

**PREPARATION, CHARACTERIZATION AND *IN VITRO* - *IN VIVO* EVALUATION OF GLICLAZIDE LOADED POLYMERIC MICROSPHERES**

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ABSTRACT

Type 2 diabetes mellitus is a heterogeneous disease which involves both defective insulin secretion and peripheral insulin resistance. Despite the availability of other agents for treatment of type 2 diabetes mellitus gliclazide, is preferred in diabetic therapy because of its selective inhibitory activity towards pancreatic K⁺-ATP channels, antioxidant property, low incidence of producing severe hypoglycemia and other haemobiological effects. In the present study gliclazide microspheres were prepared using combination of Ethyl cellulose (20 cps), Methocel K4M, Methocel K15M and Eudragit-RSPO by solvent evaporation method. They were evaluated for their microencapsulation efficiency, in vitro drug release, and surface morphology, compatibility between drug and polymers and in vivo anti-diabetic study. The microspheres were almost spherical and slightly rough. The encapsulation efficiency was in the range of 68-92%. The drug release was also found to be slow which extended for almost 8 hours. FTIR and DSC analysis indicated the good compatibility between the drug and polymers. In vivo study of the gliclazide microspheres in albino rats demonstrated significant antidiabetic effect of gliclazide. In case of gliclazide microspheres the hypoglycemic effect obtained was for more than 10 hours whereas pure gliclazide produce antidiabetic effect for only 4-5 hours suggesting that microspheres of gliclazide may be a potential candidate for safe and effective controlled drug delivery for the treatment of type 2 diabetes mellitus.

Key words: Diabetes, microspheres, gliclazide, polymers, antidiabetic effect.

INTRODUCTION

In recent years attention is being increased on the manner in which the drugs are delivered. Drugs are being incorporated into the polymers for controlling and targeting the drug release. However, microspheres constitute an important part of these particulate drug delivery systems by good worth of their small size and competent carrier capacity^[1-2]. Diabetes mellitus is a disease given to a group of disorders characterized by absent or deficient insulin secretion or peripheral insulin resistance resulting in hyperglycemia. Currently the presence of unusually high glucose levels in the blood is the only decisive factor on which analysis of diabetes mellitus is based

^[3]. Gliclazide, a second generation sulphonylurea derivative, is preferred in therapy because of its selective inhibitory activity towards pancreatic K⁺-ATP channels, antioxidant property, low incidence of producing severe hypoglycemia and other haemobiological effects. The daily dose of gliclazide is usually given in two fractions generally in between 40 and 80 mg at the beginning of treatment, but the dose can be increased when the condition is severe. Gliclazide is well absorbed from GIT; more or less 80% is absorbed. One dose of gliclazide has a half-life less than 10 hours with the peak absorbance occurring at about 4-6 hours. Like most sulphonylureas, gliclazide binds primarily to plasma

albumin (85-99%), allowing it to be distributed uniformly throughout the body^[4-7].

MATERIALS & METHOD

Gliclazide was obtained as a gift sample from Renata Ltd. Bangladesh. Ethocel (20 cps), Methocel K4M premium and Methocel K15M were a gift sample from Colorcon, Bangladesh. Eudragit-RSPO was obtained from Bizcon International, Bangladesh. All other reagents and chemicals used were of analytical grade.

Preparation of Microspheres: Microspheres were prepared by simple emulsion solvent evaporation method. In a 250 ml beaker light liquid paraffin was taken up to 100 ml along with 1% span 80 and stirring was started at 1000 rpm and continued for 5 minutes. Then required amount of gliclazide powder respective to the batch was added with external phase very slowly and stirring continued for another five minutes. Calculated quantity of polymers were dissolved in 1:1 ratio of ethanol and dichloromethane and added drop wise to the external phase and stirred for 3 hrs at 1000 rpm. After microspheres have been formed they were washed four times with n-hexan in a repeated manner and allowed to dry at room temperature for 24 hours to get free flowing microspheres^[8]. By changing the drug polymer ratio, 12 batches of microspheres were prepared (Table 1).

EVALUATION

Drug Entrapment Efficiency: 20 mg of microspheres were taken in a glass mortar and pestle and crushed into powder. Then the crushed powdered microspheres were suspended in 5 ml of ethanol and sonicated for 2 hours. After 2 hours, the volume was made up to 100 with 7.4 phosphate buffer solutions, filtered and the filtrate was analyzed for the drug content. The drug entrapment efficiency was calculated using the following formula:

$$\text{Drug Entrapment Efficiency} = (\text{Actual Drug Content} / \text{Theoretical Drug Content}) \times 100$$

In vitro Release Studies: The drug release study was performed in USP-II dissolution apparatus at $37^\circ\text{C} \pm 0.5^\circ\text{C}$ and 100 rpm using 900 ml of phosphate buffer (pH 7.4) as dissolution medium. 20 mg of gliclazide microspheres were used for the test. 10 ml sample was withdrawn at predetermined time intervals and filtered through a 0.45 micron filter paper. The absorbance of the solutions was measured at 226 nm for drug gliclazide by using a Shimadzu UV-1201 UV/Vis double beam spectrophotometer (Shimadzu, Japan). Percentage of drug release was

calculated using an equation obtained from the standard curve. The dissolution study was continued for 8 hours. Percentage drug dissolved at different time intervals was calculated using the Lambert-Beer's law equation.

