International Journal for Pharmaceutical Research Scholars (IJPRS)

V-3, I-4, 2014 ISSN No: 2277 - 7873

RESEARCH ARTICLE

Formulation and Evaluation of Eudragit RS100 Nanoparticles Containing Glibenclamide

Patel CJ[1*], Patel DV[2]

[1]*Research Scholar, Shri J.J.T.University, Jhunjhunu, Rajasthan, India.*

[2]*B. K. Mody Govt. Pharmacy College, Rajkot, Gujarat, India.*

Manuscript No: IJPRS/V3/I4/00432, Received On: 19/11/2014, Accepted On: 27/11/2014

ABSTRACT

The Glibenclamide is a drug of choice for the physician for Non-Insulin Dependent Diabetes (NIDD). The drug in oral conventional dosage form has the dosage regime of three times a day. The repeated administration in a day may cause noncompliance by the patients. Thus, it is emphasized to prolong/sustain release delivery of the drug to avoid repeated administration. The objective of the present study was to develop Glibenclamide loaded nanoparticles using Eudragit RS100 as release control polymer. Different ratio of Drug: Eudragit RS100 was tried. The prepared nanoparticles were evaluated for particle size, zeta potential, % yield, Association efficiency, CPR. *In-vivo* anti-hyperglycemic activity of the FN8 batch was studied. The formulation containing 1:4 ratio of Drug:Eudragit RS100 was selected as best formulation.

KEYWORDS

Glibenclamide, Eudragit RS100, Nanoparticle, Factorial Design, Optimization

INTRODUCTION

Oral ingestion has been the most convenient and commonly employed route for drug delivery. The oral route of drug administration has received more attention with respect to the research on physiological and drug constraint as well as design and testing of products. This is because there is more flexibility in dosage form design for the oral route than there is for parental route. The reason that the oral route achieved such popularity may be in part attributed to its ease of administration as well as the traditional belief that by oral administration, the drug is well absorbed as the food stuff that are ingested daily.[1] Interest has grown in the design of drug-containing formulations which deliver drugs to specific 'targets' in the body as well as providing drug over longer periods of time at controlled rates. A nanoparticle is a submicroscopic solid particle with a size ranging 10 nm to 1 μm. They can be prepared from emulsion, micelles, interfacial polymerization, preformed polymers, and coacervation. Nanoparticles occupy a unique position in drug delivery technology due to their attractive properties. In particular, nanoparticles have several advantages in pharmaceutical applications. In addition, they offer drug targeting possibilities and a sustained release action.[2]

***Address for Correspondence:**
Chirag J. Patel
Research Scholar, Shri J.J.T.University,
Jhunjhunu, Rajasthan, India.
E-Mail Id: chirag999patel@yahoo.co.in

Introduction to Drug Glibenclamide

Glibenclamide is a potent sulfonylurea and has established potential benefits such as lower dose, rapid onset, lower insulin levels and less-pronounced glucagon tropic effects, insulin-sensitizing and insulin-mimetic affects. However it is a poorly soluble drug (b8 μg/ml in

pH 7.4 phosphate buffers) with relatively high permeability through CaCo-2 cell monolayer's which warrants it to be classified under BCS Class II classification. Glibenclamide is effectively absorbed from the gastrointestinal tract, but the presence of food and certain dietary supplements interfere with its dissolution and in turn its absorption. Glibenclamide may be more effective if given 30 min prior to meal. The application of nanotechnology drug delivery for improving the dissolution characteristics of Glibenclamide is still in the early hours.[3] Glibenclamide is a second-generation sulphonylurea that is an orally bioavailable hypoglycemic agent used in the management of type 2 diabetes. It is administered in low doses (5 mg), is quickly cleared from the body, and its active metabolites have a considerable hypoglycemic effect.[4] Different research has reported that Glibenclamide has a low bioavailability, which is attributed to its poor dissolution properties.[5-7]

Different methods have been reported to determine Glibenclamide levels in various biological fluids, such as plasma and serum[8-10] in pharmaceutical formulation analyses[11-13] or in simultaneous determination of anti-diabetic drugs.[14]

