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FORMULATION, ASSESSMENT AND COMPATIBILITY ANALYSIS OF DIFFERENT POLYMERS LOADED MICROSPHERES BY NON AQUEOUS SOLVENT EVAPORATION TECHNIQUE: *IN VITRO-IN VIVO* STUDY OF GLIBENCLAMIDE AS A MODEL DRUG

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Keywords:

Glibenclamide, Microspheres, Emulsion Solvent Evaporation Method, Hypoglycemic, Korsmeyer Model

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ABSTRACT: Glibenclamide is an oral anti-hyperglycemic agent designed intended for the management of non-insulin-dependent diabetes mellitus (NIDDM). In certain conditions conventional drug release pattern is not suitable similar to Diabetes mellitus, cardiovascular diseases and many more diseases, this present study has taken a challenge to formulate controlled release microspheres by using different polymers. An effort has been given to prepare controlled release microspheres along with Ethyl cellulose, Eudragit RS/RL100 and Methocel K15, 100M by using non-aqueous emulsion solvent evaporation method. UV-Spectrophotometric was applied to assay the drug content and *in vitro* dissolution studies according to USP paddle method and was carried out in Phosphate Buffer (pH 7.4) for 8 hours. The *in-vitro* release kinetics was studied in different mathematical release models. The best data fitted with the highest correlation coefficient (R2) for microspheres was obtained for Korsmeyer release model. The maximum and minimum release of drug was observed 90.99% and 71.98%. Percent of Actual drug entrapment varied from 7.89% to 15.36% and percent of Drug entrapment efficacy varied from 69.23% to 98.21%. The SEM, FTIR and DSC studies used to of confirmed good spheres and smooth surface as well as interaction along with drug and polymer. Different biochemical tests have done on albino rats which showed good results and it also proved that formulations have given sustained release effects. *In-vitro-in vivo* studies showed that Glibenclamide microspheres might be a good candidate as sustained drug delivery system for treating type II diabetic.

INTRODUCTION: In modern years, increasing interest has been focused on the manner during which the drugs are delivered. Drugs are being incorporated into polymeric systems and devices for controlled and targeted drug release.

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Controlled drug delivery systems are mainly promising as these increase patient compliance because of the reduced frequency of administration, improved safety and efficacy of the drug and reduced undesirable effects. These systems are designed at controlling the rate of drug delivery, sustaining the duration of therapeutic activity and for targeting the drug to a tissue. Drug release from these systems should be at a desired rate, predictable and reproducible. A variety of controlled release systems such as coated pellets, matrix tablets, osmotically controlled release

systems, microcapsules, microspheres, nanoparticles, implants and infusion devices have been designed for various routes of drug administration. Controlled release formulations in tablet form are many but over the years the Microsphere formulations have immense popularity owing to their superiority over the former in several respects. A multiple-unit dosage form has more homogenous individual plasma profiles, shorter lag time and lower variability as compared to single unit formulations. The uniform distribution of these multiple unit dosage forms along the GIT could result in more reproducible drug absorption and reduced risk of local irritations than the use of single unit dosage forms. Risks such as spontaneous drug release from a single-unit tablet due to damaged coating or its attachment in the stomach or intestine causing an irritation of the gastric or intestinal mucosa are reduced by the use of multiunit forms. Thus, it results in a decrease in drug dose and side effects [1].

People with type 2 diabetes (non-insulin dependent diabetes) encompass a lack of a hormone called insulin. Insulin is formed by the pancreas and is the chief hormone liable for overprotective sugar levels in the blood. It usually makes the cells of the body take away excess sugar from the blood. In type 2 diabetes insulin is formed inadequately in response to surges of blood sugar, eg following a meal. Glibenclamide is an oral anti-diabetic agent that is extensively used in the management of non-insulin dependent diabetes mellitus (type II). It works mainly by stimulating the beta cells in the pancreas that produce insulin. This causes the beta cells to produce more insulin. This helps to decrease the amount of sugar in the blood of people with type 2 diabetes.

This is a second generation sulphonyl urea with the purpose of is more effective than those of first generation drugs. Its biological half-life is 4-6 hrs as a consequence; it requires repeated administration to keep plasma concentration. This cause bothers to the patient and also leads fluctuations in plasma drug concentration so as to may possibly reason lesser therapeutic effects or toxic effects. Therefore, advance of controlled release dosage forms know how to clearly be advantageous if decreased dosage necessities, as a result augment patient compliance [2].

Microspheres constitute an important part of this particulate drug delivery system by virtue of their small size and efficient carrier characteristics. However, the success of this novel drug delivery system is limited due to their short residence time at the site of absorption. It would therefore be advantageous to have means for providing an intimate contact of the drug delivery system with absorbing gastric mucosal membranes [1]. Along with a range of methods developed designed for formulation of micro spheres, emulsion solvent evaporation technique is one of the typically extensively used one as of its simplicity of fabrication devoid of compromising the action of drug [3].

This method facilitate for alter the liquids to solids, varying the colloidal and surface properties, as long as environmental protection and controlling the liberate distinctiveness of unlike coated materials. This has been made by developing the new drug entities, discovering of new polymeric materials that are appropriate for prolonging the drug release, safety, improvement in therapeutic efficacy. In general the size of the microencapsulated products is considered as larger than 1 micrometer and up to 1000 micrometers in diameter [4].

The solvent evaporation process occupies the use of emulsification of a solution containing polymer and drug with an additional medium in which the drug and polymer will not dissolve. The system was relatively simple and has been used to prepare microspheres of a mixture of compounds by means of numerous different polymeric materials [5-6].

Hydroxypropyl methylcellulose (HPMC), a semi-synthetic derivative of cellulose, has its popularity for the formulation of controlled release (CR) dosage forms as a swellable and hydrophilic polymer. Its nontoxic property, ease of handling, ease of compression, ability to accommodate a large percent of drug, negligible influence of the processing variables on drug release rates, and relatively simple tablet manufacturing technology make it an excellent carrier material [7-8].

Ethyl cellulose, a non-biodegradable and bio-compatible polymer, one of the extensively studied encapsulating materials for the controlled release of pharmaceuticals, was preferred as the retardant material [9].

Methacrylate copolymers (Eudragits) have recently received increased consideration for modified dosage forms because of their inertness, solubility in relatively non-toxic solvents and availability of resins with different properties. In the present investigation Eudragit RL is used as a rate retardant polymer. Eudragit RL is a water insoluble polymer which is widely used as a wall material for controlled release microparticles. The permeability of Eudragit RS and RL in aqueous media is due to the presence of quaternary ammonium groups in their structure; Eudragit RL has a greater proportion of these groups and as such is more permeable than Eudragit RS [10-12].

