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FORMULATION AND CHARACTERIZATION OF ETHYL CELLULOSE, EUDRAGIT RSPO AND EUDRAGIT RLPO LOADED MICROSPHERES WITH METFORMIN AND GLIPIZIDE: *IN VITRO* AND *IN VIVO* STUDIES

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ABSTRACT

Objective: The aim of the current exploration is to formulate and evaluate the metformin and glipizide, both are type II anti-diabetic agents, loaded microspheres with an objective to sustained release as well as improve bioavailability. **Materials and method:** Microspheres were prepared by solvent evaporation method using different rate retardant polymers including eudragit RSPO, eudragit RLPO and ethyl cellulose. Prepared microspheres were characterized by percent drug loading and drug entrapment efficiency, FTIR, DSC and SEM. UV-Spectrophotometric method was applied to assay the microspheres and *in vitro* dissolution studies according to USP paddle method were carried out in phosphate buffer (pH 6.8) for 8 hours. **Results:** The *in vitro* release kinetics was studied in different mathematical release models. The best data fitted with the highest correlation coefficient (R^2) for microspheres was obtained for Korsmeyer-Peppas model. The maximum and minimum release of metformin and glipizide were observed 87.02% to 59.35% and 27.31% to 11.37% for single and 85.28% to 60.05% and 26% to 15.95% for combination polymers loaded microsphere after 8 hours dissolution respectively. Percent of drug loading was varid from 7.09% to 17.06% and percent of drug entrapment efficacy varied from 57.87% to 88.51%. The SEM, FTIR and DSC studies used to of confirmed good spheres and smooth surface as well as interaction between drug and polymer. Different biochemical tests have also done on albino rats which showed better control of blood sugar and it proves that formulations have given sustained release effects. **Conclusion:** *In vitro* and

in vivo studies showed that metformin and glipizide loaded microspheres might be a good candidate as sustained drug delivery system for treating type II diabetic.

KEYWORDS: Metformin, glipizide, microspheres, eudragit RSPO, eudragit RLPO.

INTRODUCTION

Metformin HCl and Glipizide are antihyperglycemic agent widely used in the management of non-insulin dependent diabetes mellitus (NIDDM). Metformin is a hydrophilic drug and is slowly and incompletely absorbed from the gastrointestinal tract, and its bioavailability is reported to be 50% to 60% and has relatively short plasma elimination half-life of 1.5 to 4.5 hours.[1] On the other hand glipizide is a BCS class II drug, which has low solubility and high permeability and it has a short biological half-life (3.4±0.7 hours). Sulfonylureas act mainly by increasing endogenous insulin secretion, while biguanides act chiefly by decreasing hepatic gluconeogenesis and increasing peripheral utilization of glucose.[2] Chronic hyper glycemia of diabetes mellitus is associated with long-term damage, dysfunction and failure of various organs. Therefore a long lasting treatment is required with proper diet and multi drug therapy. It is a progressive illness and most patients will eventually need more than two oral agents to maintain adequate glucose control.[3] For that, multiple drug dosing can be avoided by preparing microspheres of combination drug formulation of glipizide[4] and metformin HCl. Sustained release formulations that would maintain plasma levels of drug for 8 to12 hrs might be sufficient for once a day dosing for metformin and glipizide and it is needed to prolong duration of action and to improve patient compliance.

Glipizide generally undergoes hydrolysis in presence of moisture, while metformin HCl is hygroscopic in nature. These properties may affect the stability of both molecules when given in single layer tablet dosage form. Thus both of drugs shows incompatibility with each other therefore, there is a substantial need to develop such a system to avoid intimate contact between the two drugs i.e. sustained release microspheres formulation of glipizide and metformin HCl that can overcome all the limitations of both the drugs and for that microspheres of those drugs is prepared by using various polymer by solvent evaporation technique not only avoid the contact between both of drugs but also increases the solubility profile as well as bioavailability.[5] Most importantly, because of prolonged duration of action, It will produce a strict control of blood pressure and consequently less hypertension complication. The combination of metformin HCl with glipizide is more effective than individual therapy because of the synergism.[6]

A multiplicity 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.[7]

Microencapsulation process aids for converting the liquids to solids, changing the colloidal and surface properties, providing environmental protection and controlling the release characteristics of different coated materials. This has been done by developing the new drug entities, discovering of new polymeric materials that are suitable for prolonging the drug release, safety, improvement in therapeutic efficacy. Microspheres can also offer advantages like limiting fluctuation within therapeutic range, reducing side effects, decreasing dosing frequency and improving patient compliance. Generally the size of the microencapsulated products (micro particles) is considered as larger than 1 micrometer and up to 1000 micrometers in diameter. For the preparation of microspheres, 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.[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. 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.[9]

The key observation of the present work was to prepare and estimate oral sustained release micro-particulate microspheres drug delivery system of metformin and glipizide 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.

MATERIALS AND METHODS

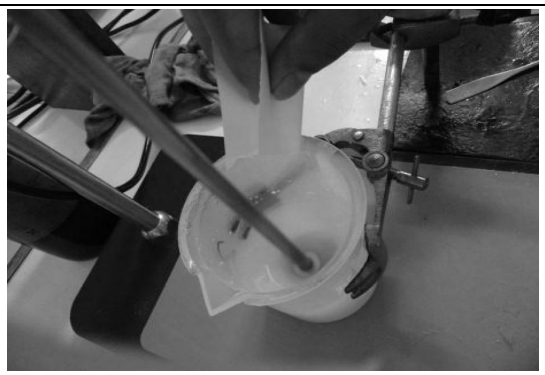
Materials

Square Pharmaceuticals limited, Bangladesh has given the active drugs, metformin HCl and glipizide as gift sample. Eudragit RS PO and eudragit RL PO (Evonik Industries, Germany), ethyl cellulose (Colorcon, India), ethanol, dichloromethane, n-hexane, tween 80, liquid paraffin (Merck, Germany), potassium dihydrogen phosphate purified (Merck, India), sodium hydroxide pellets purified (Merck, India), distilled water (Pharmaceutical research lab, UAP).

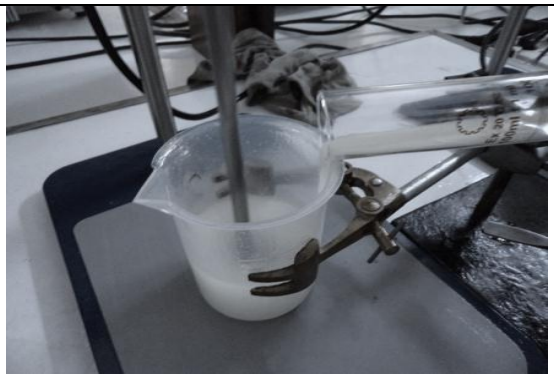
Method

Preparation of metformin HCl and glipizide microspheres

The microspheres were prepared according to **Table 1 and Fig. 1** by emulsion solvent evaporation method. The process was carried out from dispersion of metformin HCl and glipizide in 50 ml of light liquid paraffin(LLP) using 1% tween 80. At first, LLP was emulsified in a plastic beaker with tween 80 for few minutes with the help of mechanical stirrer equipped with a three-blade propeller at 450 rpm. By this time the polymers solution (internal phase) was prepared by dissolving properly weighed polymer(s) in combination of ethanol and dichloromethane at a ratio of 2:8 in a volumetric flask with the help of vortex machine stirrer for 20-30 minutes. After proper mixing prepared polymeric phase was added drop wise to the external phase. Stirring was performed for 3.5 hours. After stirring, the microspheres were decanted and washed by n-haxen and allowed for drying in natural air. The prepared microspheres were then sieved and transferred to glass vials and stored in desiccators for further experiment.



