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Formulation and Evaluation of Gliclazide Loaded Controlled Release Microspheres

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Abstract

The aim of this study was to formulate and evaluation of gliclazide loaded controlled release microspheres. The microspheres were prepared by quasi emulsion solvent diffusion technique using Eudragit RLPO, Eudragit RSPO alone and with combinations. The effect of drug-polymer ratio and polymer-polymer ratio on percent yield, mean particle size, encapsulation efficiency and *in vitro* release were evaluated. *In vivo* test of the optimized formulation was performed on streptozotocin induced type-2 diabetic rat model. The formulated microspheres showed higher encapsulation efficiencies. The *in vivo* study revealed that the hypoglycemic effect obtained by microspheres was more than 25 h suggesting microspheres are a valuable system for sustained delivery of gliclazide.

Keywords: Microspheres, Eudragit RSPO, quasi emulsion solvent diffusion, hypoglycemia.

Introduction

Microspheres, a novel microparticulate carrier used as prolonged or controlled drug delivery system (Siepmann and Siepmann 2006) and to improve bioavailability of drug (Dhovan et al. 2004). Microsphere can offer advantages like limiting fluctuation within therapeutic range, reducing side effects, decreasing dosing frequency and improving patient compliance (Davis 1985, Ritschel 1989). They spread out uniformly in the gastrointestinal tract thus avoiding exposure of the mucosa to high concentration of drug and ensuring more reproducible drug absorption. The risk of dose dumping also seems to be lower than with a single unit dosage form (Siepmann and Siepmann 2006).

Gliclazide, 1-(3-azabicyclo-[3, 3, 0]-oct-3-yl)-3-(p-tolyl sulfonyl) urea is a potent second generation sulfonylurea oral hypoglycemic drug which is most frequently used in the treatment of long term type-II diabetes (Dhovan et al. 2004). Previous studies show that gliclazide possesses good general tolerability, low incidence of hypoglycemia, (Davis 1985, Ritschel 1989) low rate of secondary failure, (Devrim and Canefe 2006) potential for slowing the progression of diabetic retinopathy (Davis 1985). Hence, gliclazide appears to be a drug of choice in long–term sulfonylurea therapy for the control of non-insulin dependent diabetes mellitus (Devrim and Canefe 2006, Hong et al. 1998). But unfortunately it causes gastrointestinal disturbances if present in larger concentration in gastrointestinal tract. Therefore, to avoid the above problem controlled release of gliclazide is necessary.

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For an oral hypoglycemic drug to be effective, rapid absorption from the gastrointestinal tract is required. However, the absorption rate of gliclazide from the gastrointestinal tract is slow and varied among the subjects (Dhovan et al. 2004). Several studies on healthy volunteers and diabetic patients revealed that the time to reach plasma concentration (t_{max}) ranged from 2 to 8 hrs following a single oral administration of 80 mg of gliclazide tablets (Davis 1985). Slow absorption of gliclazide has been suggested to be due to either poor dissolution owing to its hydrophobic nature or poor permeability of drug across the gastrointestinal membrane (Dhovan et al. 2004). The conventional formulation required twice daily administration. In a large randomized study on type-II diabetes patients, once daily gliclazide modified release 30-120 mg was found as effective as twice daily gliclazide 80-320 mg in reducing glycosylated hemoglobin (HbA1C), with fewer side effects and less risk of hypoglycemia and hence there is always a need for the development of sustained/controlled release patient compliant formulation of gliclazide (Mailhot 1993).

Thus an attempt was made to prepare oral controlled release microsphere of gliclazide utilizing Eudragit RSPO and Eudragit RLPO alone or in combination by quasi emulsion solvent diffusion method. The effects of different formulation variables (drug-polymer ratio and polymer-polymer ratio) on the physical characteristics of the prepared microspheres and the *in vitro* drug release rate were investigated. Moreover, the hypoglycemic effect of the prepared microspheres on the streptozotocin induced type-2 diabetic rat model were investigated.