Introduction to Polymer Eudragit RS 100

Eudragit RS Nanoparticles showed sustained release of the drug at the acidic conditions (pH 1.0) approximately 30% of the drug was released initially and the drug release was found to be approximately linear. Eudragit is employed as a coating material, usually for coating pallets or microparticles that are filled in to capsules or compressed into tablets. Eudragit RS has been used as a sustained release coating material. Water can penetrate in the Eudragit RS material and dissolve the encapsulated material, which then diffuses in the aqueous phase and finally into bulk solution. Eudragit serves as a matrix in which the active medicament is embedded. The matrix structure is obtained by direct compression and wet granulation. Eudragit may additionally be used to form the matrix layers of transdermal delivery system. They have also been used to prepare novel gel formulation for rectal administration.[15-17]

MATERIALS AND METHOD

Glibenclamide was gift sample from Zydus Pharm. Ltd., India. Eudragit RS100 was gift sample from Evonic Roehm Pharma Polymers, India. All other materials and solvents used in the study were of LR Grade.

Drug-Excipient Compatibility Study

For the compatibility study of drug-polymer and stability of drug during formulation process Fourier transform infra-red (FT-IR) spectroscopy analysis was piloted. Potassium bromide (KBr) pellet method was used to record FT-IR spectrums of moisture free samples. FT-IR spectrum of pure Glibenclamide, physical mixture of Eudragit RS100 and Glibenclamide with excipients and nanoparticles were analyzed. The formulation was kept for stability study before going for the FT-IR study.

Preparation of Glibenclamide Nanoparticles

Nanoparticles containing Glibenclamide were prepared using nanoprecipitation method. Nanoparticles were prepared by using different drug to polymer ratio. The different ratio of drug and polymer is as shown in Table 1 100 mg drug was dissolved in 10 ml chloroform and then the solution was diluted with water to make up the required volume. A cosolvent was needed in order to make the inner phase more homogeneous. Then polymer and 150 mg of propylene glycol were dissolved in 4 ml of methanol, and this solution was added to the drug solution to form dispersion. The dispersion was added to 40 ml of aqueous ethanol solution (70%). After 5 minutes of mixing, the organic solvents were removed by evaporation at 35°C under normal pressure, nanoparticles were separated by using cooling centrifuge (10000 rpm for 30 min), supernatant was removed and nanoparticles were washed with water and freeze dried. [18-19]

The various batches of nanoparticles were prepared as shown in Table 1.

Table 1: Composition of Glibenclamide-Eudragit RS 100 nanoparticle

Sr. No.	Formulation Code	Glibenclamide (mg)	Eudragit RS 100 (mg)
1	FN1	50	50
2	FN2	100	50
3	FN3	150	50
4	FN4	200	50
5	FN5	250	50
6	FN6	50	100
7	FN7	50	150
8	FN8	50	200
9	FN9	50	250
10	FN10	50	300

Evaluation of Nanoparticles

Particle Size Analysis and Zeta Potential

Particle size distribution of prepared nanoparticle formulations was studied by Laser Diffraction Particle Size Analyzer (SHIMADZU & METROHM). The data obtained after the observation were analyzed accordingly. The zeta potential of the samples was measured by a Zetatrac (METROHM).

Percentage Process Yield

The percentage yield of different formulations was determined by weighing the nanoparticles after freeze drying. The percentage process yield was calculated as follows:

Percentage process yield = (W_1/W_2) x 100

Where, W_1 – Total weight of nanoparticles

W_2 – Total initial weight of solids

Percentage Association Efficiency

The nanoparticle association efficiency of Glibenclamide was determined upon separation of nanoparticle from the aqueous preparation medium containing the non-associated drug by centrifugation (16,000×g, 30min, 15 ◦C). Concentrations of Glibenclamide in the supernatant (C_2) were determined by UV-visible spectrophotometry at 230 nm after suitable dilution. The entrapment efficiency was calculated according to the following equation:

Percentage association efficiency = (C_1 - C_2) / C_3 × 100

Where, C_1 – Total amount of drug taken for the formulation

C_2 – Concentration of drug in supernatant layer

In-vitro Release Studies

At the start of the study *in-vitro* release studies were carried out by dialysis bag method. An amount of nanoparticle suspension equivalent to 60 mg pure Glibenclamide was filled in dialysis bag (10 ml) (Hi media). In the acid stage, dialysis bag was placed in a round bottomed cylindrical vessel of USP dissolution test apparatus II; containing675 ml of 0.1 N HCl. Stirring speed was 100 rpm and the temperature was maintained at 37 $\pm$ 0.5^{0}C as per given in *BP 2009*. Aliquots were withdrawn at predetermined time intervals and immediately replaced with the fresh medium equilibrated at 37^{0}C. After 2 h, 225 ml of 0.2 M tribasic sodium phosphate was added to change the pH of test medium to 7.4. The sink condition was maintained throughout the experiment. The withdrawn samples were diluted and analyzed for drug content using U.V. spectrophotometer

at 230 nm keeping 0.1 N HCl and phosphate buffer pH 7.4 as blank depending on the time interval for sample taken. All the determinations were made in triplicate.

