Research Article

Design and *in-vitro* Evaluation of Floating Microspheres Containing Lactulose Using Emulsion Solvent Evaporation Technique

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ABSTRACT

The present investigation was concerned with formulation and evaluation of floating microspheres for Lactulose using Ethyl cellulose & HPMC as a release retarded material were prepared using emulsion solvent evaporation technique. Eleven different batches of microspheres were prepared by varying the concentration of drug polymer ratio from 1:0.5 to 1:5. The microspheres were characterized for drug content, percentage yield, particle size analysis and surface morphology. The results of all the physiochemical tests of all formulations were found to be favorable. In-vitro floatability studies reveled that most of the microspheres (64 - 94%) were floatable. The in-vitro % drug release was found to be in range of 89.18 to 97.89 %. At the end of 14 hrs. Formulation F showed best appropriate balance between buoyancy and drug release rate. Optimized formulation F was evaluated for FTIR, DSC and SEM. DSC and FTIR studies showed that the nature of pure drug Lactulose remains unaffected till the completion of process of microspheres formation. SEM photographs showed that the Floating microspheres were spherical in nature with smooth surface and uniform distribution of the drug within the microsphere.

Keywords: Floating microspheres, Lactulose, In-vitro floatability, buoyancy.

INTRODUCTION

Floating microspheres are gastro-retentive drug delivery systems based on non-effervescent approach. Hollow microspheres are in strict sense, spherical empty particles without core. [1] These microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers, ideally having a size less than 200 um. Solid biodegradable microspheres incorporating a drug dispersed or dissolved throughout particle matrix have the potential for controlled release of drugs. [2] Gastro retentive floating microspheres are low-density systems that have sufficient buoyancy to float over gastric contents and remain in stomach for prolonged period. As the system floats over gastric contents, the drug is released slowly at desired rate resulting in increased gastric retention with reduced fluctuations in plasma drug concentration. When microspheres come in contact with gastric fluid, the gel formers like polysaccharides and polymers hydrate to form a colloidal gel barrier that controls the rate of fluid penetration into the device and consequent drug release. As the exterior surface of the dosage form dissolves, the gel layer is maintained by the hydration of the adjacent hydrocolloid layer. The air trapped by the swollen polymer lowers the density and confers buoyancy to the microspheres. However, a minimal gastric content needed to allow proper achievement of buoyancy. [3, 4] Lactulose drug is used by mouth or rectally to treat or prevent complications of liver disease (hepatic encephalopathy). Lactulose is a colonic acidifier that works by decreasing the amount of ammonia in the blood. This medication is also used in constipation for the soft stools.

MATERIALS AND METHODS

Materials

Lactulose was obtained as a gift sample from Abbot Pharma Pvt Ltd, Mumbai. HPMC, Ethyl cellulose obtained from Rankem chemicals, Dichloromethane obtained from Research Lab Ltd, Poona. All the reagents and materials were of analytical or pharmacopoeia grade.

Methods

Formulation of floating Microspheres

Preparation of Floating Microspheres of Lactulose by Solvent Evaporation Technique Floating microspheres containing Lactulose were prepared using emulsion solvent evaporation technique. For the preparation of floating microspheres, the rate controlling polymers in varying concentration (Drug: polymer ratio, 1:0.5, 1:1.1, 1:1.5, 1:2, 1:2.5, 1:3, 1:3.5, 1:4, 1:4.5 and 1:5)., The drug and polymer mixture (1:0.5 to 1:5) was dissolved in a Dimethyl formamide and dichloromethane (100 ml) containing 0.01% of tween 80, the resultant solution was stirred with a stirrer for 1 hour at 500 rpm. Various batches of floating microsphere was shown in Table no. 1the formed floating microspheres were filtered and washed with water and dried at room temperature and stored in a desiccators until further use.[6]

Evaluation of Floating Microspheres

Particle size analysis

Particle size analysis plays an important role in determining the release characteristics and floating property. The sizes of floating microspheres were

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measured by laser diffraction particle size analyzer. Firstly, 1gm of floating microspheres was floated in 200 ml of containing 0.02% of Tween 20 in aqueous solution and stirred at 37 ± 0.5°C. Second, particle size distribution was obtained when a laser light passed through the microspheres and then diffracted the intensity in an angular distribution. The data obtained were evaluated using volume distribution diameter (d) values of 10%, 50% and 90%. The mean particle size was then calculated.[7]

