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Research Article

Preparation and evaluation of mucoadhesive microspheres of repaglinide for treatment of diabetes mellitus type II

Arvind Parmar*, Ashwani Mishra and Anupam Pathak

Department of Pharmacy, Barkatullah University, India

***Correspondence Info:**
Arvind Parmar,
Department of Pharmacy,
Barkatullah University, India
Email: ashwanipharma@gmail.com

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Abstract

Type 2 diabetes mellitus (T2DM) is the most common form of diabetes constituting 90% of the diabetic population. The number of patients with diabetes in India is currently around 40.9 million and is expected to rise to 101 million by 2030.Majority of the conventional formulations available have some drawbacks and less bioavailability, to overcome their deficiency an attempt was made to formulate mucoadhesive of Repaglinide for the treatment of Diabetes mellitus type –II ,Prepared formulations were subjected for different evaluation parameters like Particle Size Analysis, Production Yield, Bulk Density, Tapped Density and Compressibility Index, Swelling Index, Mucoadhesivity study, Entrapment efficiency, Differential Scanning calorimeter and in vitro drug release study. Best formulation was chosen who passed all the evaluation parameters.

1. Introduction

Microspheres constitute an important part of drug delivery systems by virtue of their small size and efficient carrier characteristics. A well designed controlled drug delivery system can overcome some of the problems of conventional therapy and enhance the therapeutic efficacy of a given drug. One such approach is using microspheres as carriers for drugs. It is the reliable means to deliver the drug to the target site with specificity, if modified, and to maintain the desired concentration at the site of interest without untoward effects.[1-2]

They are made up of polymeric, waxy, or other protective materials that are like synthetic biodegradable polymer and modified natural products such as starches, gums, proteins, fats and waxes. Such designed microspheres incorporating a drug dispersed or dissolved throughout the particle matrix have the potential for the controlled release.

Mucoadhesion is commonly defined as the adhesion between two materials, at least one of which is a mucosal surface. Mucoadhesive dosage forms may be designed to enable prolonged retention at the site of application, providing a controlled rate of drug release for improved therapeutic outcome.[3-7]

Type 2 diabetes mellitus (T2DM) is the most common form of diabetes constituting 90% of the diabetic population. The number of patients with diabetes in India is currently around 40.9 million and is expected to rise to 101 million by 2030.[8]

Repaglinide is an oral antihyperglycemic agent used for the treatment of non-insulin-dependent diabetes mellitus (NIDDM). It belongs to the meglitinide class of short-acting insulin secretagogues, which act by binding to β cells of the pancreas to stimulate insulin release.[9-11] In the present study Mucoadhesive Microspheres formulation was preferred over conventional tablet or capsule formulations, as it has several advanteges like it control the release pattern thus decreasing the dosing frequency by entrapping Repaglinide for the treatment of Diabetes Mellitus type II.

2. Material and Methods

Repaglinide were obtained as a gift sample, Liquid paraffin, carbopol, HPMC and span 80 were purchased from

Chemical Drug House, New Delhi. Other chemical and solvent were of Analytical Grade,

2.1 Experimental work

2.1.1 Formulation and characterization [12-14]

Formulation of mucoadhesive microspheres of repaglinide:-

The mucoadhesive microspheres were prepared using emulsification solvent evaporation technique. The polymeric solution was prepared by dissolving carbopol and HPMC in distilled water. The drug was dispersed in the polymeric solution forming the internal phase. The prepared drug and polymer solution was added drop wise by a syringe with a needle gauge 22 to liquid paraffin (external phase) containing span 80(%v/v) and was emulsified by stirring at 500 rpm. The stirring was continued at temperature 80^0c until the polymer solvent was evaporated. The produced microspheres were decanted and washed 5 times with *n* –hexane and dried overnight. As shown in Table no.1.

Table no. 1- Different Formulation of Mic rospheres.

S.N.	Formulation code	HPMC K4m + Carbopol (w/v)	HPMC K15m + Carbopol (w/v)	HPMC K100m + Carbopol (w/v)	Liquid paraffin (ml)	Span 80 (%)	Stirring speed (rpm)
1.	F-1	1:1	-	-	200	0.5	500
2.	F-2	1:2	-	-	200	1.0	500
3.	F-3	1:3	-	-	200	1.5	500
4.	F-4	-	1:1	-	200	0.5	500
5.	F-5	-	1:2	-	200	1.0	500
6.	F-6	-	1:3	-	200	1.5	500
7.	F-7	-	-	1:1	200	0.5	500
8.	F-8	-	-	1:2	200	1.0	500
9.	F-9	-	-	1:3	100	1.5	500

2.1.2 Characterization of Mucoadhesive Microspheres

Various characterization parameters were performed to study the particle size, mucoadhesivity, production yield, tapped density, bulk density, swelling index, *in-vitro* drug release of the prepared microsphere formulations.