Kinetic modeling of drug release

After implementation of *in vitro* dissolution of all the batches for eight hours, the data was treated with zero order^[9] and Higuchi equations^[10] (equation 1-2 respectively).

$$M_t = M_0 + k_0 t \dots\dots\dots(1)$$

$$M_t = M_0 - k_H t^{1/2} \dots\dots\dots(2)$$

In these equations, M_t is the cumulative amount of drug released at any specified time (t) and M_0 is the dose of the drug incorporated in the delivery system. k_0 and k_H are rate constants for Zero order and Higuchi model respectively. These models fail to explain drug release mechanism due to swelling (upon hydration) along with gradual erosion of the microspheres. Therefore the dissolution data were also fitted to well-known Korsmeyer kinetic equation to establish the mechanism of drug release.

$$\log(M_t/M_\infty) = \log k + n \log t \dots\dots\dots(3)$$

where, M_∞ is the amount of drug release after infinite time; k is the release rate constant; and n is the diffusional exponent or release exponent pinpointing of the mechanism of drug release. For a microsphere having spherical shape, while n is below 0.43 or equal to 0.43, the Fickian diffusion phenomenon dominates, and n between 0.46 and 0.84 is an anomalous transport (non-Fickian diffusion), often termed as first-order release. If the n value reaches 0.85 and above, the release can be characterized by case II and super case II transport, which means the drug release rate does not change over time and the release is characterized by zero order release. In this case, the drug release is dominated by the erosion and swelling of the polymer^[11,12].

Surface Morphology: The shape and surface morphology are important consideration for microsphere characterization. Surface morphology of the microspheres was analyzed by using scanning electron microscopy (SEM).

Drug- Excipient compatibility study by FTIR: The main aim of FTIR spectroscopic analysis was to determine the chemical functional groups and to investigate the composition in the samples. FTIR spectra were employed to be confirmed about the compatibility of gliclazide with various polymers used to prepare the microspheres.

Differential Scanning Calorimetry (DSC) Analysis: The compatibility between drug and the

polymer was further tested by Differential Scanning Calorimetry (DSC). Samples were sealed in Aluminum pans and DSC thermogram was reported at a constant flow rate of 20 ml/min over the temperature range of 10 to 400 C.

ANTIDIABETIC EVALUATION

Experimental Animals and Drugs: The approval of the Institutional Animal Ethics Committee was obtained before starting the study. The study was conducted in accordance with standard institutional guidelines. Albino rats of both sex (80 -95 g) were used for the study. They were housed in polypropylene cages under standard laboratory conditions (12 h light/12 h dark, 21±2° C). The animals were fed on standard pellet diet and glucose water^[13].

Induction and Treatment of Diabetes: Initially all the rats were weighed by an electric balance. Then initial blood glucose level was checked by a Rightest Glucometer GM 100 of Bionime Corporation. All the rats were found within normal Glucose level. After weighing the rats their body weight was recorded. Then according to the body weight the dose (120 mg/kg) of alloxan needed for individual rat was weighed in an electric balance. After that a solution of alloxan was prepared by dissolving the alloxan in WFI. To prepare the suspension distilled water was used for both pure drug and microsphere of Ethocel and Methocel K15M containing Gliclazide and microsphere of Ethocel and Eudragit RSPO containing Gliclazide. Hydroxy Propyl Methyl Cellulose (HPMC) was used as a suspending agent to keep the pure Gliclazide and microsphere suspended.

The animal model of type-2 diabetes mellitus (NIDDM) was induced in overnight fasted animals by a single intraperitoneal injection of 120 mg/kg body weight alloxan. During experiment no food or drinks were allowed but water with glucose. Then the rats were allowed 72 hours to develop diabetes. After 72 hour blood glucose level was checked and the rats were isolated those developed diabetes. A level of 200 mg/dl was considered to develop diabetes. Pure drug and Gliclazide microsphere was given orally in the predetermined doses and blood glucose level was checked upto 8 hour at every 1 hour interval and at 12th and 24th hour by the glucometer.

The rats were divided into 4 groups comprising of 5 animals in each group as follows:

Group I: Positive control, received only glucose water after diabetes induction.

Group II: Received pure gliclazide of 2 mg per kg body weight.

Group III: Received 2 mg equivalent gliclazide loaded polymeric microsphere FB3.

Group IV: Received 2 mg equivalent gliclazide loaded polymeric microsphere FC3^[14-18].

RESULTS AND DISCUSSION

The gliclazide microspheres were prepared by simple solvent evaporation technique using ethocel-methocel K4M, ethocel-methocel K15M and ethocel- Eudragit-RSPO as a polymer. The viscosity grade of ethocel was 20 cps. 1:1 ratio of ethanol and DCM was used to prepare polymer solution. Light paraffin was used as external phase and 1% span 80 surfactant to external phase was found to be crucial to minimize aggregation of microspheres.

Drug Entrapment Efficiency: Figure 1 reflects a direct relationship of polymer concentration and polymer type with drug entrapment efficiency. The bar diagram is showing that as the amount of polymer is increasing the entrapment efficiency is also increasing. 50% polymer loaded Ethocel-K4M microsphere shows only 73.68% gliclazide entrapment efficiency on the other hand when the polymer amount increases to 80% the drug entrapment efficiency also increases to 87.37%. The types of polymer also have direct effect to the drug loading efficiency of microspheres. FA1 and FB1 is 50% drug loaded microsphere containing Ethocel-K4M and Ethocel-K15M respectively. FA1 shows 73.68% drug entrapment on the other hand FB1 shows 81.58% drug entrapment efficiency. FB4 showed the highest drug entrapment which was 92.63%. The difference between the two polymers in terms of entrapment efficiency is also clear for FB and FC where, FC is the formulation of Ethocel and Eudragit-RSPO combination.