Addition of drug to mixture of LLP and 1% Tween 80



Addition of prepared polymer mixture (Internal phase)

Filtration of Microspheres After washing

Storage in airtight container after drying

Figure 1: Steps of preparation of metformin HCl and glipizide microspheres by non aqueous emulsion solvent method.

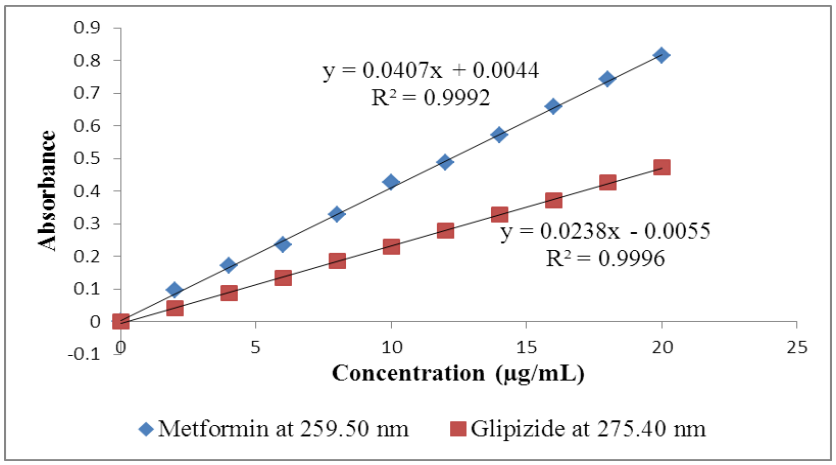
Table: 1: Formulation of metformin HCl and glipizide microspheres.

Formulation Code	Drug (mg)	Polymers (mg)			D:P Ratios
	Met +Gli	EC	Eudragit RS PO	Eudragit RLPO	
ECF1	1	1	-	-	1:1
ECF2	1	2	-	-	1:2
ECF3	1	3	-	-	1:3
EURSF7	1	-	1	-	1:1
EURSF8	1	-	2	-	1:2
EURSF9	1	-	3		1:3
EURLF10	1	-	-	1	1:1
EURLF11	1	-	-	2	1:2
EURLF12	1	-	-	3	1:3
CF13	1	1	1	-	1:1:1
CF14	1	2	2	-	1:2:2
CF15	1	3	3	-	1:3:3
CF16	1	1	-	1	1:1:1
CF17	1	2	-	2	1:2:2
CF18	1	3	-	3	1:3:3

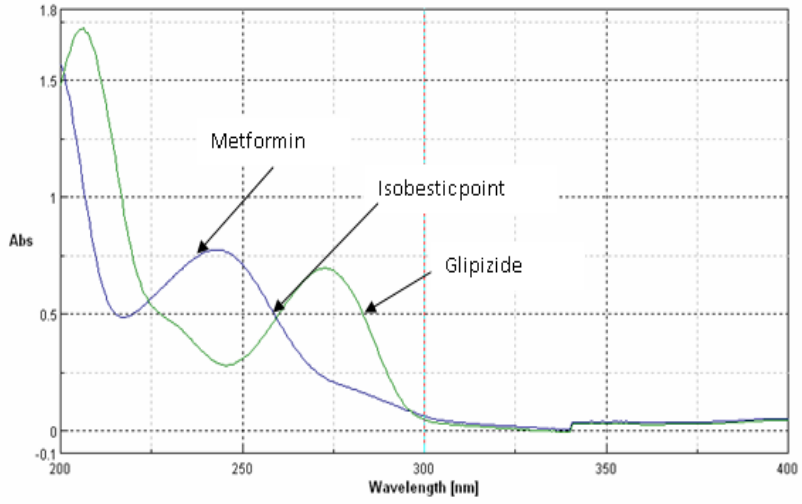
Where, Met= Metformin HCl, Gli= Glipizide, EC= Ethyl Cellulose, D= Drug, P=Polymer.

Preparation of standard curve of metformin HCl and glipizide

Standard stock solution containing metformin HCl and glipizide were prepared by dissolving 20 mg of drug in 100 ml distilled water and phosphate buffer (pH 6.8) using a vortex mixer to prepare a clear solution respectively. This solution is considered as stock solution. From the stock solutions again 10ml was withdrawn and taken in another 100ml volumetric flask. The solution was then adjusted up to 100ml by using distilled water and phosphate buffer (pH6.8) respectively. From those solutions 1ml, 2ml, 3ml, 4ml, 5ml, 6ml, 7ml, 8ml, 9ml, and 10ml drug solution were withdrawn and taken in test tubes individually. Then distilled water and phosphate buffer (pH6.8) were added to the test tubes to make the volume up to 10ml of each test tube respectively. Finally absorbance was recorded at 232.70 nm and 275.40 nm for metformin HCl and glipizide respectively by using the UV spectrophotometer shown in **Fig. 2**.



a



b

Figure 2: Standard curve of a) metformin HCl at 259.50 nm and glipizide at 275.40 nm b) overlay spectra of metformin HCl and glipizide respectively

Assay of percent drug entrapment efficiency and drug loading

Few microspheres were taken in a mortar and were triturated properly until fine powder was formed. 20 mg of powder was taken in a screw cap test tube. 5ml buffer was added to the powdered microsphere and was vortex for 10 minutes. Then the solution was filtered and taken in a 100ml volumetric flask. The volume of the solution adjusted to 100ml with buffer solution the absorbance was taken at 233nm. From the absorbance value the percent of drug entrapment efficiency and drug loading was determined using standard curve. The drug entrapment efficiency was calculated by using the following formula-

$$\% \text{ Drug Entraptment Efficiency (DEE)} = \frac{\text{Actual Drug Loading}}{\text{Theoratical Drug Loading}} \text{X100}$$

$$\% \text{ Drug loading (DL)} = \frac{\text{Actual Drug Loading}}{\text{Microspheres to be taken}} \text{X100}$$

***In vitro* dissolution study of metformin HCl and glipizide loaded microspheres by absorption ratio method (Q-ratio method)**