1. Particle Size Analysis

Particle size was determined by optical microscope. Microsphere was examined on an optical microscope by using calibrated ocular micrometer and determined particle size of every formulation. The mean particle size was calculated by measuring nearly 200 particles of each formulation. Image of microspheres taken from Leica DM 1000 microscope are given blow.

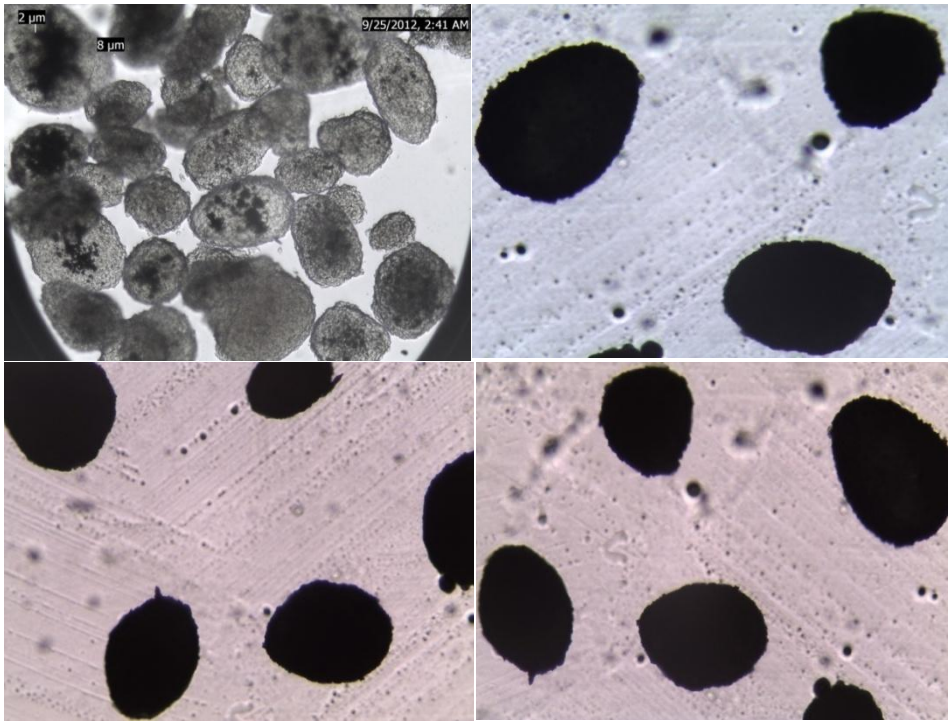

Fig 1: Microspheres Image from Leica DM 1000 Microscope

2. Production Yield:

The production yield of microspheres of various batches using the weight of final product after drying with respect to initial total weight of the drug and polymer used for preparation of mucoadhesive microspheres and percent production yields are calculated as per the formula mentioned below.As shown in Table No.3

$$\% \text{ Production Yield } = \frac{\text{Actual weight of microspheres}}{\text{Total weight of exipients and drug}} X100$$

3. Determination of Bulk Density, Tapped Density and Compressibility Index.

A) Bulk density:

The bulk density determined by small quantity of microsphere (m) samples is carefully introduced into 10ml graduated cylinder, without compacting, read the unsettled apparent volume (V_0) to nearest graduated unit. Calculate the bulk density in g/ml by the formula.

$$\text{Bulk density} = \frac{\text{Mass of microspheres (m)}}{\text{Initial volume (Vo)}}$$

B) Tapped density :-

Tapped density determined by taking small quantity of microsphere sample carefully introduced into 10 ml graduated cylinder. Cylinder was dropped at 2 sec. intervals on hard wood surface 100 times from height 1 inch. Tapped density of each sample was obtained by dividing weight of sample in gm. By final tapped volume in cm^3 of sample contain in cylinder.As shown in Table No.4

$$\text{Tapped density} = \frac{\text{Weight of microspheres}}{\text{Volume of microspheres after tapping}}$$

C) Compressibility Index:

It was determined by taking small quantity of microsphere sample in 10 ml measuring cylinder. The height of the sample was measured before and after tapping.