In Vitro Drug Release: To inspect the effect of polymer concentration on release pattern of gliclazide from microspheres twelve formulations were made (Table 1). Formulation-A1 best fits with Korsmeyer ($R^2 = 0.978$) and Higuchi ($R^2 = 0.973$) kinetic models to the same extent. The value of release exponent was found 0.6 which indicates that the release pattern of gliclazide from FA1 microspheres followed anomalous transport mechanism, which appears to indicate a coupling of the diffusion and erosion mechanism. FA2, FA3 and FA4 also followed Korsmeyer model ($R^2 = 0.977$, $R^2 = 0.971$ and $R^2 = 0.967$). The values of n for FA2, FA3 and FA4 were in between 0.46 and 0.84 (Table 2) which indicate that the drug was released by anomalous transport. The same phenomena was repeated for all the Formulations of series B except that FB4 followed

zero order release mechanism ($R^2=0.985$). Microspheres of FB1, FB2 and FB3 followed Korsmeyer model ($R^2 = 0.983$, $R^2 = 0.984$ and $R^2 = 0.987$ respectively) and anomalous transport mechanism. In case of Formulation C, FC1 and FC2 followed Higuchi release pattern but FC3 and FC4 followed Korsmeyer model ($R^2 = 0.973$ and 0.965 respectively) and there is a clear indication of diffusion and erosion mechanism from the diffusional exponent value of FC3 and FC4.

Effect of Polymer type and concentration: From the zero order release profile it is observed that the total percent release of gliclazide from FA1, FA4 were 98.26%, 81.32%, from FB1, FB4 were 89.70% and 75.67% and from FC1 and FC2 percent release was found 99.04% and 87.35% respectively at the end of eight hour (Figure 2). From the Figure 2, it is observed that when polymer concentration was minimum, drug release from the Formulation-A1, B1 and C1 was more. In all these cases the increase of the amount of polymers causes a gradually less release of the drug.

From the Figure 2 another observation was that, when the type of polymer was changed, the percent drug release was also changed where, Methocel K15M showed the highest drug retaining capacity. Ethylcellulose is cellulose either distinguished by its versatility. Ethylcellulose toughens, hardens and reduces or even eliminates the surface tack of compositions in which it is compatible. Microencapsulation of drug particles with Ethylcellulose provides another means of controlling solubility rates. Methocel K4M and K15M premium are of CR grades. They differ from each other in viscosity and molecular weight. In Eudragit-RSPO the ammonium groups are present as salts and make the polymers permeable and lend themselves to pH independent swelling (in the physiological range) and enable sustained release of active ingredient in the formulation.

Scanning electron microscopy (SEM): The surface morphology of micro particles was observed by scanning electron microscopy and representative micrographs are shown in Figure 3. The images of FA1 are shown at three different magnifications (Figure 3: A, B and C). Figure 3A shows almost spherical shapes with a slight rough surface of particles. No significant fusion among particles was observed. Surface morphology also revealed presence of porous microparticles. It also shows high surface drug due to its high drug loading (which is 50%) and therefore there was also a burst release (Figure 3B). The photomicrographs also showed the presence of loose crystals on the surface of a few microparticles

and also revealed presence of pore due to higher drug loading which is 50% (Figure 3C).

The images of FB3 are shown at three different magnifications (Figure 3: D, E and F). Figure 3D shows almost spherical shapes with a rough surface of particles. No significant fusion among particles was observed. A slightly porous surface was observed. It also shows lower surface drug due to its lower drug loading (which is 25%) and therefore there was also a burst release but a much lower amount compared to the formulation FA1. The photomicrographs also showed the presence of loose crystals on the surface of a few microparticles (Figure 3F).

Both the formulation showed more or less rough surface. But Formulation A1 of Ethocel and Methocel K4M showed very rough surface compared to the Formulation B3 of Ethocel and Methocel K15M combination microsphere.

Infrared Spectroscopy: FTIR spectra were employed to confirm the compatibility of gliclazide with various polymers used to prepare the microspheres of gliclazide. Peak at 1347.3 and 1164 cm^{-1} for pure drug was found as the evidence of Sulfonyl (S=O) group stretching. At 1709.92 cm^{-1} a sharp peak was observed because of C=O stretching. In the spectra of formulation A2, B3 and C3 there was not even a slight change. At 2950 cm^{-1} peak was observed because of C-H stretching which shifted towards lower wavelength of 2949 for FA2 indicating a very slight interaction. Formulation B3 and C3 showed no change. For C-H bending a strong peak is observed at 1436 cm^{-1} . Symmetric and asymmetric stretching of C-H bond was visible at 2837.34 and 2868.2 cm^{-1} respectively which is also seen to be unchanged in case of FA2, FB3 and FC3. Peak appeared at 3274 cm^{-1} and at 3193 cm^{-1} because of N-H Primary and secondary stretching respectively (Figure 4). The aforementioned characters ensure the chemical integrity of gliclazide with polymers in microspheres. Slight shift of peak in some rare cases indicated very weak interaction between gliclazide and polymers. The above findings suggest that there were either very weak or almost negligible interactions between the drug and polymers, so the predominant release retarding mechanism was only due to the property of polymer that retarded the drug.