Kinetic Modeling

In order to understand the kinetic and mechanism of drug release, the result of *in-vitro* drug release study of nanoparticles were fitted with various kinetic equation like zero order (equation 3) as cumulative percentage release vs. time, higuchi's model (equation 4) as cumulative % drug release vs. square root of time. r^2 and k values were calculated for the linear curve obtained by regression analysis of the above plots.

$$C = k_0 t$$

Where k_0 is the zero order rate constant expressed in units of concentration/time and t is time in h.

$$Q = k_H t^{1/2}$$

Where, k_H is higuchi's square root of time kinetic drug release constant.

To understand the release mechanism *in-vitro* data was analyzed by peppas model (equation 5) as log cumulative drug release vs. log time and the exponent n was calculated through the slope of the straight line.

$$M_t / M_\infty = bt^n$$

Where M_t is amount of drug release at time t, M_∞ is the overall amount of the drug, b is constant, and n is the release exponent indicative of the drug release mechanism. If the exponent n = 0.5 or near, then the drug release mechanism is Fickian diffusion, and if n have value near 1.0 then it is non-Fickian diffusion.

Stability Study

Effect of Different Temperature on Eudragit RS100 Nanoparticle Size Distribution

The prepared Eudragit RS100 nanoparticles batch FN8 were subjected to storage at two different temperature conditions i.e. 4^0C and 25°C for 12 months. The stability of Eudragit RS100 nanoparticles was determined by studying the particle size distribution.

In-vivo Anti-Diabetic Studies

The *in vivo* release behavior of the formulation was studied by measuring anti-hyperglycemic activity in normal healthy male albino rats (weighing 250 to 300 g each) using the glucose-measuring instrument. Rats were caged under controlled temperature and 12 h light/dark cycle. They were fed with standard laboratory chow and water. The experiments were designed and conducted in accordance with the guidelines of institutional animals' ethics committee. For the induction of diabetes, rats were kept on fasting for 24 h prior to alloxan injection. On the day of administration, alloxan tetrahydrate was freshly dissolved in 0.01 M (pH 4.5) citrate buffer and subcutaneous injection was given at the dosage of 250 mg/kg. Blood glucose concentration was checked by the glucose oxidase and Glucometer (Roche) after 1 week of alloxan injection. The animals with glucose concentration exceeding 250 mg/dl were considered diabetic. To evaluate the anti-hyperglycemic activity of drug formulation, the male wistar albino rats were divided into three groups, each group consisting of six animals. One group served as control, second group served as diabetic control while in third group, again it was divided in two groups of three animals, one group received Glibenclamide solution and another group received nanoparticles containing Glibenclamide orally (5 mg/kg of Glibenclamide) once daily during experiment. Glibenclamide nanoparticles were administered orally to third group by stomach intubation. Blood samples were collected at particular time intervals upto 24 h and the blood glucose level was measured as described.

RESULTS AND DISCUSSION

Drug-Excipient Interaction Study

The results of the FT-IR spectroscopy analysis conducted for the analysis of drug-polymer interaction shows no chemical interaction between Glibenclamide and Eudragit RS100.

