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Original Research Article

Characterization of Gliclazide-Loaded Combination of Polymer Microspheres Prepared by Emulsification Solvent Evaporation Method

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Abstract: The study investigated the production of microencapsulated gliclazide by the emulsion-solvent evaporation method. Microspheres were prepared using combination of polymers Eudragit®RS PO and ethyl cellulose by solvent evaporation method and characterized for different properties. Compatibility studies were performed by differential scanning calorimetry, fourier trans form infrared spectroscopy (FTIR) and scanning electron microscopy techniques. The resulting microspheres were white free flowing in nature. The particle size of microspheres ranged from 162-497µm and the encapsulation efficiencies ranged from 51.61-99.61%. The encapsulation efficiency was found to be dependent on nature of polymer used in the formulation. The infrared spectra confirmed the stable character of gliclazide in the drug-loaded microspheres. Scanning electron microscopy revealed that the microspheres were spherical in nature. From the *in vitro* drug dissolution studies showed that the sustaining effect of microspheres depended on the polymer concentration, amount of dispersant and the type of polymer used in the formulation. The mechanism of drug release from the microspheres was non-fickian type. Results suggest that combination of polymers containing gliclazide microspheres could be prepared successfully by using an emulsion solvent evaporation technique using polymethacylate polymers, which will not only sustain the drug release but also manage the complicacy of the diabetes in better manner.
Keywords: Gliclazide, Polymethacylate, Eudragit®RS PO, Ethyl cellulose, FTIR, Drug Release.

INTRODUCTION

Naturally occurring biodegradable polymers have attracted considerable attention for several years in sustained drug delivery to produce microspheres carrier system and can precisely control the release rates and target drugs to a specific body site with enormous impact in formulations and development of novel drug delivery systems [1].Microspheres form an important part of oral and parenteral controlled release delivery systems with varied applications and are prepared using various polymers [2-7]. However, the success of these microspheres is limited due to their short residence time at the site of absorption. It would be advantageous to have means for providing an intimate contact of the drug delivery system with the absorbing membranes [8-11]. Microspheres of gliclazide is a second generation sulphonylurea derivative and 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. Gliclazide is well absorbed by the body, approximately 80% is absorbed. One dose of gliclazide has a half-life of 11-12 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 [15]. A special dosage form is needed for them that can provide continuous therapy with high margin of safety. However, there are numerous drugs for treating type II diabetes, sulphonylureas and biguanides are used commonly by a wide section of patients. The microspheres of gliclazide with combination of polymers were formulated by selected techniques and then characterized by evaluating preformulation and post formulation parameters in this study.

MATERIALS AND METHODS

MATERIALS

Gliclazide was received as a courtesy from ACI pharmaceuticals, Dhaka, Bangladesh. Eudragit® RS PO (EVONIK, Germany), ethyl cellulose (Colorcon, India), Light liquid paraffin (MERCK, Germany), *n*-hexane (MERCK, Germany), Span 80 (MERCK, Germany), distilled water (Research lab). All other reagents and solvents used were of pharmaceutical or analytical grade.

METHODS

Gliclazide microspheres were prepared by solvent evaporation techniques along with the combination of polymer Eudragit® RS PO and ethycellulose (grade 20cps) [13, 16-18]. Briefly, internal phase using ethanol and dichloromethane (1:1) was prepared. Gliclazide suspended in light liquid paraffin oil with 1% span 80 was prepared using a stirrer for 5-10 minutes (external phase). Internal phase was incorporated to external phase drop wise and stirred for 3 hours at 1000 rpm (revolutions per minute). After 3 hours, prepared microspheres were washed with *n*-hexane repeatedly and were allowed to dry for 24 hours at room temperature (20 to 25°C). Subsequently, the microspheres were stored in glass vials in desiccators.6 different formulations of microspheres were prepared with varied drugs and polymer ratios (Table 1).