Materials and Methods

Materials

Gliclazide was obtained as a gift sample from Lupin Ltd. (Pune, India). Eudragit RSPO and Eudragit RLPO were obtained as gift sample from Sun Pharma (Vododara, India). Polyvinyl alcohol was purchased from Merck (Mumbai, India). Streptozotocine and nicotinamide were purchased from Loba Chemie (Mumbai, India). All other reagents used were of analytical grade.

Method

Preparation of Microsphere

The modified quasi emulsion solvent diffusion technique was used for the preparation of gliclazide loaded microspheres using Eudragit RLPO, Eudragit RSPO alone and in combination as retardant materials [7]. Polyvinyl alcohol was used as surfactant to stabilize the microspheres. Eudragit RLPO and Eudragit RLPO alone or in combination were dissolved in 20 mL dichloromethane at 45^{0}C temperature in the different ratio as mentioned in the Table 1. The drug was added to the above solution and mixed with continuous stirring using magnetic stirrer (Remi, Mumbai, India) at 500 rpm. The above solution was poured into 80 mL distilled water containing polyvinyl alcohol (0.05%w/v) with continuous starring at 900 rpm using magnetic stirrer. The temperature of the system was thermally controlled at 20^{0}C. After 1 h of stirring, the microspheres were separated by filtration using ordinary filter paper (0.25 µm) and the microspheres were washed twice with 50 mL of distilled water. The formulated microspheres were dried in room temperature for 24 h.

Table 1. Formulations and physical properties of microspheres

Formulation code	Polymer used	Drug to polymer ratio	Encapsulation efficiency (%)*	Particle size (μm)*
FL1		1:01	85.19±1:1	327±2.3
FL2	Eudragit RLPO	1:02	93.5±1:7	259±1.5
FL3		2:01	94.17±2.1	231±1.9
FS1		1:01	79.7±1.3	235±1.6
FS2	Eudragit RSPO	1:02	88.87±1.5	173±2.2
FS3		2:01	89.21±1.4	153±1.4
Drug to polmer ratio 1:1				
		Polymer ratio		
FSL1	Eudragit RLPO + Eudragit RSPO	1:02	91.51±1.6	225±1.3
FSL2		1:04	90.9±1.1	201±1.9
FSL3		1:06	89.59±1.8	185±2.3

*n = 3 (Mean±SD

Characterization of Microspheres

Particle Size Analysis

Particle size of the microspheres was measured by laser light scattering technique (Mastersizer 2000, Malvern. UK). The sizes of the completely dried microspheres of different formulations were measured by dry sample technique using dry sample adapter. The completely dried particles were placed on the sample tray with an in built vacuum and compressed air system was used to suspend the particles. The laser obscuration range was maintained between 1% and 2%. The volume-mean diameter (V_d) was recorded. After measurement of particle size of each sample, the dry sample adopter was cleaned thoroughly to avoid cross contamination. Each batch was analyzed in triplicate, but the average values were considered in data analysis.

Drug Encapsulation Efficiency

Drug encapsulation efficiency of the prepared microspheres was carried out by crushing the microspheres using mortar and pastel. The powder microspheres equivalent to 8 mg gliclazide was taken in 100 mL volumetric flask containing 50 mL of phosphate buffer (pH 7.4) and shaken for 48 h in magnetic stirrer. The samples were filtered through membrane filter (0.45 μm) and after suitable dilution; the drug content was analyzed spectrophotometer using UV-Visible spectrophotometer (Shimadzu, UV-1700, Japan) at 225 nm. The percentage drug incorporation efficiency was calculated using the following equations: Practical drug content/Theoretical drug content) × 100 (Harrower 1994).

Drug-polymer Compatibility Study

The drug-polymer compatibility was carried out using FTIR spectroscopy. The FTIR spectra of blank and drug loaded microspheres were taken at room temperature in KBr pellets by applying 6000 kg/cm^2 pressure using Perkin Elmer FTIR model 883 (Pyris Diamond, USA) between range of 500 to 4000 cm^{-1}.

Scanning Electron Microscopy (SEM) Study

The surface topography of the prepared microspheres was examined by scanning electron microscope (Hitachi, S-3600N, Japan). The samples were fixed on brasss stub using double- sided tape and then gold-coated in vacuum by a sputter coater. The SEM pictures were then taken at an excitation voltage of 20 kV.