Evaluation of Nanoparticle

Table 2: Tabulated study results of various evaluation parameters

Sr. No.	Formulation code	Particle size (nm)	Zeta potential (mV)	Process yield (%)	Association efficiency (%)
1	FN1	353.7 ± 21.89	25.34 ± 1.21	63.80 ± 0.96	39.24 ± 0.65
2	FN2	354.4 ± 18.65	25.74 ± 1.03	63.67 ± 0.59	37.42 ± 1.53
3	FN3	404.6 ± 49.38	20.43 ± 1.13	62.48 ± 0.84	36.15 ± 0.97
4	FN4	423.8 ± 28.95	24.86 ± 1.00	63.81 ± 0.39	33.14 ± 1.28
5	FN5	402.2 ± 8.93	24.23 ± 1.48	63.09 ± 0.74	25.92 ± 1.06
6	FN6	375.4 ± 40.25	27.86 ± 0.94	61.47 ± 0.65	44.96 ± 1.14
7	FN7	477.9 ± 32.37	28.34 ± 1.07	63.40 ± 0.33	54.43 ± 1.72
8	FN8	474.2 ± 34.48	29.36 ± 1.63	64.97 ± 0.67	59.54 ± 1.83
9	FN9	436.8 ± 26.71	28.92 ± 1.23	68.41 ± 0.92	57.11 ± 1.65
10	FN10	423.1 ± 17.47	28.38 ± 1.27	67.12 ± 0.72	56.33 ± 1.17

Particle Size Analysis and Surface Morphology

The particle size of nanoparticles was examined for all the prepared formulations. The particle size of nanoparticles varied among the formulation due to variation in the composition of formulations. The mean particle size of nanoparticle formulations was in the range of 353 – 477 nm. Nanoparticles have relatively higher intracellular uptake as compared to microparticles. Drug release and polymer degradation may also be affected by particle size distribution. From the SEM analysis of the Eudragit RS 100 nanoparticles one can conclude that shape of the nanoparticle was approximately spherical with irregular surface properties. There was also presence of drug particles on the surface of prepared nanoparticles which may have been absorbed during the preparation of nanoparticles. As seen from the Figure 1, particle size remained within the narrow range for FN1 to FN5 where concentration of drug was changed for each formulation. But when FN6 to FN10 were compared for particle size distribution, the mean diameter tend to increase with increase in the polymer concentration to some extent.

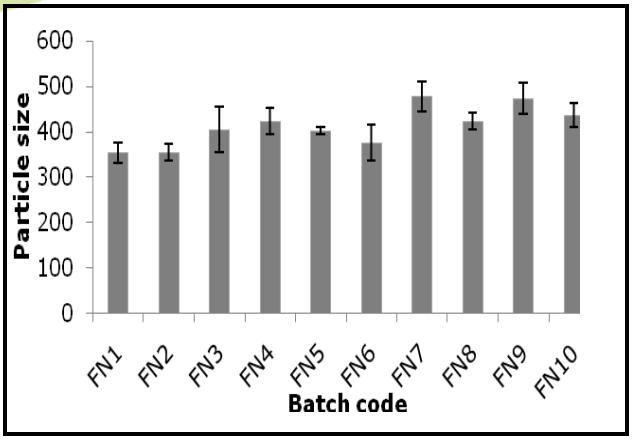

Figure 1: Comparison of particle size of batches FN1 to FN10

Zeta Potential Analysis

An important characteristic of nanoparticles is the surface charge which determines the

physical stability in the formulation, in vivo distribution and targeting ability of nanoparticles. The zeta potential is the measure of the amount of charge on the particle and represents an index of particle stability. A physically stable nanosuspension stabilized by electrostatic repulsion should have a minimum zeta potential value of ± 30 mV. The zeta potential also indicates whether the charged active material is encapsulated within the center or adsorbed onto the surface of the nanoparticles. Thus consideration of the zeta potential is important in preventing aggregation of the particles. The values of zeta potential for different batches were compared in Figure 2. The zeta potential values for the prepared formulaitons were in the range of 20 mV to 30 mV. Zeta potential for the prepared nanoparticles was indicating that increase in polymer concentration has no considerable effect on the value of zeta potential as it remained in narrow range for FN6 to FN10.