Differential Scanning Calorimetry (DSC) Analysis: The compatibility between drug and the polymer was further tested by Differential Scanning Calorimetry (DSC). Samples were sealed in Aluminum pans and DSC thermogram was reported at a constant flow rate of 20 ml/min over the temperature range of 10 to 400 C . DSC thermogram

of pure Gliclazide showed endothermic peaks at 171.64 C corresponding to its melting point (T_m) with the onset temperature at 168.38 C. Gliclazide microsphere of Ethylcellulose and Methocel K15M combination (FB3) showed peak at 165.80 C describing a shift of Gliclazide peak to a lower temperature. The slightly lower T_m could be attributed to the amorphous form of Gliclazide in the microsphere. So it shows the absence of considerable incompatibility between the drug and polymers. The thermogram of Gliclazide, Ethocel and Eudragit RSPO microspheres showed a peak at 167.13 C with onset temperature of 161.12 C. The peak as compared to the pure drug decreased a little representing a negligible interaction between Gliclazide and polymers. In case of Formulation A2 (microsphere formulation of Ethocel and Methocel K4M containing Gliclazide) thermal curve was found at 159 C. This marked decrease in T_m happened probably because of the presence of interaction between the drug and polymers containing the drug. Comparing the entire DSC curve against pure Gliclazide only interactions were found between the drug and polymers in case of FA2.

In Vivo Antidiabetic Study: To evaluate the anti-diabetic effect of pure Gliclazide and Gliclazide loaded microspheres the blood glucose levels were measured in alloxan induced diabetic rats at 01-08 hours and at 12 and 24 hours. The results are given in Table 3.

Positive Control group was the group 1. It was the group of rats those developed diabetes after alloxan injection and kept untreated by any anti-hyperglycemic agent serving only glucose water during experiment. They did not show any significant hypoglycemia when blood glucose level was checked along with the anti-diabetic agent treated group (Group 2, 3 and 4) at every time interval. Pure Gliclazide was administered to group 2 rats to see the hypoglycemic effect of pure Gliclazide. From the Table 5 it can be observed that initially there was a rapid reduction of blood glucose level of rats making the glucose level almost half of the initial blood glucose level at one hour which reached maximum at 3-4 hours and made the glucose level to normal. At 5, 6 and 7 hour the average blood glucose level started to rise again (119.98 mg/dl 139.96 mg/dl and 162.32 mg/dl respectively) leading to average blood glucose level above normal at 8 to 12 hours (186.14 mg/dl, 237.56 mg/dl). The % reduction in glucose level by pure Gliclazide at 1 hour was 43.03% (Table 3) and the average % reduction became maximum at 5 hours (66.77%). At 12 hour there was no significant hypoglycemic effect of pure Gliclazide (237.56 mg/dl) and at 24 hours average blood glucose level

became 317.68 indicating the severe hyperglycemia in group 2 rats.

Formulation 1 was the microsphere of Ethocel (20 cps) and Methocel K15M containing Gliclazide. F1 was administered to Group 3 rats in the dose equivalent to pure Gliclazide. Table 3 shows that average blood glucose level reduction of F1 at 1 hour was 237.24 mg/dl which was 357.86 mg/dl initially when the rats were untreated. At 3 and 5 hours the glucose level observed was 182.38 mg/dl and 136.68 mg/dl respectively. Figure 6 shows the gradual reduction in blood glucose level compared to pure Gliclazide. From the Table 3 initially there was a 33.89% blood glucose reduction. A 25% glucose reduction is considered as a severe hypoglycemic effect ^[19]. For the formulation 1 significant hypoglycemic is maintained for 10-12 hours. Figure 3.7 indicates that % reduction in blood glucose was maintained for 10-11 hours. Formulation 2 was the microsphere of Ethocel (20 cps) and Eudragit-RSPO containing Gliclazide. F2 was administered to Group 4 rats in a dose equivalent to pure Gliclazide. Table 3.23 shows that average blood glucose level reduction of F2 at 1 hour was 233.175 mg/dl which was 344.18 mg/dl initially when the rats were untreated. At 6 and 8 hours the glucose level observed was 1182.925 mg/dl and 102.375 mg/dl respectively which are quite normal. Figure 3.26 and 3.27 shows the gradual reduction in blood glucose level compared to pure Gliclazide. From the Table 3.24 initially there was a 32% blood glucose reduction and hypoglycemic effect was maintained for almost 10 hours. A 25% glucose reduction is considered as a severe hypoglycemic effect ^[19]. For the formulation 1 significant hypoglycemic is maintained for 10-12 hours. Figure 3.27 indicates that % reduction in blood glucose was maintained for 10-11 hours. At 24 hours there is a raised blood glucose level again which is almost close to initial level for all the group of rats. The sustained hypoglycemic effect observed over a longer period of time in case of Formulation 1 and Formulation 2. Gliclazide microsphere showed significantly more effectiveness in reducing blood glucose level than when only pure Gliclazide was administered as a suspension form ^[20].

CONCLUSION

The preparation of Gliclazide microspheres for sustain drug delivery was successfully performed by solvent evaporation method using Ethylcellulose and Methocel K4M, Ethylcellulose and Methocel K15M and Ethylcellulose and Eudragit RSPO combination in different ratio of drug and polymer. *In vitro* dissolution study showed the sustained release of Gliclazide from the microsphere for about 8 hours.

From the *in vitro* dissolution data it is established that the drug dissolution profile could be slowed down by increasing the retardant polymer amount in the formulations. Scanning electron microscopy showed the rough and slightly porous surface of microspheres. FT-IR spectral analysis indicated that characteristic peak of Gliclazide pure drug was retained in the spectra of all the formulations indicating the intactness of the drug in all the formulations. From Differential Scanning Calorimetric test it can be concluded that there was no significant change in melting point, Glass transition temperature and no significant crystallinity.

