Research Article

FORMULATION AND EVALUATION OF GLIPIZIDE LOADED NANOPARTICLES

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ABSTRACT

Objective: Glipizide is low soluble, high permeable and short half life antidiabetic drug. The aim of the study was to develop and formulate the sustained release Glipizide loaded nanoparticles and evaluate it.

Method: Emulsification-solvent evaporation technique was used to develop the nanoparticles with 3^2 full factorial design. Drug and polymer was dissolved in methanol/dichloromethane mixed solvents. High pressure homogenizer was used to reduce the particles size in nano level. The optimized nanoparticles formulation were studied for FT-IR, particle size, zeta potential, encapsulation efficiency, XRD, *in vitro* release study and *in vivo* evaluation etc.

Results: The effects of dependent variables drug-polymer ratio (X_1) and surfactant concentration (X_2) on particle size and encapsulation efficiency were studied. The drug and polymer were not interacting with each other. The particles were smooth, spherical and and homogeneous external aspects. The crystallinity of nanoparticles was less than pure glipizide. The selected formulation for dissolution study shows 209.6 nm size and 95.66±1.70 percent encapsulation efficiency. *In vitro* release was found to be much sustained up to seven days (64.79±2.68) and follow first order kinetic. The sustained release naoparticles decreased the blood glucose level up to 132.66±9.83 mg/dL in seven days study period.

Conclusion: The sustained release nanoparticles of glipizide could be able to manage type II diabetes mellitus with reduced dose frequency, decreased side effects and improve patient compliance.

Keywords: Type II Diabetes, Polycaprolactone, Nanoparticles, Drug release study.

INTRODUCTION

An oral hypoglycemic agent Glipizide is second generation sulfonyl urea used for the treatment of non-insulin dependent diabetes mellitus. It acts by stimulating the release of insulin from the pancreas. Biopharmaceutically Glipizide is a class II drug, which has low solubility and high permeability. Its short biological half-life (3.4±0.7 hours) necessitates that it be administered in 2 or 3 doses of 2.5 to 10 mg per day [1,2]. Thus it is a potential candidate for the development of extended release formulations. Extended release formulations released the drug slowly for maximum period of time may improve the therapeutic effect, bioavailability and drug stability. It also reduces side effects of respective drugs and dosing frequency [3]. Nanoparticulate drug delivery system (1-1000 nm) is usually intended for oral, parenteral or topical rout with the ultimate objective being the alteration of the pharmacokinetic profile of the active molecule [4]. To sustain the release of drug there are different polymers available. Of these poly-Ɛ-caprolactone (PCL) is a biodegradable, biocompatible and semicrystalline polymer. Degradation of PCL in comparison to polyglycolic acid and other polymers is slow making it suitable for long-term delivery extending over a period of more than one year. PCL is soluble in chloroform, dichloromethane, carbon tetrachloride, benzene, toluene, cyclohexanone and 2-nitropropane at room temperature. It has a low solubility in acetone, 2-butanone, ethyl acetate, dimethylformamide and acetonitrile and is insoluble in alcohol, petroleum ether and diethyl ether [5]. Release kinetics of biodegradable polymers are controlled by diffusion, erosion or a combination thereof and are depend on the polymer's properties like molecular weight, copolymer ratio, crystallinity, drug properties, preparation conditions, particle size, surface morphology, drug loading and the dissolution conditions [6]. The sustained release nanoparticles can be prepared by an emulsion solvent extraction/evaporation technique. In the solvent evaporation method, the required amount of polymer and drug are dissolved in an organic phase which is emulsified under homogenization with surfactant to form an oil in water emulsion. Stirring is continued to evaporate the organic phase, the formed nanoparticles separate and dried [7]. The aim of this study was to formulate and optimize Glipizide loaded PCL nanoparticles to achieve a sustained release profile with maximum encapsulation efficiency. A 3^2 full factorial design was employed to study the effect of independent variables, polymer to drug ratio (X_1) and surfactant concentration (X_2) on the dependent variables encapsulation efficiency and size of the naoparticles. The optimized batch depending on encapsulation efficiency was characterized for Field-Emission Scanning Electron Microscopy, Fourier Tansform Infrared Spectroscopy, X-ray Diffraction analysis, *In-vitro* dissolution study, Drug release kinetics and *In-vivo* study.