V_0: Unsettled apparent volume

Vf: Final tapped volume.

$$\text{Compressibility index} = \frac{(\text{Vo} - \text{Vf})}{Vo} \text{x } 100$$

4. Determination of Swelling Index of Microspheres

For estimating the swelling index, weighed 100 mg of microspheres were allowed to swell in 0.1 N HCL for 24 h. The excess surface adhered liquid drops were removed by blotting and swollen microspheres were weighed by using microbalance. The degree of swelling was calculated by the following formula. As shown in Table No.5

$$\text{Swelling index} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Final weight}}$$

5. Mucoadhesivity of Microspheres

The mucoadhesive properties of the microspheres were evaluated in PBS 7.4. A 2x2-cm piece of got stomach mucosa was tied onto a glass slide (3x1-inch) using thread. Microspheres were spread (100) onto the wet, tissue specimen, and the prepared slide was hung onto one of the groves of a USP tablet disintegrating test apparatus. The disintegrating test apparatus was operated such that the tissue specimen was given regular up and down movements in a beaker containing the 0.1N HCL (pH 1.2). At hourly intervals up to 6 hours, the number of microspheres still adhering onto the tissue was counted. Percent mucoadhesion was calculated by the following formula. As shown in Table No.6

$$\% \text{ Mucoadhesion } = \frac{\text{No. of microspheres remains on stomach mucosa}}{\text{No. of microspheres applied on stomach mucosa}} X100$$

6. Determination of Entrapment Efficiency

Entrapment Efficiency was determined by 100mg of dried microspheres were crushed using pestle and mortar. After that microspheres were placed in 100ml 0.1 N HCL (pH 1.2) and shaken for 1 hour at 37 ± 0.5^0 c. Sample was withdrawn and filtered to obtain clear solution and analyzed for drug content spectrophotometrically at 247nm. As shown in Table No.7

$$\text{Entrapment Efficiency} = \frac{\text{Calculated drug content}}{\text{Theoretical drug content}} X100$$

7. *In-Vitro* Release Study:

The in vitro dissolution studies were performed at three different pH values: (i) 1.2 pH (0.1N HCL) and (ii) 7.4 pH (PBS). *In vitro* drug release studies were carried out using US paddle type- II dissolution apparatus at 37± 0.5 C with constant stirring rate of 50 rpm. Mucoadhesive microspheres of repaglinide were used for the test. An accurately weighed sample was suspended in dissolution media consisting 900 ml of 0.1 N (pH 1.2) HCl and dissolution was carried out for 2 h. The dissolution medium was then replaced with pH 7.4 phosphate buffer (900 ml) and drug release study was carried out for further 10h. A sample volume of 5 ml was withdrawn from each dissolution vessel at regular intervals and replaced with equal volume of fresh dissolution medium. The sample was filtered through Whatman filter paper and analyzed spectrophotometrically at 247 nm. As shown in Figure No.2

8. Drug release kinetics:

The drug release kinetics was studied by various kinetic models such as zero order, first order, Higuchi model and Koresmeyer peppas release kinetics. As shown in Table No.8

3. Result and Discussion

3.1 Particle Size Analysis

Particle size of microspheres prepared with HPMC K4m and different concentration of carbopol and span 80 (F_1-F_3) was found to be in the range of 218-247µm; while range of particle size prepared with HPMC K15m and different carbopol and span 80 concentration (F_4-F_6) was found to be 375- 480µm and the particle size of HPMC K100m and different carbopol and span 80 concentration (F_7-F_9) was found to be 326-590µm. The viscosity of surfactant and polymer in medium results in increasing particle size.

The mean particle size of mucoadhesive microsphere was found in the following range as shown in Table –

Table No.2– Mean Particle Size of Mucoadhesive Microspheres

S. No.	Formulation	Average Particle size in (µm)
1	F -1	216
2	F -2	241
3	F -3	247
4	F -4	375
5	F -5	462
6	F -6	480
7	F -7	326
8	F -8	496
9	F -9	590

2. Production Yield:

Production yield of microspheres prepared with HPMC K4m and different concentration of carbopol and span 80 concentration (F_1-F_3) was found to be in the range of 75-81%; while range of production yield of microspheres prepared using HPMC K15m with different carbopol and span 80 concentration (F_4-F_6) was found to be 68-79% and production yield of microspheres prepared with using HPMC K100m with different carbopol and span 80 concentration (F_7-F_9) was found to be 72-80% as shown in Table.

Table No.3- Production Yield of Mucoadhesive Microspheres of Repaglinde

S. No.	Formulation	% Production Yield
1	F -1	75
2	F -2	79
3	F -3	81
4	F -4	68
5	F -5	71
6	F -6	79
7	F -7	72
8	F -8	79
9	F -9	80

4. Bulk Density, Tapped Density and Compressibility Index:

A) Bulk Density:

The bulk density of prepared microspheres with HPMC K4m and different concentration of carbopol and span 80 concentration (F_1-F_3) was found to be in the range of 0.2957-0.3681 gm/cm^3; while range of bulk density of microspheres prepared with HPMC K15m and different carbopol and span 80 concentration (F_4-F_6) was found to be 0.3090-0.3726 gm/cm^3; and bulk density of microspheres prepared with HPMC K100m and different carbopol and span 80 concentration (F_7-F_9) found to be 0.300-0.3800 gm/cm^3.

