSC). The effect of stirring speed (250, 1000, 1500 and 2500 rpm) and drug: polymer ratio (1:1, 1: 2, 1:3 and 2:1) on particle size, size distribution, zeta potential, drug loading, encapsulation efficiency and drug release was also studied.*
Results: *Stable NPs were successfully prepared without any incompatibility, as indicated by TEM and DSC studies, respectively. As polymer and drug concentrations, and stirring speed increased, particle size, drug loading and encapsulation efficiency also increased. Increase in polymer concentration sustained drug release but reverse was obtained as drug concentration increased.*
Conclusion: *Controlled release biodegradable glibenclamide NPs can be efficiently prepared by emulsification solvent evaporation method suitably modulating processing variables.*

Keywords: *Poly (lactic-co-glycolic) acid, Nanoparticles, Glibenclamide, Transmission electron microscopy, Differential scanning calorimetry*

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INTRODUCTION

Nanoparticles (NPs) are one of the multiparticulate delivery systems used for prolonged and/or controlled drug delivery, improved bioavailability, stability, and targeted drug delivery to specific sites [1-4]. NPs can also offer advantages such as limiting fluctuation within a therapeutic range, reducing side effects, decreasing dosing frequency, and improving patient compliance.

Poly (lactic-co-glycolic) acid (PLGA) is a biodegradable polymer that has been used extensively for preparation of controlled release nanoparticulate drug delivery systems. The release kinetics of PLGA and other biodegradable polymers are controlled by diffusion, erosion or combinations thereof, and are dependent on the polymer molecular weight, copolymer ratio and crystallinity.

Glibenclamide (GB) is a second-generation sulfonylurea oral hypoglycaemic agent used in the treatment of non-insulin dependent diabetes mellitus. It is administered in low doses (5 mg) and its active metabolites have a significant hypoglycemic effect. However, glibenclamide's low bioavailability has been attributed to its poor dissolution properties. Nanoparticle formulation can be used to formulate poorly soluble drugs to enhance bioavailability using an erosion-based biodegradable polymer such as PLGA [5-12].

The present investigation aims to formulate and characterize PLGA controlled release nanoparticles containing GB by emulsification solvent evaporation method for improved bioavailability.

EXPERIMENTAL

Poly (lactic-co-glycolic) acid (PLGA, copolymer ratio, 50:50, viscosity, 0.41 dL/g) was purchased from Birmingham Polymers Inc. (Birmingham, USA) while glibenclamide (GB) was received as a gift from Alembic Pharma, India. All other chemicals used were of analytical grade and obtained from E. Merck (India).

Methods of Preparation of GB-loaded NPs

GB-loaded PLGA nanoparticles (NPs) were prepared by emulsification solvent evaporation method. An attempt was made to optimize the nanoparticle formulation using various formulation parameters including varying drug/polymer ratio organic solvent (methanol/dichloromethane) ratio, stirring speed (250 – 2500 rpm) and surfactant ratio (PVA/polysorbate-80) in a fixed concentration (0.5 %w/v), using a modified emulsification solvent evaporation technique. PLGA and GB were dissolved in 25 ml of solvent mixture containing methanol to dichloromethane in a ratio 2:1, using a vortex shaker to form a homogeneous solution of drug and polymer. This homogeneous solution was added slowly to 120 ml of aqueous surfactant solution containing 0.5 % w/v polyvinyl alcohol and polysorbate-80, and homogenised using a high pressure homogenizer (Ika, Japan) to obtain an emulsion [13-15]. The emulsion formed was stirred with a laboratory magnetic stirrer (Remi, India) for 5 h at 25 ºC followed by centrifugation (Remi, India) for 22 min. The supernatant was discharged and the pellets obtained were washed using the same volume of distilled water as the supernatant and then centrifuged for 6 min. Washing was repeated three times and the resulting NPs were freeze-dried (Lyphlock, Labconco).

Determination of drug incorporation efficiency

The freeze-dried NPs (10 g) were dissolved in dichloromethane (50ml), a common solvent for both drug and polymer. The amount of drug in the solution was determined using Perkin-Elmer ultraviolet spectrophotometer at 243.5 nm. Drug content (%w/w) and drug entrapment (%) were calculated using Eqs 1 and 2, respectively.

$$\% \text{ Drug content} = (M/Mr)100 \quad \ldots\ldots\ldots\ldots\ldots (1)$$

where M is the mass of drug in the NPs and Mr the mass of NPs recovered.

% Drug entrapment = (D/Df)100 ..... (2)

where D = amount of drug in the nanoparticles and Df = total amount of drug used for the preparation of the nanoparticles.