Table-1: Formulation of gliclazide microsphere using combination of EU-RSPO & ethyl cellulose

Formulations	Gliclazide, EU-RSPO and ethylcellulose ratio	Gliclazide (mg)	EU-RSPO (mg)	Ethyl cellulose (mg)	Ethanol (mL)	DCM (mL)	Span 80	Paraffin oil (mL)
F1	1:0.25:0.25	1000	250	250	5	5	1%	Up to 100mL
F2	1:0.5:0.5	1000	500	500	5	5	1%	
F3	1:1:1	1000	1000	1000	5	5	1%	
F4	1:1.5:1.5	1000	1500	1500	5	5	1%	
F5	1:2:2	1000	2000	2000	5	5	1%	
F6	1:2.5:2.5	1000	2500	2500	5	5	1%	

Determination of gliclazide and drug release behavior

100mg of microsphere was weighed from each batch of formulation for dissolution purpose. Dissolution apparatus (type 2) at 100 rpm at temperature of 37 ± 0.5°C using 900ml volume of ml pH 7.4 used as the medium, equivalent 40mg of drug was taken. The dissolution process was carried out for 6 hours. Samples of 10ml were withdrawn at predetermined intervals of 15 minutes, 30 minutes, 1 hour, 2 hour, 3 hour, 4hour, 5 hour and 6 hour. The volume withdrawn was replaced by fresh volume of dissolution medium to maintain constant volume of medium. The filtered sample were analyzed at λ_{max} 226nm using a UV-VIS spectrophotometer (Shimadzu UV-mini 1240, Japan) and the percentage of drug content was determined. The dissolution study for each batch was performed in triplicate.

Morphology Study by scanning electron microscope (SEM)

Scanning electron microscopy has been employed to study the morphology and surface topography of the microspheres. The samples for SEM study were prepared by mounting each formulation on a double - adhesive tape stuck to an aluminium stub which were then coated with gold (200 A°) under reduced pressure (0.001 torr) for 15 minutes using an ion sputtering device. The gold coated samples was scanned using scanning electron microscope (S-3400N, Hitachi, Japan) under varying magnifications and photomicrographs were obtained.

FTIR Study

The infrared spectra of the pure drug and optimized microsphere formulation were obtained to prove the chemical integrity of the drug in the microspheres. About 5mg samples from each formulation were powdered and intimately mixed with 250mg of pure dry powdered potassium bromide and the mixture was pressed into a disc using a special mould and a hydraulic press. The mixtures were then taken into a diffuse reflectance sampler and IR spectra recorded by scanning in the wavelength region of 450 to 4000 cm^{-1} in a FTIR Spectrophotometer (model 460 Plus, Jasco, Japan).

Thermal analysis by differential Scanning calorimetry (DSC)

DSC was performed on gliclazide drug loaded microspheres using differential scanning calorimetry (DSC) (Seiko DSC 6300, Japan.). Samples were then sealed in platinum pans and the DSC thermo grams were recorded at a constant heating rate of 20°C/min over the temperature range of 20°C to 600°C for 28 minutes.

DATA ANALYSIS

All the data used in this study have been analyzed and presented (graphs) by using GraphPad Prism 6.0 (GraphPad Software Inc., USA).

RESULTS

Drug loading and incorporation efficiency

Figure 1 shows the drug loading content and incorporation efficiency of formulations (F1-F6). In this present study, the loading content's range was in between 16.60-46.04 wt.%. Furthermore, the incorporation efficiency of the formulations did not follow any specific pattern of changes in association with drug loading (Figure 1).

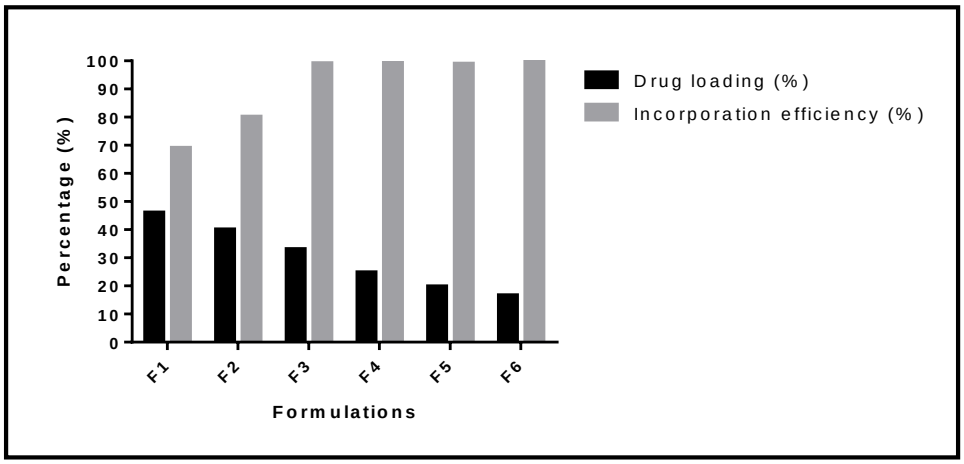


Fig-1: Drug loading and incorporation efficiency of the formulations

Effect of combination of EU-RSPO and ethylcellulose on release kinetics of gliclazide from microspheres

