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Role of polymeric excipients on controlled release profile of Glipizide from PLGA and Eudragit RS 100 Nanoparticles

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Abstract

Polylactic-*co*-glycolic acid (PLGA) 50:50 and Eudragit RS 100 nanoparticles entrapping glipizide along with excipients were prepared using single emulsion solvent evaporation method. The objective was to develop single oral dose glipizide nano particles for reducing blood sugar level in diabetes induced experimental animals. Incorporation of Polyethylene glycol (PEG) (0.5%), Hydroxypropyl methylcellulose (HPMC) (0.5%) and Tween 20 (0.5%) in the organic phase during particle formulation improved release profile of glipizide from the polymer particles. Entrapment efficiency of glipizide in all the polymeric formulations was around ~70 %. Around 80 % of glipizide was released from both PLGA and Eudragit RS 100 nanoparticles when 0.5% of PEG and Tween 20 were added during preparation. Incorporation of amphiphilic polymer during particle formulation not only improved entrapment efficiency of glipizide but also resulted in uniform stabilized nanoparticles having desired control release characteristics. Both PLGA and Eudragit nanoparticles were biocompatible to SW 480 adenocarcinoma human cell line at concentration ranges from 12.5 to 500 µg/ml. The efficacy of glipizide loaded particle formulations were evaluated in female out breed Wistar rats. Significant reduction of blood glucose level was observed ($p \leq 0.05$) for 24 hours from a single oral dose using stabilized nanoparticles formulations.

Keywords: Nanoparticle, glipizide, excipients, PLGA, Eudragit RS 100, *in vitro* release.

1. Introduction

Polymer like PLGA and Eudragit nanoparticles have diverse biomedical application particularly in the area of diagnosis, tissue engineering and as drug delivery agents[1-7]. To date, several polymeric micro and nanoparticles have been used for the delivery of drugs and therapeutic proteins[8-10]. Two major problems hinder the biological application of polymeric nanoparticles based delivery system. One is the optimal formulation with appreciable load of the drug with desired sustained release profile[11]. The other is the toxicity of nano particulate system[12]. The major toxicological concern with respect to nanomaterials particularly to be redox active[13-15] and some particles transport across mammalian cell membranes and especially into mitochondria[16]. It is thus imperative to evaluate the toxicological parameters associated with nanoparticles before evaluating their therapeutic potential for drug/biomolecules delivery.

Glipizide is an oral hypoglycemic agent, which is a commonly prescribed drug for the treatment of type II diabetes mellitus[17]. It is a weak acid (pK_a = 5.9), practically insoluble in water and acidic environment but highly permeable according to biopharmaceutical classification system (BSC)[18]. Oral absorption is uniform, rapid and complete with a bioavailability of 100 % and the elimination half life is 2-4 hours[18]. Glipizide have a short biological half life (3.4 ± 0.7 hour) requiring it to be administered in 2-3 doses of 2.5 to 10 mg per day[19]. As the glipizide have a short biological half life (3.4 ± 0.7 hour), requiring it to be administered in 2-3 doses per day. Several study reported Polylactic-co-glycolic acid (PLGA) and Eudragit RS 100 polymers has the sustained release properties. Due to sustained release properties of PLGA and Eudragit RS 100 polymers, are used for controlling the release of glipizide from glipizide loaded nanoparticles to maintain therapeutic effect for a longer duration of time after single dose administration.

Though a number of multi-particulate systems have been proposed for per oral controlled delivery of glipizide, most of them are polymeric drug delivery systems[20,21] and the major problem is the slow release of glipizide from the polymeric matrix due to its hydrophobic nature. To the best of our knowledge the role of such excipients (PEG, HPMC and Tween 20) on release of small molecular hydrophobic drugs like glipizide from polymeric nanoparticles system has not addressed so far in the literature.

Due to the hydrophobic nature of glipizide, more hydrophilic excipients (PEG, HPMC and Tween 20) were used during formulation. During particle preparation, co-encapsulation of glipizide with PEG, HPMC and Tween 20 the hydrophilic group is exposed towards the external aqueous phase which improves the hydrophilicity of the polymeric nanoparticles and enhance the release of glipizide from the polymeric nanoparticles. Additionally, PEG and HPMC are polymeric osmoagents which have the ability to improve the release of drug osmotically[22].

The objective of the present investigation was to improve the release of entrapped glipizide from the PLGA 50:50 and Eudragit RS 100 nanoparticles by incorporation of polymeric excipients. The *in vitro* cytotoxicity of these nanoparticles was evaluated in SW 480 adenocarcinoma human cell line. Efficacy of polymeric formulations was evaluated *in vivo* in terms of lowering of blood glucose level in Wistar rats through oral administration.

2. Materials and methods

2.1 Materials

Poly Lactide-co-Glycolide (PLGA) [112-66-1] was purchased from Birmingham Polymer Inc. USA; Eudragit RS 100 from Corel Pharma Chem, India; Glipizide [29094-61-9], Polyethyleneglycol(PEG) [25322-68-3], Hydroxypropylmethyl Cellulose

(HPMC) [H7509], Tween 20 [9005-64-5], Polyvinyl alcohol (PVA) [P8136], Sucrose [S1888], sodium bicarbonate [S5761], DMEM F-12 HAM, FBS, L-glutamine, Penicillin and streptomycin from Sigma Chemicals Co. USA. The glucose assay kit was purchased from Span Diagnostic Ltd, India [B01122]. Dichloromethane (HPLC grade) [15105], Disodiun Hydrogen orthoPhosphate [27785], Sodiun di-hydrogen ortho Phosphate [14105/01] from Qualigens and acetonitrile, Methanol (HPLC grade) were purchased from Spectrochem (New Delhi, India).