The fundamental opinion of the present effort was to prepare and estimate oral controlled release micro-particulate drug delivery system of glibenclamide using different polymers by water-in-oil emulsion solvent diffusion method by means of high entrapment capacity and extended release. Moreover, such small single units enable a more reproducible dispersion throughout the gastrointestinal tract leading to a reduction of drug release variations and an improved bioavailability. Multiple-unit system generally disperse freely in the gastrointestinal fluids, maximizes absorption, minimizes side effects and reduce inter and intra patient variability.

MATERIALS AND METHODS:

Materials: Glibenclamide as an donation sample from Popular Pharmaceutical limited, Bangladesh, Ethyl Cellulose (Colorcon Asia Pvt. Limited, India), Eudragit RS100 (Evonik, Germany), Eudragit RL100 (Evonik, Germany), Methocel K100M (Colorcon Asia Pte. Limited, India), Methocel K15M (Colorcon Asia Pte. Limited, India), Magnesium stearate (Merck, Germany), Ethanol (Merck, Germany), Dichloromethane (Merck, Germany), Light liquid paraffin (Merck, Germany), cyclohexane (Merck, Germany), Span 80 (Merck, Germany), Sodium hydroxide (Merck, Germany), Potassium dihydrogen phosphate (Merck, Germany), n-hexane (Merck, Germany) etc.

Methods:

Preparation of Glibenclamide microspheres by emulsion solvent evaporation technique:

The microspheres were prepared according to **Table 1** by solvent evaporation method. The process was initiated from dispersion of Glibenclamide in 70 ml of light liquid paraffin (LLP) using 1% span 80. At first, LLP was emulsified in a plastic beaker with Span 80 for few minutes with the help of stirrer at 500-1500 rpm. By this time the polymer solution (internal phase) was prepared by dissolving properly weighed polymer(s) in combination of ethanol and dichloromethane at a ratio of 5:5 in a volumetric flask. Then appropriately weighed Glibenclamide was added in the internal phase slowly and stirrer for 20-30 minutes. After proper mixing prepared polymeric phase was added drop wise to the external phase. Stirring was performed for 2.5 hours. After stirring, the microspheres were decanted and washed by n-haxen and allowed for drying in natural air. The microspheres transferred to glass vials and placed in the desiccators for further experiment.

TABLE 1: FORMULATION OF GLIBENCLAMIDE MICROSPHERES PREPARED BY EMULSION SOLVENT EVAPORATION METHOD

Formulation Code	Polymers (gm)			
	D:EC:EURL100	D:EC:EURS100	D:EC:MK15M	D:EC:MK100M
F1	1:1:1	-	-	-
F2	1:2:2	-	-	-
F3	1:3:3	-	-	-
F4	-	1:1:1	-	-
F5	-	1:2:2	-	-
F6	-	1:3:3	-	-
F7	-	-	1:1:1	-
F8	-	-	1:2:2	-

F9	-	-	1:3:3	-
F10	-	-	-	1:1:1
F11	-	-	-	1:2:2
F12	-	-	-	1:3:3

D=Drug, EU=Eudragit, M=Methocel

Assay methods of prepared microspheres:

Approximately 20 mg equivalent amount of Glibenclamide Microspheres was taken in a 100 ml volumetric flask and dissolved with minimum quantity of methanol. Few ml of phosphate buffer pH 7.4 was added and sonicated for 15 minutes in a sonicator to make a clear solution and made it 100 ml with phosphate buffer. Then the solution was finally filtered. Approximately 10 ml of the above solution was taken (if further dilution is necessary) in another volumetric flask and made it 100 ml with phosphate buffer. Absorbance value was determined using UV spectrophotometer at a wavelength of 242 nm. Using the absorbance value, the amount of drug entrapped was determined with the help of standard curve. Percent drug loading and drug entrapment efficiency was calculated by using the following equation

% drug Loading = (Actual drug loading / Weighed quantity of microspheres) X 100

Drug entrapment efficiency (%) = (Actual drug loading / Theoretical drug loading) X 100

***In-vitro* drug release study of prepared Glibenclamide microspheres:**

In vitro dissolution study was performed in a paddle type dissolution Apparatus. 900 ml of Phosphate buffer (pH 7.4) was used as dissolution media, paddle speed was 100 rpm and temperature was maintained fixed at 37^0C. Approximately 20 mg equivalent amount of Glibenclamide microspheres from each batch was transferred in each dissolution basket. The dissolution process was carried out for 8 hours and 10 ml dissolution sample was withdrawn at a predetermined intervals of 1 hour, 2 hour, 3 hour, 4 hour, 5 hour, 6 hour, 7 hour, 8 hour. Each and every time 10 ml dissolution sample was compensated by fresh 10 ml of phosphate Buffer. Dissolution samples were withdrawn with the help of 10 ml syringe was kept in test tube. The dissolution samples were then analyzed spectrophotometrically in UV-VIS spectrophotometer at a wavelength of 242 nm.

Successive fractional dissolution time:

To characterize the drug release rate in different experimental conditionals like $T_{25\%}$, $T_{50\%}$ and $T_{80\%}$ were calculated from dissolution data according to the following equations:

$$T_{25\%} = \left(\frac{0.25}{k}\right)^{\frac{1}{n}}$$

$$T_{50\%} = \left(\frac{0.50}{k}\right)^{\frac{1}{n}}$$

$$T_{80\%} = \left(\frac{0.80}{k}\right)^{\frac{1}{n}}$$

Another fractional tool MDT (Mean dissolution time) can be calculated by the following equation:

$$MDT = \left(\frac{n}{n+1}\right).K^{\frac{-1}{n}}$$

MDT value is used to characterize the drug release rate from the microspheres and the retarding efficiency of the polymer. A higher value of MDT indicates a higher drug retarding ability of the polymer and vice versa. The MDT value is also considered to be a function of polymer loading, polymer nature and physic-chemical properties of the drug molecule[13].

Surface Morphology Study by Scanning Electron Microscope (SEM):

Surface nature of microspheres was examined with the help of Scanning Electron Microscope (JEOL, JSM-6490 LA, Japan).The microspheres were dried completely before examination SEM was done at different magnifications of 20 kV X 40, 20 kV X 100, 20 kV X 200, 20 kV X 500 to examine the surface picture and size of the microcapsules that changed from formula to formula shown in **Fig. 4**. The working distances were 10 and 11 inches.