The *in vitro* dissolution studies were carried out in 900 ml of phosphate buffer (pH 6.8) maintained at 37 ± 0.5° and 100 rpm by using United States Pharmacopoeia paddle type (Type-II) dissolution test apparatus (Electrolab, India) under sink conditions. Accurately weighed amount of microspheres containing 100 mg was taken from each batch of formulation and were added to the dissolution medium and 10 ml aliquots were withdrawn and replaced with an equal volume of fresh dissolution medium at a predetermined intervals of 30min, 1st hour, 2nd hour, 3rd hour, 4th hour, 6th hour and 8th hour. After suitable dilution, the samples were analyzed spectophotometrically at 259.50 nm and 275.40 nm. The concentration of metformin HCl and glipizide in test samples was corrected and calculated using the following equations of absorption ratio method (Q-ratio method). [10]

$$\mathbf{Cx} = \frac{(\mathbf{Qm} - \mathbf{Qy}) \times \mathbf{A1}}{(\mathbf{Qx} - \mathbf{Qy}) \times \mathbf{ax1}} \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

$$\mathrm{Cy} = \frac{(\mathrm{Qm} - \mathrm{Qx}) \times \mathrm{A1})}{(\mathrm{Qy} - \mathrm{Qx}) \times \mathrm{ay1})} \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where,

$$Qm = \frac{A2}{A1}$$

$$Qx = \frac{ax2}{ax1}$$

$$Qy = \frac{ay2}{ay1}$$

Cx = Concentration of metformin HCl in gm/lit

Cy = Concentration of glipizide in gm/lit

A2 =Absorbance at 275.4nm

A1 =Absorbance at 259.50nm

ax1= absorptivity of metformin HCl at 259.50nm

ay1= absorptivity of glipizide at 259.50nm

ax2= absorptivity of metformin HCl at 275.40nm

ay2= absorptivity of glipizide at 275.40nm

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.[11]

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 20.0 kv X 75, 20.0 kv X 90, 20.0 kv X 95, 20.0 kv X 140, 20.0 kv X 300, 20.0 kv X 600, 20.0 kv X 1000. The working distance was 10 an 11 inches.

Fourier Transform Infrared (FT-IR) Spectroscopy studies

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. FTIR spectrum of pure metformin and glipizide and also 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 100 mg 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 microspheres

The DSC measurements were performed on a DSC-60 (SHIMADZU) differential scanning calorimeter with a thermal analyzer (TA-60WS). Pure metformin and glipizide and formulated microsphere sample was placed in aluminum pan and sealed before heating under nitrogen flow (300 ml/min) at a scanning rate of 10 °C min - I from 30°C to 550 °C. An empty aluminum pan was used as reference.

***In vivo* study**

Experimental animals

At first Albino rats (about 165-200 gm) were collected from the animal house of Jahangirnagor University and kept 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[8]

The rats were divided into 4 groups comprising of 6 animals in each group as follows

Group I (Negative Control)	: Normal rats treated with saline daily and served as the negative control.
Group II (Positive control)	: Animals treated with single dose of Streptozotocin (65 mg/kg) by the intra peritoneal route to induce diabetes and served as a positive control.
Group III (Pure metformin)	: Diabetic rats were treated with 250 mg /kg body weight of pure metformin.
Group IV (Pure glipizide)	Diabetic rats were treated with 2.5 mg/kg and body weight of pure glipizide.
Group V (Formulation ECF2)	: Diabetic rats were treated with 2.5 mg/kg and 250 mg/kg body weight of metformin and glipizide containing microspheres respectively.
Group VI (Formulation CF17)	: Diabetic rats were treated with 2.5 mg/kg and 250 mg/kg body weight of metformin and glipizide containing microspheres respectively.

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 and glyco-hemoglobin were assayed using diagnostic reagent kit manufactured by Crescent diagnostics ltd. and Stanbio diagnostics ltd.[12-14]

RESULTS AND DISCUSSION

Percent drug loading (DL) and drug entrapment efficiency (DEE) of prepared microspheres by emulsion solvent evaporation method

Drug loading and the drug entrapment efficiency (DEE) of the prepared microspheres were carried and the graphical presentation are given bellow in **Fig. 3**.The percent drug loading and the drug entrapment efficiency were found to be in the range of 9.5% to 17.5% and 82.56% to 94.59 % respectively. Increase in concentration of polymers resulted decrease in encapsulation efficiency of microspheres and vice versa. The encapsulation efficiency was

also found to be dependent on nature of polymer used in the formulation. On the other hand percent of drug loading increased with increased in concentration of polymers in microspheres.

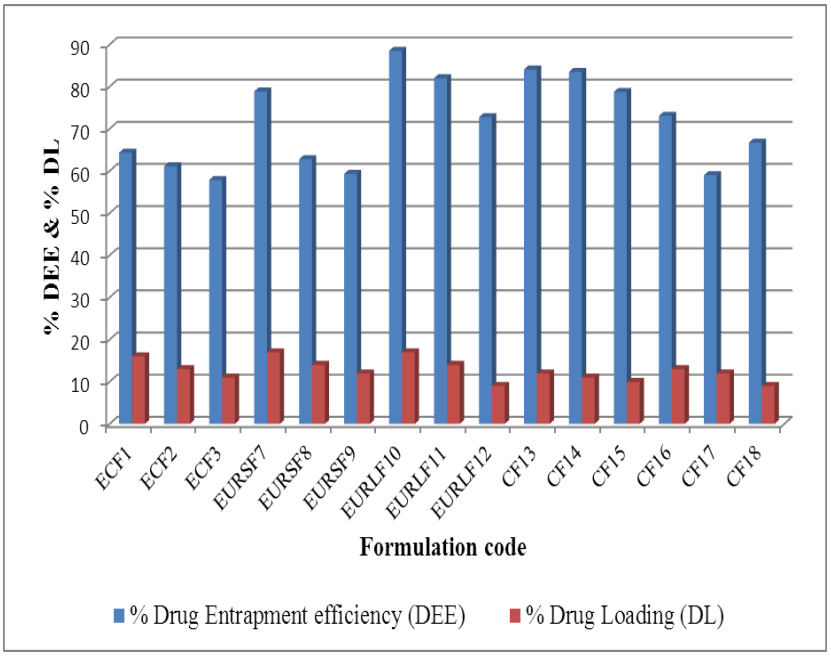


Figure 3: Percent drug entrapment efficiency of different formulations polymer loaded microspheres

***In vitro* dissolution study of metformin and glipizide microspheres prepared by emulsion solvent evaporation method**

To find out the mechanism of drug release, the controlled release metformin and glipizide loaded 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), 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. It was 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.

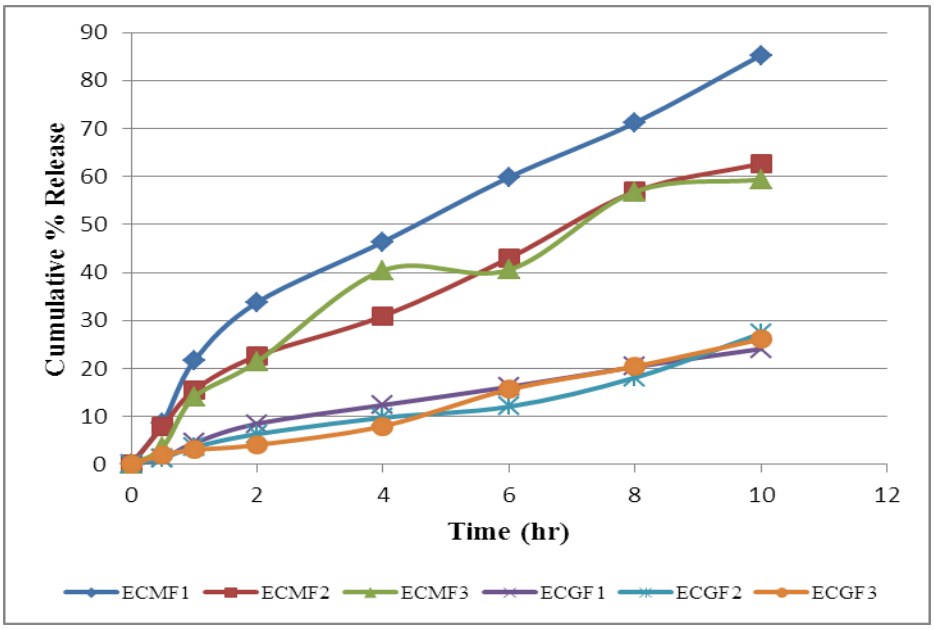
Table 2: Interpretation of release rate constants and correlation coefficient (R^2) values of different formulations of metformin and glipizide loaded microspheres using different polymers by emulsion solvent evaporation technique respectively