Release kinetics of microspheres of EU-RSPO and ethylcellulose containing gliclazide from each of the six batches (F1 to F6) were calculated by dissolution process. Different release profiles were plotted to find out the best fitted models and to observe the release patterns of gliclazide from the EU-RSPO and ethylcellulose micro spheres (Figure 1). From the zero order release kinetic, it can be observed that the release rate of gliclazide were 61.24%, 56.24%, 51.57%, 47.24%, 42.85%, and 39.2% within 6 hours for F1, F2, F3, F4, F5, and F6 respectively (Figure 2A). The results showed that the release rate of gliclazide from the microspheres can be modulated by adjusting the ratios of polymer and drug in the formulation. In addition, increasing the ratio of polymer from 33.33% to 83.33% resulted in a marked decrease in drug release rate (Figure 2).

Table 2 depicts the observed release rate constants and r-squared values (R^2) with different mathematical models.

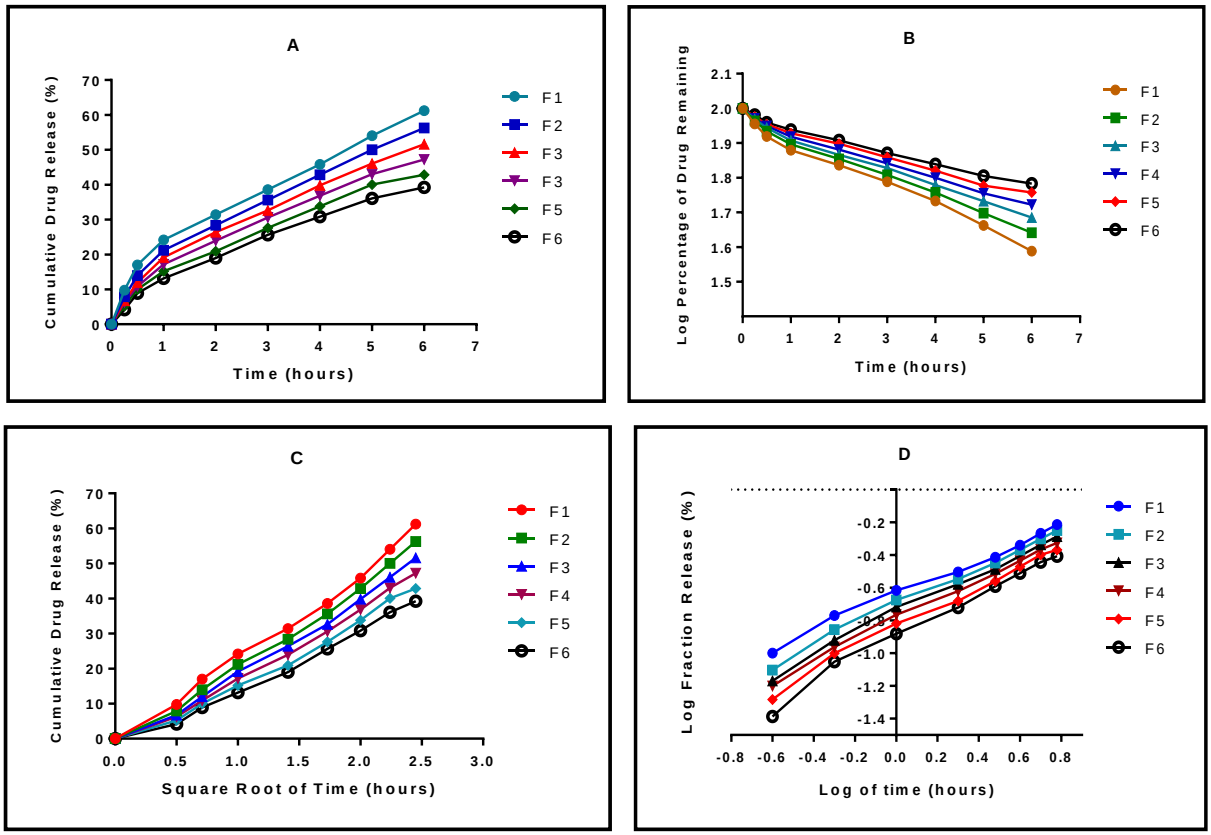


Fig-2: *In vitro* release kinetics of gliclazide from combination of Eudragit®RS PO and ethylcellulose microspheres ;(A) Zero Order Plot (B) First Order Plot (C) Higuchi Plot (D) Korsmeyer Plot