Drug-polymer compatibility study by Fourier transforms infrared (FTIR) spectroscopic analysis:

The FTIR technique is to measure the absorption of various infrared radiations by the target material, to produces an IR spectrum that can be used to identify functional groups and molecular structure in the sample shown in **Fig.6**. FTIR spectrum of

pure glibenclamide and formulated microspheres were recorded by using FT-IR 8400S (SHIMADZU, Japan). Appropriate quantity of KBr and micorspheres (in the ratio 100:2) were mixed by grinding in an agate mortar. Disk was made with about 100mg mixture under hydraulic pressure of 600 kg. Then the FTIR spectra were recorded between 4000 to 400 cm^{-1}. The resolution was 2 cm^{-1}.

Drug-polymer compatibility study by Differentiate scanning calorimetry (DSC) study of microsphere:

The DSC measurement was performed on a DSC-60(SHIMADZU) differential scanning calorimeter with a thermal analyzer (TA-60WS). Exactly 3.6 mg of glibenlamide was placed in scaled aluminium pan, before heating under nitrogen flow (300 ml/min) at a scanning rate 10 c min^{-1} from 30 c to 400 can empty aluminium pan was used as reference.

Experimental animals:

At first Albino rats consider about 165-200 gm were get hold as of the animal house of Jahangirnagor University and kept back in a area with a 12-hour day-night cycle, at even temperature of 22°C and humidity of 45-64%. During the investigational study rats were fed on pellets (Rat feed, Bangladesh) with free access to distilled water.

Induction of experimental diabetes:

Rats were turned into diabetic via a single intra-peritoneal injection of freshly ready streptozotocin (STZ-65 mg/kg body weight) in 0.1M citrate buffer (pH 4.5) in a volume of 1ml/kg body weight. Standard rats received 1 ml citrate buffer as vehicle. After 48 h of streptozotocin administration, blood glucose levels were estimated in rats following overnight fasting. Rats with a blood glucose ranging between 200–300 mg/dl were considered diabetic and used for the experiments.

Drugs and Chemicals:

Streptozotocin was procured from Sigma Aldrich Co., St. Louis, MO, USA, 0.1M citrate buffer (pH 4.5) was procured from Merck, Germany. All other biochemical's and chemicals used for the experiment were of analytical grade.

Experimental Design:

The rats were divided into six groups comprising of six animals in each group as follows:-

Group I: Negative control Rats, received only saline water

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

Group III: Received pure Glibenclamide of 5 mg per kg body weight after diabetes induction.

Group IV: Received Glibenclamide loaded polymeric microsphere Formulation F2 after diabetes induction.

Group V: Received Glibenclamide loaded polymeric microsphere Formulation F22 after diabetes induction.

Collection of blood from the rats:

After the experimental course of therapy, the rats were sacrificed by cervical dislocation under mild chloroform anesthesia. Blood was collected on decapitation and serum was separated by centrifugation (for 20 min at 2000 rpm).

Estimation of biochemical parameters in serum or plasma:

Serum glucose, Cholesterol, triglycerides, urea, creatinine, Bilirubin, Glyco-hemoglobin, SGOT, SGPT and alkaline phosphatase were assayed using diagnostic reagent kit manufactured by Crescent diagnostics Ltd, Atlas Medical diagnostics Ltd and Stanbio diagnostics Ltd. [14-16].

Statistical Analysis:

Results are expressed as Mean ± SD of five individual experiments and the statistical significance was evaluated by one way analysis of variance (ANOVA) using SPSS version (10.0). A value of $p<0.05$ was considered to indicate a significant difference between groups. Comparison with in the group were achieved by student's test and compared at $p<0.05$.

***In vivo* anti-hyperglycemic studies:**

In-vivo evaluation of different biochemical analysis in albino rats to see that Glibenclamide alone or combination with different polymers containing microspheres has better glycemic control than conventional dosage form. *In vivo* efficiency of the

optimized drug loaded microspheres of formulation F2 and F22 were performed in healthy normal Albino rats by measuring the hypoglycemic effect produced after oral administration using the glucose- measuring instrument. The drug was administered at a dose equivalent to 5 mg/kg pure drug, and different polymer loaded microspheres were used for the study. To evaluate the anti-diabetic effect of pure drug and drug loaded microspheres the blood glucose levels were measured in streptozotocin induced diabetic rats for 48 hours.

RESULTS AND DISCUSSIONS:

Actual drug loaded and drug entrapment efficiency (DEE) of prepared microspheres:

Drug loading and the drug entrapment efficiency (DEE) of the prepared microspheres were carried and the graphical presentation are given bellow in **Fig. 1**. The actual drug loaded and the drug entrapment efficiency were found to be in the range of 8.10% to 14.11% and 81.54% to 98.21% respectively.

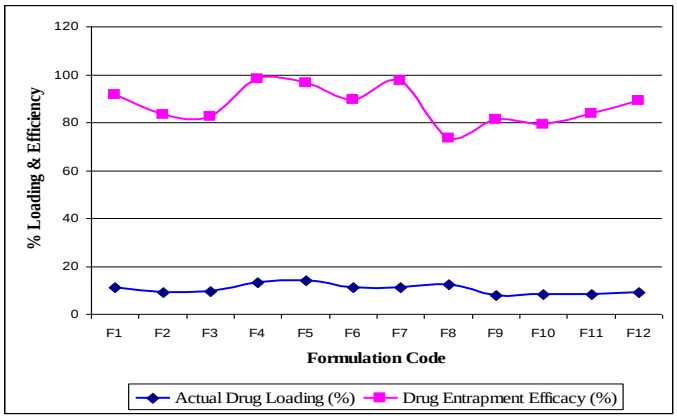


FIG. 1: COMPARATIVE PERCENT RELEASE STUDY OF ACTUAL DRUG LOADING AND DRUG ENTRAPMENT EFFICIENCY OF DIFFERENT FORMULATIONS

In-vitro drug release study of prepared Glibenclamide microspheres:

To find out the mechanism of drug release, the controlled release Glibenclamide microspheres were treated in different mathematical models like Zero order (cumulative percentage of drug release versus time), First order (log percentage of drug remaining versus time), Higuchi model (cumulative percentage of drug release versus square root of time), Hixon-crowell model (cubic root percentage release versus time), Korsmeyer model (log cumulative percentage of drug release versus log time).