Table 1: Formulation of the floating microspheres of Lactulose

Formulation code	Lactulose	Ethyl cellulose	HPMC
A	500	125	125
B	500	250	250
C	500	375	375
D	500	500	500
E	500	625	625
F	500	750	750
G	500	875	875
H	500	1000	1000
I	500	1125	1125
J	500	1250	1250

Angle of repose

Flow property of floating microspheres is usually assessed by determining angle of repose of the floating microspheres. It is the maximum angle that can be obtained between the free flowing surface of floating micro balloons heap and the horizontal plane. The angle of repose of floating microspheres was determined by fixed funnel method. The floating microspheres were allowed to fall freely through a funnel until apex of conical pile just touched the tip of the funnel.[8] The angle of repose θ was determined according to the following formula

$\theta = \tan^{-1} h/r$

Where, h = height of pile r = radius of the pile formed by the floating microspheres.

Bulk density

The sample equivalent to 2 gm was accurately weighed and filled in a 100 ml graduated cylinder and the powder was leveled and the unsettled volume, Vo was noted. The bulk density was calculated by the formula. Weight of sample in gms Bulk density (g/ml) = Volume occupied by the sample

Tapped density

2 gm sample was poured gently through a glass funnel in to a 100ml graduated cylinder. The cylinder was tapped from height of 2 inches until a constant volume was obtained. Volume occupied by the sample after tapping were recorded and tapped density was calculated. Weight of sample in gms Tapped density (g/ml) = Volume occupied by the sample

Percentage compressibility

The same tapping method was used to determine percentage compressibility index. The percentage compressibility index was calculated according to following formula. Tapped density – Bulk density % Compressibility Index = x100 Tapped density

Hausner's ratio

Hausner's ratio is an indirect index of ease of powder flow. It is calculated by the following formula, Tapped density Hausner's Ratio = Bulk density

Percentage yield

The percentage yield of different formulations was determined by weighing the floating microspheres after drying. The percentage yield was calculated as follows.[9] Total weight of floating microspheres % Yield = x 100 Total weight of drug and polymer

Drug entrapment

The various batches of the floating microspheres were subjected to estimation of drug content. The floating microspheres equivalent to 50 mg of Lactulose from all batches were accurately weighed and crushed. The powdered of microspheres were dissolved in ethanol (10 ml) in volumetric flask (100ml) and made the volume with 0.1 N HCl. This solution is then filtered through Whatmann filter paper No. 44. After filtration, from this solution accurate quantity (10 ml) was taken and diluted up to 100 ml with 0.1 N HCl. From this solution, accurate volume (2 ml) was pipette out and diluted up to 10 m1 with 0.1 N HCl and the absorbance was measured at 276 nm against 0.1 N HCl as a blank.[10]

Percent drug content

The microsphere was powdered, accurately weighed a quantity of the powder equivalent to 25mg of Lactulose, transfer to a 500-ml volumetric flask using 300 ml of methanol, the resulting suspension was heated to 60o and shacked for 15 minutes. Cool, dilute to 500.0 ml with methanol dilute a suitable volume of the filtrate with sufficient methanol to produce a solution containing 0.01% w/v of Lactulose. Measure the absorbance of the resulting solution at the maximum at about 276 nm. [11]

Floating ability of microspheres

Microspheres (300mg) were spread over the surface of a USP dissolution apparatus type II filled with 900 ml of 0.1 N HCl containing 0.02% Tween 80. The medium was agitated with a paddle rotating at 100 rpm for 12 h. The floating and the settled portions of microspheres were recovered separately. The microspheres were dried and weighed. Buoyancy percentage was calculated as the ratio of the mass of the microspheres that remained floating and the total mass of the microspheres. [12]

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% Buoyancy = of / (Qf + Qs) Where Qf and Qs are the weight of the floating and the settled microspheres respectively.

***In-vitro* release studies**

In-vitro release of Lactulose from floating microspheres was carried out using the USP dissolution test apparatus (Type-I). A weighed amount of floating microspheres equivalent to 25 mg of drug were filled into a capsule and placed in the basket. Dissolution media used was 900 ml of 0.1 N HCl (pH 1.2) maintained at 37 ± 0.5°C and stirred at 100 rpm. At predetermined time intervals, 10 ml of sample was withdrawn and replaced with equal amount of 0.1 N HCl (pH 1.2). The collected samples were filtered and suitably diluted with 0.1 N HCl and analyzed spectrophotometric ally at 276 nm to determine the amount of drug released in the dissolution medium.