In vitro Drug Release Study

In vitro drug release study of the prepared microspheres were carried out using USP XXIV

paddle type apparatus (Campbell Electronic, Mumbai, India) using 900 mL of 0.1M hydrochloric acid (pH 1.2) and phosphate buffer (pH 7.4) as dissolution medium. The temperature of the above solution was maintained at 37±0.5°C and stirred at 50 rpm. At a predetermined time intervals, 5 mL of samples were withdrawn and replaced with the same volume of fresh media. The samples were then filtered through membrane filter (0.45 μm) and diluted suitably. The amount of drug present in the solution was then analyzed spectrophotometrically at 225 nm using UV-Visible spectrophotometer (Shimadzu, UV-1700, Japan).

In vivo Study

In vivo study of the gliclazide microspheres were performed on Wister albino rats of both sex between 2-3 months of age and weighing 180-240 g. The approval of Animal Ethics Committee was obtained before starting the study. The study was conducted in accordance with standard institutional guidelines. Hyperglycemia was induced in overnight fasted rats by a single intraperitoneal injection of streptozotocin at a dose of 65 mg/kg dissolved in ice cold 0.1M Citrate buffer (pH 4.5) (Campbell et al. 1991) after 15 minutes nicotinamide at a dose of 110 mg/kg was administered intraperitoneal (Kawashima et al. 1989). After 48 h of injection blood glucose level was monitored using glucose estimation kit (Auto Pak, Johnson and Johnson, India). The rats with blood glucose level 300 mg/dl or more were considered diabetic and were employed for further study. In the experiment, diabetic rats were divided into three groups of five rats in each. Group 1: Diabetic rats administered gliclazide solution (2 mg/kg). Group 2: Diabetic rats administered formulation FLS2 (dose equivalent to 2 mg/kg) by using oral feeding needle. Group 3: Diabetic rats administered tablet (dose equivalent to 2 mg/kg). For the estimation of blood glucose level, blood samples were withdrawn by the retro orbital puncture at predetermined time at 1 h intervals up to 25 h, and were analyzed by glucose oxidase and peroxidase (GOD/POD) method using commercial glucose estimation kit.

Results and Discussion

Quasi emulsion solvent diffusion method is widely used to prepare microspheres due to its following advantages: simplicity, low cost and encapsulation of both hydrophilic and lipophilic drugs. In the present work, modified quasi emulsion solvent diffusion technique was used with dichloromethane solution of drug and polymer as dispersed phase and solution of polyvinyl alcohol in water used as continuous phase. The aqueous and organic solvents are counter- diffused into and out of the droplets, respectively. The diffused water within the droplets might be decrease the drug and polymer solubility. On the other hand, the continuous dichloromethane diffusion resulted co-precipitation and solidification of drug-polymer mixture and thereby formation of matrix type microspheres.

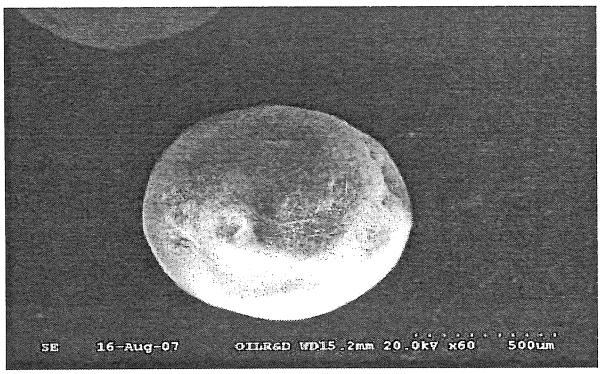
The temperature between internal phase (45°C) and external phase (20°C) were maintained to prevent the coalescence of microspheres as reported by Prajapati et al. (1989).