MATERIALS AND METHOD

Materials

Glipizide was kindly gifted by Harind Pharmacal Pvt. Ltd. Mumbai. Polycaprolactone (Mn 70,000-90,000) and streptozocin purchased from Sigma-Aldrich, USA. Dichloromethane and methanol purchased from Merck, Mumbai. Polyvinyl alcohol (PVA) procured from SD Fine Chem Ltd., Mumbai, India. The experimental work was performed by using triple distilled water filtered with 0.22 µ membrane filter.

Method

The drug and polymer were dissolved in methanol/dichloromethane mixed solution. This solution was added drop by drop using controlled flow rate syringe pump (Infusor, Universal Medical Instruments, India) (3ml/min) in PVA aqueous solution with high speed homogenization (Omni GLH Homogenizer) for 10 minutes of 4 cycles and 30 Sec interval. Further the solution was high pressure homogenized (Panda GEA Niro Soavi, Italy) at 500 bar for 5 cycles. The nanosuspension was stirred at 500 rpm for 6 h to evaporate organic solvents. The obtained nanoparticles were recovered by centrifugation (R243A, Remi) at 18000 rpm for 30 min and washed thrice with distilled water. The washing water was removed by a further centrifugation and nanoparticles were freeze dried (Lyophilizer, Labosene, Scanvac Coolsafe, Denmark) [8].

Fourier transform infrared spectroscopy

The samples were homogeneously mixed with potassium bromide and infrared spectrums were traced in the region of 4000-400 cm^{-1} by using an infrared spectrophotometer (IR- 8400, Shimadzu Co. Ltd., Singapore).

Table 1: Different batches with their experimental coded level of variables for 3^2 Factorial Design

Batch code	Variables in coded form	
	X_1	X_2
NP1	-1	0
NP2	0	+1
NP3	0	-1
NP4	+1	0
NP5	0	0
NP6	+1	-1
NP7	+1	+1
NP8	-1	-1
NP9	-1	+1

Table 2: Conversion of coded unit in actual units.

Variable Levels	Low (-1)	Medium (0)	High (+1)
X_1= Polymer concentration (mg)	100	200	300
X_2= Surfactant concentration (%,w/v)	0.3	0.5	0.7

Particle size and zeta potential determination

The obtained nanoparticles were suspended in distilled water by sonication and vortex mixing for 30 seconds and the particle size (Z-average mean) and zeta potential were determined by using Nano series Malvern Instruments, UK.

Encapsulation efficiency and drug content determination

Accurately weighed nanoparticles were dissolved in dichloromethane. 100 ml phosphate buffer (pH 7.4) solution was added and stirred constantly to extract glipizide in it after the evaporation of dichloromethane. Removed the precipitated polymer from phosphate buffer by filtration and measured the amount of drug in filtrate using Ultraviolet spectroscopy (U2900, Hitachi, Japan) at 275.5 nm. Encapsulation efficiency (%) and drug content (%, w/w) were represented by Eqs. (1) and (2) respectively [9].

$$\text{Encapsulation Efficiency (EE \%)} = \frac{\text{Mass of drug in nanoparticles}}{\text{Mass of drug used in formulations}} \times 100 \quad (1)$$

$$\text{Drug content (\%, w/w)} = \frac{\text{Mass of drug in nanoparticles}}{\text{Mass of nanoparticles recovered}} \times 100 \quad (2)$$

Field Emission-Scanning Electron Microscopy

The shape and surface characteristics of nanoparticles were examined and photographed using Field Emission-Scanning Electron Microscopy (FE-SEM) (S4800, Hitachi, Japan). Appropriate samples were mounted on stub, using double sided adhesive carbon tapes. Samples were gold coated and observed for morphology, at acceleration voltage.

X-ray diffraction analysis

X-ray diffraction of samples was carried out using Model-D8 Advance, Brucker AXS GmbH, Germany diffractometer. An Cu Kα source operation (40 kV, 40 mA) was employed. The diffraction pattern was recorded over a 2θ angular range of 3-50° with a step size of 0.02° in 2θ and a 1 Sec counting per step at room temperature.