2.2 Preparation of Nanoparticles

PLGA (50:50) and Eudragit RS 100 nanoparticles entrapping glipizide were prepared by a solvent evaporation method[23]. This involves preparation of oil in water (O/W) emulsion between the glipizide and polymer solution in dichloromethane (DCM) and an external aqueous phase (EAP) containing PVA and sucrose. The emulsion was stirred over night to evaporate residual DCM. Sonication at 40 % duty cycle for 3 minutes was used to prepare the emulsion. After complete evaporation of excess DCM, the particulate suspension was centrifuged at 15000 rpm for 20 minutes and washed thrice with Milli Q water for complete removal of excess PVA. The samples were lyophilized to produce a free flowing powder. Initially, the phase volume ratio and (sonication) energy input were standardized to produce the desired size particle (~ 200 nm). To improve the release profile of glipizide from the polymeric nanoparticles, three different polymeric excipients were incorporated in the organic phase during preparation of the nanoparticles. Type and concentration of excipients were varied during formulation keeping constant phase volume ratio and energy input. Detailed compositions of different formulations used for particle preparation are given in table 1.

2.3 Characterization of PLGA and Eudragit RS 100 nanoparticles

The size distributions of the glipizide loaded nanoparticles were analyzed using a particle size analyzer (Master sizer, Malvern instruments, UK). For a typical experiment, about 20 mg of nanoparticles was suspended in 5 ml of Mill Q water and analyzed with an obscuration index (measurement of the amount of light lost due to the introduction of the sample into the light path) ranging from 5 to 10 %. Zeta potential of the different formulations was estimated by a Zeta sizer (Malvern Instruments, UK).

2.4 Entrapment efficiency of glipizide

Entrapment efficiency of glipizide loaded nanoparticles was estimated by UV/Vis spectroscopy. A known amount of nanoparticles (10 mg, dry powder, prepared as above) was dissolved in 1 ml of chloroform to extract the solubilized glipizide from the polymer particles. The samples were centrifuged at 13,000 rpm for 15 minutes and the supernatant was used for spectroscopic analysis. The Entrapment efficiency (EE) was calculated using the formula

$$\% \text{ EE} = \frac{\text{Practical load}}{\text{Theoretical load}} \text{ X } 100$$

2.5 In vitro cytotoxicity study of PLGA and Eudragit RS 100 nanoparticles

2.5.1 Cell culture

SW480 cells (ATCC, CCL-228), a primary adenocarcinoma cell line of the human colon, was used for cytotoxic assay. SW480 cells were cultured in Dublecco's Modified Eagle's Medium Nutrient Mixture F-12 HAM with 2mM L-glutamine supplemented with 10 % fetal bovine serum (FBS), 45 IU ml^{-1} penicillin and 45 IU ml^{-1} streptomycin at 37ºC in 5 % CO_2 .

2.5.2 Cytotoxicity assay

The Alamar blue (AB) assay was carried out for quantitative measurement of cell viability[24,25]. Cells were plated at a seeding density of 1 x 10^5 cells/ml for the 24 hour test, 6 x 10^4 cells/ml for the 48 hour test, 4 x 10^4 cells/ml for the 72 hour and 2 x 10^4 cells/ml for the 96 hours in 96 well plates. Note that, due to the nature of the assay, and the need for lower cell numbers for the longer duration exposure experiments (to allow sufficient room for the cells to proliferate. The plates were kept in a CO_2 incubator for 24 hours for proper attachment of cells on the surface of the 96 well plates. Before exposure of nanoparticles, the plates were washed with 100 µl of phosphate buffer saline (PBS), where upon 100 µl of different concentrations (12.5 - 500 µg/ml) of nanoparticles (PLGA 50:50 and Eudragit RS 100) were added to the respective well of each plate. After exposure for 24, 48, 72 and 96 hour, cell viability was assessed by the AB assay according to the manufacture's guidelines. Briefly, control media or test exposures were removed; the cells were rinsed once with PBS and 100µl of AB medium (5% v/v solution of AB) prepared in fresh media (without FBS or supplements) were added to each well. After 3h of incubation, AB fluorescence was measured at the excitation and emission wavelengths of 540 nm and 595 nm respectively, in a microplate reader (TECAN GENios, Grodig, Austria). In order to ensure that the presence of nanoparticles did not influence the assay readout, the fluorescence intensity of AB media in the absence and presence of nanoparticles was compared, and no significant difference was observed, suggesting that the particles do not interact with the AB. Three independent experiments were performed.

2.6 In vitro drug release study

In vitro release of glipizide nanoparticles were carried out at 37°C. Approximately 10 mg nanoparticles were suspended in 1 ml of phosphate buffer saline (pH 7.4) taken in a 1.6 ml microfuge tube and placed in an incubator shaker for the period of study (37°C, 200 rpm). Drug samples were collected at different time intervals after centrifugation at 13,000 rpm for 10 minutes and the amount of drug released in the supernatant was estimated by UV/Visible spectroscopy (at 276 nm) method[36]. The pellet was reconstituted, resuspended in 1 ml of fresh phosphate buffer saline (pH 7.4) and kept in a shaker for further sampling.

2.7 In vivo studies

Animals were maintained according to the guidelines established by the Institute of Animal Ethics Committee (IAEC) of the National Institute of Immunology, New Delhi. The efficacy of glipizide loaded nanoparticles of different formulation were evaluated in female outbreed Wistar rats (n = 6), weighing between 200 g to 250 g. Glipizide loaded PLGA, and Eudragit RS 100 nanoparticles were suspended in Milli Q water and administered orally with the help of an oral cannula. Care was taken to ensure that the particle suspension entered through the esophagus. Animals were divided into 6 groups of six animals and were provided with standard diet and water *ad libitum.* Group I served as control, Group II were given a suspension of glipizide loaded PLGA nanoparticles (Formulation A1) orally at a dose level of 800 μg/Kg body weight, Group III were given suspension of glipizide loaded PLGA nanoparticles (Formulation A5) orally at a dose level of 800 μg/Kg body weight, Group IV were given suspension of glipizide loaded Eudragit RS 100 nanoparticles (Formulation B1) orally at a dose level of 800 μg/Kg body weight. Group V were given suspension of glipizide loaded Eudragit RS 100 nanoparticles (Formulation B5) orally at a dose level of 800 μg/Kg body weight.