Particle size and size distribution were analyzed by dynamic laser light diffraction technique using Mastersizer-2000, Malvern, UK. Volume-mean diameter of the prepared microspheres produced by taking different drug-polymer ratios are summarized in Table 1. Particle size analysis of the microspheres containing Eudragit RLPO, Eudragit RSPO alone and in combination revealed that the mean particle diameter was affected by drug-polymer ratio. A reduction in microsphere size was observed with decreasing polymer amount and increasing in drug amount. As the drug amount was increased and the polymer amount increased, a more viscous internal phase occurred. During the emulsification process, the internal phase was dispersed in the outer phase and large microspheres were produced. When the amount of drug was increased and the amount of polymer decreased (drug-polymer ratio 1:2, 1:1 and 2:1), the size of the microspheres

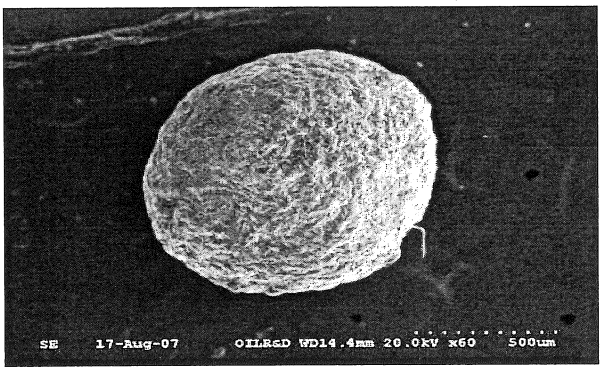
decreased due to reduced viscosity of the internal phase. These findings are similar to those reported by Kim et al. (1994). The mean particle size of microspheres containing Eudragit RLPO was less as compared with the microspheres containing Eudragit RSPO. The mean particle size of microspheres prepared by using combination of Eudragit RLPO and Eudragit RSPO decreased with decreasing of Eudragit RLPO amount and increasing of Eudragit RSPO amount.

The encapsulation efficiency of the microspheres was affected by the drug-polymer ratio. As the Eudragit RLPO amount decreased and drug amount increased, the encapsulation efficiency decreased; this is due to the fact that lower amount polymer produce small size droplets with increased surface area, such that the amount of diffusion of drug from such microspheres will be more, resulting in more encapsulation efficiency. Whereas, the encapsulation efficiency of the microspheres prepared with Eudragit RSPO increased with decreasing polymer amount and increasing drug amount. This is due to the fact that E udragit RSPO contain les amount of quaternary ammonium groups as compare with Eudragit RLPO. The effect of drug-polymer ratio on the encapsulation efficiency of microspheres was similar with the microspheres prepared by using Eudragit RLPO alone.

The SEM photograph (Figure 1) revealed the spherical nature of the microspheres with rough on the surface and no pores were observed. Pores were detected on the surface of microspheres after dissolution indicating leaching of the drug through the channels of the polymer. The spherical nature and size of the microspheres had not been changed after dissolution.



(a)



(b)

Figure 1. Scanning electron micrographs of gliclazide microspheres a) before and b) after *in vitro* release study.

The FTIR spectrum of gliclazide (Figure 2) revealed the presence of peaks at 1,710 cm^{-1} due to the presence of carboxyl group, peaks at 1,162 cm^{-1} due to sulphonyl group and peaks at 3,272 cm^{-1} was due to amino group. Major frequencies of functional groups of pure drug remain intact in microspheres containing different polymers; hence, there was no interaction between the gliclazide and the polymers used in the study.

