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FORMULATION AND EVALUATION OF MICROSPONGE DRUG DELIVERY OF NATEGLINIDE FOR TREATMENT OF DIABETES MELLITUS

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ABSTRACT
Microsponges are tiny, uniform, micro-porous polymeric beads and spherical in shape. It has the interconnected voids. The particle size of it ranges between 5-300µm. The porous surface of non- collapsible structure of microsponges helps to deliver the active ingredient in controlled manner. Nateglinide is anti-Diabetic drug. The plasma half-life of Nateglinide is 1.5 hrs which increases the dosing frequency. Therefore the purpose of present investigation was to design suitable controlled release Nateglinide microsponges which can reduce the dosing frequency. In the present work, Nateglinide loaded Eudragit microsponges were prepared using quasi emulsion solvent diffusion method. Different drug: polymer ratios were used to formulate the microsponges. The compatibility of the drug with polymer was established. Surface morphology of the microsponges was examined using scanning electron microscopy. Production yield, loading efficiency, particle size analysis, and in-vitro release studies were carried out. In-vitro release study showed that the release of drug was in controlled manner and it was increased with increase in drug to polymer ratio up to certain limit.

KEYWORDS: Microsponges, Nateglinide, Eudragit RS100, Controlled release, Quasi- Emulsion.

INTRODUCTION
Many of conventional delivery systems require high concentrations of active agents to be incorporated for effective therapy because of their low efficiency as delivery systems. Thus novel drug delivery systems have been increasingly investigated to achieve targeted and controlled release of drugs. Microsponges are highly cross-linked, patented, porous, polymeric microspheres that acquire the flexibility to entrap a wide variety of active ingredients that are mostly used for prolonged topical administration and recently for oral administration. Microsponges are designed to deliver a pharmaceutically active ingredient efficiently at minimum dose and also to enhance stability, elegance, flexibility in formulation, reduce side effects and modify drug release profiles. Microsponges are prepared by several methods utilizing emulsion systems as well as by suspension polymerization in a liquid-liquid system. The most common emulsion system used is Quasi-emulsion solvent diffusion method.[1-3] Drug dissolution is the single most important factor in the absorption of it, especially from the most widely used conventional solid dosage form, tablets and capsules.[4-6] Diabetes is the metabolic disorder characterized by hyperglycaemia, glycosuria, hyperlipaemia, negative nitrogen balance and ketonemia. TypeII diabetes (also known as non-insulin dependent diabetes mellitus (NIDDM) or adult onset diabetes) is one of the most serious medical conditions affecting our nation today. The number of people who have it has been rising widely .For this the fast delivery of drug is required to the diabetic patient.[7] Nateglinide is anti-Diabetic drug. Nateglinide (NTG) is an amino-acid derivative that lowers blood glucose level by stimulating insulin secretion from the pancreas. This action is dependent upon functioning beta-cells. The subsequent depolarization of the beta cells opens the calcium channel, producing calcium influx and insulin secretion theextend of insulin release is glucose dependent and diminishes at low glucose levels. The tablets have to be taken 10-20 minutes before meal.[8] The Nateglinide is BCS class II drug -i.e. high permeability low solubility. The plasma half-life of Nateglinide is 1.5 hrs which increases the dosing frequency. Thus the present study is aimed at developing microsponge based novel drug delivery system containing Nateglinide. The microsponges of Nateglinide were formulated and evaluated they were filled in capsule and subjected to *in-vitro* studies for various attributes.

MATERIAL AND METHODS

Materials

Nateglinide was obtained as gift sample from Cipla Ltd. MIDC, Kurkumbh 413 802 Pune. Polyvinyl Alcohol and Triethyl citrate were obtained as gift sample from Glenmark Pharmaceuticals, Sinnar, Nashik. All other chemicals and solvents were of analytical grade.

Drug-Excipient Interactions

The physicochemical compatibilities of the drug and polymer were tested by FT-IR spectrophotometry. FT-IR spectra of the drug alone and drug-polymer physical mixtures (1:1 w/w) were derived from Alpha T-BRUKER, FT-IR.[9,10]

Formulation of Nateglinide loaded microsponge

The microsponges of Nateglinide were prepared by using quasi emulsion solvent diffusion technique. The internal phase was prepared by dissolving Drug and polymer in ethanol in the ratio 1:1, 3:1, 5:1, 7:1, 9:1, 11:1, 13:1 followed by addition of triethyl citrate. The internal phase was then poured into aqueous solution of polyvinyl alcohol, the external phase, and kept for continuous stirring and heating. After 3hrs of continuous stirring and heating, the microsponges were formed due to evaporation of alcohol. Then the microsponges were filtered and dried at 40^0c for 12hrs. For this purpose mini magnetic stirrer (DBK instruments) was used. The composition of microsponge formulations are given in table 1.[11,12,13]

Table 1: Composition of various microsponge formulations.