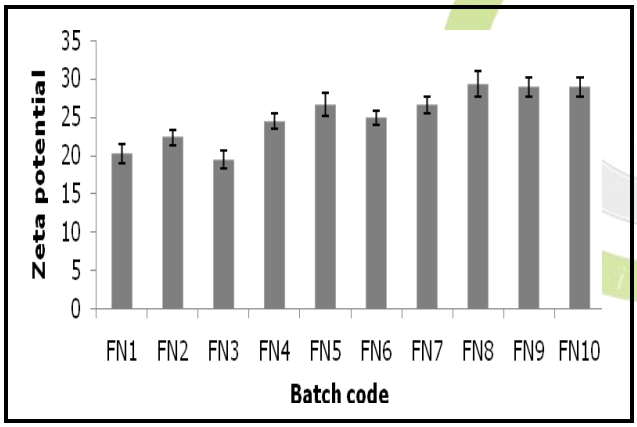

Figure 2: Comparison of zeta potential of batches FN1 to FN10

Percentage Process Yield

The percentage yield of different formulation was determined by weighing the nanoparticles after drying. The percentage yields of different formulation were in the range of 61.47 + 0.65 % to 68.41 ± 0.92 %, Figure 3. Determination of process yield was carried out by calculating the weight of the nanoparticles and the total amount of solid used. Ratio of which gives the process yield of the prepared formulation. It can be seen that the process yield decreases very slightly with increase in drug concentration, FN1 to FN5 and there was marked increase in process yield from FN6 to FN9, then after increase in concentration of polymer didn't increase the process yield.

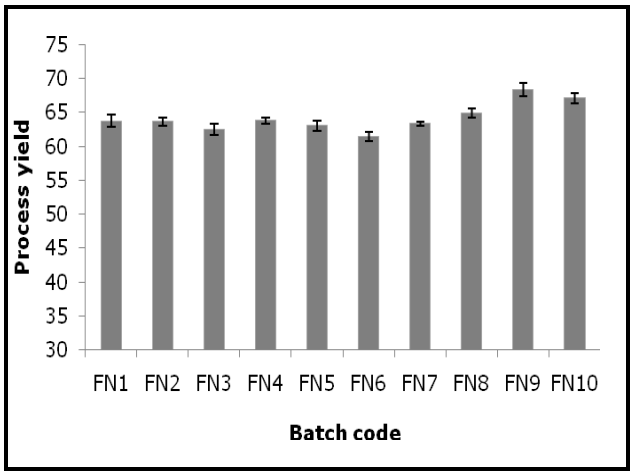

Figure 3: Comparison of process yield of batches FN1 to FN10

Percentage Association Efficiency

The indirect method was used to determine drug association efficiency. After preparing the fresh nanosuspension, it was centrifuged and the free drug present in the supernatant was analyzed by UV-Visible spectrophotometry using a calibration curve. By subtracting form initial amount of drug, association efficiency was calculated. The method is suitable for determining association efficiency of nanosuspension when fairly high concentration of free drug is present in the supernatant after centrifugation to be detected using UV-visible spectrophotometer.

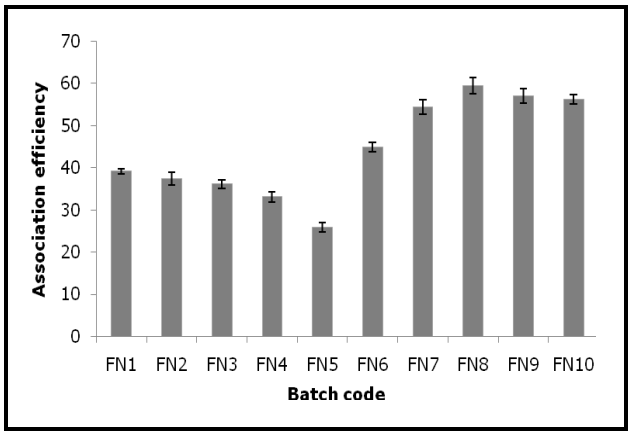

Figure 4: Comparison of association efficiency of batches FN1 to FN10

The drug association efficiency of different formulations was in the range of 25.92 ± 1.06 % to 59.54 + 1.83 %. Drug association efficiency was decreased with the increase in drug content and it increase with increasing polymer concentration. Water could penetrate in Eudragit RS100 which facilitates the diffusion of a part of entrapped drug to surrounding medium during preparation of nanoparticles.

In-vitro Release Studies

Release of Glibenclamide from nanoparticles was evaluated in 0.1 N HCl (pH 1.2) and phosphate buffer pH 7.4. Eudragit RS 100 is of low permeability and insoluble in acidic medium. It is an anionic copolymer of methacrylic acid, methyl methacrylate containing free carboxylic and ester groups. It has very low permeability results from high intermolecular attraction between its molecules. Hydrogen bonding between the hydroxyl groups of the carboxylic moiety and the carbonyl oxygen of ester group increase the degree of compactness of the polymer and decrease its porosity and permeability. Water can penetrate in the Eudragit RS material and dissolve the encapsulated material, which then diffuses in the aqueous phase and finally into bulk solution. Approximately 30% of the drug was released initially. Further drug release from the Nanoparticles matrix was controlled by the polymer.