Characterization of Microspheres

Fourier transforms infra-red spectroscopy (FT-IR) analysis.

The fourier transform infra-red analysis was conducted for the analysis of drug polymer interaction and stability of drug during microencapsulation process. Spectrum of pure Lactulose, Ethyl Cellulose and floating microspheres were recorded. [13, 14]

Differential scanning calorimetry

The DSC thermogram of microsphere was recorded using Differential scanning calorimeter (Metller Toledo DSC. Japan). Samples were accurately weighed onto aluminum pans and then hermetically sealed with aluminum lids. Thermograms were obtained at a scanning rate of 10°C/min conducted over a temperature range of 30-300° C in the environment of liquid nitrogen.

Accelerated Stability studies

From the prepared floating microspheres, batch C which showed appropriate balance between the buoyancy and the percentage release was selected for stability studies. The floating microspheres (F) were placed in borosilicate screw capped glass containers and stored at temperature (40 ± 2°C) with relative humidity (75±5 RH) for a period of 90 days. The samples were assayed for drug content at regular intervals of 30 days. [15, 16]

Surface Morphology

Study From the formulated batches of floating microspheres, formulation (F) which showed an appropriate balance between the buoyancy and the percentage release were examined for shape using scanning electron microscope. [17]

RESULT AND DISCUSSION

Table 2: Evaluation of different batches of floating microsphere

Sr. No.	Batch No.	Mean particle size(µm)	Angle of Repose (°)	Bulk Density gm/cm 3	Tapped Density gm./cm 3
1	A	230.6± 0.06	21.03± 0.15	0.540 ±0.02	0.444± 0.05
2	B	236±0. 62	22.42±0. 12	0.512 ±0.03	0.408± 0.05
3	C	243.3± 0.85	19.70± 0.24	0.476 ±0.05	0.487± 0.09
4	D	230.6± 0.06	21.03± 0.15	0.540 ±0.02	0.443± 0.05
5	E	226±0. 62	22.42±0. 12	0.516 ±0.03	0.418± 0.05
6	F	238.6± 0.06	20.03± 0.15	0.547 ±0.02	0.424± 0.05
7	G	241.6± 0.06	21.03± 0.15	0.530 ±0.02	0.434± 0.05
8	H	245±0. 62	23.42±0. 12	0.519 ±0.03	0.428± 0.05
9	I	233.3± 0.85	19.70± 0.24	0.466 ±0.05	0.467± 0.09
10	J	188.6± 0.01	23.87± 0.19	0.467 ±0.06	0.426± 0.05

Compressibility index	Hausner Ratio	% Yield	Entrapment Efficiency (%)	% drug content
21.62± 0.00	1.237± 0.00	44.7 ±0.41	62.94 ± 0.09	93.97 ±0.04
25.49± 0.02	1.155± 0.03	59.04± 0.35	69.43 ± 0.92	94.95 ±0.06
22.58± 0.01	1.213± 0.02	79.29 ±0.88	72.21 ± 0.90	95.96 ±0.07
21.52± 0.00	1.236± 0.00	46.7 ±0.41	63.94 ± 0.09	93.87 ±0.03
24.44± 0.02	1.155± 0.03	62.04± 0.35	70.43 ± 0.92	96.98 ±0.04
21.56± 0.00	1.227± 0.00	79.26 ±0.41	85.94 ± 0.09	99.94 ±0.07
21.72± 0.00	1.239± 0.00	45.7 ±0.41	69.94 ± 0.09	96.97 ±0.06
24.59± 0.02	1.145± 0.03	61.04± 0.35	65.43 ± 0.92	97.91 ±0.08
23.48± 0.01	1.223± 0.02	70.26 ±0.88	78.21 ± 0.90	95.93 ±0.05
18.59± 0.01	1.178± 0.01	74.85± 0.20	68.04 ± 0.56	97.99 ±0.06