B) Tapped Density:

The tapped density of microspheres prepared with HPMC K4m and different concentration of carbopol and span 80 concentration (F_1-F_3) was found to be in the range of 0.351-0.4263 gm/cm^3; while range of tapped density of microspheres prepared with HPMC K15m and different carbopol and span 80 concentration (F_4-F_6) was found to be 0.377-0.4176 gm/cm^3 and tapped density of prepared microspheres with HPMC K100m and different carbopol and span 80 concentration (F_7-F_9) was found to be 0.360-0.444 gm/cm^3

C) Compressibility Index:

The Compressibility index of micrspheres prepared with HPMC K4m and different concentration of carbopol and span 80 concentration (F_1-F_3) was found to be in the range of 13.636-16.667 %; while range of Compressibility index of microspheres prepared with HPMC K15m and different carbopol and span 80 concentration (F_4-F_6) was found to be 10.526-18.182 % and Compressibility index of microspheres prepared with HPMC K100m and different carbopol and span 80 concentration (F_7-f_9) found to be 0.833-16.667 %.

Table No.4– Bulk and tapped density of different formulations

Sr. No.	Formulation code	Bulk density(gm/cm^3)	Tapped density (gm/cm^3)	Compressibility index
1	F-1	0.3125	0.375	16.667
2	F -2	0.2957	0.351	15.789
3	F -3	0.3681	0.4263	13.636
4	F -4	0.3090	0.377	18.182
5	F -5	0.340	0.3923	13.33
6	F -6	0.3736	0.4176	10.526
7	F-7	0.300	0.360	08.33
8	F -8	0.3288	0.3946	16.667
9	F-9	0.3809	0.444	14.286

5. Swelling Index:

Swelling property of microspheres prepared with using HPMC K4m and different concentration of carbopol and span 80 concentration (F_1-F_3) was found to be in range 72.40-82.63 %; while swelling property of microspheres prepared with HPMC K15m and different carbopol and span 80 concentration (F_4-F_6) was found to be 74.96-79.31 % and the swelling property of microspheres developed with HPMC K100m and different carbopol and span 80 concentration (F_7-F_9) was found to be 69.97-80.19 %.

Table No. 5- Swelling index of Mucoadhesive Microspheres:

S. No	Formulation code	Swelling index (%)
1.	F-1	82.63
2.	F-2	76.31
3.	F-3	72.40
4.	F-4	79.31
5.	F-5	77.81
6.	F-6	74.96
7.	F-7	80.19
8.	F-8	73.87
9.	F-9	69.97

6. Mucoadhesivity of Microspheres:

Mucoadhesivity of microspheres prepared with using HPMC K4m and different concentration of carbopol and span 80 concentration (F_1-F_3) was found to be in the range of 76-84%; while mucoadhesivity of microspheres prepared with HPMC K15m and different carbopol and span 80 concentration (F_4-F_6) was found to be 72-80 % and the mucoadhesivity of microspheres developed with HPMC K100m and different carbopol and span 80 concentration (F_7-F_9) was found to be 69-79 %.

Table No. 6- Mucoadhesivity of Microspheres

Time in (hr)	Formulation code	Mucoadhesivity in (%)
1.	F-1	76
2.	F-2	79
3.	F-3	84
4.	F-4	80
5.	F-5	75
6.	F-6	72
7.	F-7	77
8.	F-8	73
9.	F-9	69

7. Drug Entrapment:

Entrapment efficiency of microspheres prepared with using HPMC K4m and different concentration of carbopol and span 80 concentration (F_1-F_3) was found to be in the range of 71-82 %; while entrapment efficiency of microspheres prepared with HPMC K15m and different carbopol and span 80 concentration (F_4-F_6) was found to be 60-76 % and the entrapment efficiency of microspheres developed with HPMC K100m and different carbopol and span 80 concentration (F_7-F_9) was found to be 75-86 %.