The release data was plotted. From the linear portions of the curve slope correlation coefficients (R^2) were calculated. With the Korsmeyer plot, linearity was noted highest in all formulations using all data points. The data yielded apparently straight line with Korsmeyer plot ($R^2 > 0.99$) while a bit with zero order, first order kinetics and Higuchi plot. No linearity was noted with Hixon-crowell kinetics.

It is observed that drug released from sustained release microsphere followed Korsmeyer release log cumulative percentage of drug release versus log time. The mechanism of drug release was calculated according to Peppas equation. The calculated "n" values along with the correlation coefficients (R^2) have been shown in **Table 2**. The values of n depend upon the polymer concentration. The calculated "n" values suggest that the mechanism of drug release followed non-Fickian transport [17-18].

TABLE 2: RATE CONSTANT (K) AND CORRELATION COEFFICIENT (R^2) OF FORMULATIONS OF PREPARED MICROSPHERES

Formulation Code	Rate constant (K,n) and correlation coefficient (R2)							
	Zero Order		First Order		Higuchi		Korsmeyer-peppas	
	K_0	R^2	K_1	R^2	K_h	R^2	n	R^2
F1	8.47	0.966	-0.109	0.929	25.76	0.978	0.73	0.992
F2	8.65	0.968	-0.193	0.756	25.55	0.981	0.74	0.984
F3	9.79	0.976	-0.11	0.95	29.77	0.98	0.83	0.998
F4	9.145	0.98	-0.075	0.969	27.63	0.962	0.665	0.988
F5	10.46	0.994	-0.099	0.939	31.48	0.957	0.78	0.998
F6	9.464	0.991	-0.08	0.937	28.41	0.95	0.718	0.992
F7	9.82	0.951	-0.124	0.991	30.47	0.951	0.65	0.998
F8	9.68	0.969	-0.118	0.985	28.55	0.981	0.76	0.994
F9	9.04	0.981	-0.121	0.991	27.62	0.966	0.71	0.996

F10	10.78	0.974	-0.11	0.976	32.74	0.977	0.62	0.983
F11	9.28	0.979	-0.119	0.935	25.55	0.971	0.74	0.986
F12	8.95	0.976	-0.115	0.926	26.66	0.942	0.52	0.994

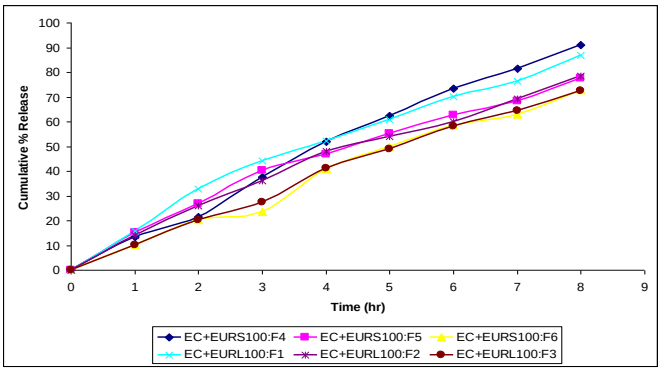
Effect of different polymers on the release of Glibenclamide from microspheres prepared by Emulsion solvent evaporation method:

Glibenclamide microspheres were prepared by polymeric concentration variation to study the effect of combination of polymers on the release of drug from microspheres. Formulations F1 to F3 were prepared by using Ethyl cellulose and Eudragit RL100. After the end of 8 hours of dissolution, the release drug from microspheres was 91.51%, 82.11% and 83.31% respectively that is shown in **Fig. 2a**. Formulations F4 to F6 were prepared by using Ethyl cellulose and Eudragit RS100. After the end of 8 hours of dissolution, the release drug from microspheres was 98.21%, 96.51% and 89.31% respectively that is shown in **Fig. 2a**. Formulations F10 to F12 were prepared by using Ethyl cellulose and Methocel K100M. After the end of 8 hours of dissolution, the release drug from microspheres was 83.21% 81.54% and 88.95% respectively that is shown in **Fig. 2b**. Formulations F7 to F9 were prepared by using Ethyl cellulose and Methocel K15M. After the end of 8 hours of dissolution, the release drug from microspheres was 92.31%, 97.51% and 81.23% respectively that is shown in **Fig. 2b**. A comparative study of formulated microspheres, marketed product and pure drug has shown in **Fig. 2c**.

It is noticeable that the entrapment efficiency of Eudragit RS100 microspheres was higher than that of the Eudragit RL100 microspheres. Eudragit RL100 contains higher amount of quaternary ammonium groups, which facilitates the diffusion of a part of entrapped drug to the surrounding medium during preparation of microspheres. Eudragit RS100 has thick polymeric surfaces due to the presence of lower amount of quaternary ammonium groups, which restrict the migration of drug particles to the surrounding medium.

This suggested that the release of Glibenclamide from Eudragit RS 100 microsphere exhibits diffusional characteristics, closely following Higuchi model and is highly correlated with Korsmeyer-Peppas model release kinetics. This differences in drug release behavior suggested structural differences of the wall materials, and it is dependent on the content of the quaternary ammonium groups. Good release retardant effect obtained from ethyl cellulose because of it is hydrophobic nature, less permeation of dissolution medium there by decrease of drug diffusion. Methocel on the basis of it high water absorption, fast hydration and swelling to form an outer pseudo-gel layer controlling drug release from the inner to the outer side of the microspheres. Thus the results showed that the release rate of drug from the microspheres can be modulated with adjusting the ratios of polymer/drug in the formulation.

All the formulations were best fitted with Korsmeyer model as shown in **Table 2**. The data obtained were also put in Korsmeyer-Peppas model in order to find out n value, which describes the drug release mechanism. The n value of microspheres of different drug to polymer ratio was ranged between 0.45-0.83, indicating that the mechanism of the drug release was diffusion controlled and erosion.