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The mean particle size of floating microspheres formulation which shows high percentage of entrapment was in the range of 188.6±0.01-245±0.62µm. Formulation F showed relatively higher percentage of large size and formulation J showed relatively small size floating microspheres. Smaller the microspheres, floating ability will be less and faster will be the release rate of drug from microspheres, While larger the size, floating ability will be more and sustained will be the release of drug. Angle repose of floating microspheres was observed in range of 19.70±0.24-23.87± 0.19. The bulk density value of different microspheres ranged from 0.466±0.05- 0.540 ±0.02gm/cm3. The Tapped density values of microspheres ranged from 0.408±0.05- 0.467±0.09 gm. /cm3. The percentage compressibility value less than 30 for all formulation suggested excellent flow property of floating microspheres. The percentage yields of different formulation were in range of44.7±0.41- 79.26 ±0.41% Drug entrapment efficiency was decreased with the increased drug concentration and increased with increasing polymer concentration in floating microspheres. This may be due to solubility of Ciprofloxacin in water and water can penetrate in polymer which facilitates the diffusion of a part of entrapped drug to surrounding medium during preparation of floating microspheres. The drug content of different batches of floating microspheres was found in the range of 93.87±0.03- 99.94±0.07%. Floating ability of floating microsphere Floating ability of different formulations was found to be differed according to polymer ratio. F formulations showed best floating ability (74 – 96%) in 10 hours. Other formulation showed less floating ability (64 - 94%) in 10 hours.

Table 3: The percentage floating ability of floating microspheres.

Formulation code	1 hr.	3 hr.	5 hr.	10 hr.
A	94	84	76	70
B	90	82	72	64
C	93	89	79	71
D	94	84	76	70
E	94	86	72	62
F	96	90	80	74
G	92	82	70	60
H	90	80	72	64
I	92	84	74	66
J	88	78	68	58

In-Vitro drug release study

Floating microspheres showed sustained release of the drug in acidic condition (pH 1.2) and the drug release was found to be approximately linear. Approximately 20% of the drug was released initially. Furthermore, drug release from the floating microspheres matrix was controlled by the polymer. Ethyl cellulose is not a water soluble polymer and it does not show pH dependency. As the polymer content was increased and the drug loading was decreased, the release of drug was decreased significantly. In order to increase the release rate of drug, the ratio of drug and polymer is decreased and increased respectively. Formulation F showed best appropriate balance between buoyancy and drug release rate.

Surface Morphology study

The floating microspheres were examined by Surface Morphology illustrating the microscopies of formulation at lower and higher magnification. The floating microspheres were spherical with no visible major surface irregularity. Few wrinkles and inward dents were appeared at the surface and some crystal shape particles were appeared. It may due to collapse of floating microspheres during the in- situ drying process. The surface morphology of both formulations was examined at higher magnification (500X, 1000X) which illustrates the smooth surface of floating microspheres. Some small pores and cavities were present on the surface of floating microspheres, probably arising as a trace of solvent evaporation during the process.

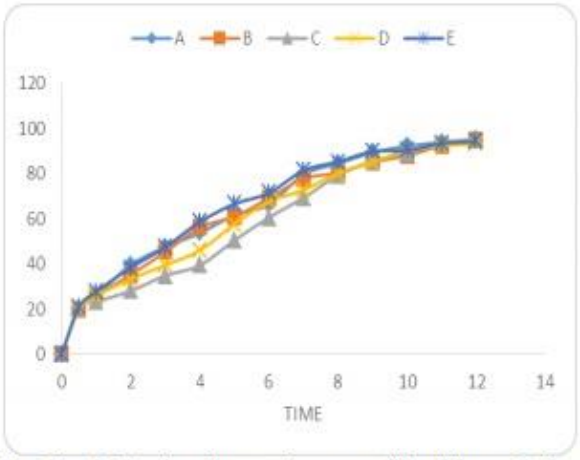

Fig. No: 1. *In-vitro* drug release profile of formulation A-E.