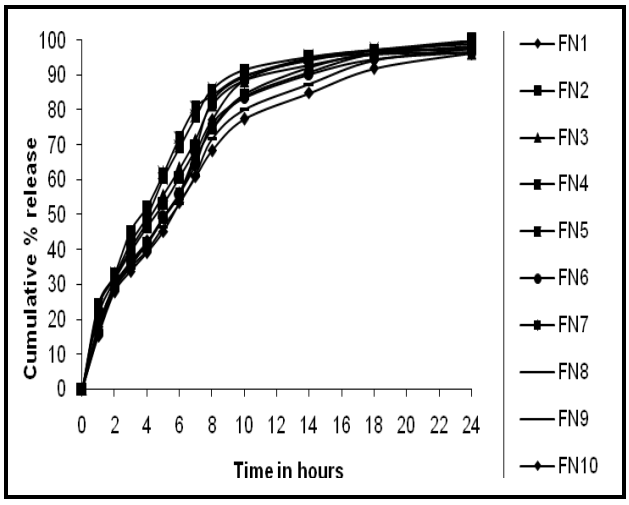

Figure 5: Dissolution profiles of batches FN1 to FN10

As the 70%-80% drug for most formulations was released within 8 to 10 h the nanoparticles could not give a day long therapeutic effect which was not desired for this study. The drug release from the nanoparticles was showing steady and controlled rate after the change in pH of the dissolution medium. This could be because as Eudragit RS 100 is not a water soluble polymer and does not show pH dependency. Therefore the rate of drug release was in controlled manner. Release data has been shown in Figure 5.

If we compare the release of drug from different formulations after 2 h it was found that as the concentration of Eudragit RS 100 increases CPR also increase. The reason behind that could be more amount of drug was available on the surface of the nanoparticle as the concentration of drug increases. This could be supported by the results of the of the association efficiency as they were showing the increase in value with increase in concentration of drug. Concentration of polymer was changed for FN5 to FN10 and drug concentration was kept constant. When the results of the drug release were compared it was evident that there was an effect of polymer concentration on the amount of drug release from the nanoparticles. As the polymer concentration was increased the drug release was decreased. **FN8** was found to be promising formulation out of all prepared formulations as it has the highest association efficiency as well as zeta potential and process yield was also good compare to others.

Kinetic Modeling

The results of kinetic study of batches FN1 to FN10, represented in Table 3 were derived after subjecting the *in-vitro* release profile results to zero-order, higuchi and peppas model to derive various kinetic parameters described here. The release profiles when subjected to higuchi model, r^2 value is maximum for all formulation indicating the release follows Higuchi model and mechanism of release is diffusion. It could be concluded from the values of n from the Korsmeyer-peppas model that the release of drug from nanoparticles followed non-Fickian

Table 3: Kinetic Study of Batches FN1 TO FN10

Formulation code	Zero order	Higuchi's model	Korsmeyer-Peppas model
	r^2 value	r^2 value	n value
FN1	0.886	0.968	0.572
FN2	0.955	0.987	0.532
FN3	0.982	0.991	0.495
FN4	0.840	0.953	0.493
FN5	0.827	0.949	0.482
FN6	0.902	0.978	0.503
FN7	0.896	0.975	0.569
FN8	0.891	0.973	0.579
FN9	0.911	0.981	0.591
FN10	0.915	0.984	0.592

Table 4: Stability study data for FN8

Batch	Condition*	Days			
		0	30	90	180
FN8	1	100 ± 0.73	99.45 ± 0.24	98.12 ± 0.34	97.74 ± 0.74
	2	100 ± 0.23	98.31 ± 0.74	97.65 ± 0.64	96.34 ± 0.35

*Condition: 1, Long term stability study; 2, Accelerated stability condition.

#%RDC – Percentage of remaining drug concentration

diffusion mechanism. This contrast between drug release mechanisms between two models could be due to the initial rapid release of the drug which might have affected the n value.