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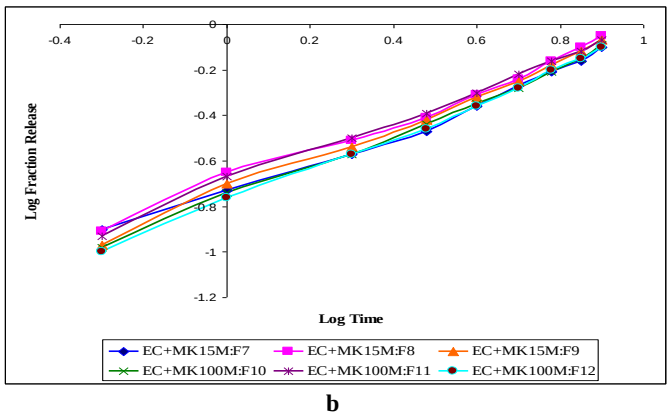

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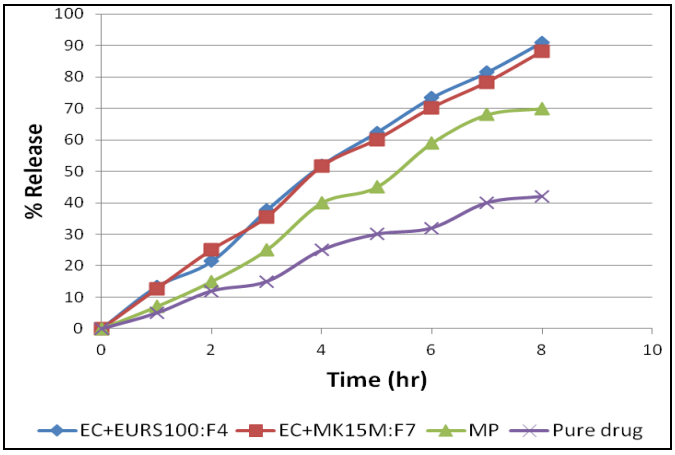


c

FIG. 2: RELEASE KINETICS OF a) CUMULATIVE PERCENT RELEASE VS TIME PLOTS OF GLIBENCLAMIDE MICROSPHERES OF FORMULATION F1, F2, F3, F4, F5 AND F6, b) CUMULATIVE LOG FRACTION RELEASE VS LOG TIME PLOTS OF GLIBENCLAMIDE MICROSPHERES OF F7, F8, F9, F10, F11AND F12 c) CUMULATIVE PERCENT RELEASE VS TIME PLOTS OF GLIBENCLAMIDE MICROSPHERES OF FORMULATION F4, F7, MARKETED PRODUCT (MP) AND PURE DRUG AFTER 8 HOURS RESPECTIVELY

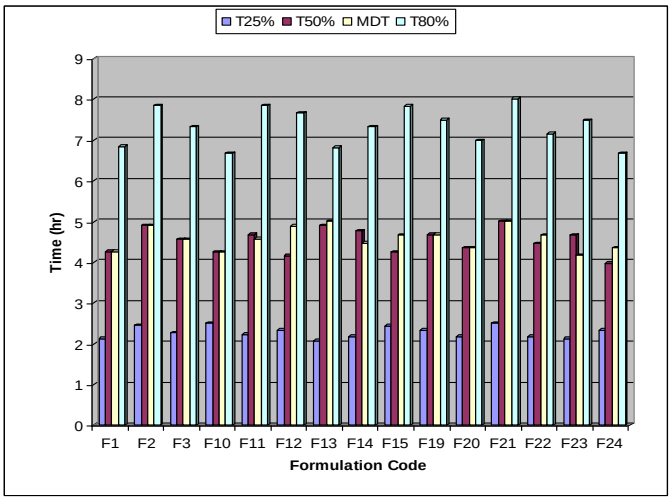


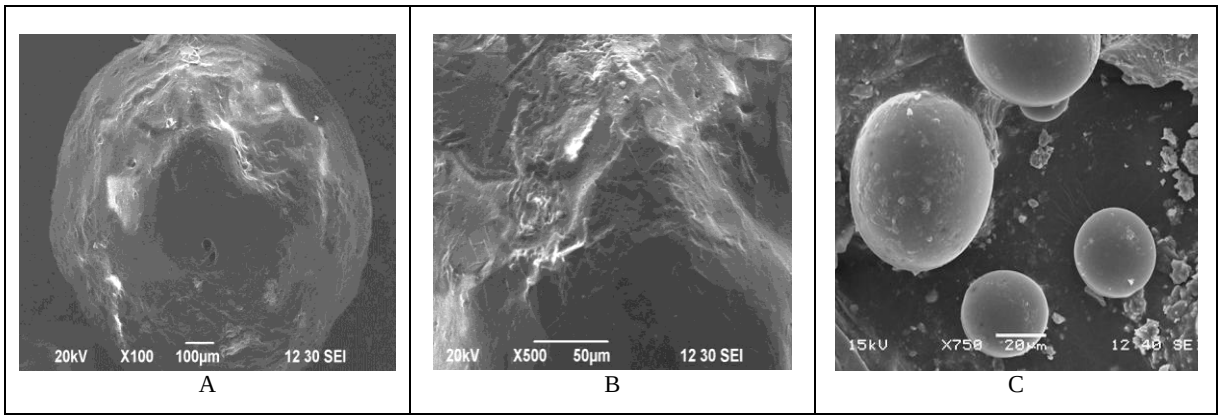
FIG. 3: SUCCESSIVE FRACTIONAL DISSOLUTION TIME ($T_{25\%}$, $T_{50\%}$, MDT AND $T_{80\%}$) OF FORMULATIONS F1, F2, F3, F10, F11, F12, F13, F14, F15, F19, F20, F21, F22, F23 AND F24 RESPECTIVELY

Successive fractional dissolution time:

To characterize the drug release rate in different experimental conditionals were calculated from dissolution data. MDT of formulations A1, A2 and A3 were found 4.60 hours, 5.16 hours and 5.23 hours respectively. MDT of formulations B1, B2 and B3 were found 4.78 hours, 4.83 hours and 4.00 hours and MDT of formulations C1, C2 and C3 were found 3.59 hours, 4.39 hours and 4.75 hours respectively that is shown in **Fig. 3**. The figure clearly indicates that higher the polymer level, higher the value of$T_{25\%}$, $T_{50\%}$, MDT and $T_{80\%}$.

Surface Morphology study and analysis of the size of microspheres by Scanning electron Microscope (SEM):

The surface morphology of the microspheres was explored by SEM. As seen in **Fig. 4**, they were sphere-shaped as well as revealed porous surfaces. The SEM of drug-polymer laden microspheres had uneven shell owing to elevated application of drug in the microspheres. Surface analysis of the microspheres following liberate study showed larger pores signifying that the drug was unconfined from side to side pores and the means of drug release was diffusion controlled.