Group VI were given suspension of standard glipizide orally at a dose level of 800 μg/Kg body weight. The blood samples were withdrawn from the retro-orbital plexus of each rat pre-treatment and at 1, 2, 4, 6, 12, and 24 hours post-treatment. The serum was separated and stored at –20 °C for estimation of glucose level. Glucose levels in the serum were estimated by the Glucose Oxidase Peroxidase method[26].

2.8 Statistical analysis

Statistical analyses were carried out using one-way analyses of variance (ANOVA) followed by Dunnett's multiple comparison tests. Statistical significance was accepted at $P \leq 0.05$ for all tests. Cytotoxicity was expressed as mean percentage inhibition relative to the unexposed control ± standard deviation (SD).

3. Results and Discussion

3.1 Characterization of glipizide loaded PLGA and Eudragit RS 100 Nanoparticles

PLGA and Eudragit RS 100 nanoparticles entrapping glipizide were prepared by the solvent evaporation method. To prepare ~ 200 nm sized particles and to provide emulsion stability, 1% polyvinyl alcohol (PVA) was added to the external aqueous phase (EAP) during the particle formulation. Different concentrations of excipients PEG, HPMC, Polysorbate 20 (Tween 20) were added during particle preparation to improve the release of glipizide from the polymer particles. Detailed composition of the different formulation was presented in Table-1. The polymeric particles were characterized by measurement of hydrodynamic diameter and zeta potential of all the nanoformulations and results are shown in Table-2.

PLGA nanoparticles are more stable in the gastrointestinal tract than other colloidal carriers, such as liposomes, and the use of polymeric materials enable the modulation of physicochemical characteristics (e.g. hydrophobicity, zeta potential), drug release

properties (e.g. delayed, prolonged, triggered), and biological behavior (e.g. targeting, bioadhesion, improved cellular uptake) of the nanoparticles[27]. From previous studies it was concluded that by adjusting the phase volume ratio (Organic phase and External aqueous phase) and energy input in terms of sonication/homogenization, different size polymeric particles can be generated[23,26]. In this study the sonication time (40 % duty cycle for 3 minute) was increased and the phase volume ratio adjusted (1:4) in order to generate ~ 200 nm polymer particles as shown in Table-2. The encapsulation efficiency of glipizide in both the polymeric particles was around 70%, shown in Table-2 and the size distribution of PLGA and Eudragit RS 100 nanoparticles are shown in Figure 1a and b. No significant difference in encapsulation of glipizide in different formulations was observed. Amphiphilic stabilizers thus helped in improved entrapment of the drug in nanoparticles along which monodisperse stable polymeric particles. Similar effect of excipients on emulsion stability and entrapment efficiency of protein/antigen have been reported[26,28,29]. This suggested that these excipients mostly stabilize the aqueous/organic emulsion droplet during primary emulsification step of particle formulation. As primary emulsion stability controls the features of the polymer particles, these excipients helped in stable particle formulation.

The zeta potential of plain PLGA particles was nearly -7 mV and upon addition of different surfactants this value decreased to -15 mV which was an indication of an improvement of the colloidal stability and a reduction in the tendency of the nanoparticles to agglomerates. However, the zeta potential of plain Eudragit RS 100 nanoparticles was 23 mV resulting in an almost stable colloidal suspension. Upon addition of surfactant during particle preparation, the zeta potential value slightly decreased, indicating aggregation of nanoparticles.

Zeta potential is the degree of repulsion between adjacent, similarly charged particles. After addition of non-ionic surfactants to nanoparticle formulation the change of zeta potential occur due to change in force of attraction/repulsion between the particles. It has also been reported that zeta potential of PLGA and PEG-PLGA nanoparticles is differ because of the PEG concentration reduces the overall negative surface charge[30] and also hydrophobic interaction has important role in the change of zeta potential with the nonionic surfactant[31].

3.2 In vitro release of glipizide from polymeric nanoparticles

Glipizide released from nanoparticles at different time intervals was analyzed spectroscopically. The *in vitro* release of glipizide from PLGA particles was faster than the Eudragit RS 100 nanoparticles without any burst release in the initial 30 minutes, after which, sustained release of glipizide was observed (Figure 2 and 3). In the presence of different excipients (PEG, HPMC and Tween 20), the release pattern of glipizide from PLGA particles varied considerably. Due to the hydrophobic nature of glipizide, more hydrophilic excipients were used during formulation. Co-encapsulation of glipizide with PEG, HPMC or Tween 20 improved the release profile of encapsulated glipizide in both the polymeric particles (PLGA and Eudragit RS 100). *In vitro* release data showed a significant difference ($p \leq 0.05$) between the plain and co-encapsulation of glipizide with PEG (formulation A1 and A2), HPMC (formulation A3 and A4), and Tween-20 (formulation A5 and A6), in the case of the PLGA 50:50 nanoparticles at all the time points except 30 minutes. However, a significantly different ($p \leq 0.05$) release profile was observed in the case of Eudragit RS 100 nanoparticles for all the time points except 24 hour for formulation B2, B3, B4, and B6 as compared to formulation B7 (glipizide loaded plain Eudragit RS 100 nanoparticles). The release pattern was

optimized by addition of different concentrations of excipients during particle preparation (Table-1), namely by incorporation of 0.5% of Tween 20 and PEG, ~ 80% of glipizide was released within 24 hours. Among the two excipients, 0.5 % Tween 20 showed a better release profile than 0.5% PEG and this effect was observed in both the polymer particles. At lower concentration of excipient shows better release than higher concentration is due to higher concentration of PEG and Tween 20 accelerates agglomeration of nanoparticles at longer duration of time period than lower concentration, which affects the release pattern of glipizide.