Formulation code	Rate constants and R-squared values							
	Zero order		First order		Higuchi		Korsmeyer-Peppas	
	K_0	R^2	K_1	R^2	K_H	R^2	n	R^2
ECF1	9.83	0.983	-0.218	0.919	29.81	0.96	0.62	0.991
ECF2	9.73	0.979	-0.211	0.951	29.7	0.974	0.61	0.995
ECF3	9.8	0.981	-0.209	0.969	30.06	0.98	0.64	0.994
EURSF7	9.97	0.979	-0.221	0.929	30.57	0.98	0.63	0.994
EURSF8	10.35	0.988	-0.211	0.957	31.33	0.963	0.67	0.991
EURSF9	10.67	0.984	-0.204	0.964	32.54	0.973	0.66	0.993
EURLF10	10.4	0.989	-0.225	0.925	31.51	0.966	0.75	0.991
EURLF11	9.39	0.951	-0.221	0.944	29.16	0.976	0.67	0.995
EURLF12	8.48	0.988	-0.218	0.965	25.54	0.959	0.7	0.994
CF13	11.13	0.993	-0.222	0.945	33.51	0.958	0.78	0.995
CF14	10.6	0.981	-0.225	0.954	32.37	0.973	0.77	0.995
CF15	9.86	0.987	-0.231	0.924	29.05	0.912	0.76	0.992
CF16	1.764	0.978	-0.008	0.951	5.854	0.942	0.62	0.978
CF17	1.944	0.978	-0.009	0.979	6.357	0.919	0.72	0.982
CF18	2.317	0.975	-0.013	0.982	8.766	0.957	0.83	0.987

Effect of different polymers on the release of metformin HCl and glipizide loaded microspheres prepared by emulsion solvent evaporation method

Metformin HCl and glipizide loaded microspheres were prepared by polymeric concentration variation in ratios (1:1, 1:2 and 1:3) to study the effect of single and combination polymer(s) on the release of drug from microspheres. Formulations ECF1 to ECF3 were prepared by using ethyl cellulose. After the end of 8 hours of dissolution, the percent of release of metformin and glipizide from microspheres was **85.28**%, 62.68% and **59.35**% and **28.07**%, 27.31% and **26.12**% respectively shown in **Fig.4a**. Formulations EURSF7 to EURSF9 were prepared by using eudragit RS100.After the end of 8 hours of dissolution, the percent of release of metformin and glipizidefrom microspheres was **80.99**%, **69.44**% and **61.73**% and **22.39**%, **21.36**% and **11.37**% respectively shown in **Fig.4b**. Formulations EURLF10 to EURLF12 were prepared by using eudragit RL100. After the end of 8 hours of dissolution, the percent of release of metformin and glipizidefrom microspheres was **87.02**%, **77.36**% and **76.82**% and**24.09**%, **20.63**% and **17.36**% respectively shown in **Fig.4c**. Formulations **CF13** to **CF15** were prepared by using combination polymers of ethyl cellulose and eudragit

RS100. After the end of 8 hours of dissolution, the percent of release of metformin and glipizide from microspheres was **82.02**%, **66.62**% and 60.05% and **25.38**%, **20.96**% and 15.77% respectively shown in **Fig.4d**. Formulations **CF16** to **CF18** were prepared by using combination polymers of ethyl cellulose and eudragit RL100. After the end of 8 hours of dissolution, the percent of release of metformin and glipizide from microspheres was **85.28**%, **80.78**% and 62.2% and **26.01**%, **21.37**% and 19.95% respectively shown in **Fig. 4d**. And **Fig.** 4f has shown comparative study among the different formulations.



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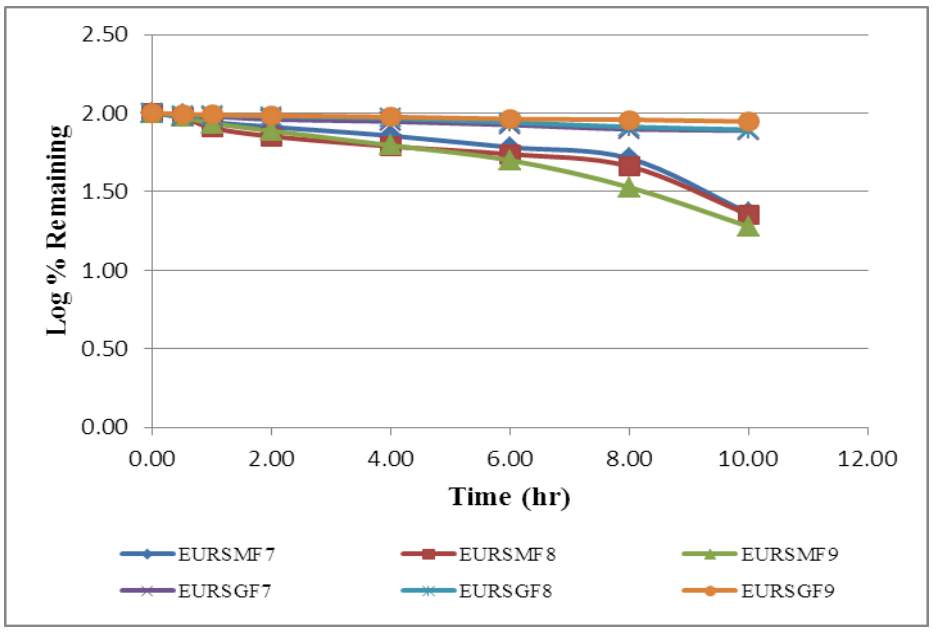

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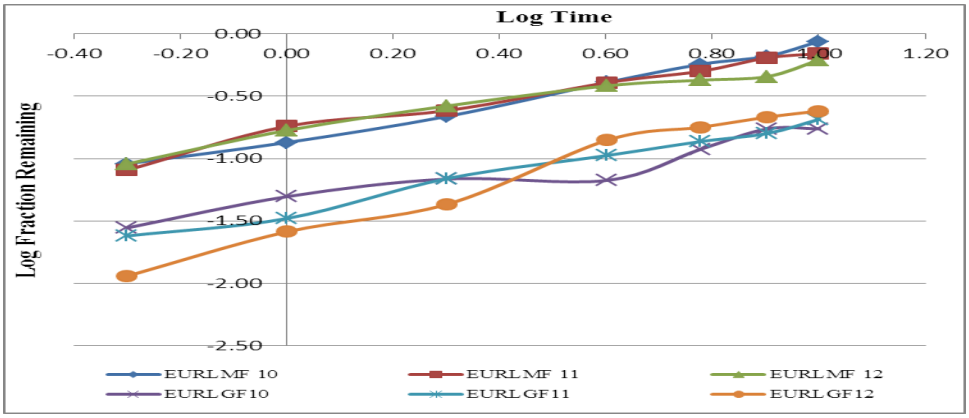