A B C

FIG. 4: SURFACE MORPHOLOGY OF F4 MICROSPHERES BY SCANNING ELECTRON MICROSCOPE (SEM) (A) MAGNIFICATION AT X100 SEI (B) MAGNIFICATION AT X 500 SEI AND (C) MAGNIFICATION AT X 750 SEI

Drug-polymer compatibility study by Fourier transform infrared (FTIR) spectroscopy: The drug-polymer interaction was studied by FTIR analysis. A FTIR spectrum of Glibenclamide alone

and its combination with polymers has shown in **Fig. 5**. The IR Spectra of Glibenclamide was recorded and it has showed short absorption peak due to -OH group present in the drug molecules. In this case -NH absorption peak present in the form of amine because of its weak characters exhibits a weak absorption at 3311.19 cm-1. The aliphatic-CH absorption peak is seen from 2929.06 cm-1. The amide C=O present in the molecules gave a short absorption peak at 1010.89 cm-1. These peaks can be considered as characteristic peaks of Glibenclamide and were not affected and prominently observed in FTIR spectra of Glibenclamide along with polymers and clearly indicated the stable nature of microspheres prepared with these polymers during encapsulation.

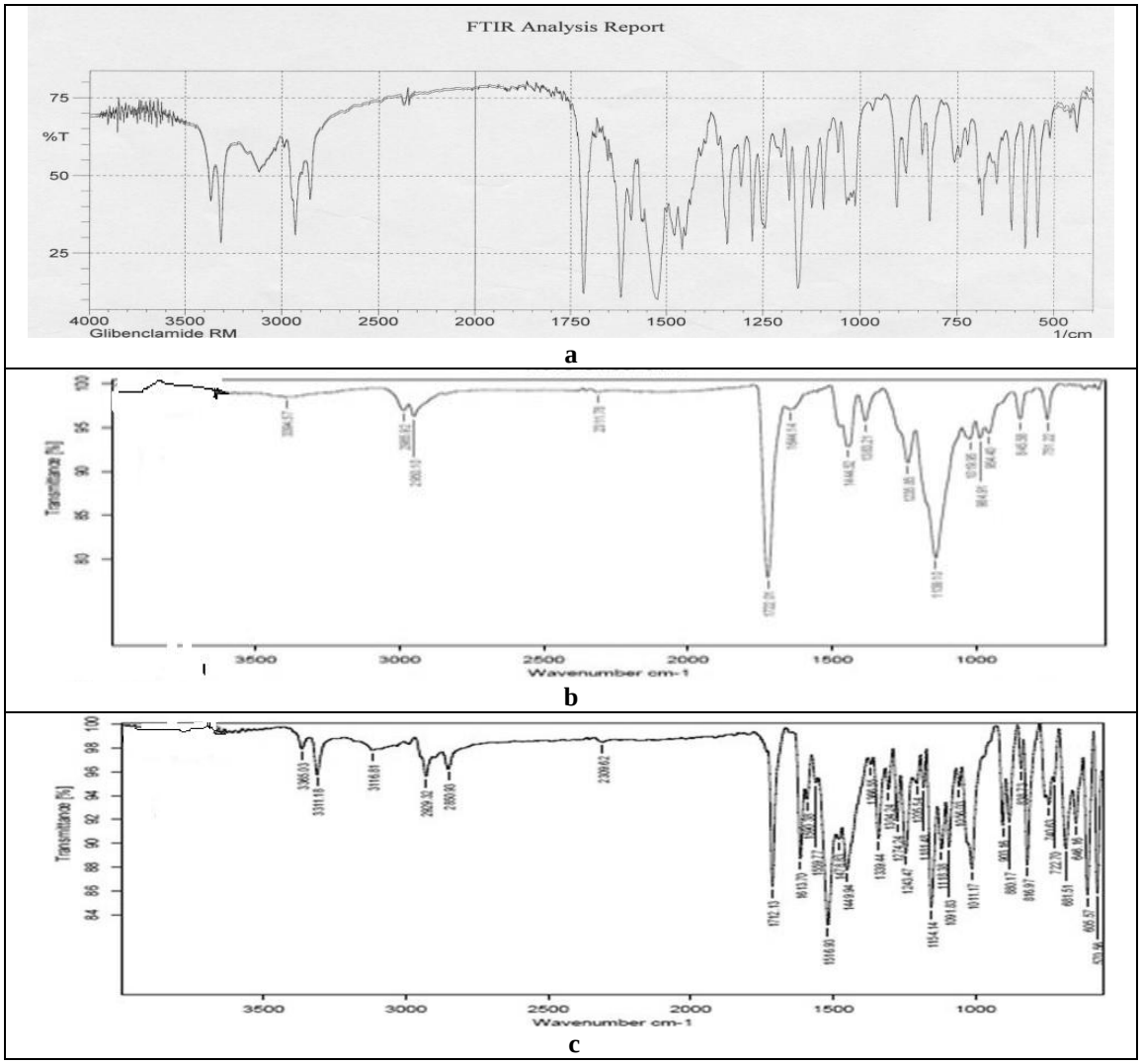


FIG. 5: FTIR SPECTRUM OF A) PURE GLIBENCLAMIDE B) EUDRAGIT RS100 C) FORMULATION F4

Drug-polymer compatibility study by Differentiate scanning calorimetry (DSC) study of microspheres:

DSC is a fast and reliable method for understanding polymorphic transitions when screening drugs and polymers for compatibility, obtaining information about possible interactions. It was evident from the DSC profile (**Fig. 6**) that Glibenclamide exhibited a sharp endothermic peak at 172.99°C, which corresponds to the reported melting temperature of the drug but drug loaded formulation F22 microspheres loss of its sharp appearance. It appears that there is a significant reduction of drug crystallinity in the microspheres. The absence of detectable crystalline domains in drug loaded microspheres clearly indicates that drug was dispersed completely in the formulation, thus modifying the microspheres to an amorphous, disordered-crystalline phase.