All the three excipients (PEG, HPMC and TWEEN 20) are amphiphilic in nature so that they are soluble both in the organic and aqueous phase. During particle preparation the hydrophilic group is exposed towards the external aqueous phase and improves the hydrophilicity of the polymeric nanoparticles which enhances the release of glipizide from the polymeric nanoparticles. Additionally, PEG and HPMC are polymeric osmoagents which have the ability to improve the release of drug osmotically[22]. Due to the absence of amphiphilic surfactant in the formulation of A7 and B7 enhanced release was absence due to the solubility limit of the glipizide in buffer. The mechanism of enhanced release due to amphiphilic excipients reflects solubility enhancement of glipizide.

Tween 20 is relatively non-toxic[32] and is used as an emulsifier during particle preparation for improving the stability of emulsion and as a surfactant in a number of domestic, scientific, and pharmacological applications. The release profile can also be controlled by altering the pH of PLGA film by incorporation of basic amines (ammonium acetate/magnesium acetate) which can change the rate of degradation of polymer as results improve the release of drugs from the polymer particles[33].

Incorporation of stabilizers not only helped in improved entrapment of the drug in the particles but also helped in continuous release of the drug from the particles.

3.3 In vitro cytotoxicity study of PLGA and Eudragit nanoparticles

Cytotoxicity of the PLGA and Eudragit RS 100 nanoparticles was studied in the SW480 cells, a primary adenocarcinoma cell line of colon. As these nanoparticles are proposed for the oral delivery of anti-diabetic drugs like glipizide, the cell line was chosen to evaluate the cytocompatibility to the intestinal model. The assay was carried out by using alamar blue (AB), a water-soluble dye that has been previously used for quantifying in vitro viability of various cells[14,15]. When added to cell cultures, the oxidized form of the AB enters the cytosol and is converted to the reduced form by mitochondrial enzyme activity by accepting electrons from NADPH, FADH, FMNH, and NADH as well as from the cytochromes. This redox reaction is accompanied by a shift in colour of the culture medium from indigo blue to fluorescent pink, which can be easily measured by colorimetric or fluorometric analysis[14]. No significant cytotoxicity was observed at a concentration range of 12.5 to 500 µg/ml (Figure 4 and 5). In recent days, it was given most attention to the nanomaterials toxicity to human health, so it is important to understand the adverse toxicology of these polymeric nanoparticles. Particles less than 1000 nm was easily cross the cell membrane and follow different kinetic within the cells according to the surface functional group. In our recent study of PNIAPM nanoparticles with ~ 70nm particles, although internalized and localized in lysosomes, did not show any toxicological response to HaCaT (keratinocyte cells) and SW 480 (Primary adenocarcinoma cells)[15]. Similarly, both PLGA and Eudragit RS 100 nanoparticles of ~ 200 nm size did not produce any adverse toxicological response to the SW 480 cells at exposure concentration between 12.5 to 500 µg/ml, as result shows excellent biocompatibility to SW 480 cells *in vitro*.

3.4 In vivo study of glipizide loaded PLGA and Eudragit RS 100 nanoparticles

The efficacy of the glipizide loaded PLGA and Eudragit RS 100 nanoparticles was evaluated in female outbreed Wistar rats at doses of 800 µg/Kg body weight. Formulation A1, A5, B1 and B5 were selected for testing *in vivo* as the *in vitro* release of glipizide from these formulations was found to be better than the other formulations. It was observed that all the formulations (A1, A5, B1 and B5) reduced the blood glucose level in a sustained manner up to 24 hours (figure 6). A significant ($p \leq 0.05$) reduction in blood glucose level was observed in all the formulations as compared to control group. Reduction of blood glucose level was observed significantly ($p \leq 0.05$) in all the formulations at 4h, 6h, 12h and 24h time points as compared to standard glipizide (soluble form), as shown in Figure 6. An *in vivo* and *in vitro* correlation was established with all the formulations. The sustained release profile of glipizide from the polymeric nano-formulation was improved by the incorporation 0.5% HPMC, TWEEN 20 and PEG, which helps in the controlled manner of absorption and receptor attachment for the therapeutic effect of glipizide, as a result, improve and sustain the reduction of blood glucose level for a longer duration of time period from a single dose. These results indicated that by optimizing the release profile of glipizide using different surfactant, a single daily oral dose of glipizide entrapped PLGA 50:50 and Eudragit RS 100 nanoparticles can maintain blood sugar level up to 24 hours. This nanoparticulate glipizide formulation was much better than that observed with conventional glipizide which maintains blood glucose level for 4 to 6 hours from a single moral dose.

4. Conclusions

High entrapment efficiency, biocompatibility of polymeric nanoparticles and continuous release of the entrapped drug are the most essential prerequisite for the development of

polymeric nanoformulations for oral delivery of glipizide. The present investigation explored the possibility of controlled oral delivery of glipizide by combining the advantages of the PLGA and Eudragit RS 100 polymer particulate system using different excipients. Addition of PEG, HPMC and Tween 20 in the organic phase, during the particle preparation improved the release of glipizide from PLGA and Eudragit RS 100 nanoparticles. Cytotoxicity study in SW 480 cells indicates the biocompatibility of both the nanoparticles. Addition of different excipients resulted in sustained release of glipizide from both the nanoparticles up to 24 hours. Excipients helped in emulsion stability, uniform sized particle formulation and high entrapment efficiency of glipizide in nanoparticles.