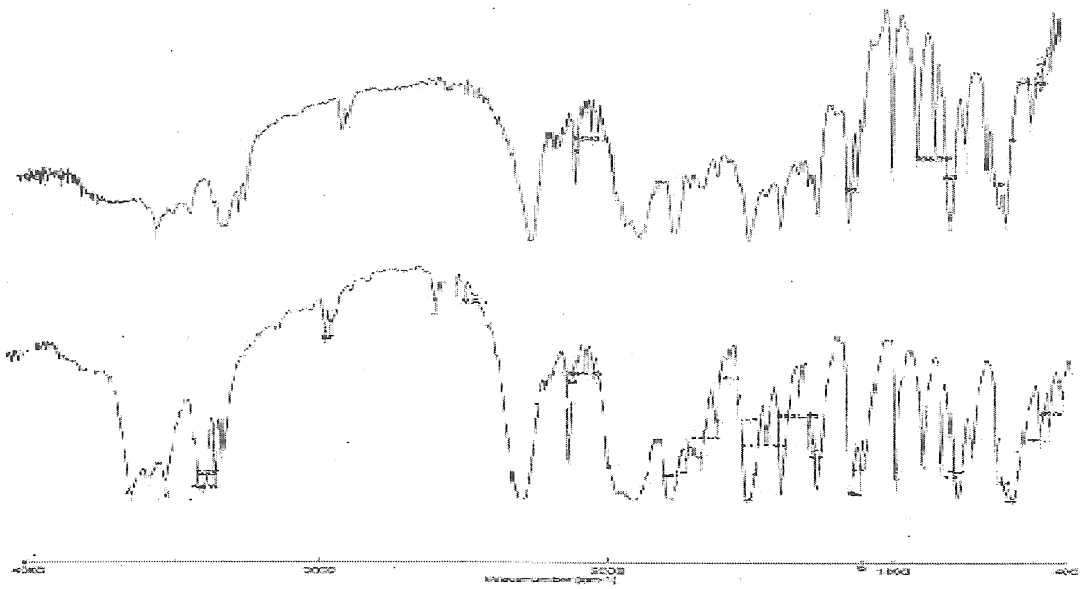


Figure 2. FTIR spectra of gliclazide and formulation FLS2

The *in vitro* release studies of some selective formulation carried out in both 0.1M hydrochloric acid (pH 1.2) and phosphate buffers (pH 7.4). The selection of the formulation for *in vitro* release study was based on the morphology, mean particle size, and encapsulation efficiency of the microspheres. The release profile of gliclazide microspheres in 0.1M hydrochloric acid (pH 1.2) and phosphate buffer (pH 7.4) were presented in Figure 3 and Figure 4, respectively. As the amount of polymer increased in the formulation, the release rate decreased insignificantly ($p<0.05$). Increasing in amount of polymer in the formulation resulted in increase in particle size, thereby increasing in matrix thickness formed by the polymer, resulted in the increasing the distance that the drug passed through the surface of the microspheres. Similar arguments were made by Kawata et al. (1986) and Chiao and Price (1994). Gliclazide release from microspheres at 0.1M hydrochloric acid (pH 1.2) was slow as compared to release at phosphate buffer (pH 7.4). The reason for this low release might be the acid resistant nature of the polymer and gliclazide is a weak acid drug and its solubility is higher at high pH, as expected. The release of drug from the microspheres was more sustained in microspheres that prepared with Eudragit RSPO as compared with Eudragit RLPO. This is due to the fact that Eudragit. RSPO content less amount of quaternary ammonium group (4.5-6.8%) as compare with Eudragit RLPO (8.8-12%). Therefore, to get the optimum sustained release of drug from the microspheres these two polymers were combined in different ratio. The release of the drug decrease significantly ($p<0.05$) as polymer ratio (Eudragit RLPO: Eudragit RSPO) increased from 1:2 to 1:4, but there is no significant ($p<0.05$) decrease was observed when the polymer ratio increased from 1:4 to 1:6. This was observed in 0.1M hydrochloric acid (pH 1.2) as well as in phosphate buffer (pH 7.4). Therefore, the formulation FSL2 was

selected for the further in vivo study on the basis of good morphological characteristics, particle size, high encapsulation efficiency, and in vitro sustained release of drug in 0.1M hydrochloric acid (pH 1.2) and phosphate buffer (pH 7.4).