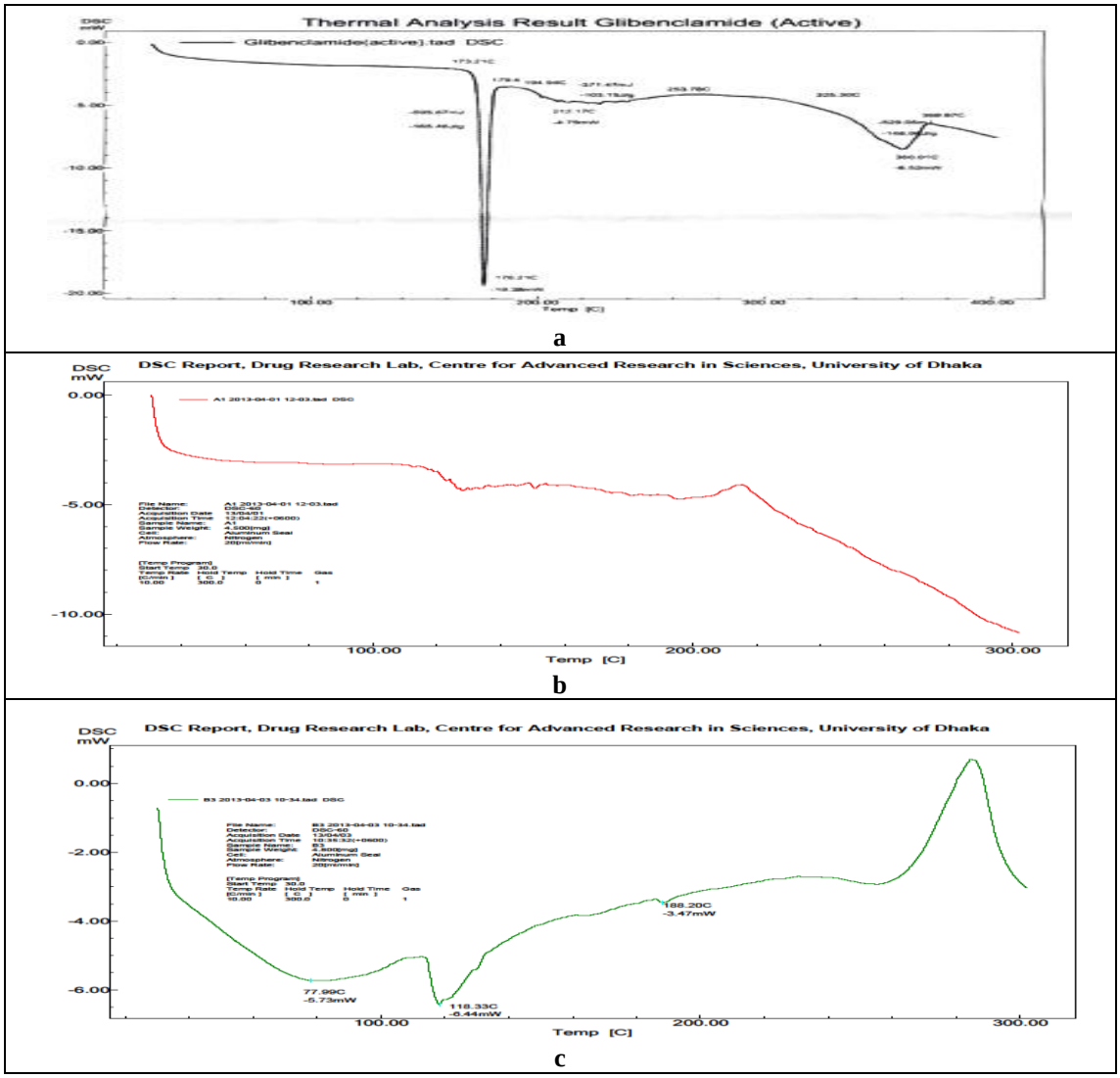


FIG. 6: DSC OF A) PURE GLIBENCLAMIDE B) EUDRAGIT RS 100 C) FORMULATION F4

Study of *in-vivo* anti-hyperglycemic and different biochemical effects on Albino rats:

To evaluate the anti-diabetic effect of pure Glibenclamide and drug loaded microspheres the blood glucose levels were measured in streptozotocin induced diabetic rats for 48 hours. Group II was the "Positive Control" where six rats were developed diabetes after streptozotocin 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 III, IV and V) at every time interval (shown in **Table 3**). Pure drug was administered to Group III in a suspension form at the same dose. A rapid reduction in blood glucose levels was observed and maximum reduction of (56.44%) was observed within 4 hours after oral administration. At 12 hours there was no significant hypoglycemic effect of pure drug (253.00 mg/dl) and at 48 hrs average blood glucose level became 361.00 mg/dl indicating the severe hyperglycemia in Group II rats.

Table 3 has shown the gradual reduction in blood glucose level in group IV and Group V compared to pure drug. In the case of drug loaded microsphere, the reduction in blood glucose level was slow and reached maximum reduction within 4-8 hrs after oral administration. This reduction in blood glucose level was sustained over longer periods of time. It was suggested that a 25% reduction in blood glucose levels is considered a significant hypoglycemic effect. This hypoglycemic effect (25%) was maintained only for 4-6 hrs after oral administration of the drug, whereas in the case of microspheres of

Glibenclamide, significant hypoglycemic effect (25%) was maintained for a period of 4 to 24 hours. The sustained hypoglycemic effect observed over a longer period of time in the case of microsphere is due to the slow release and absorption of drug over longer periods of time. Glibenclamide sustained release formulation is significantly more effective than the immediate release formulation in reducing fasting plasma glucose levels.

TABLE 3: SHOWING AVERAGE REDUCTION IN GLUCOSE LEVEL (MG/DL) IN NEGATIVE CONTROL, POSITIVE CONTROL, PURE DRUG GLIBENCLAMIDE AND DIFFERENT POLYMER CONTAINING MICROSPHERES OF F7 AND F4

Time (hour)	Negative Control (Group I)	Positive Control (Group II)	Pure Drug (Group III)	Formulation F7 (Group IV)	Formulation F4 (Group V)
0	70±5.329	391±3.444	368±1.989	385±6.342	381±2.096
1	70±5.556	327±2.324	310±1.978	325±4.324	319±5.329
2	70.1±5.663	313±2.457	289±4.890	309±4.123	298±5.556
4	70.2±3.445	305±3.854	210±3.890	260±1.543*	245±3.687
6	70.25±3.687	305±4.578	211±3.698	222±2.365	195±2.378*
8	70.29±3.567	305±2.096	200±5.489	200±3.897	176±3.568
10	70.3±2.478	306±5.708	240±2.489	183±2.156*	153±3.698
12	70.3±3.568	307±6.348	253±5.789	165±2.096	149±2.278*
14	70.31±3.598	307±2.497	258±6.143	144±2.496	127±6.123
24	71±2.456	309±2.099	260±6.345	123±3.999	111±6.347
26	71±1.456	309±3.999	266±4.576	102±3.547	90±5.487
28	71.2±2.786	311±3.547	258±4.590	97±3.654	87±5.324
30	71.2±2.487	312±4.586	270±3.654	89±2.409	79±5.327
32	71.2±2.409	316±4.680	300±3.908	81±4.680	75±3.879
34	71.3±5.678	320±5.698	328±5.008	75±5.690	71±3.908
48	71.35±3.4447	321±5.321	361±4.214	60±3.265	70±2.453

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

STZ diabetic rats were found to have significantly elevated serum creatinine and urea levels as compared to non diabetic control rats. This is because STZ diabetic rats have diminished ability to filter urea and creatinine from blood and excrete them in urine. This is another characteristic change in diabetes. Whereas after treatment both the values where comparable to those which received glibenclamide treatment. There was no significant difference in values obtained for group I and group V shown in **Fig. 7**.