The efficacy of the glipizide loaded PLGA and Eudragit RS 100 nanoparticles were evaluated in wistar rats. A significant reduction of blood glucose as compared to control group was observed for all formulations and the effect was sustained up to 24 hours. This represents a significant improvement on standard glipizide (soluble form) which exhibits a glucose reduction up to 4 hours. A single oral dose of such nanoparticles entrapped glipizide could be helpful in controlling glucose level for more than 24 hours. The study suggested that stability of emulsion during formulation, and incorporation of PEG and Tween 20 as an enhancer has major role in controlled release of glipizide from polymeric particles. Similar strategy can be used to stabilize for single dose based polymeric formulation for oral delivery of hydrophobic drugs.

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Table Legands

Table 1. Composition of different PLGA/Eudragit RS 100 nanoparticle formulations.

Table 2. Effect of different formulation on particle size, zeta potential and encapsulation efficiency.

Figure Legands

Figure 1.a. Size distribution of PLGA 50:50 nanoparticles

b. Size distribution of Eudragit RS 100 nanoparticles

Figure 2. *In vitro* release of glipizide from PLGA nanoparticles in different time points. A1, A2, A3, A4, A5, A6 and A7 are glipizide loaded PLGA nanoparticles formulations with different type and concentration of PEG, HPMC and Tween 20. Data shown in Mean ±SD (n=6).

Figure 3. *In vitro* release of glipizide from Eudragit RS 100 nanoparticles in different time points. B1, B2, B3, B4, B5, B6 and B7 are glipizide loaded Eudragit

RS 100 nanoparticle formulations with different type and concentration of PEG, HPMC and Tween 20. Data shown in Mean ±SD (n=6).

Figure 4. Cytotoxicity results of PLGA 50:50 nanoparticles in SW 480 cells. Data shown in Mean ± SD (n=3).

Figure 5. Cytotoxicity results of Eudragit RS 100 nanoparticles in SW 480 cells. 96 hour. Data shown in Mean ± SD (n=3).

Figure 6. *In vivo* study of glipizide loaded PLGA and Eudragit RS 100 nanoparticles in wistar rats. Gropup (GR) 1- Control group; Group 2- glipizide loaded PLGA nanoparticles (Formulation A1); Group 3- glipizide loaded PLGA nanoparticles (Formulation A5); Group 4- glipizide loaded Eudragit RS 100 nanoparticles (Formulation B1); Group 5- glipizide loaded Eudragit RS 100 nanoparticles (Formulation B5); Group 6- standard glipizide; all the formulations are given orally at a dose of 800 µg/Kg body weight. Data shown in Mean ± SD (n=6).

Table 1.

No. of formulations	External Aquuous Phase	Organic Phase	% Surfactant Used
A1	PVA 1% Sucrose 10% Milli-Q water	PLGA 50:50- 200mg DCM: 4 ml Glipizide 100mg	PEG : 0.5 %
A2	PVA 1% Sucrose 10% Milli-Q water	PLGA 50:50- 200mg DCM: 4 ml Glipizide 100mg	PEG : 2.5 %
A3	PVA 1% Sucrose 10% Milli-Q water	PLGA 50:50- 200mg DCM: 4 ml Glipizide 100mg	HPMC: 0.5 %
A4	PVA 1% Sucrose 10% Milli-Q water	PLGA 50:50- 200mg DCM: 4 ml Glipizide 100	HPMC: 2.5 %
A5	PVA 1% Sucrose 10% Milli-Q water	PLGA 50:50- 200mg DCM: 4 ml Glipizide 100	Tween 20 : 0.5%
A6	PVA 1% Sucrose 10% Milli-Q water	PLGA 50:50- 200mg DCM: 4 ml Glipizide 100mg	Tween 20 : 2.5%
A7	PVA 1% Sucrose 10% Milli-Q water	PLGA 50:50- 200mg DCM: 4 ml Glipizide 100mg	-
BI	PVA 1% Sucrose 10% Milli-Q water	Eudragit RS 100- 200mg DCM: 4 ml Glipizide : 100 mg	PEG : 0.5 %
B2	PVA 1% Sucrose 10% Milli-Q water	Eudragit RS 100- 200mg DCM: 4 ml Glipizide : 100 mg	PEG: 2.5 %
B3	PVA 1% Sucrose 10% Milli-Q water	Eudragit RS 100- 200mg DCM: 4 ml Glipizide : 100 mg	HPMC: 0.5 %
B4	PVA 1% Sucrose 10% Milli-Q water	Eudragit RS 100- 200mg DCM: 4 ml Glipizide : 100 mg	HPMC: 2.5 %
B5	PVA 1% Sucrose 10% Milli-Q water	Eudragit RS 100- 200mg DCM: 4 ml Glipizide : 100 mg	Tween 20 : 0.5%
B6	PVA 1% Sucrose 10% Milli-Q water	Eudragit RS 100- 200mg DCM: 4 ml Glipizide : 100 mg	Tween 20 : 2.5%
B7	PVA 1% Sucrose 10% Milli-Q water	Eudragit RS 100- 200mg DCM: 4 ml Glipizide : 100 mg	-

Table 2.