Table-2: Release rate constants and R-squared values (R^2) for different release kinetics of Eudragit® RS PO and ethycelullose based gliclazide microspheres

Formulations	Zero order		First order		Higuchi		Korsmeyer	
	K_0	R^2	K_1	R^2	K_H	R^2	n	R^2
F1	7.497	0.998	- 0.049	0.983	24.36	0.992	0.539	0.989
F2	7.058	0.999	- 0.044	0.987	22.93	0.993	0.586	0.992
F3	6.553	0.999	- 0.039	0.990	21.3	0.993	0.612	0.993
F4	6.074	0.998	- 0.035	0.991	19.75	0.993	0.615	0.995
F5	5.599	0.996	- 0.031	0.988	18.19	0.989	0.638	0.991
F6	5.148	0.995	- 0.028	0.990	16.72	0.989	0.671	0.988

Successive fractional dissolution times

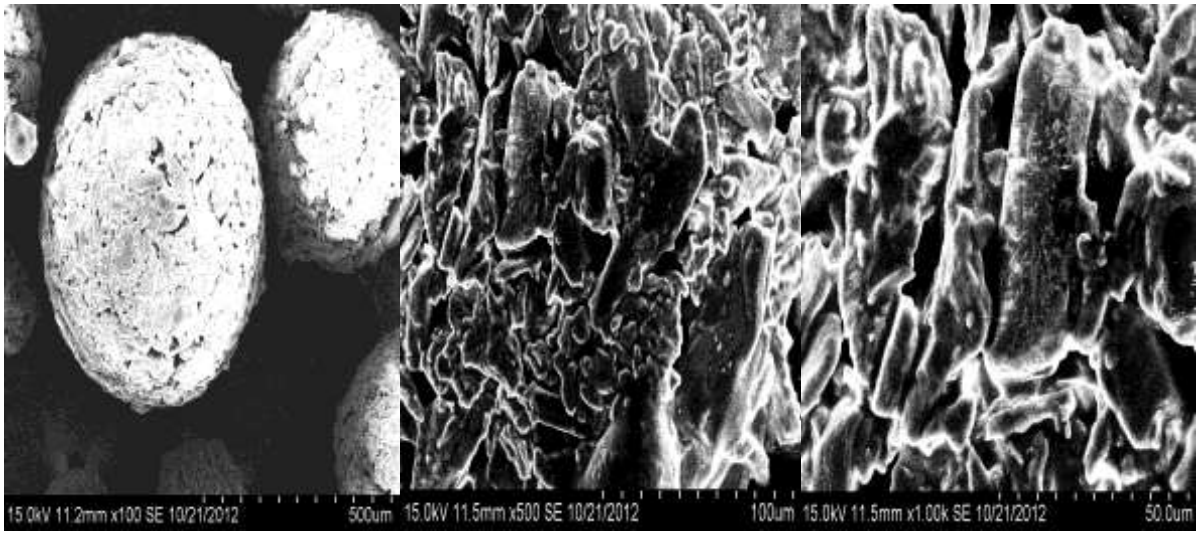
Table-3: Successive fractional dissolution times of formulations (F1-F6)

Formulations	Successive dissolution time (hours)			
	$T_{25\%}$	$T_{50\%}$	MDT	$T_{80\%}$
F1	5.486	6.017	5.821	6.406
F2	7.620	8.4065	8.123	8.985
F3	8.609	9.5702	9.229	10.28
F4	8.900	9.976	9.61	10.778
F5	10.258	11.609	11.148	12.626
F6	11.183	12.795	12.257	14.018

The observed successive fractional dissolution times of gliclazide from formulations (F1-F6) have been mentioned in table 3.