Table No.7- Drug Entrapment Efficiency

Sr. No.	Formulation	Entrapment Efficiency (%)
1	F-1	71
2	F -2	79
3	F -3	82
4	F -4	60
5	F -5	65
6	F -6	76
7	F-7	75
8	F -8	81
9	F-9	86

8. *In-Vitro* Release Study:

In vitro drug release study of repaglinide loaded mucoadhesive microspheres of optimized formulation of F_3 was performed in 0.1 N HCL for inital 2 hour and PBS 7.4 pH for remaining 10 hour. The sizes of microspheres of F3 was small at low polymer concentration and have a larger surface area exposed to dissolution medium, giving rise to faster drug release. The production yield of F_3 was 81 % which gives a greater total mass of microspheres as compare to other formulation which resulted in increased surface area of this batch releasing more drug release per unit time. The release of repaglinide was not likely to be dissolution controlled mechenism because repaglinide is water insoluble.

The drug release could be attributed to the diffusion of repaglinide from the mucoadhesive microspheres through the pore and channels on and close to the surface of the microspheres.

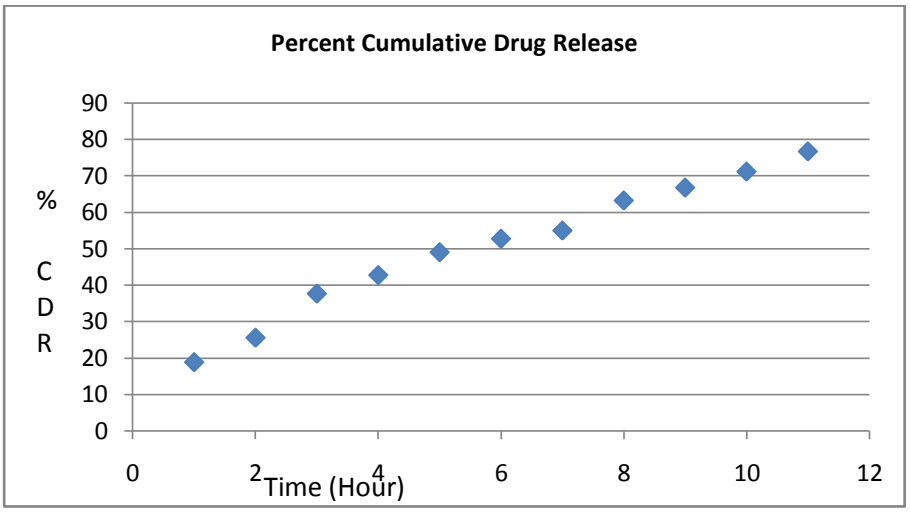

Figure No. 2: % Cumulative drug release of microspheres in 7.4 pH PBS

Analysis of drug release data:

The data obtained for *in vitro* release where fitted into equations for the zero order, first order, Higuchi and peppas release model. The interpretation of data was based on the value of the resulting regression coefficient.

The zero order rate describe the system where the drug release rate is independent of its concentration graph-3 shows the cumulative amount of drug release Vs time for zero order kinetic. The first order rate describes the release from system where the release rate is concentration dependent, which is shown in graph-4. The Higuchi model explain the release of drug from and insoluble matrix as a square root of time dependent process based on fickian diffusion graph-5 illustrate Higuchi release model.

The calculated regression coefficient for zero order, first order, Higuchi and peppas was shown in table no 8. It was found that the *in vitro* drug release of repaglinide mucoadhesive microspheres was best explained by Higuchi as the plot showed the highest linearity. Therfore the release seems to fit the Higuchi's model.

To determine the exact mechanism of drug release, the data were fitted according to Korsemeyer-peppas release exponent n for the optimized formulation was 0.586 indicating release by fickian diffusion.

From the value of r^2 obtained as shown below in the table it was found that the maximum r^2 value is shown in Higuchi release kinetics. Thus, the optimizied formulation followed the Higuchi release kinetics i.e. diffusion controlled release system.

Table No.8: Regression value of formulation F-3 for different Release Kinetics Models

S. No.	r^2	Kinetic model
1	0.948	Zero order
2	0.985	First order
3	0.992	Higuchi model
4	0.991	Peppas model

DSC:

DSC of pure drug Repaglinide, polymer HPMC, Carbopol, Physical Mixture of drug and excipients and of formulation was performed. The DSC thermogram of Repaglinide showed a sharp endothermic peak at 131.92^0 C. HPMC (K100m) exhibits a broad endotherm at 147.18^0C. HPMC (K15m) shows a sharp endothermic peak at 106.58^0C.HPMC (K4m) decomposes at 161.7^0C.carbopol shows two peak out of which one is broad endothermic at 260.47^0C and second may be due to H-Bonding.the physical mixture shows two endothermic and two exothermic peaks.the drug repaglinide in the physical mixture exhibits a sharp endotherm at 135.60^0C. the DSC of F-3 shows 4 peaks out of which two are endothermic and two are exothermic.