***In vitro* release study**

Accurately weighed samples were suspended in 100 ml phosphate buffer saline (pH 7.4). The solution was stirred at 50 rpm with temperature adjusted to 37±1 $^\circ$C. At planned time intervals 1 ml samples were withdrawn and make the volume up to 10 ml. Centrifuged this solution at 20,000 rpm for 30 min. Aliquots of supernatant were examined by a UV spectrophotometer at 275.5 nm. The settled nanoparticles in centrifuge tube were re-dispersed in 1 ml fresh phosphate buffer saline (pH 7.4) and returned to the dissolution media.

***In vitro* release kinetic evaluation**

The dissolution data of each batch were fitted to various kinetic equations and mechanism of drug release investigated. Equation (3), (4), (5), (6) and (7) are Zero order, First order, Higuchi model Hixon-Crowell and Korsmeyer-Peppas model respectively.

$$Q_t = K_0\, t \quad (3)$$

$$\ln Q_t = \ln Q_0 - K_1 t \quad (4)$$

$$Q_t = K_h\, t^{1/2} \quad (5)$$

$$Q_0^{1/3} - Q_t^{1/3} = K_{HC} t \quad (6)$$

$$M_t / M_\alpha = K_p t^n \quad (7)$$

Where, Q_t is the percentage of drug released at time t, Q_0 is the initial amount of drug present in the formulation and K_0, K_1, K_h, K_{HC}, K_P are the constants of equations. Regression coefficient (R^2) was determined from the slope of the following plots: Cumulative % drug release Vs Time (Zero order kinetic model), Log cumulative of % drug remaining Vs Time (First order kinetic model), Cumulative % of drug release Vs Square root of Time (Higuchi model), Cube root of % drug remaining in matrix Vs Time (Hixon-Crowell model) and Log cumulative % drug release Vs Log time (Korsmeyer- Peppas model) [10,11]. In Korsmeyer- Peppas model, first 60% of drug release was fitted and release exponent "n" was calculated which is indicative of drug release mechanism. According to Korsmeyer theory, if 'n' is 0.45 then drug release will follow Fickian diffusion mechanism, for 0.45 < n < 0.89 follows Anomalous (non-Fickian) diffusion, for n = 0.89 case II transport and for n > 0.89 diffusion mechanism will super case II transport [12].

***In vivo* evaluation**

Healthy Wistar rats weighing 250-300 g were used to study. Rats were adjusted to their environment for 4 days before dosing. Streptozocin was dissolved in 100 mmol/L citrate buffer (pH 4.5) and 60 mg/kg fresh solution was injected intraperitoneally to overnight fasted rats. After 48 h blood samples were checked using Gluco-strips (SD Codefree™, SD Biostandard Diagnostic Pvt. Ltd.) and rats with blood glucose values between 255-400 mg/dL were considered diabetic. Six animals in each group like rats were divided into the following groups. The group I consisted of normal control rats treated with 1% gum acacia suspension, given orally, t.i.d. for up to 7 days; group II was diabetic control, treated same like group I; group III consisted of diabetic rats treated with Glipizide (800 µg/kg, t.i.d.) for 7 days; group IV diabetic rats were treated with formulation (approximately 800 µg/kg, t.i.d.) for 7 days. Blood glucose levels were measured in all rats using Glucostrips at 24 h intervals over the 7 days treatment period. All experiments were conducted in triplicate and expressed as the mean ± SD. One-way analysis of variance (ANOVA) was used followed by Tukey's test. Differences were considered to be statistically significant at a level of $p \leq 0.05$.

RESULT AND DISCUSSION

Glipizide loaded PCL nanoparticles were prepared by using simple oil in water method. Glipizide and PCL were soluble in methanol /

dichloromethane combined solvent system. This organic phase added slowly drop by drop in PVA containing water phase. At the same time high speed homogenizer shake this solution vigorously to form emulsion and slowly suspension as the emulsion globules start to convert in solid particles as solvent evaporates and due to the hydrophobic nature of the drug and polymer. This formed nano system processed through high pressure homogenizer to reduce the size of suspended particles in nano level. After collecting the nanosuspension system was kept to evaporate the organic solvents by stirring, a further collection of the particles