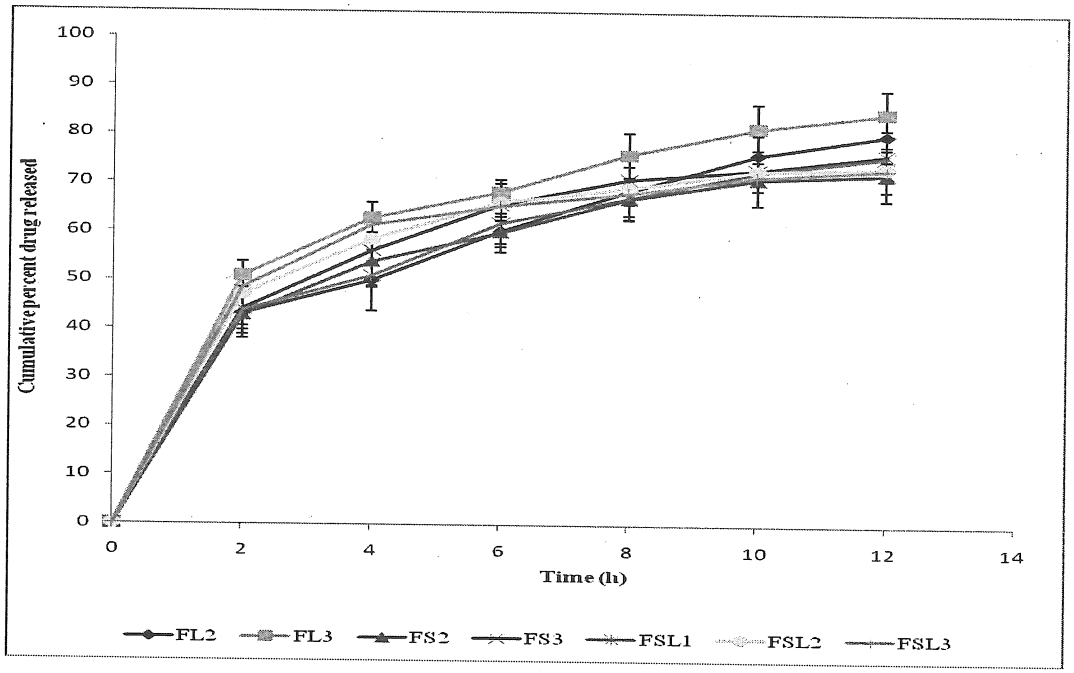


Figure 3. *In vitro* release profile of gliclazide microspheres in 0.1M hydrochloric acid (pH 1.2)

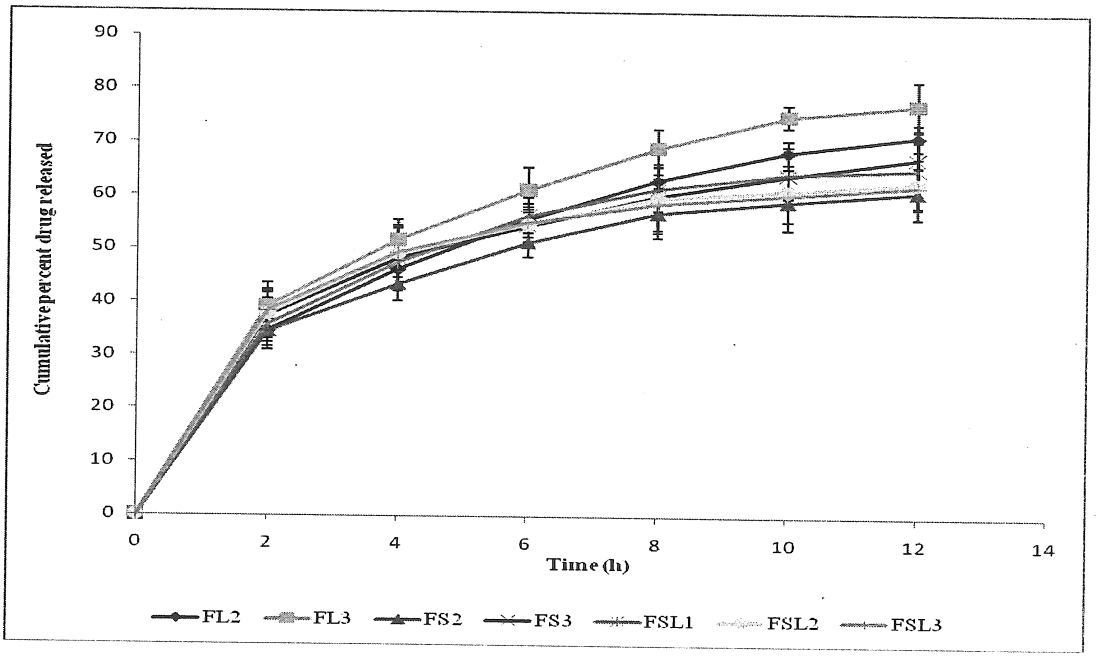


Figure 4. *In vitro* release profile of gliclazide microspheres in phosphate buffer (pH 7.4)