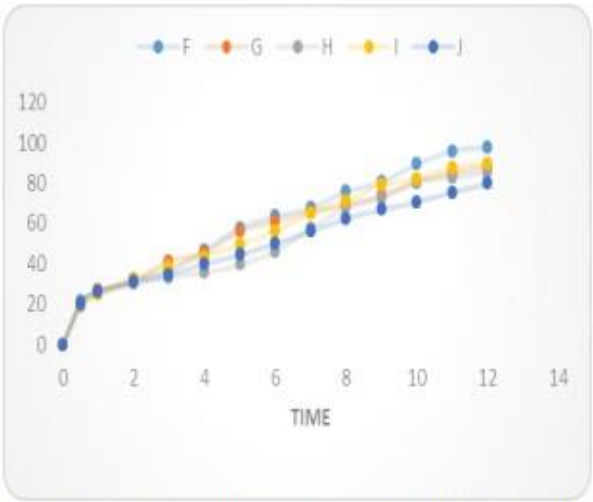

Fig. No: 2. In-*vitro* drug release profile of formulation F-J

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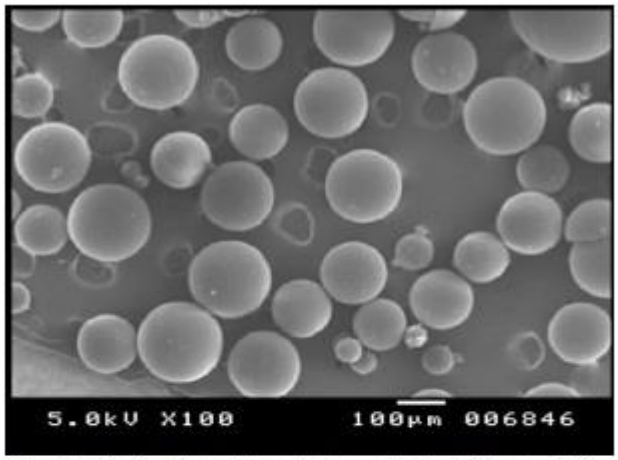

Fig. No: 3. Surface Morphology study of formulation F at x100.

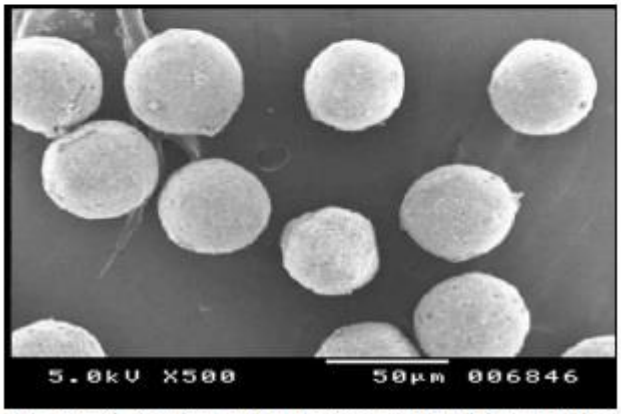

Fig. No: 4. Surface Morphology study of formulation F at x500.

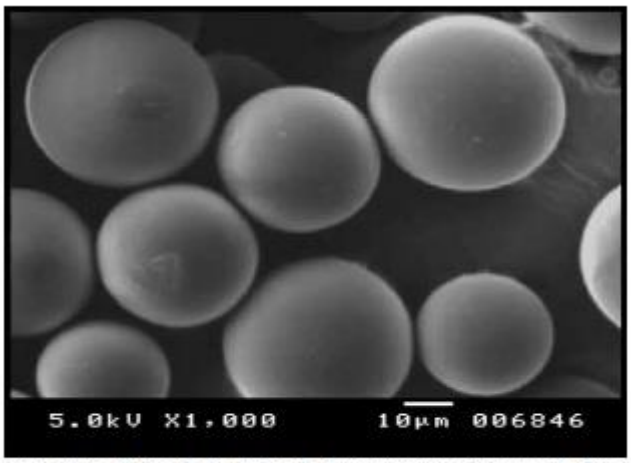

Fig. No: 5. Surface Morphology study of formulation F at x1000.

Accelerated Stability Studies

Stability study was carried out for optimized batch (F) by exposing it to temperature 40ºC and 75±5% RH for 90 days. The sample was analyzed for drug content at the regular intervals of 30 days. It was found that no remarkable change in the drug content of F formulation. This indicated that F was stable for following temperature.

Table 4: Accelerated Stability study data for formulation F.

Days	Color changes	% Drug Content 40±2 °C	% Max Drug Release
0	White	99.94±0.07	97.89±1.82
30	No change	99.1 ± 0.11	97.19±0.58
60	No change	99.0 ± 0.16	97.14±0.26
90	No change	98.9 ± 0.22	97.11±0.32

Fourier transforms infrared spectroscopy (FT-IR) analysis

The FT-IR spectra of the pure drug and physical mixture of drug – polymers were recorded to check interaction between drug and polymers. The characteristic peak of Lactulose was appeared in the spectra of physical mixture without any makeable change in the position. It indicates that there was no chemical interaction between Lactulose and polymers. The FT-IR spectra of Lactulose, physical mixture of drug-polymer and floating microspheres (Batch F) were recorded. The drug, Lactulose present in the formulation F was confirmed by FT-IR spectra. The characteristics peaks due to –OH stretching at 3000-3700, C=O at 3300-3600, aryl alkyl ether and –NH2bending at1500-1700 groups present in Lactulose appeared in floating microspheres spectra (Batch F), without any remarkable change in their position after successful encapsulation, indicating no chemical interaction between Lactulose, HPMC and ethyl cellulose. It also confirmed the stability of drug during microencapsulation process.