After checking those compatibility parameter optimized two formulations was chosen for *in vivo* anti-diabetic study. Both the formulation of Ethocel and Methocel K15M and Ethocel and Eudragit-RSPO combination polymer showed sustain antidiabetic activity compared to the pure Gliclazide. So from the result, we can conclude that drug retardant polymers and its concentration affect all the evaluation parameter significantly. Hence the prepared polymeric microspheres of Gliclazide may prove to be potential candidate for safe and effective sustained drug delivery for the treatment of diabetes.

Table 1: Formulation of Gliclazide microsphere using combination of Ethocel and Methocel K4M, Ethocel and Methocel K15M and Ethocel and Eudragit-RSPO

Ingredients	FA1	FA2	FA3	FA4	FB1	FB2	FB3	FB4	FC1	FC2	FC3	FC4
Gliclazide (mg)	1000 mg											
Ethocel (mg)	500	1000	1500	2000	500	1000	1500	2000	500	1000	1500	2000
Methocel K4M (mg)	500	1000	1500	2000	-	-	-	-	-	-	-	-
Methocel K15M (mg)	-	-	-	-	500	1000	1500	2000	-	-	-	-
Eudragit-RSPO (mg)	-	-	-	-	-	-	-	-	500	1000	1500	2000
Ethanol (ml)	5 ml											
DCM (ml)	5 ml											
Span 80 (%)	1 %											
Liquid paraffin (ml)	100 ml											

Table 2: Release rate constants and R-squared values for different release kinetics of twelve formulations of gliclazide loaded polymeric microspheres

Formulation	Zero Order		Highuchi		Korsmeyer	
	K_0	R^2	k_H	R^2	n	R^2
FA1	11.51	0.964	35.55	0.973	0.586	0.978
FA2	10.57	0.969	32.55	0.970	0.564	0.977
FA3	10.01	0.969	30.58	0.962	0.542	0.971
FA4	8.831	0.972	26.96	0.968	0.545	0.967
FB1	10.38	0.977	31.56	0.961	0.570	0.983
FB2	9.767	0.981	29.71	0.966	0.587	0.984
FB3	9.054	0.983	27.46	0.961	0.585	0.987
FB4	8.614	0.985	25.92	0.949	0.649	0.981
FC1	10.36	0.950	32.25	0.979	0.457	0.962
FC2	9.986	0.953	30.84	0.967	0.469	0.954
FC3	9.615	0.960	29.45	0.958	0.473	0.973
FC4	9.0	0.950	27.66	0.955	0.456	0.965

Table 3: Showing reduction in glucose level (mg/dl) for positive control, pure Gliclazide and Gliclazide loaded microspheres of FB3 and FC3

Time (hour)	Average Glucose Level Reduction (mg/dl)			
	Positive control	Pure Drug	F 1	F 2
0	311.9±5.92	361.44±5.44	358.76±6.90	344.17±9.49*
1	312.3±4.36	205.82±13.25	237.24±12.03**	233.17±8.68**
2	316.16±7.77	157.7±11.26	207.64±10.17	214.87±7.71
3	318.77±8.67	137.4±9.89	182.38±5.70	202.75±8.21
4	325.53±11.26	110.08±10.94	158.52±4.35***	174.75±11.94
5	329.33±3.77	119.98±8.90	136.68±5.21**	140.07±7.67**
6	329.83±4.78	139.96±7.37	118.92±5.35***	118.92±9.32**
7	335.57±10.57	162.32±10.81	106.28±6.14	108.32±7.70
8	338.9±11.93	186.14±11.03	96.8±7.49	102.37±9.68
12	351.77±12.06	237.56±9.66	109.38±6.78	132.72±9.19
24	392.77±7.39	317.68±9.97	291.94±11.87**	309.82±18.05

Values are given as mean±standard deviation for group of five rats. Values are statistically significant at *p<0.05