Particle size analysis and zeta potential measurement

Freeze-dried NPs were reconstituted in distilled water. The size of the NPs was determined by Zetasizer (Nano ZS, Malvern Instruments, Malvern, UK) based on dynamic light scattering technique. The polydispersity index (PI), which is a dimensionless number indicating the width of the size distribution, was also measured. The mean particle size of the formulation was determined by photocorrelation spectroscopy with a zeta master (Malvern Instruments, UK) equipped with the Malvern PCS software. Each sample was diluted with phosphate buffer (pH 7.4) and the surface charge (zeta potential) of the NPs determined by measuring their electrophoretic mobility of the NPs by the zeta sizer (Malvern Instruments, UK).

Differential scanning calorimetric (DSC) studies

The physical state of glibenclamide encapsulated in NPs was characterized by differential scanning calorimetry (DSC, Shimadzu, DSC-50, Japan). Each sample containing the drug, equivalent to 10 mg, was sealed separately in a standard aluminium pan, the samples were purged with nitrogen gas at a flow rate of 10 ml/min, and heated at 10 °C/min from 0 to 350 °C

Transmission electron microscopic studies

NPs were also evaluated for size with a transmission electron microscope (Philips/FEI Inc, Barcliff, Manor, NY, USA). For this purpose, a sample of NPs was suspended in water (0.5 mg/ml) and sonicated for 30 s. One drop of this suspension was placed over a carbon-coated copper TEM grid (150 mesh, Ted Pella Inc., Rodding, CA) and negatively stained with 1 % uranyl acetate for 10 min and then allowed to dry. Images were visualized at 120 kV under the microscope.

***In vitro* drug release studies**

The *in vitro* release of GB-loaded NPs were performed using USP type II dissolution test apparatus (Electrolab, India) in 900 ml of medium (0.1M hydrochloric acid) for the first 2 h and then in phosphate buffer (pH 7.5) from 3 - 72 h at 37 ± 0.5 °C and stirring rate of 100 rpm. Samples (5 ml) were collected periodically and replaced with equal volume of fresh dissolution medium on each occasion. After filtration through Whatman Grade No. 41 Quantitative Filter Paper (pore size 25 µm), the concentration of GB was determined spectrophotometrically at 243.5 nm on UV-Visible spectrophotometer (Jasco V530, Japan). *In vitro* release profile was analyzed by various kinetic models (zero order, first order and Higuchi) in order to determine the mechanism of drug release [16].

Statistical analysis

The results are expressed as mean ± standard deviation (SD, n = 3). The data were statistically analyzed by one-way analysis of variance using Graph Pad Instat®, version 3.05 (USA), and significant difference was set at $p < 0.05$.

RESULTS

Entrapment efficiency, drug content, particle size and characteristics

The entrapment efficiency (EE), drug content (DC) and particle size (PS) of the formulations (with their codes) is given in Table 1. As the concentration of polymer increased, entrapment efficiency and particle size also increased. Lower particle size were

observed when stirring speed was increased. TEM photographs (Fig 1) indicate that NPs were spherical in shape and in the nanosize range with discrete spherical outline. DSC thermograms of native PLGA, GB and GB-loaded NPs showed the characteristic peaks of the respective compounds, as shown in Figure 2. An endothermic peak of PLGA occurred at around 48 ºC due to glass transition (Tg). The peak of PLGA was slightly shifted to 46.5 ºC in drug-loaded NPs compared to that of native PLGA. The endothermic peak of native GB occurred at 172.8 ºC. In formulation F2, no similar peaks were observed, indicating that the drug was molecularly dispersed in the polymer matrix.

In vitro release profile and kinetics of the GB NPs are shown in Figure 3 and Table 2, respectively. As polymer concentration increased, drug release decreased. Also, when the drug concentration of the NPs was increased as in case of formulation F2 (drug: polymer ratio 1:2) as compared to formulation F1 (drug: polymer ratio 1:1), drug release increased. In order to determine the release pattern of GB from prepared NPs, various *in vitro release* kinetic models such as zero order, Higuchi and first order were analysed. In all formulations, the highest regression coefficient was observed using zero order model (Table 2).

Table 1: Effect of drug/polymer ratio (D:P), stirring speed (SP), entrapment efficiency (EE), drug content (DC) and particle size (PS) on GB-loaded NPs.