and freeze dried to make free flowing, water free drug loaded nanoparticles. The IR spectrum of glipizide loaded nanoparticles clearly illustrates that the following peaks of glipizide were observed in it. –NH stretching (3323.46 cm^{-1}), =CH stretching (2860.53 cm^{-1}), C=O (1689.70 cm^{-1}), C=C aromatic (1653.05 cm^{-1}), C-H deformation (1467-1430 cm^{-1}), SO$_2$-NH (1354 cm^{-1}). Therefore, the drug-polymer interaction study indicates no interaction between drug, polymer and excipients in drug-loaded nanoparticles. The effect of polymer and surfactant concentration on particle size was shown in Fig. 1A.
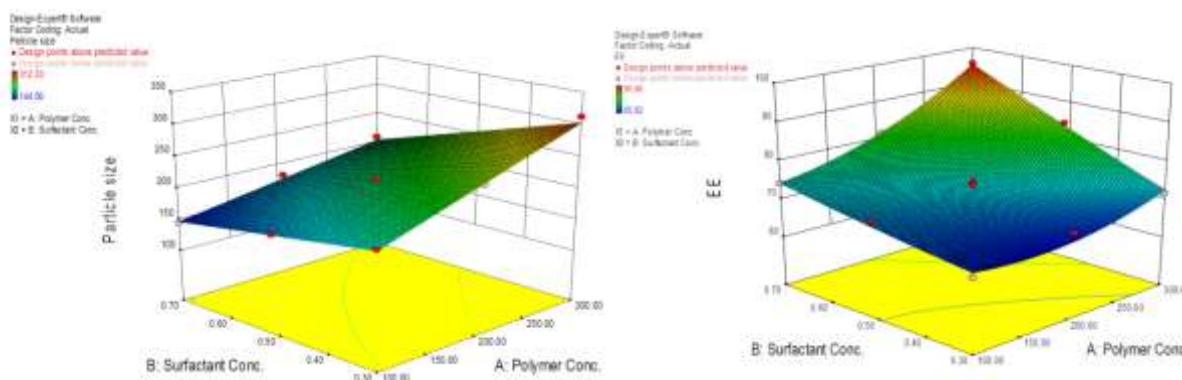

Fig. 1A: Influence of dependent factors on particle size and Fig. 1B: Encapsulation efiiciency

From figure it was concluded that as the amount of polymer increased the size of particles also increased. The surfactant concentration also equally affects on the particle size. The minimum size of the particles was 144.56 nm of NP9 batch and maximum size was 312.33 nm of NP6 batch (Table 1). The maximum glipizide encapsulated formulation (NP7) particle size was 209.6 nm and shown in fig. 2. The high polymer

concentration gives more size emulsion globules, because of the hindrance in the breaking of globules due to more viscosity [13]. The surfactant helps to produce new area for the formation of globule and decreased the size of particles [14]. Thus from the results it was observed that as the polymer concentration decreased and surfactant concentration increased the particle size also decreased.
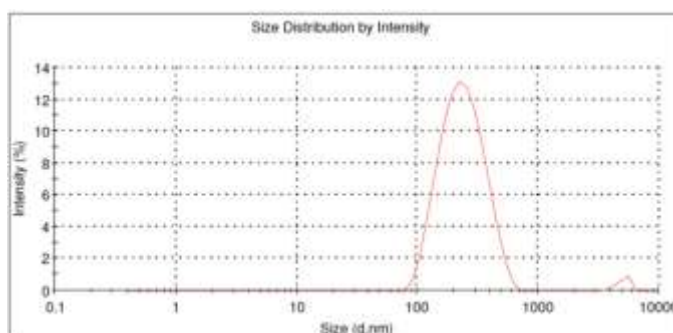

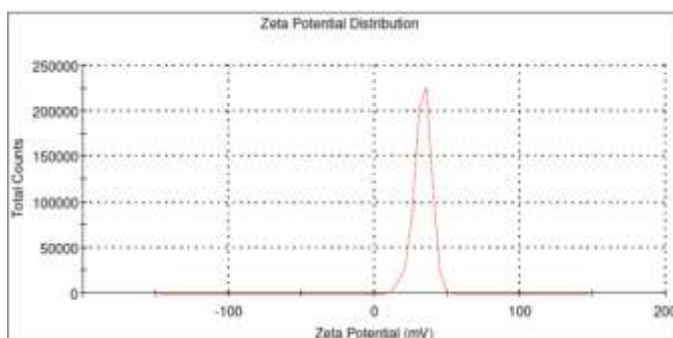**Fig. 2: Particle size distribution curve of most encapsulated batch NP7.**

Fig. 3: Zeta potential distribution curve of batch NP7.