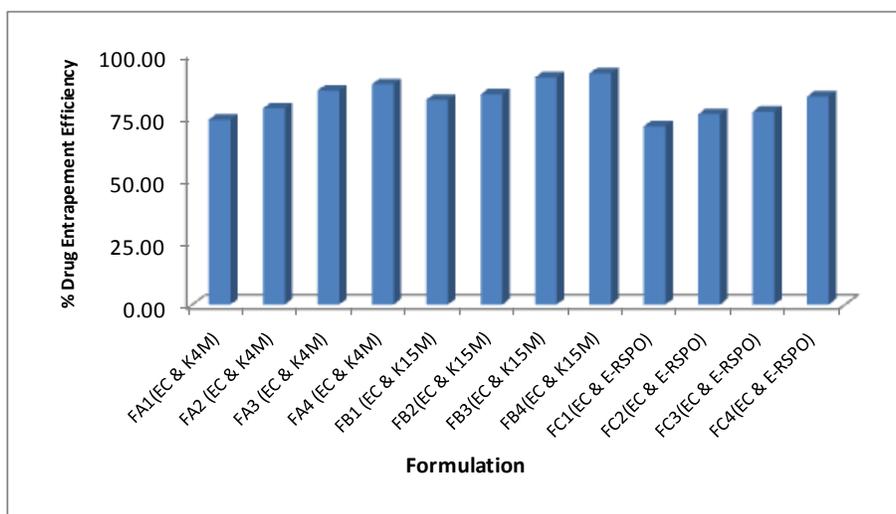


Figure 1: Drug Entrapment Efficiency of all formulations

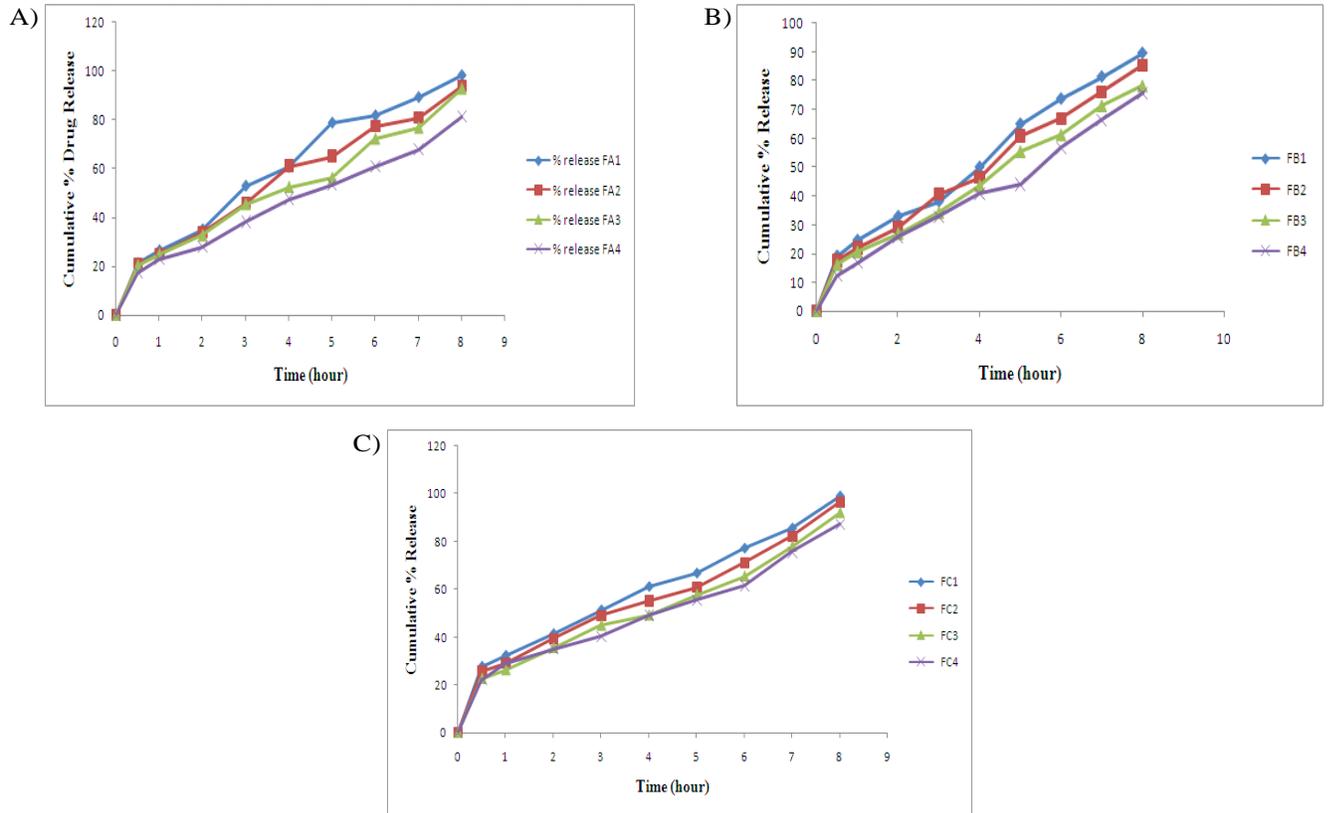


Figure 2: Zero order plot of release kinetics of A) Ethylcellulose-Methocel K4M, B) Ethylcellulose-Methocel K15M and C) Ethylcellulose-Eudragit-RSPO microspheres containing gliclazide

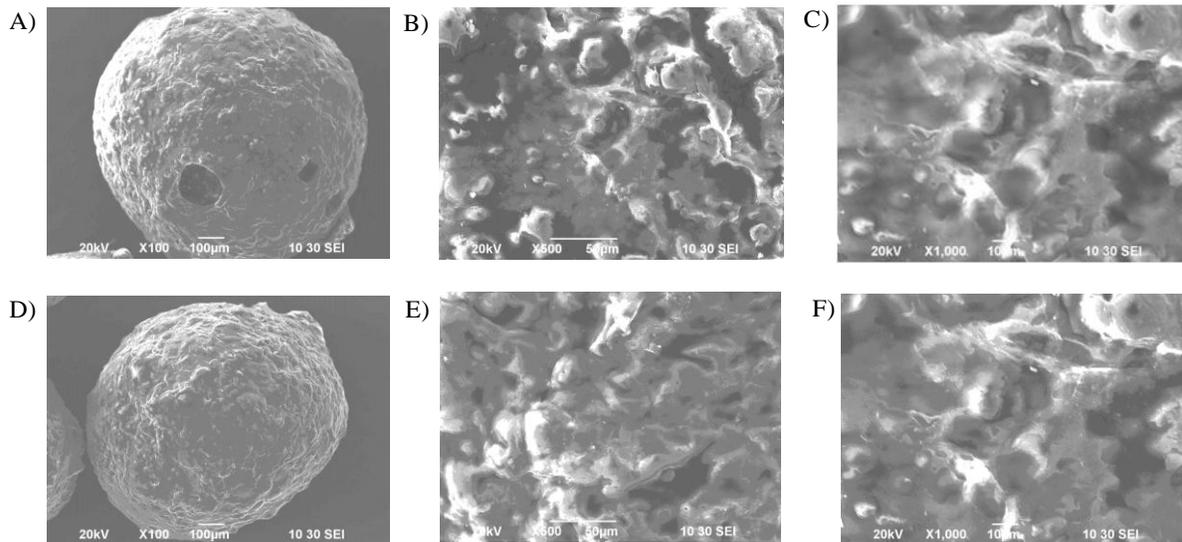


Figure 3: Effect of Ethocel and Methocel K4M on surface morphology of FA1 microspheres (A) Magnification at X100 SEI (B) Magnification at X 500 SEI and (C) Magnification at X 1000 SEI and the effect of Ethocel and Methocel K15M on surface morphology of FB3 microspheres (D) Magnification at X100 SEI (E) Magnification at X 500 SEI and (F) Magnification at X 1000 SEI