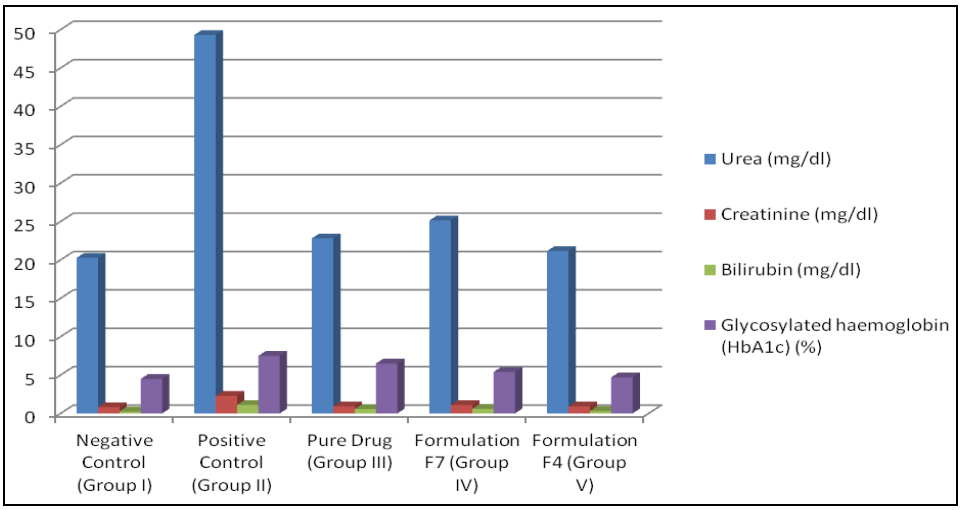


FIG. 7: EFFECT OF DIFFERENT FORMULATION ON UREA, CREATININE, BILIRUBIN AND GLYCOSYLATED HAEMOGLOBIN (HBA1C) IN SERUM OR PLASMA OF CONTROL AND EXPERIMENTAL GROUPS

Various parameters of blood lipid profiles were tested in the normal and diabetic rats. The levels of TC and TG were significantly elevated and levels of serum HDL was decreased in diabetic control group as compared to normal control rats. In case of insulin deficiency, there is increased lipolysis leading to hyperlipidemia. In insulin deficient diabetes, the concentration of free fatty acids is elevated as a result of free fatty acid outflow from fat depots, where the balance of free fatty acid esterification- triglyceride lipolysis cycle is displaced in favor of lipolysis. It has shown from **Fig. 8** that after treated with formulation F7 and F4, the alteration in lipid metabolism was partially attenuated as evidenced by decreased serum TG and TC levels in diabetic rats.

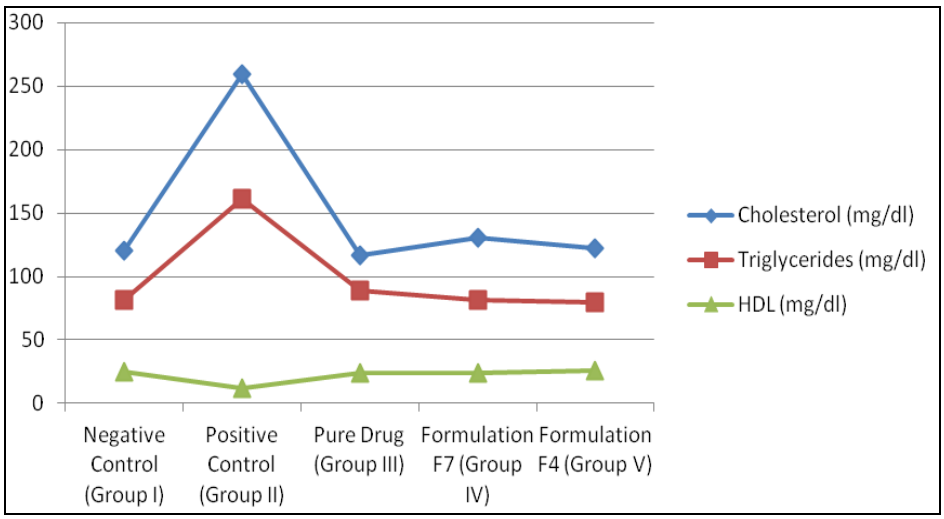


FIG. 8: EFFECT OF DRUG ON LIPID PROFILE OF CONTROL AND EXPERIMENTAL GROUPS

CONCLUSION: The present study was conducted to design Glibenclamide sustained release Microspheres by Emulsion solvent evaporation. The microspheres prepared with different polymers had prolonged drug release for 8 hours or longer and would be capable of reducing the frequency of administration. As the concentration of polymer increases, the release rate decreases gradually and the release studies showed that highest concentration of polymer gave the best sustained effect. When the release data was plotted in Zero order, First order, Korsmeyer, Higuchi, Hixon-Crowel equations, all the formulations prepared by the best data fitted with the highest correlation coefficient (R^2) for microspheres was obtained for Korsmeyer release model.

The maximum and minimum release of drug was observed 90.99% and 71.98% respectively. Percent of Actual drug entrapment varied from 7.89% to 15.36% and percent of Drug entrapment efficacy varied from 69.23% to 98.21%. The release exponent $0.89 < n > 0.45$ for all the formulations showed non-fickian release of drug which refers to a combination of both diffusion and erosion controlled drug release. The SEM photograph of microspheres confirmed good spheres and smooth surface of the microspheres. The IR and DSC studies used to of confirmed the interaction along with drug and polymer. Different biochemical tests such as triglycerides, urea, cholesterol, glycohemoglobin, bilirubin etc. showed good results and it also proved that Glabenclamide formulations has given sustained release effects. Microspheres are able to limiting fluctuation within a therapeutic range, dropping side effects, diminishing dosing rate, and improving patient conformity. *In-vitro-in vivo* studies showed that Glibenclamide microspheres might be a good candidate as sustained drug delivery system for treating type II Diabetic.

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AUTHORS' CONTRIBUTIONS: ID performed and designs the experiment and prepared the

manuscript and SR supervised the work. Authors read and approved the final manuscript.

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