Effect of EU-RSPO and ethylcellulose on the surface morphology

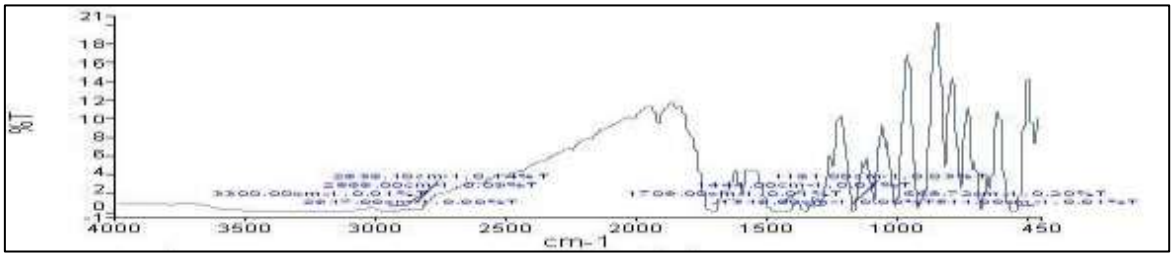
The resulting microsphere formulated by solvent evaporation method was found to be spherical and free flowing in nature. It was noticed that particle size increased with increase in polymer concentration. A SEM image of F3 was taken and surface of these microspheres analyzed morphologically. The image showed rough surface of microspheres of F3 (Figure 3A). Furthermore, small amount of surface drug and slight porous were observed (Figure 3C). This was due to small amount of drug loading (which was only 33.33% in case of F3).



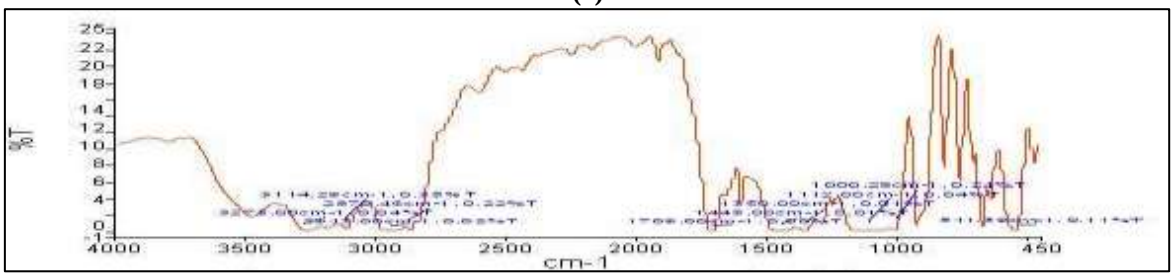
A **B** **C**

Fig-3: Effect of Eudragit® RS PO and ethylcellulose on surface morphology of F3 microspheres; (A) Magnification at X100 SE (B) Magnification at X500 SE and (C) Magnification at X1000 SE

Fourier transforms infrared spectrometry (FTIR) study



(1)



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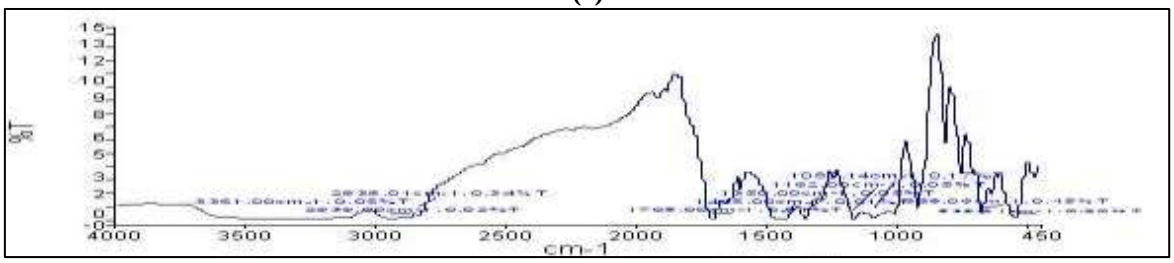

(3)

Fig-4: FTIR Spectra of (1) pure gliclazide, (2) gliclazide and Eudragit® RS PO, (3) gliclazide and ethylcellulose

The recorded FTIR spectrum of pure drug (alone) and (with) polymers has been illustrated in figure 4.

Differential scanning calorimetric (DSC) study

The observed DSC thermo gram of pure gliclazide and gliclazide in association with ethylcellulose and Eudragit® RS PO have been shown in figure 5 and figure 6 respectively.