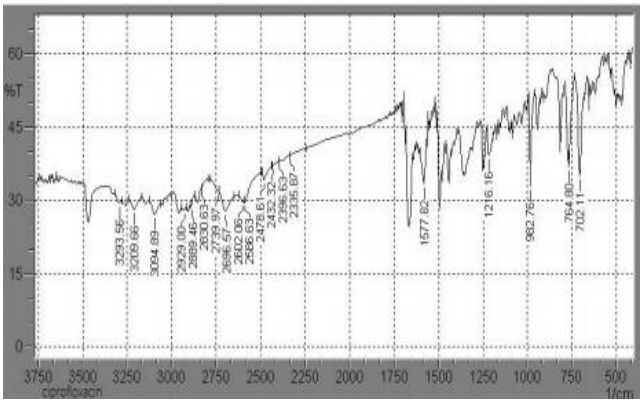

Figure 6: FTIR Spectra of Lactulose

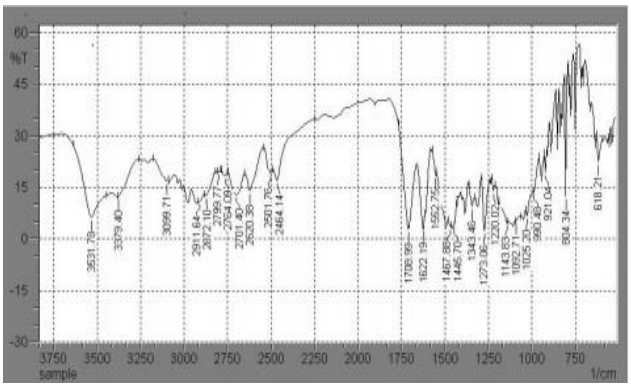

Figure 7: FT-IR spectrum of floating microspheres (Optimized Batch F).

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Differential scanning calorimetry (DSC)

The DSC thermogram of microsphere was recorded using Differential scanning calorimeter (Metller Toledo DSc. Japan). Physical mixture of drug and polymer was analyzed by DSC in order to ascertain if there were any interaction between the active ingredient and the polymer. This showed that pure drug gives sharp endothermic peak near 156.74 degree. Thus Ethyl had no visible effect on the drug peak, there was no such peak change for endothermic to exothermic process. This suggested that drug and polymer were compatible with each other. Min conducted over a temperature range of 30- 300°C in the environment of liquid nitrogen.

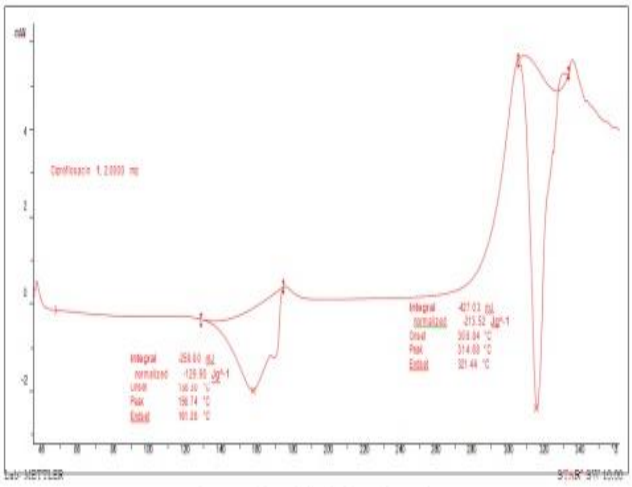

Figure 8: DSC of Lactulose

CONCLUSION

Floating microsphere for prolonged drug release of Lactulose were formulated and evaluated. Tablets were prepared by using various hydrophilic and hydrophobic polymers such as HPMC, Ethyl cellulose. HPMC and Ethyl cellulose a low density polymer that has been widely used. The ideal properties of floating microspheres were a high buoyancy and sufficient release of drug in 0.1 N HCl, so it was necessary to select an appropriate balance between buoyancy and drug release rate. From these findings, it was observed that formulation F possesses these properties. In stability study, there was no remarkable change in content of F formulation during 90 days in which it was stored at (40 ± 2°C) temperatures with relative humidity (75±5 RH).

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