No. of Formulation	Particle Size in nm	Zeta Potential in mV	% EE
A1	248 ± 35.5	-15.96 ± 2.9	65.72 ± 3.9
A2	232 ± 23.2	-12.43 ± 3.1	68.992 ± 2.8
A3	236 ± 21.9	-5.42 ± 3.5	70.312 ± 4.1
A4	227 ± 19.7	-11.63 ± 2.1	70.312 ± 5.3
A5	221 ± 21.4	-6.17 ± 2.3	72.296 ± 6.8
A6	239 ± 35.5	-10.27 ± 1.2	70.312 ± 3.9
A7	243 ± 21.6	-6.3 ± 2.6	67.34 ± 6.4
BI	184 ± 5.9	32.89 ± 1.2	70.312 ± 3.4
B2	198 ± 7.8	13.57 ± 3.5	72.296 ± 3.8
B3	213 ± 9.1	23.18 ± 1.5	70.312 ± 4.6
B4	219 ± 8.2	21.23 ± 2.1	72.296 ± 2.3
B5	221 ± 11.8	7.06 ± 3.5	72.3 ± 2.7
B6	189 ± 13.6	22.99 ± 2.9	71.632 ± 1.9
B7	234 ± 25.3	23.71 ± 3.2	68.992 ± 4.5

Figures

Figure 1a.

b.

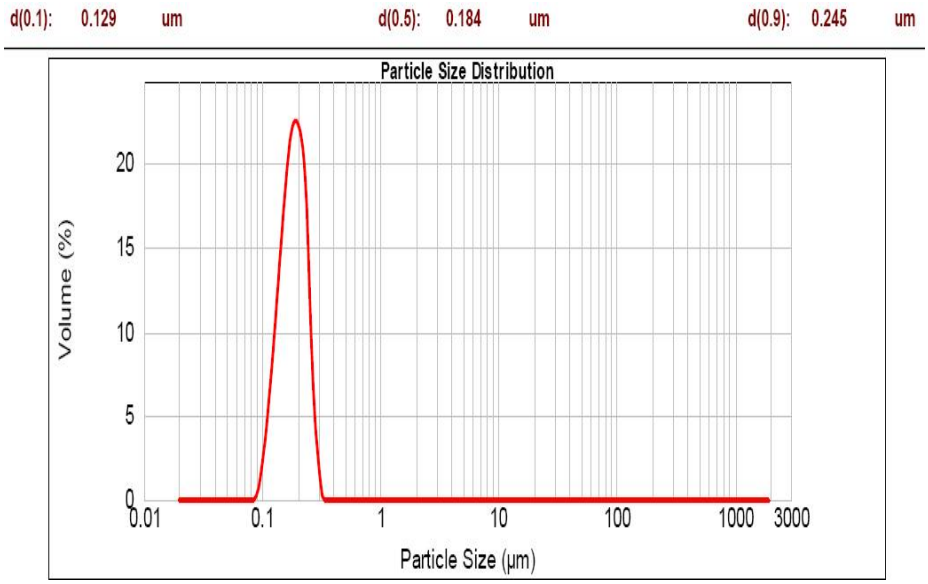


Figure 2.

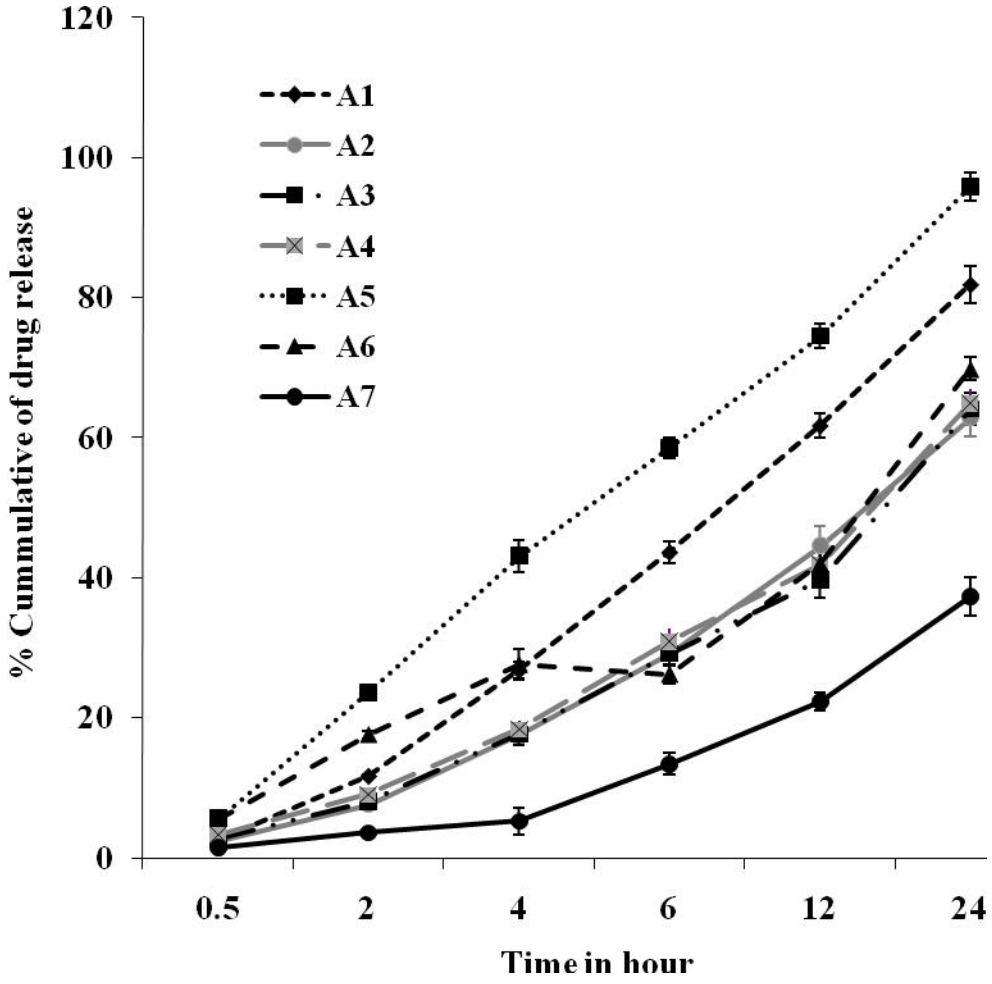

120
100
80
60
40
20
0
% Cummulative of drug release
A1
A2
A3
A4
A5
A6
A7
0.5
2
4
6
12
24
Time in hour

Figure 3.