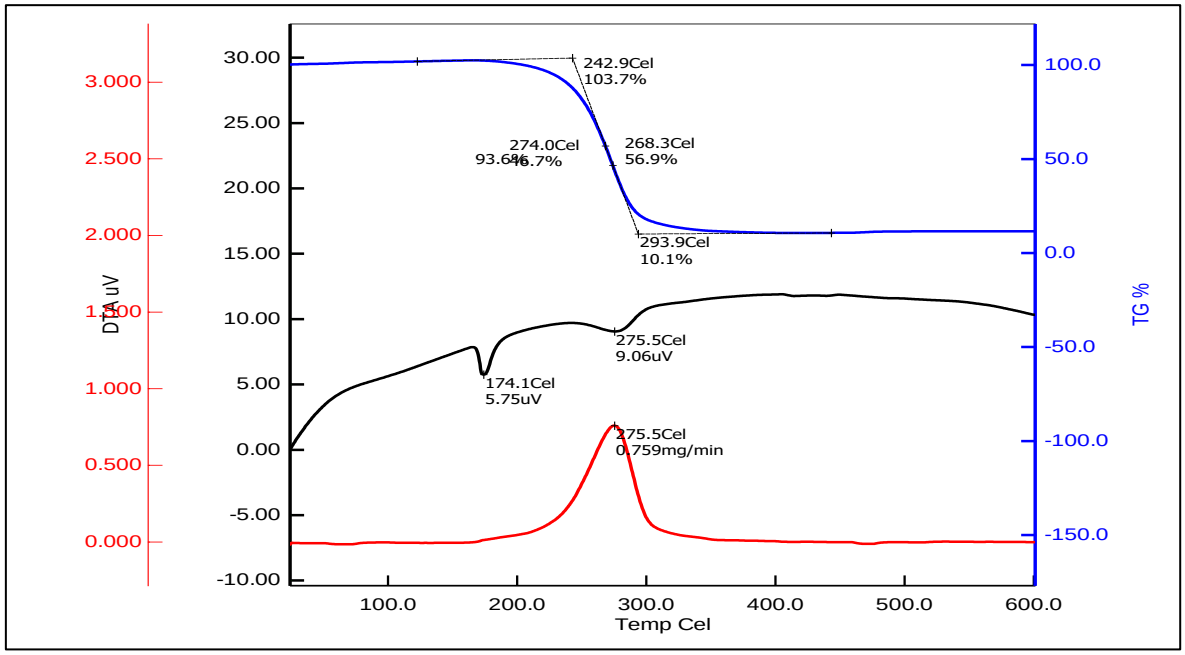

Fig-5: Differential scanning calorimetry of pure gliclazide

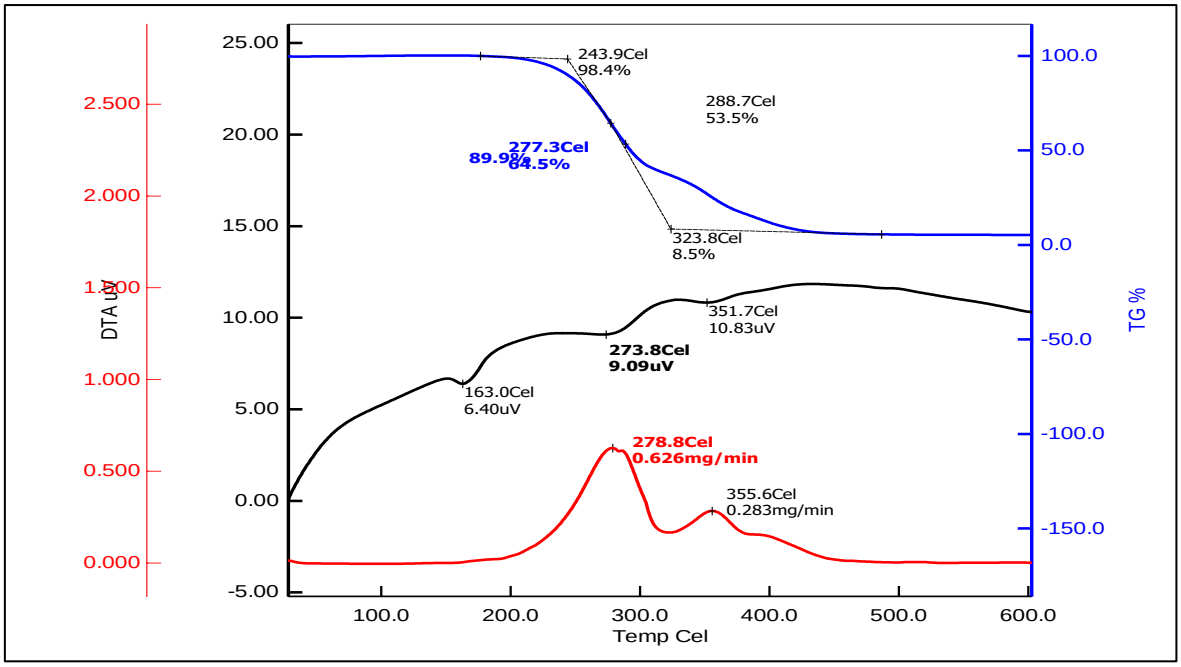

Fig-6: DSC of gliclazide, ethylcellulose and Eudragit® RS PO

Thermal analysis by DSC

The melting point of pure gliclazide was found at 174.4ºC following endothermic reaction with onset and end set at 170.58°C and 177.23ºC respectively with a glass transition lag around 6.65°C (Figure 5) and the same was observed for other formulations with no change in both melting point and glass transition lag.

Special peaks were found indicating melting point of ethycellulose at 273.8ºC, Eudragit® RS PO at 163.0°C. Thermogravimetric analysis **(**TGA) is also commonly employed to determine characteristics of materials such as polymers and also to determine degradation temperatures. The degradation temperature obtained from the figure 6 for gliclazide was 293.9ºC, for combination of gliclazide, ethylcellulose and Eudragit® RS PO was 323.8°C.

DISCUSSION

Increase of particle size with increase in polymer concentration may have occurred due to the fact that as polymer concentration increases it produces a significant increase in the viscosity in a fixed volume of solvent, thus leading to an increase of the emulsion droplet size and finally a higher microsphere size [19-20].

The encapsulation efficiency of the drug depended on the solubility of the drug in the solvent and continuous phase. An increase in the concentration of polymer in a fixed volume of organic solvent resulted in an increase in encapsulation efficiency [21]. Scanning electron microscopic photographs of microspheres are shown in figure 3. The surface of the microspheres appeared spherical but rough. Moreover, the microsphere was not aggregated.

FTIR spectra of gliclazide pure drug showed number of prominent peaks at different wave numbers, indicating a presence of functional groups and substituents. Peaks at 1444cm^{-1} and 1349 cm^{-1} because of C=C stretching inside the benzyl ring, at 1161 cm^{-1}, 1709cm^{-1} because of C=O stretching, at 2836 cm^{-1}, 2868 cm^{-1}, 2947 cm^{-1} because of C=H asymmetric and symmetric stretching in methyl groups, at 668.72 cm^{-1}, 544 cm^{-1} because of C-H bending in disubstituted benzene ring. Peaks between 2250-2700 cm^{-1} were