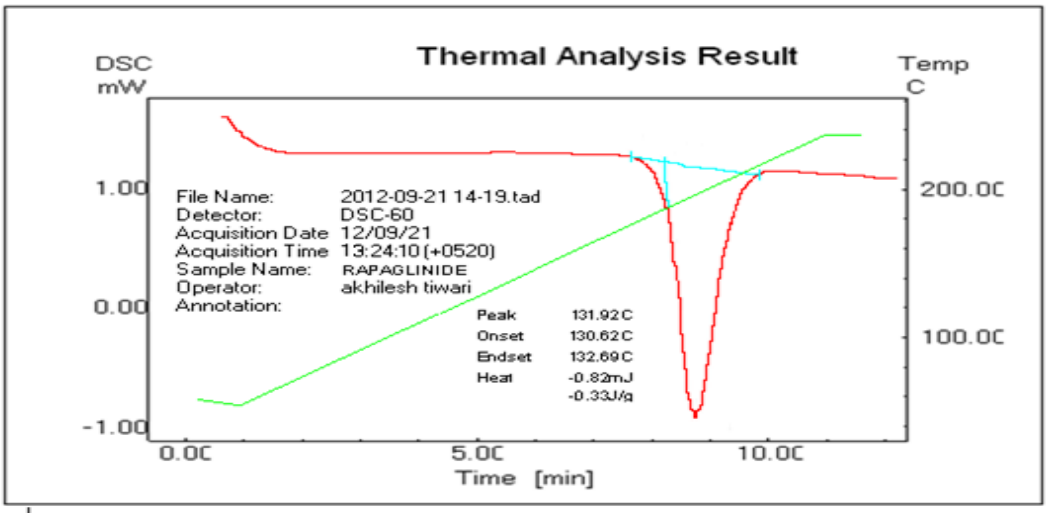

Fig 3: The DSC of pure Repaglinide

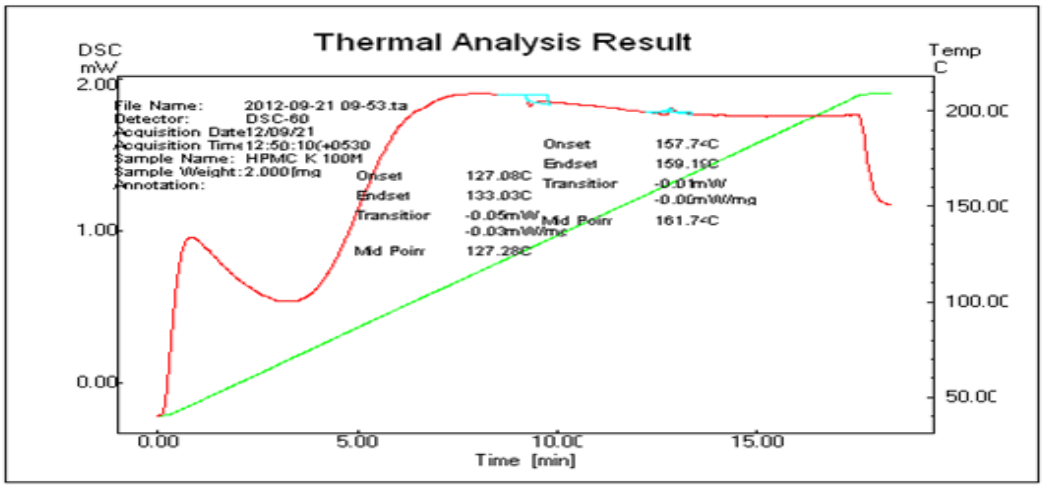

Fig 4: The DSC of hpmc K100m

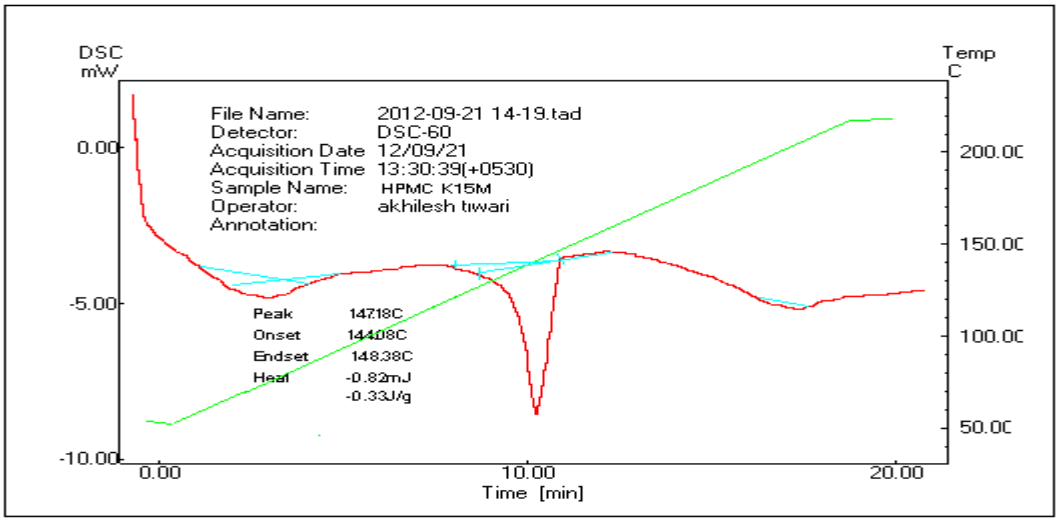

Fig 5: DSC of HPMC K15m

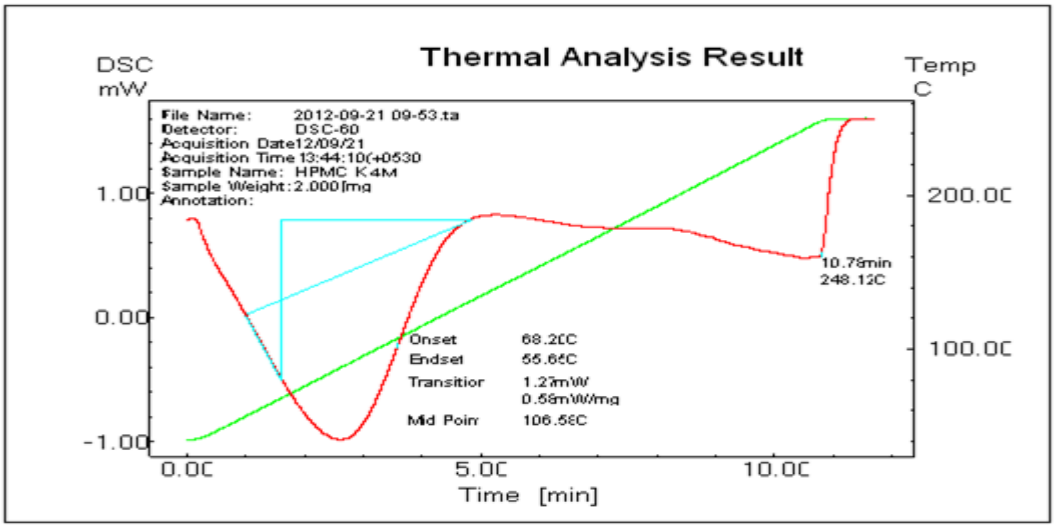

Fig 6 DSC of HPMC K4m

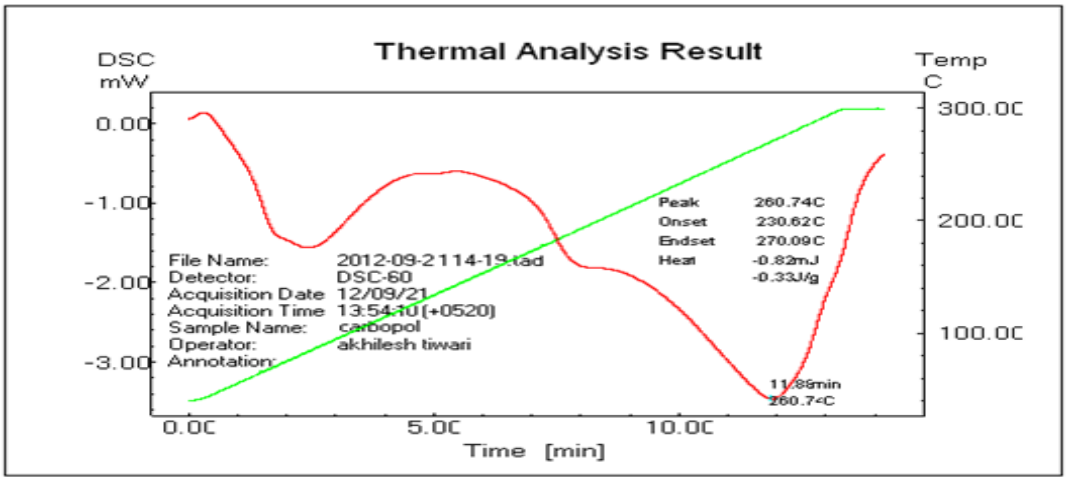

Fig 7 The DSC of carbopol 934

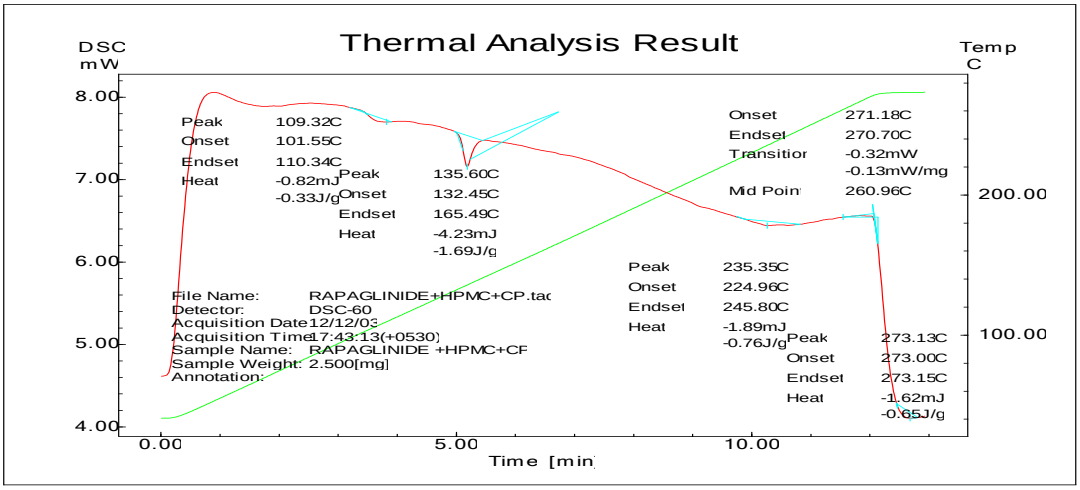

Fig 8 HPMC and Repaglinide + carbopol are shown in the fig.

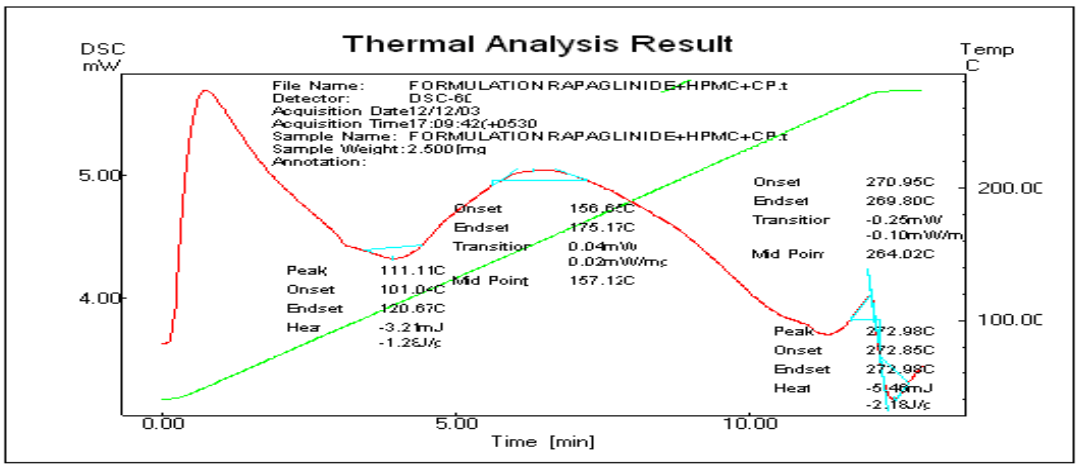

Fig 9 DSC of microspheres of repaglinide

4. Conclusion

Mucoadhesive microspheres are prepared with various polymer HPMC (K4m, K15m, K100m), Carbopol successfully by the emulsification solvent evaporation technique. The amount of drug released from microspheres could be enhanced. *In-vitro* data obtained from mucoadhesive microspheres of Repaglinide showed optimum particle size, excellent mucoadhesivity, sufficient entrapment efficiency, good production yield, good swelling property and prolonged drug release. Microspheres of different size and drug content could be obtained by varying the formulation variables, thus the prepared mucoadhesive microspheres may prove to be potential candidates for the treatment of diabetes mellitus type II as a controlled drug delivery system.

The formulations were evaluated for various micromeritics and characteristic studies. It increases the bioavailability of dosage form with prolong effect, hence improves the patients compliances.

The designed Formulation F_3 adheres in the stomach and prolongs the gastric residence time (GRT) consequently, providing controlled action. In addition, mucoadhesive microspheres enabled increased drug absorption rate, as it retained in the stomach and arrived at the absorption site. The developed formulation overcomes the drawbacks and limitations of conventional preparations. Therefore mucoadhesive microsphere will be possibly beneficial for controlled action.

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