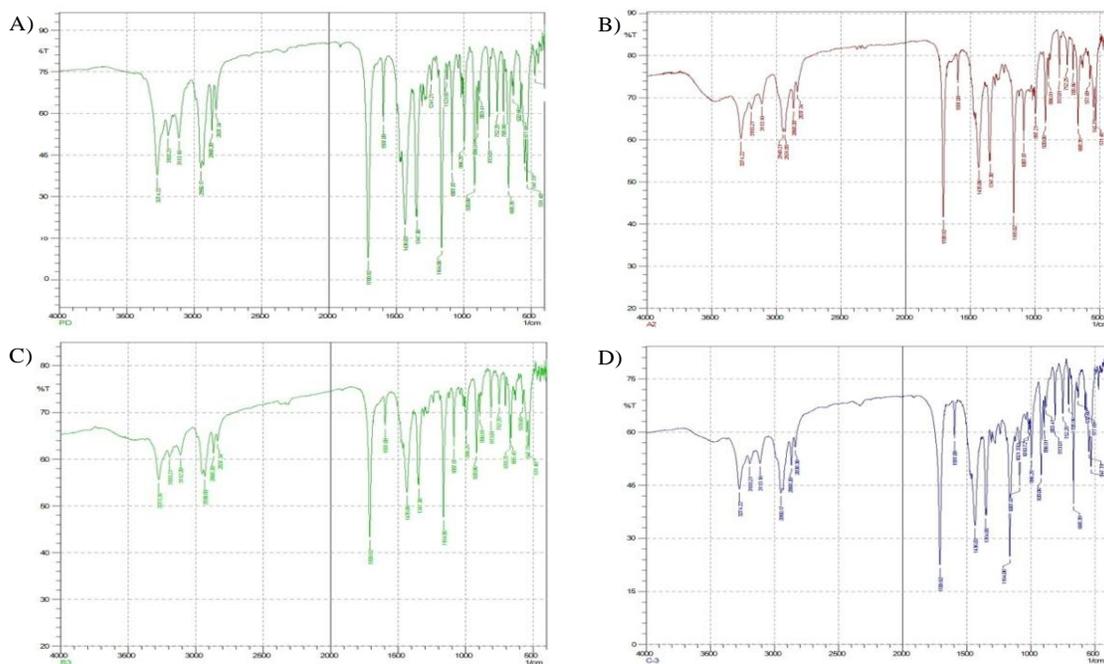


Figure 4: Image showing drug polymer interaction study by FTIR spectra (A- pure Gliclazide, B- spectrum of FA2, C- spectrum of FB3 and D- spectrum of FC3)

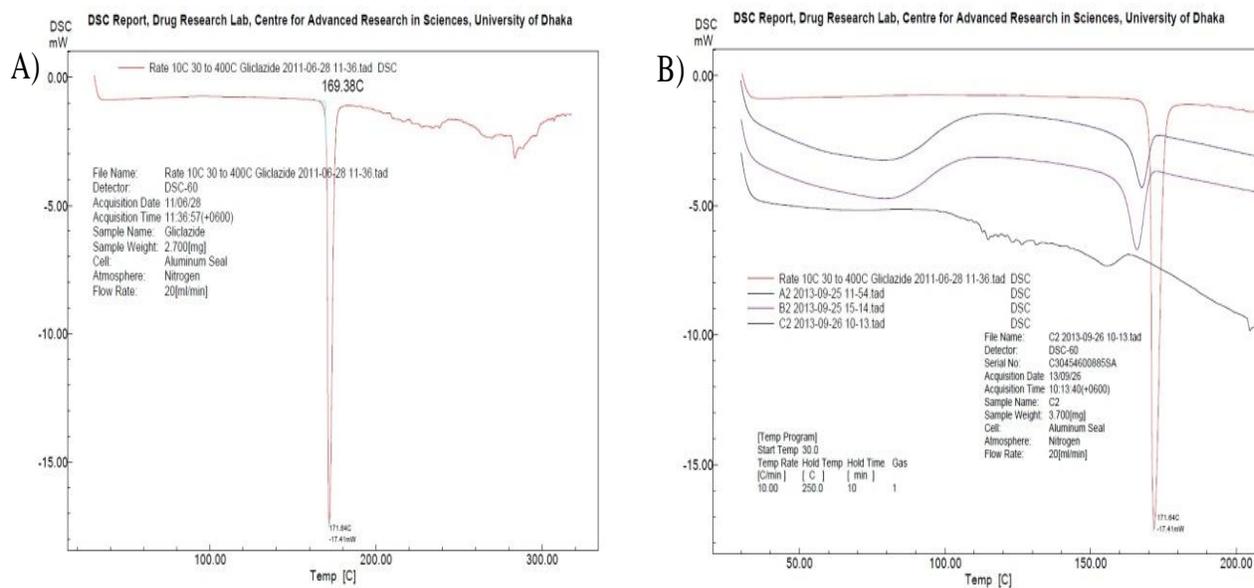


Figure 5: Thermal analysis of pure Gliclazide (A), microsphere of Gliclazide, Ethocel and Methocel K4M (FA2), microsphere of Gliclazide, Ethocel and Methocel K15M (FB3) and microsphere of Gliclazide, Ethocel and Eudragit-RSPO (FC3) (B)