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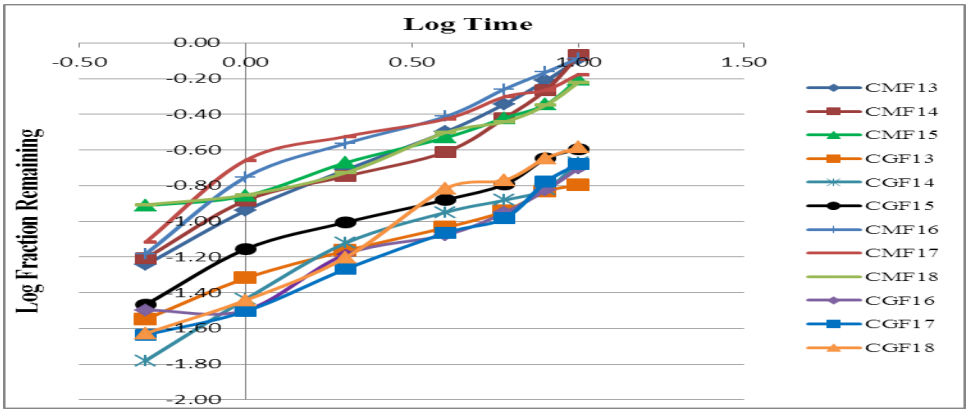
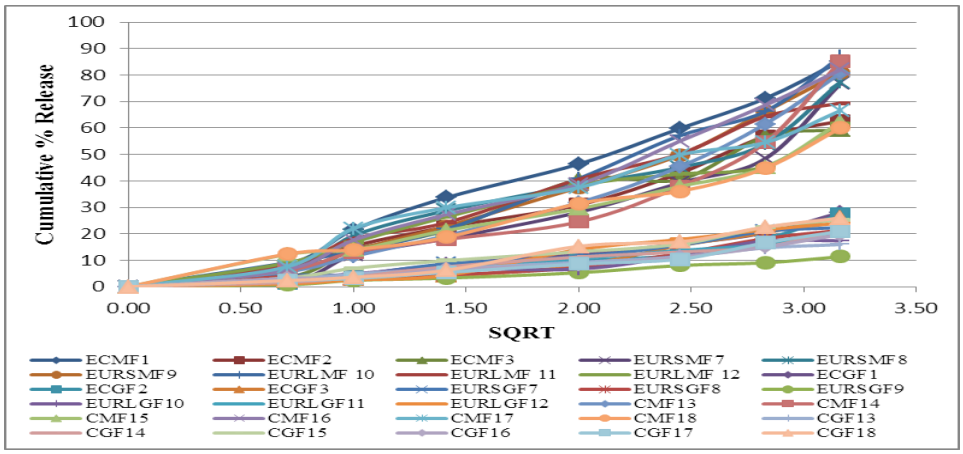


d)

Where, M= metformin HCl, G= glipizide, EC= ethyl cellulose, EU=eudragit, C= combination polymer



e)

Figure 4: Percent release of different kinetics of metformin HCl glipizide loaded microspheres along with different polymers a) Zero order model for single polymer, b) First order model for single polymer, c) Korsmeyer-Peppas model for single polymer, d) Korsmeyer –peppers model for combination polymers and e)Comparative study of different formulations respectively

It is noticeable that the entrapment efficiency of eudragit RL100 microspheres was higher than that of the eudragit RS100 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 metformin HCl and glipizide 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 was obtained from ethyl cellulose because of it is hydrophobic nature, less permeation of dissolution medium there by decrease of drug diffusion. 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.

Study of successive fractional dissolution time of metformin HCl and glipizide loaded microspheres

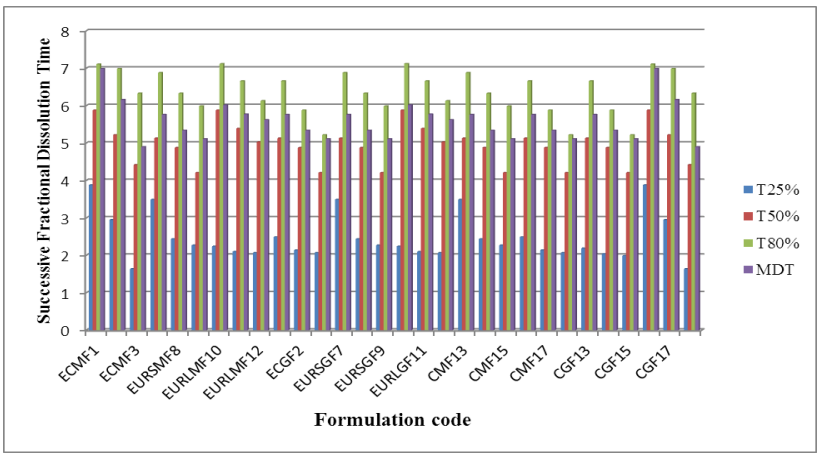


Figure 5: Successive fractional dissolution times (hrs) of different formulations of metformin HCl and glipizide loaded microspheres

To characterize the drug release rate in different experimental conditionals were calculated from dissolution data. MDT of different formulations has shown in **Fig. 5.**The figure indicates that higher the polymer level, lower the value of $T_{25\%}$, $T_{50\%}$, MDT and $T_{80\%}$ and behave according to their properties. A higher value of MDT indicates a higher drug retarding ability of the polymer and vice versa.

Observation of particle Morphology by scanning electron microscopy (SEM)

From the formulated batches different formulations which showed an appropriate balance between the entrapment efficiency and the percentage release were examined for surface morphology shown in **Fig.** 6. SEM images of F1, F13 and F17 were taken and surface of these microspheres were analyzed morphologically. Formulation F1 microspheres consisting ethyl cellulose having drug loading of about 64.36%, shown a smooth surface of microspheres and small pores present on the surface of the microspheres. Formation F13 and F17 were made by using double polymer with a ratio of drug: polymer =1:2 and having drug entrapment efficiency of about 84.02% and 66.71% respectively. So from the scanning electron microscope we can conclude that microspheres are losing its spherical shape and also having rough surface with increase of drug entrapment efficiency.