found because of N-H are asymmetric and symmetric variations in amino groups. Broad and intense peak appeared at 3300 cm^{-1} and 1473 cm^{-1} because of N-N stretching respectively (Figure 4). All these peaks are appeared unchanged in FTIR spectra of all compositions and state that drug and excipients were found compatible without interaction.

Figure 2 shows that the release of gliclazide from the microspheres and it illustrates that the rate of drug release from the microspheres depended on the polymer concentration. The decrease in release rate with increasing content of the polymer can be explained by a decreased amount of drug present close to the surface and also by the fact that the amount of uncoated drug decreases with increase in polymer concentration [22]. The release profile was in line with the theory of the effect of the particle size on dissolution [2]. As microsphere size decreased, the drug release increased as a result of higher surface area.

Eudragit polymers are a series of acrylate polymers and methacrylate polymers available in different ionic forms**.** Eudragit® RS PO is a copolymer of ethyl acrylate, methyl methacrylate and a low content of methacrylic acid ester with quaternary ammonium groups. 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 [13-14].

In order to determine the mechanism of drug release rate obtained were fitted to the zero order models. The values of release exponent (n) of the formulations were above 0.45 and the value of n was increased with the increase of concentration of polymers indicating that the drug was released from the formulations by following anomalous transport mechanism or non-fickian mechanism which appears to indicate a coupling of the diffusion and erosion mechanism [12].

CONCLUSION

The combination of Eudragit®RS PO and ethylcellulose microspheres containing gliclazide can be prepared successfully by using an emulsion solvent evaporation technique. The surface structure of the microspheres was spherical and rough. The encapsulation efficiencies were over 60%. The release pattern of the combination of Eudragit® RS PO and ethylcellulose microspheres was found to be of the non-Fickian.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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