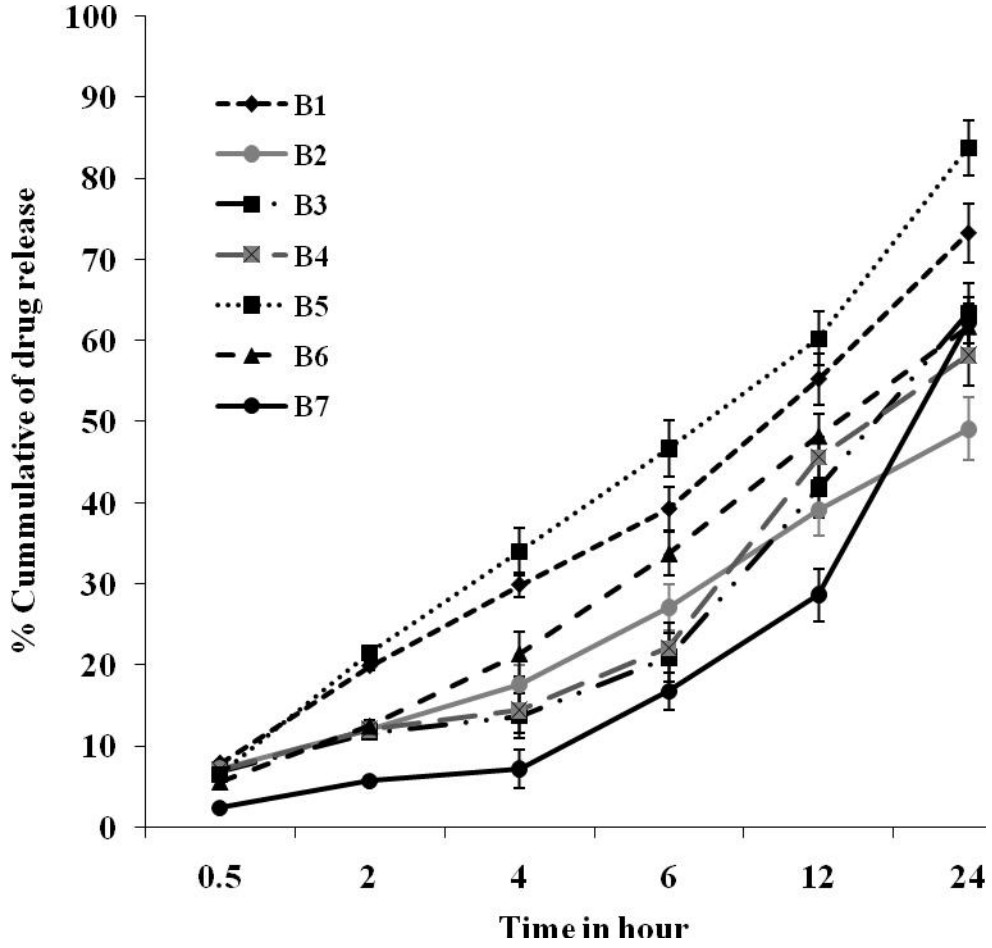

100
90
80
70
60
50
40
30
20
10
0
% Cummulative of drug release
B1
B2
B3
B4
B5
B6
B7
0.5
2
4
6
12
24
Time in hour

Figure 4.

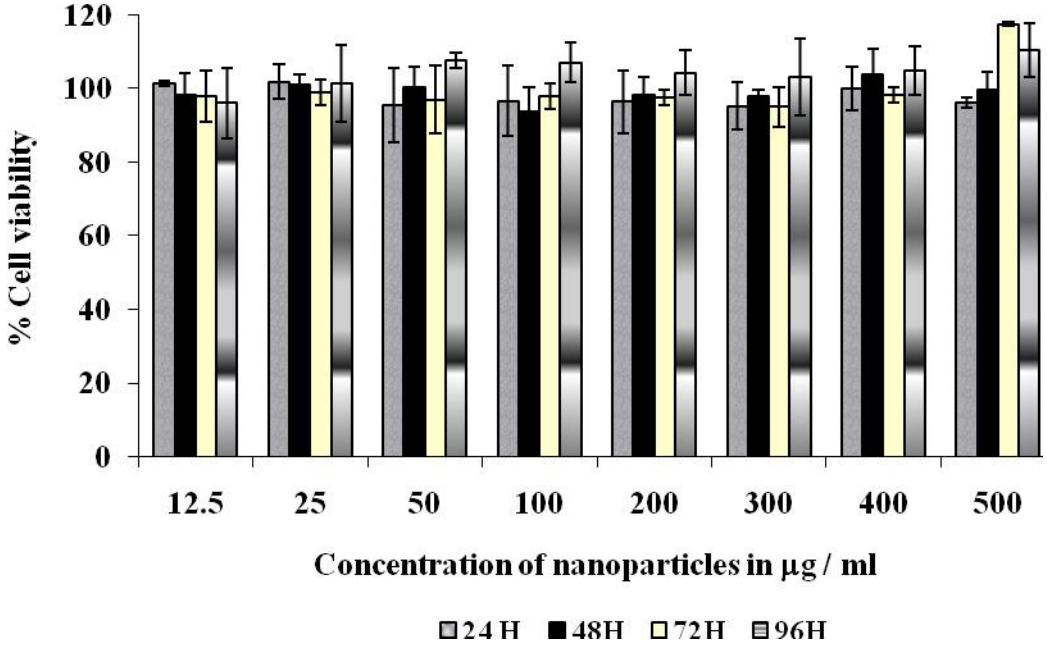

120
100
80
60
40
20
0
% Cell viability
12.5
25
50
100
200
300
400
500
Concentration of nanoparticles in µg / ml
24 H
48H
72H
96H

Figure 5.