The zeta potential value of most encapsulated formulation batch NP7 was +32.8 (Fig.3). This may be due to polycationic polymer. Zeta potential is an abbreviation for electrokinetic potential in colloidal systems. Zeta potential is electric potential in the interfacial double layer at the location of the slipping plane versus a point in the bulk fluid away from the interface. A value of zeta potential (positive) could be taken as the arbitrary value that separates low-charged surfaces from highly-charged surfaces.

The significance of zeta potential is that its value can be related to the stability of colloidal dispersion. The zeta potential indicates the degree of repulsion between adjacent, similarly charged particles in dispersion. For molecules and particles that are small enough, a high zeta potential will confer stability, i.e. the solution or dispersion will resist aggregation [15]. Both above mentioned factors also influenced on the encapsulation efficiency of nanoparticles (Fig.1B). The maximum polymer and surfactant concentration give more encapsulation efficiency and low polymer and surfactant concentration give low encapsulation efficiency. This may be due to the increased in size encapsulate the more drug, but more surfactant also accelerates the encapsulation by enhanced the binding contact between drug and polymer in emulsion stage. Therefore the encapsulation efficiency of batch NP7 was more than NP6 and NP4. Therefore from obtained results of all nine batches we can say that both the factors X_1 and X_2 affected combined on the results. The final high encapsulated batch NP7 was used for further characterization study. Fig. 4 illustrates the morphology of the particles.
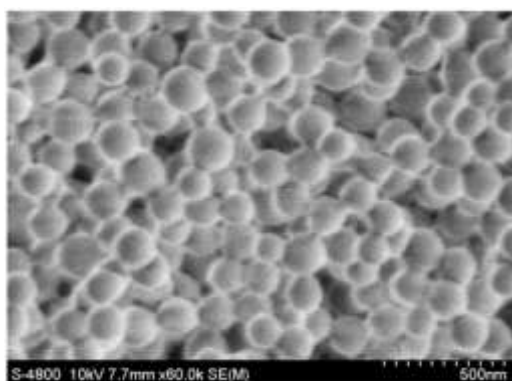

Fig. 4: FE-SEM photograph of selected formulation batch NP7.

All particles were spherical in shape and non-aggregated. The particles were non-porous, smooth and homogeneous external aspects. This may be due to the PCL solidifying property or slow release of dichloromethane during the last stage of the evaporation

process. Glipizide and PCL were crystalline and semicrystalline in nature respectively. But the crystallinity of glipizide loaded PCL nanoparticles was less (Fig. 5).
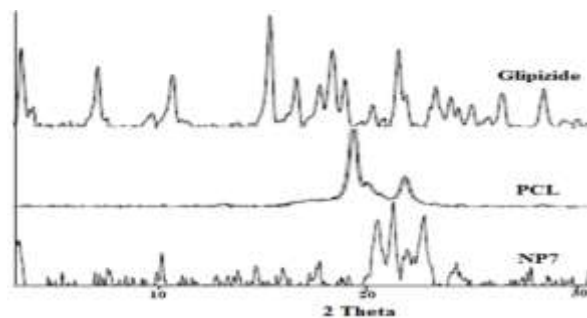

Fig. 5: X-Ray Diffraction pattern of glipizide, polycaprolactone and batch NP7.

This may be due to the polymer overlapped on glipizide which shows the drug is dispersed at the molecular level in polymer matrix or the intervention of PCL molecule arrangement in the glipizide molecules during solidification [16]. The dissolution profile of batch NP7 is shown in fig. 6. The formulation sustained the drug successfully up to 168 h.
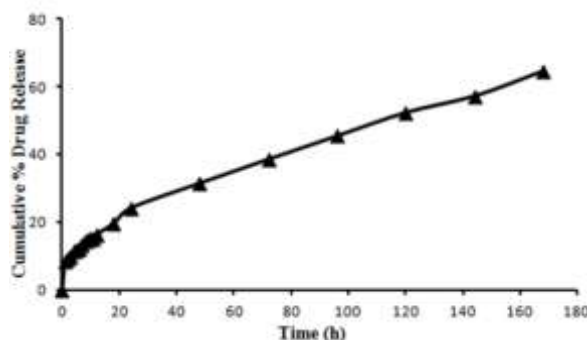

Fig. 6: Dissolution profile of final formulation NP7.