NP code	D:P ratio	SP (rpm)	EE (%)	DC (%)	PS (nm)
F1	1:1	2500	46.32 ± 2.14*	11.21 ± 2.43*	532 ± 11.29*
F2	1:2	2500	54.24 ± 3.15	26.52 ± 3.16	562 ± 5.28
F3	1:3	2500	62.63 ± 1.87*	28.51 ± 2.29*	812 ± 14.72**
F4	2:1	2500	90.52 ± 4.52**	36.73 ± 3.16*	1453 ± 13.26**
F5	1:2	250	33.21 ±3.12**	10.28 ± 1.13*	1081 ± 23.26**
F6	1:2	1000	42.24 ± 2.57*	13.14 ± 2.12*	993 ± 14.28*
F7	1:2	1500	48.25 ± 2.21*	21.23 ± 3.31*	803 ± 11.16**

*Significantly different from batch F2 at ** $p < 0.01$ and * $p < 0.05$*

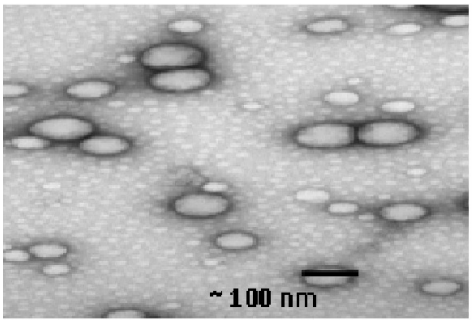

Figure 1: Transmission electron micrograph (TEM) of NP with 1:2 drug: polymer ratio

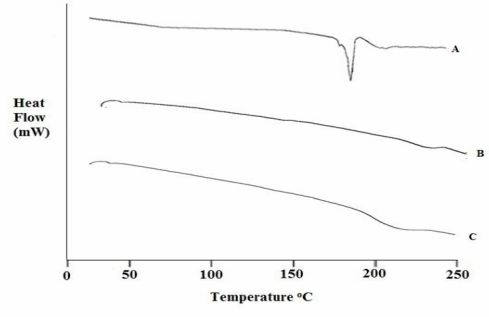

Figure 2: DSC of pure GB (A), PLGA (B), formulation F2 (drug polymer ratio 1:2) (C)

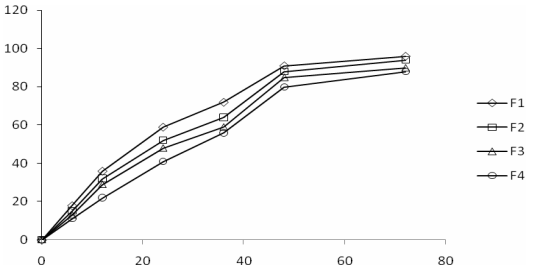

Figure 3: *In vitro* release of GB from formulation

Table 2: Release kinetics of GB-loaded NPs

Formulation	Regression coefficient (r^2)		
	Zero order	First Order	Higuchi
F1	0.9818	0.9422	0.9413
F2	0.9812	0.9462	0.9326
F3	0.9726	0.9532	0.9524
F4	0.9831	0.9321	0.9623

DISCUSSION

As the polymer content of the NPs increased, there was a corresponding increase in entrapment efficiency and particle size. This may be due to the fact that the higher the polymer content the thicker is the coat around the drug, which prevents the loss of drug during the processing of NPs. The increase in particle size with increase in polymer concentration may be due to increased viscosity of polymeric phase, which resulted in the formation of large emulsion droplets during homogenization and thus was hard to break; hence, they precipitated leading to increase in their particle size.

DSC is a fast and reliable method for understanding polymorphic transitions when screening drugs and excipients for compatibility, obtaining information about possible interactions. The absence of detectable crystalline domains of GB in drug-loaded NPs clearly indicates that GB was dispersed completely in the formulation, thus modifying the NPs to an amorphous, disordered-crystalline phase.

With increase in polymer concentration, drug release decreased due to the fact that increase in the polymer amount resulted in increase of matrix thickness of NPs, thereby increasing the distance that the drug transversed to reach the surface of the NPs. As drug concentration in the drug/polymer mixture in the NPs increased, i.e., from 1:1 to 2:1, drug release increased. This is because the amount of polymer required to coat the higher concentration of drug was not sufficient to retard drug release.

Overall, formulation F2 which has suitable particle size, drug entrapment efficiency and controlled release profile was the most satisfactory among all the NPs.

CONCLUSION

GB-loaded PLGA NPs were successfully prepared by emulsification/solvent evaporation method using varying GB/PLGA ratios. Encapsulation efficiency and drug content were adequate. Furthermore, drug release from the NPs was controlled indicating its potentials in controlled drug delivery.

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