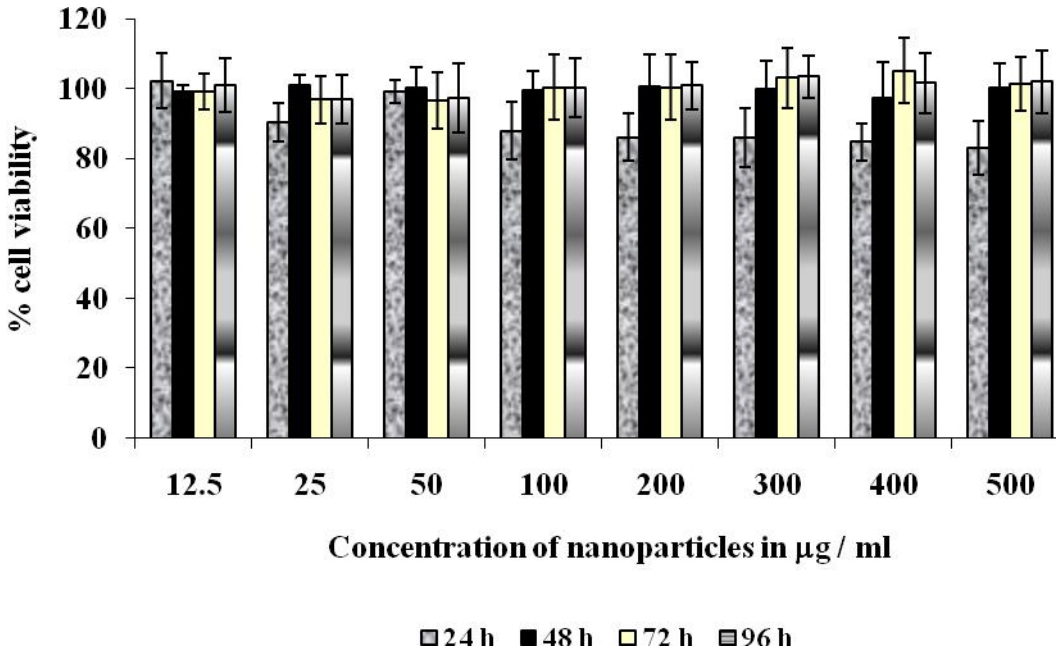
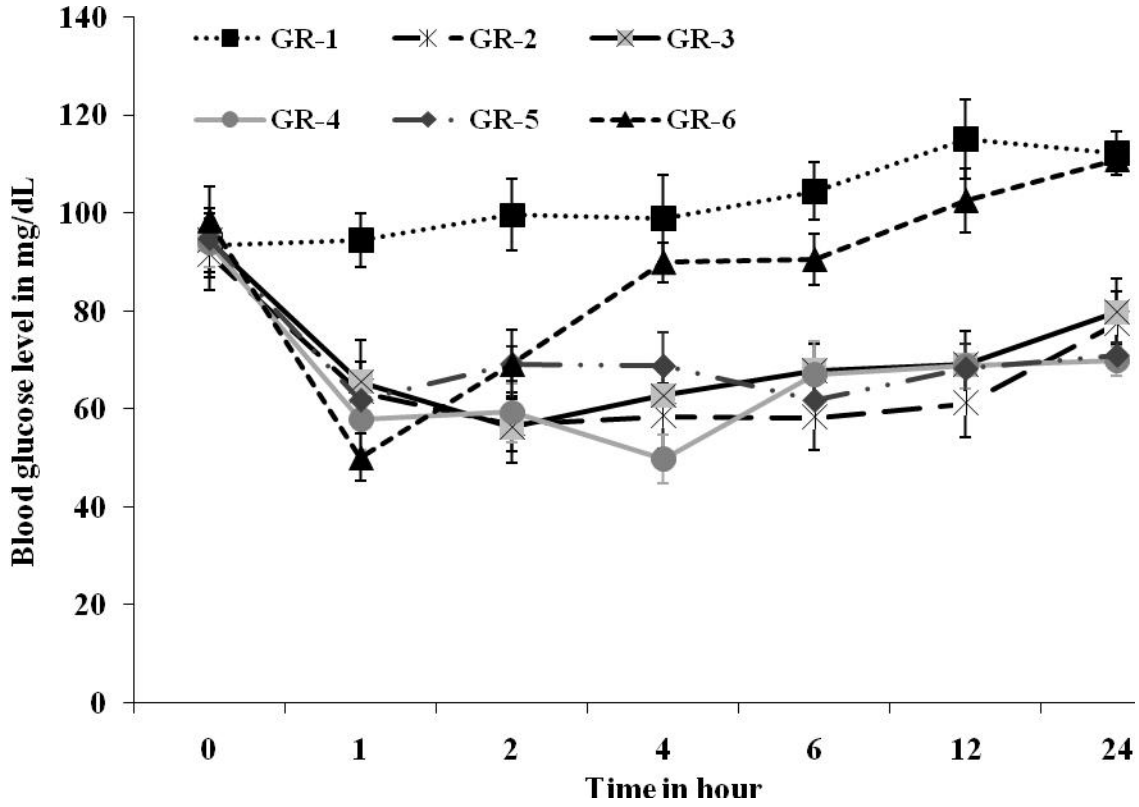

120
100
80
60
40
20
0
% cell viability
12.5
25
50
100
200
300
400
500
Concentration of nanoparticles in µg / ml
24 h
48 h
72 h
96 h

Figure 6.


GR-1
GR-2
GR-3
GR-4
GR-5
GR-6
Blood glucose level in mg/dL
140
120
100
80
60
40
20
0
0
1
2
4
6
12
24
Time in hour