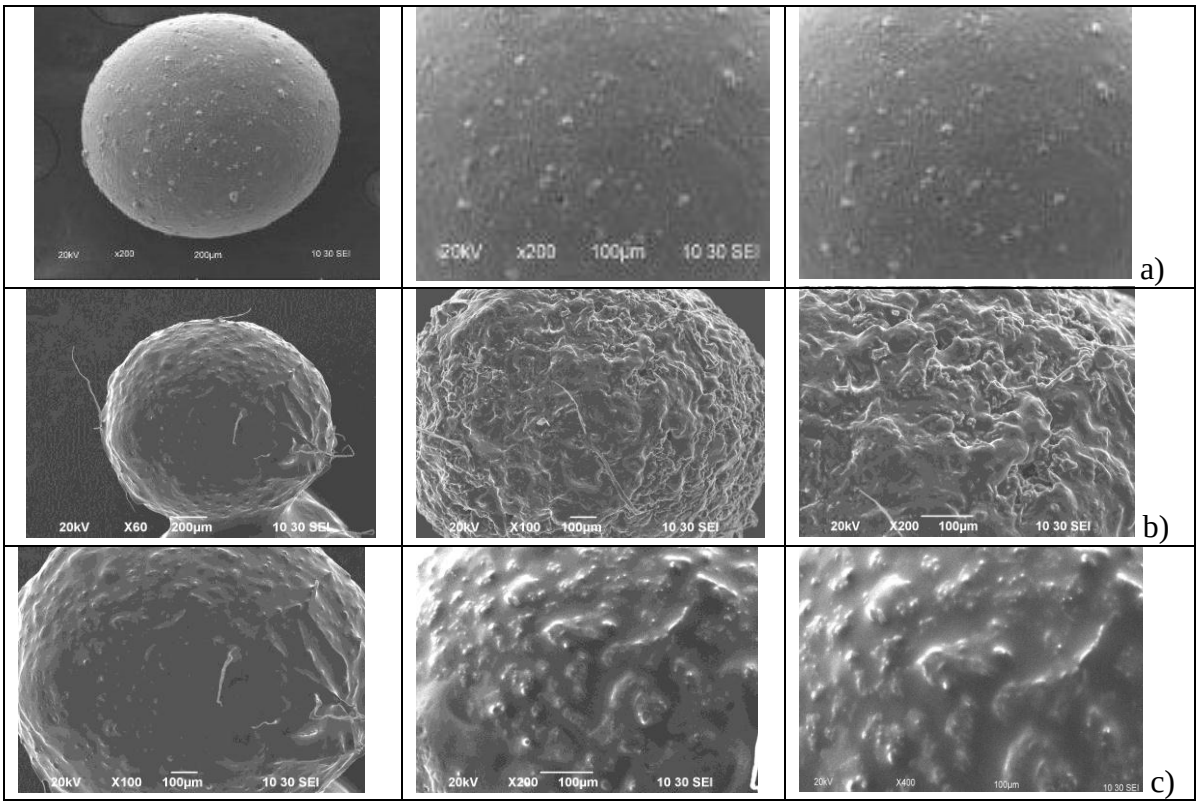


Figure 6: SEM of different formulations a) F1, b) F13 and c) F17 microspheres surface morphology at different magnification respectively

Drug Compatibility Studies by Fourier Transform Infrared Spectroscopy (FTIR)

The change in spectra of the drug in the presence of polymer was investigated which indicates the physical interaction of drug molecule with the polymer. Fourier Transform Infrared Spectroscopy (FTIR) study was conducted for pure metformin HCl and glipizide, formulation F1, F13 and F18.

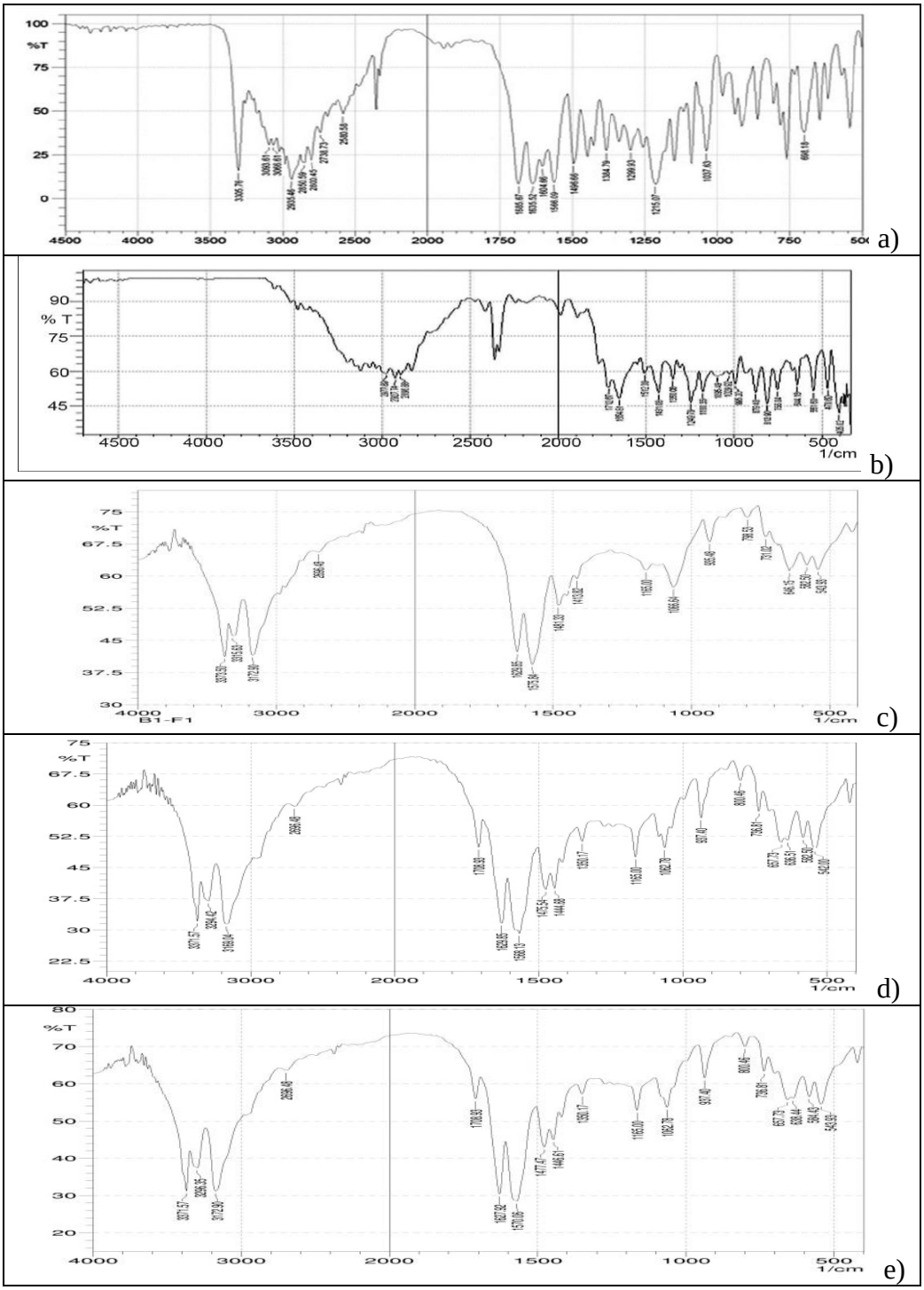
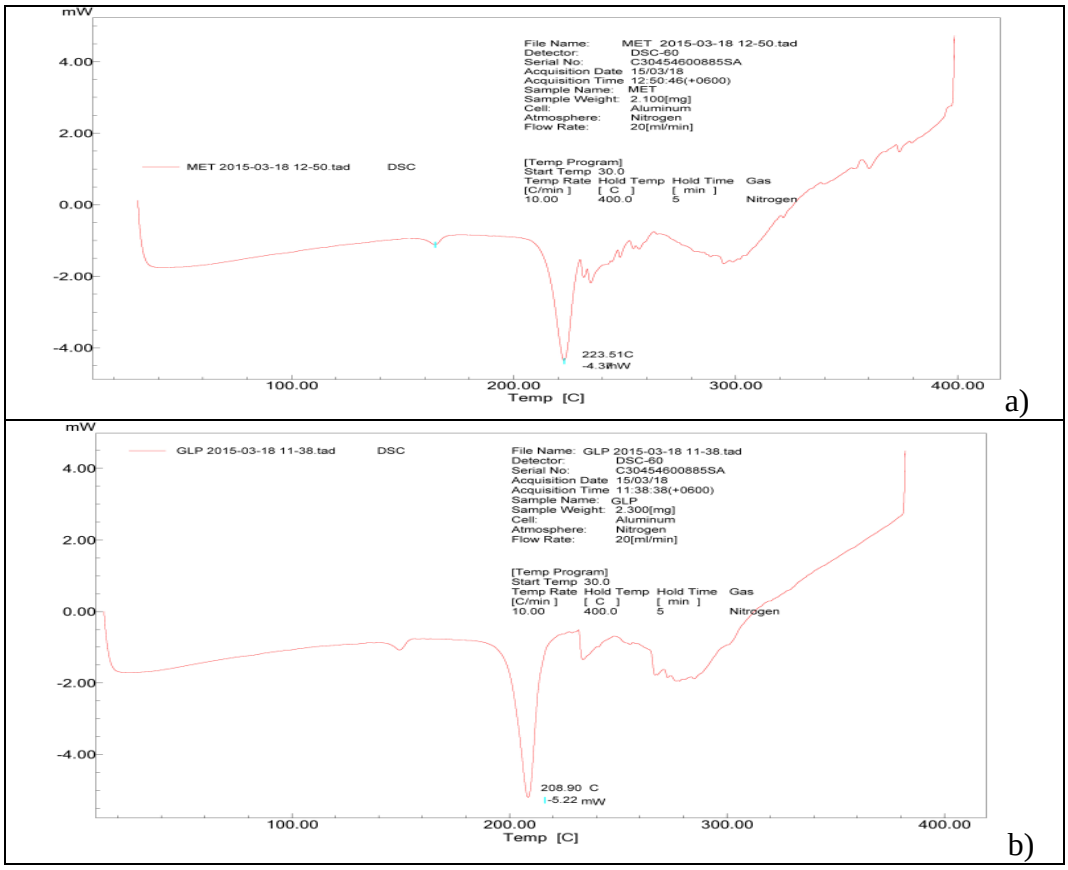