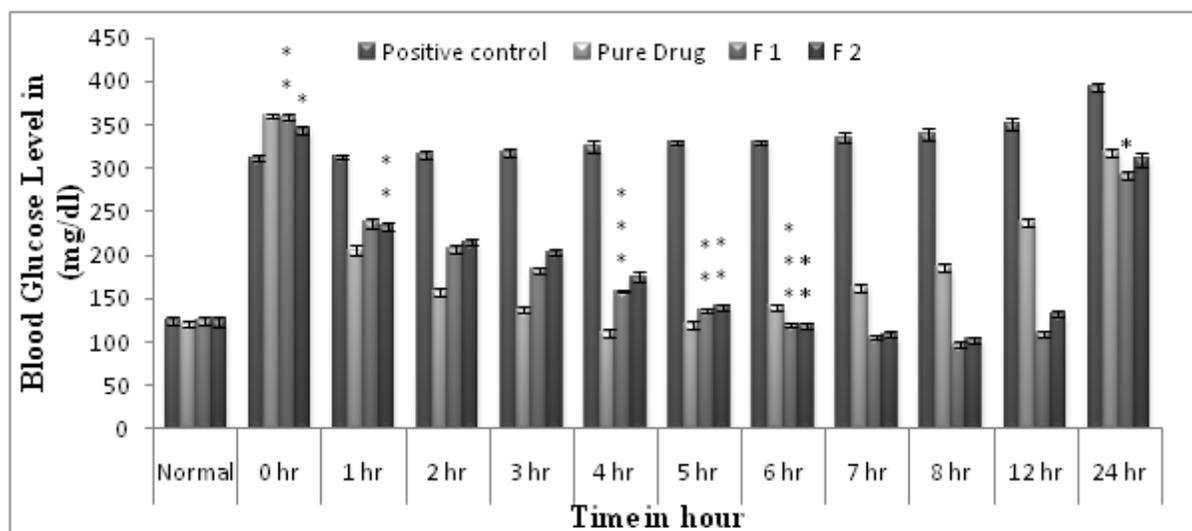


Figure 6: Diagram showing reduction in average blood glucose level with time for control group, pure Gliclazide, Formulation B3 and Formulation C3.

REFERENCES

1. Parmar H, Bakliwal S, Gujarathi N, Rane B, Pawar S. *Int J App Bio and Pharm Tech*, 2010; 1(3): 1157-11.
2. Panchal NV, Jadhav TR, Paul BN, Dana BS. *Jour Pharm Res*, 2011; 4(12): 4476.
3. Abirami P, Rajendran A. *Euro J Exp Bio*, 2012; 2(1): 9-12.
4. Dubey RR, Parikh RH. *Ame Asso Pharm Scien Pharm Sci Tech* 2004; 5(1): E5.
5. Foster RH, Plosker GL. *Pharmacoeconomics*, 2000; 18(3): 289-306.
6. Thombre AG, Denoto AR, Gibbes DC. *Jour Contr Release*, 1999; 60(2-3): 333-41.
7. Chowdary KPR, Balatripura G. *Ind J Pharm Sci*, 2003; 65: 591-4.
8. Lim ST, Martin GP, Berry DJ, Brown MB. *Jour Contr Release*, 2000; 66(3): 281-292.
9. Mockel JE, Lippold BC. *Pharma Res*, 1993; 10: 1066-1070.
10. Higuchi T. *Jour Pharm Sci*, 1963; 52: 1145-1149.
11. Korsmeyer RW, Gurny R, Doelker E, Buri P, Peppas NA. *Int J Pharm*, 1983; 15: 25-35.
12. Peppas NA. *Pharma Acta Helvetiae*, 1985; 60: 110-111.
13. Chowdary KPR, Rao NK, Malathi K. *Ind J Pharm Sci*, 2004; 66(4): 412-416.
14. Kumar KPS, Bhowmik D, Srivastava S, Paswan S, Dutta AS. *The Pharma Inno*, 2012; 1(2): 48-60.
15. Prajapati SK, Tripathi P, Ubaidulla U, Anand V. *Ame Asso Pharm Scie Pharm Sci Tech*, 2008; 9(1): 224-230.
16. Senthil A, Thakkar HR, Ravikumar, Narayanaswamy VB. *RGUHS J Pharm Sci*, 2011; 1(2): 163-171.
17. Choudhary S, Sharma M, Tripathi J, Mishra P. *Int J Adv Res*, 2013; 1(2): 35-42.
18. Pal D, Nayak AK. *Drug Delivery*, 2012; 19(3): 123-131.
19. Khan CR, Shechter Y. *Insulin, Oral Hypoglycemic Agents and the Pharmacology of the Endocrine Pancreas*. In: Gillman AG, Rall TW, Nies AS, Taylor P (eds.). *Goodman and Gillman's The Pharmacological Basis of Therapeutics*. 8th ed., Vol. 2. New York: Pergamon press, Maxwell Macmillan International Editions: 1991, pp. 1463-1495.
20. Berelowitz M, Fischette C, Cefelu W, Schade DS. *Diabetes Care*, 1994; 17(12): 1460-1464.