Stability Study

From the prepared different batches of nanoparticles, formulation FN8 was selected for the long term and accelerated stability study. The subjected formulations when observed after the specified time of storage at stipulated conditions, depending on the type of study, have shown that there was no change in physical appearance.

Further, they were evaluated for the amount of drug remained after specified time interval. The results of which were shown in Table 4. The results were indicative that Glibenclamide remained stable in both formulations for long term and accelerated stability study conditions. No significant ($p>0.05$) variation in drug content was observed at mentioned conditions.

***In-Vivo* Anti-Diabetic Studies of FN8**

The *in-vivo* study was performed by using prepared nanoparticle of formulation batch FN8. The study results are shown in Figure below. From the *in-vivo* study of nanoparticles and Glibenclamide solution, it was found the t_{min} value for nanoparticles was 7.5 h with the serum glucose level (SGL) was 47.23 ± 9.45 %. Nanoparticles when studied at 18 h, SGL was found to be 60 % which means that the drug

release has slow down and glucose level was rising with time.

CONCLUSION

From the data obtained here it could be concluded that nanoparticles of Glibenclamide using Eudragit RS 100 were successfully prepared and when evaluated for the *in-vivo* efficacy have shown the diabetic control upto 16-18 h. It suggests that by using the prepared nanopaticles we can sustain the action of the Glibenclamide.

ACKNOWLEDGEMENT

The author thank to Zydus Pharm. Ltd. For providing gift sample of Glibenclamide & also thankful to Evonic Roehm Pharma Polymers, India for providing gift sample of Eudragit RS100. We are also grateful for instruments and laboratory support provided from the Veerayatan Institute of Pharmacy.

REFERENCES

1. Chugh, I, Seth, N., & Rana, A. C. (2012). *International Research Journal of Pharmacy, 3(5),* 57-62.
2. Sung-Ho, K. & In-Sook, K. (2002). *International Journal of Pharmaceutics, 245(1),* 67-73.
3. Shah, S. R., Parikh, R. H., Chavda, J. R. & Sheth, N. R. (2013). *Powder Technology, 235(1),* 405–411.
4. Jönsson, A., Hallengren, B., Rydberg, T. & Melander, A. (2001). *Diabetes, Obesity and Metabolism, 3(1),* 403.
5. Talka, P. G. (1981). *Analytical Profiles Drug Substrates, 10(1),* 337.
6. Varma, M. M., Jayaswal, S. B. & Singh, J. (1992). *Indian Drugs, 29,* 608.
7. Chalk, J. B., Patterson, M., Smith, M. T. & Eadie, M. J. (1986). *European Journal of Clinical Pharmacology, 31(1),* 177.
8. Gedeon, C., Kapur, B., Aleksa, K. & Koren, G. (2008). *Clinical Biochemistry, 41,* 167.
9. Niopas, I. & Daftsios, A.C. (2002). *Journal of Pharmaceutics and Biomedical Analysis, 28,* 653.
10. Hsieh, S. & Selinger, K. (2002). *Journal of Chromatograph and Bio Analytical Technology of Biomedical Life Science, 772,* 347.
11. Venkatesh, P., Harisudhan, T., Choudhury, H., Mullangi, R. & Srinivas, N. R. (2006). *Biomedical Chromatography, 20,* 1043.
12. Yao, J., Shi, Y. Q., Li, Z. R. & Jin, S. H. (2007). *Journal of Chromatography, 853,* 254.
13. Chaturvedi, P. K. & Sharma, R. (2008). *Acta Chromatography, 20,* 451.
14. AbuRuz, S., Millership. J. & McElnay, J. (2005). *Journal of Chromatograph and Bio Analytical Technology of Biomedical Life Science, 817,* 277.
15. Chang RK, Peng Y, Shukla AJ, (2006). *Handbook of Pharmaceutical Excipients*, 5th ed. London (UK): Pharmaceutical press, 553-560.
16. Ginity Mc, Eds JW. (1997). *Aqueous polymeric coating for pharmaceutical dosage form*, 2nd ed. Marcel Dekker Inc. New York, 101-173.
17. Hannele E. (2005). *VTT Publications*, Finland, 563: 1-112.
18. Swarnali, D, & Preeti, K. S. (2011). *Nanomedicine*; (7) 242-247.
19. Leena Peltonen. (2002). *AAPS PharmSciTech*. (3) E1-E7.