Figure 7: FTIR Spectra of a) pure metformin HCl, b) pure glipizide, c) formulation F1, d) formulation F13, and e) formulation F18 respectively

According to **Fig. 7 (a) and (b)** FTIR studies revealed that metformin hydrochloride and glipizide showed no significant shifts of reduction in intensity of the FTIR bands. As the identical principle peaks were observed in all the cases, it is confirmed that interactions do not exist between the drug and polymer of formulation F1, F13 and F18 shown in **Fig. 7** (c), (d) and (e) respectively.

Differentiate scanning calorimetry (DSC) study of microspheres

DSC studies were performed to understand the nature of the encapsulated drug in the matrix. The physical state of drug in the polymer matrix would also influence its release characteristics. To probe this effect, DSC analysis was performed on metformin hydrochloride, glipizide, formulation F1 and F19. **Fig. 8** (a) and (b) represent the DSC thermogram of pure metformin HCl with a melting point of 223.51^{0}C and for glipizide 208.90^{O}C respectively. The DSC thermogram of formulation F1, and F19 shows the melting point at 228.00^{0}C and 224.90^{0}C respectively. So it is evident that there is no drastic change occurred to the melting point of the microspheres in comparison with pure metformin HCl and glipizide that means there is no interaction between drug and polymers according to Fig. 8 (c) and (d).



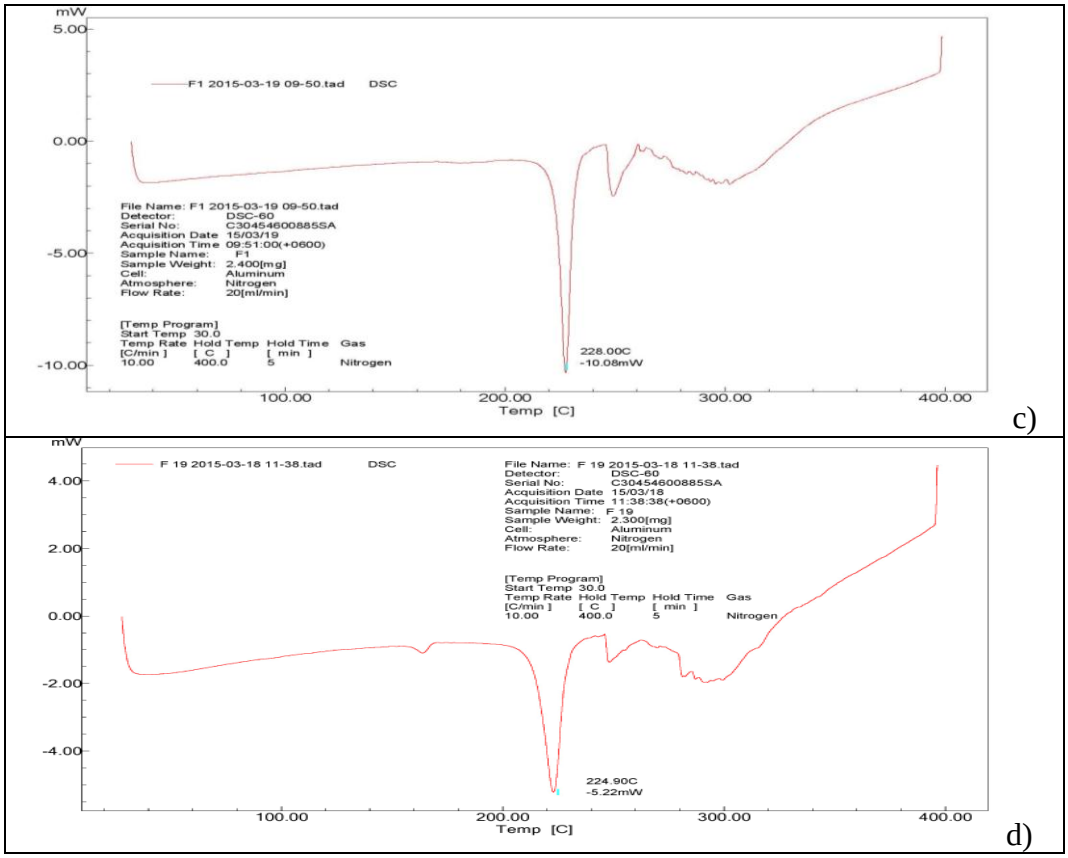


Figure 8: DSC thermogram of a) pure metformin HCl, b) pure glipizide, c) formulation F1 and d) formulation F19 respectively

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

From the **table 3** it was observed that streptozotocin induced diabetics greatly in rats. On the other hand pure drug reduced the blood glucose level in an irregular manner up to 7 hours and then blood glucose level increased to the up, because it could not give sustained release of drug. It might be due to the fact that, here metformin HCl and glipizide rapidly reduces blood glucose level. But the drug release was not sustained for a long time uniformly due to the absent of sustained release polymers.

For the formulation ECF2 and CF17, it was observed that it provided sustained release of drug up to 08 hours and that was proved by the data of reduction of blood glucose level in **table 3**. As a result blood glucose level was reduced rapidly and reduced glucose level was maintained for 08 hours smoothly. It was due to the uniform sustained release of drug from formulations containing sustained release polymers ethyl cellulose and eudragit RL 100.