The drug was administered at a dose equivalent to 2 mg/kg body weight of gliclazide. Pure gliclazide solution was administered in a suspension form at the same dose. A 25% reduction in blood glucose level is considered as significant hypoglycemic effect which was maintained from 0.5 to 10 h after oral administration of gliclazide. When pure gliclazide solution was administered a rapid reduction in blood glucose level was observed and maximum reduction of 47.9% was observed within 1 h after oral administration. Then blood glucose level was increased with increased in time but still significant hypoglycemic effect was shown up to 11 h and blood glucose level were recovered nearly to the normal level in 15 h (Figure 5). When conventional tablet of gliclazide was given, a constant decrease in blood glucose level (maximum up to 44.62%) obtained at 10 h. Then blood glucose level was increased with increased in time and but still significant hypoglycemic effect was shown up to 13 h of administration. In case of FSL2 significant hypoglycemic activity was obtained within 1 to 2 h of administration of drug and maximum reduction in blood glucose level was obtained after 3 h of administration of formulation i.e. 46.3% and after 24 h the % reduction in blood glucose level was 25.72%. Hence it showed significant hypoglycemic effect more than 24 h.

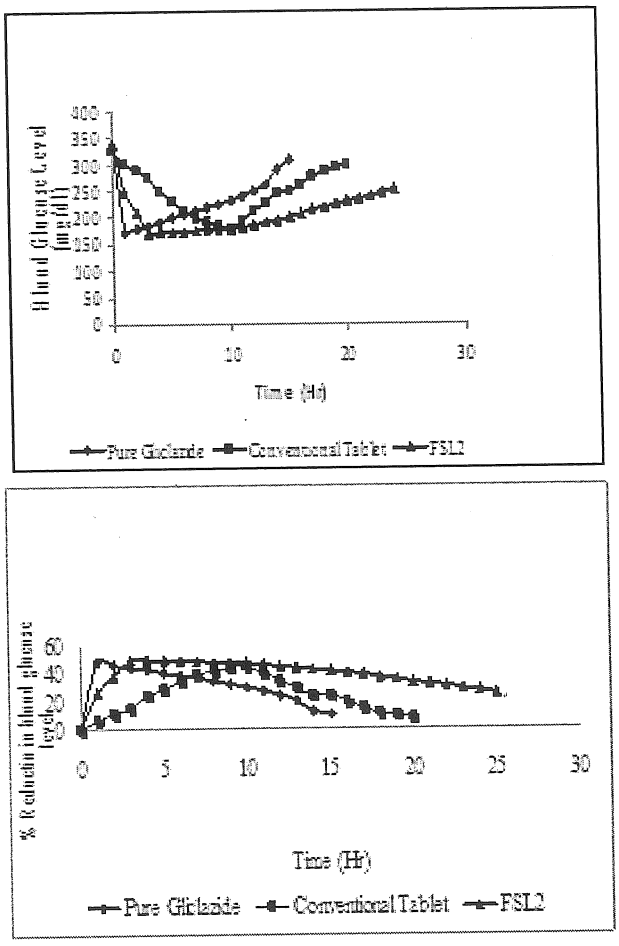


Figure 5. Comparative in vivo study of gliclazide microspheres of formulation FSL2 with pure gliclazide and conventional marketed tablet

Conclusion

Gliclazide loaded spherical microsphere were successfully prepared by quasi emulsion solvent diffusion method. The results revealed that the drug-polymer ratio and polymer-polymer ratio were affects the percentage yield, encapsulation efficiency and release of the gliclazide from the microspheres. It can be concluded from this study that gliclazide could be made in to controlled- release drug delivery system using formulation FLS2. FLS2 microspheres significantly reduced the blood glucose level in controlled manner as compared with conventional tablet. Hence, the formulation FLS2 has potential to be used as controlled release formulation.

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