In first hour 8.44±0.79 percent drug was released. This drug may be present at the surface of the particles. After the first hour the drug released very slowly. In 24 h 24.17±2.05 percent drug was released. This percent was increased up to 64.79±2.68 at 168 h. These results concluded that the degradation of PCL was a very slow process because of its semi-crystallinity and hydrophobicity [5]. To study the kinetics and mechanism of drug release the dissolution data were fitted in different kinetic equations.

Table 3: *In-vitro* release kinetics of selected formulation NP7.

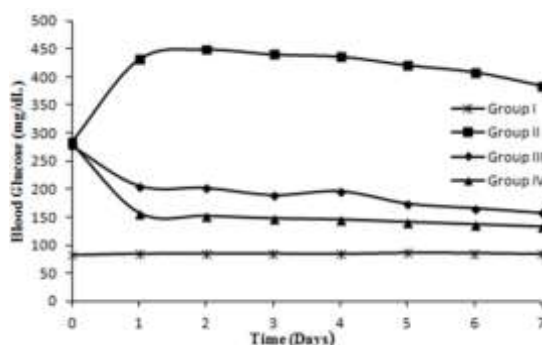| Batch	Zero order (R2)	First order (R2)	Higuchi (R2)	Hixon-Crowell (R2)	Korsmeyer-Peppas (R2)	n
NP7	0.9829	0.9961	0.9939	0.9945	0.9722	0.4176

Fig. 7: Blood glucose levels in the all four study groups for 7 days.

According to table 3, the selected formulation NP7 follows first order kinetics which explained that drug released was depending on its concentration. Furthermore, since PCL is a semi-crystalline polymer, buffer solution can penetrate into the amorphous part of the polymer matrix thus facilitating the release of the drug by diffusion. The difference in drug remaining in matrix and released drug in phosphate buffer facilitates the release of drug from nanoparticles. After that we can say function of time, porosity, diffusion rate and polymer degradation may be responsible for drug release. The value of the release exponent of selected formulation was 0.4176 which is near to the Fickian diffusion [12]. Even if values of the exponent are found that would indicate a diffusion controlled drug release mechanism.

The standard glipizide and formulation NP7 administration significantly reduced blood glucose level within 1 h (Fig 7). In group I the blood glucose level was normal throughout the study. In group II, the diabetic control, blood glucose level was very high until the last day of study. One-way ANOVA followed by Tukey's test explained that blood glucose levels in group II rats were significantly larger than group III rats treated with standard glipizide (p<0.05). In group IV, rats treated with formulation batch NP7, a continuous decreased in blood glucose level until the end of the experiment and significantly lowers than group II and group III (P<0.05). In comparison of group III and IV, group III animal's blood glucose level for 1h (162.16±11.61) was less than group IV animals (255.16±12.04). But after 1h the glucose level of group IV was continuously less than group III animals may be due to slow and constant release of glipizide from nanoparticles. A reduction in blood glucose levels of group IV animals within 1h were also not more than 25%, so there were less chances of hypoglycemic effect. From all these results it was concluded that the glipizide loaded nanoparticles may effectively decrease the blood glucose level.

CONCLUSIONS

From the present study it was concluded that the above mentioned factors are individually as well as combined influenced on the encapsulation efficiency and particle size of the glipizide loaded nanoparticles. The optimum amount of polymer and high concentration of surfactant respect to polymer concentration was required to develop the good encapsulated nanoparticles. The glipizide loaded polycaprolactone nanoparticles sustained the glipizide up to 7 days and follows first order kinetics for drug release. The formulated long-lasting biodegradable nanoparticles using an emulsification solvent evaporation technique were able to manage type II diabetes mellitus for a period up to one week. Thus, the formulated nanoparticles could be used as an effective delivery system which improves patient compliance by decreased in dosing frequency.

ACKNOWLEDGEMENT

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