Table 3: Average reduction in glucose level (mg/dl) in negative control, positive control, pure drug metformin and glipizide and formulation ECF2 and CF17

Time (hr)	Group-I: Negative Control (mg/dl)	Group-II: Positive Control (mg/dl)	Group-III: Pure Drug (mg/dl)		Group-IV: Formulation (mg/dl)	
			Metformin	Glipizide	ECF2	CF17
0	69.33	326.02	364.63	354.99	489.77	486.17
2	70.18	318.63	270.36	254.22	370.27	366.73
4	70.43	323.45	218.95	215.05	291.37	308.58
6	71.03	331.15	147.33	190.28	226.37	248.47
8	70.97	340.65	180.02	137.72	160.73	178.03
10	71.28	343.55	226.80	226.80	118.00	119.83
12	71.77	357.16	265.43	265.43	265.55	98.00
24	71.77	371.91	286.11	286.11	317.73	210.57

Fig. 9 has shown the effect of drug and drug loaded microspheres on the glycosylated hemoglobin (HbA1c) level in STZ diabetic rats. Pure drug metformin and glipizide decreased HbA1c levels as theses have significant effect on the lowering of HbA1c levels. According to figure it has shown that pure drug started lowering HbA1c level after administration of drug but after certain period the reduction effect has lost whereas the formulated microspheres has kept on lowering HbA1c level in diabetic rats up to 8 hour exhibiting effect for a longer period as compared to pure drug. The drug released from the polymeric matrix in a controlled manner through diffusion over longer period of time; thus the microspheres have exhibited reduction in blood HbA1c level for a longer period compared to pure drug.

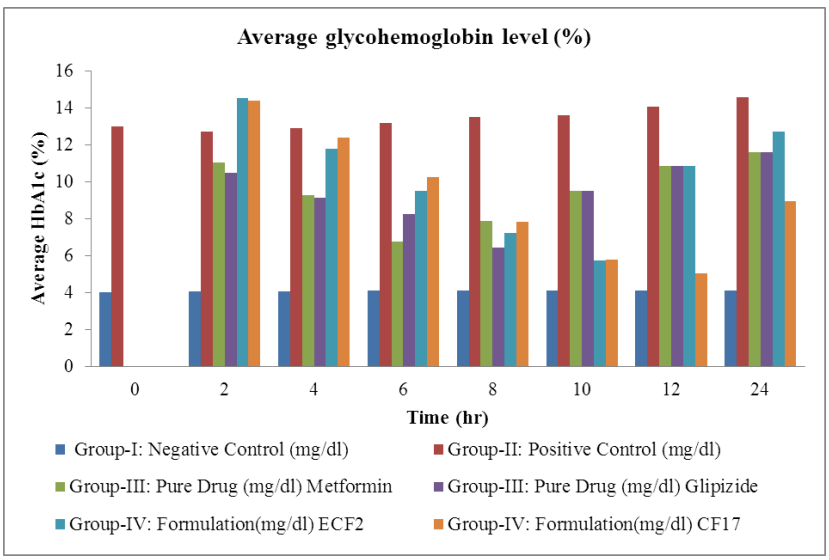


Figure 9: Effect of Different formulation on glycosylated haemoglobin (Hba1c) in serum in different groups

CONCLUSION

The present study was conducted to design metformin HCl and glipizide loaded sustained release microspheres by emulsion solvent evaporation method. *In vitro* dissolution study showed the sustained release of drugs from the microspheres for about 8 hours. From the *in vitro* dissolution data it has been established that the drug dissolution profile could be slowed down by increasing the amount of retardant polymers in the formulations and 5:5 solvent ratios ensured the better sustained release. Scanning electron microscopy showed the smooth and slightly porous surface of microspheres. From Differential Scanning Calorimetric test it can be concluded that there was change in melting point, Glass transition temperature and crystallinity. FTIR data showed absence of any new functional group and any other interaction in between drugs and polymers. In addition formulated microspheres can be chosen for *in vivo* anti-diabetic study and other biomedical study exhibited satisfactory results.

So from the results it can be concluded that, drug retardant polymers and its concentration affect all the evaluation parameters significantly. Hence the prepared polymeric microspheres of metformin HCl and glipizide loaded microspheres might be proved to be potential candidate for safe and effective sustained drug delivery from microspheres for the treatment of type-II diabetes.

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Conflict of Interests

The authors affirm that there is no conflict of interests concerning the publication of this manuscript.

REFERENCES

1. Stephen S, Jason L. Iltz, Jason T, Keith RC. Diabetes and its treatments: A review. *Clin Therap*, 2003; 25(12): 2991-3025.
2. Sweetman. Microsphere: a novel drug delivery system. 2nd ed., PHP London, 2008.
3. Tripathi KD. Metformin is a first line drug for treatment of Diabetes. Essentials of Medical Pharmacology, Jaypee Brothers Medical Publishers (P) Ltd, 2006; 254-255.

4. Jamzad S, Tutunji L, Fassihi R. Formulation and evaluation of sustained release metformin microspheres. *Int J Pharma Res*, 2005; 292(3): 75-85.
5. Passerini N, Albertini B, Gonzalez M L, Cavallari C, Rodriguez L. Preparation and characterization of ibuprofen-poloxamer 188 granules obtained by melt granulation. *Eur J Pharma Sci*, 2002; 15: 71–78.
6. Katzung BG. Basic and clinical pharmacology. 2nd ed., New York Mcgraw Hill, 2001.
7. Dewan I, Islam M M, Hasan M A, Nath J, Sultana S, Rana M S. Surface Deposition and Coalescence and Coacervation Phase Separation Methods: *In Vitro* Study and Compatibility Analysis of Eudragit RS30D, Eudragit RL30D and Carbopol-PLA Loaded Metronidazole Microspheres. *J Pharmaceutics*, 2015; 1-2.
8. Dewan I, Islam S M A, Rana S. *Characterization and compatibility studies of different rate retardant polymer loaded microspheres by solvent evaporation technique: in vitro-in vivo* study of vildagliptin as a model drug. *J Drug Deliv*, 2015; 1-12.
9. Dewan I, Miah S, Islam S M A, Rana S. Design, characterization and *in-vitro* evaluation of different cellulosic acrylic and methacrylic polymers loaded aceclofenac microspheres. *Pak J Pharma Sci.*, 2014; 27(5): 1241-1248.
10. Singh G, Kumar D, Sharma D, Singh M, Kaur S. Q-Absorbance Ratio Spectrophotometric Method for the Simultaneous Estimation of prednisolone and 5-Amino Salicylic Acid in Tablet Dosage form. *J App Pharma Sci*, 2012; 2(6): 222-226.
11. Mockel J E, Lippold B C. "Zero-order release from hydrocolloid matrices". *J Pharma Res*, 1993; 1066-1070.
12. Carpenter K J, Gotsis A, Hegsted D M. "Estimation of total cholesterol in serum by a micro method". *Clin Chem.*, 1999; 6: 233-238.
13. Werner M, Gabrielson D G, Eastman G."Ultramicro determination of serum triglyceride by bioluminescent assay". *Clin Chem*, 1981; 27: 268-272.
14. Trivelli L A, Ranney R H, Lai H T. "Clinical and biomedical features of type 2 diabetics patients". *New Eng J Medicine,* 1971; 284: 53.