Ingredients	Ratios (Drug: Polymer)						
	1:1	**3:1**	**5:1**	**7:1**	**9:1**	**11:1**	**13:1**
Nateglinide (mg)	50	150	250	350	450	550	650
Eudragit RS 100 (mg)	50	50	50	50	50	50	50
PVA (mg)	3	3	3	3	3	3	3
Ethanol (ml)	3	3	3	3	3	3	3
TEC (ml)	0.1	0.1	0.1	0.1	0.1	0.1	0.1
DW (ml)	60	60	60	60	60	60	60

Evaluation of Nateglinide loaded microsponges

Particle size determination

Particle size of microsponges was determined using Motic microscope (Motic DMB series). To determine the particle size, small amount of microsponges were taken on glass slide and slide was placed on stage of microscope. Then the coarse and fine adjustment was done to obtain the clear image. The reading of particle size was displayed on the display of computer. Same procedure was repeated for all batches.[13]

Surface Morphology

Scanning electron microscopy of optimized microsponge formulation was carried to determine the surface morphology. The sample was mounted directly onto the SEM sample holder using double sided sticking tape and images were recorded at different magnifications at acceleration voltage of 10 kV using scanning electron microscope(Supra 5, Carl Zeiss Ltd. Germany).[14,15]

Differential scanning Calorimetry

For the structural, crystal and physical state characterization of Nateglinide the DSC study was performed for pure drug, and formulation. Accurately weighed sample of drug and Formulation was placed in sealed aluminium pans before heating under nitrogen flow (20/ml min) at a scanning rate of 10^0 c per min from 25 to 300^0 c. An empty aluminium pan was used as a reference.[16,17]

Loading efficiency and Production yield

Loading efficiency

Nateglinide microsponges equivalent to 10 mg of the drug was taken in a 10 ml volumetric flask. 5 ml methanol was added and shaken for about half an hour and the volume was made up to 10 ml with methanol. 0.1 ml of the solution was taken and diluted to 10 ml with methanol. The absorbance of the resulting solution was measured at 211nm and the content of Nateglinide was calculated. The loading efficiency (%) of the microsponges was calculated by using following formula.

$$\text{Loading efficiency (\%)} = \frac{\text{DC act} \times 100}{\text{DC theo.}} \quad \ldots\ldots\ldots\ldots\ldots \quad (1)$$

Where, DC act = Actual drug content in microsponges.
DC theo. = Theoretical drug content.

Production yield

The production yield of the microsponges was determined by calculating accurately the initial weight of the raw material and the weight of the microsponges obtained.

$$\textbf{Production yield (\%)} = \frac{\text{Wpr} \times 100}{\text{W th}} \quad \ldots\ldots\ldots\ldots\ldots \quad (2)$$

Where , W pr = Practical mass of Microsponges
W th = Theorectical mass (polymer + drug).[12,18]

$$\text{Porosity}\ (\%) = \frac{\text{Bulk volume} - \text{True volume}}{\text{Bulk volume}} \times 100 \text{----------} \quad (3)$$

To measure the bulk volume, weighed amount of microsponges was poured into 1 ml pipette and the bulk volume was noted to nearest graduated unit. True volume was determined by liquid displacement method.[19,20]

In-vitro Drug Release Study
The test is designed to determine compliance with the dissolution requirement for solid dosage forms administered orally. Dissolution test was performed in dissolution test apparatus Type II (IP)/ Type I (USP) (Electrolab TDT 08L) for capsules and Type I (IP)/Type II (USP) for marketed preparation. Before the test, microsponges amount which are equivalent to dose of Nateglinide were filled in capsules. For comparison, pure drug filled in capsules and marketed formulations were taken.

Apparatus: Basket

RPM: 50

Temperature: 37 ± 5^0c.

The dissolution of microsponges filled in capsules and plain drug filled in capsules were carried out in 900ml of phosphate buffer pH6.8. Drug release was monitored for 1, 2, 3, 4, 5, 6, 7, 8,9,10,11,12,th hr. Samples(5 ml) were withdrawn at regular time intervals and sink conditions were maintained by replacing an equal amount of fresh dissolution medium. The samples were filtered through whatmann filter paper no.52, and diluted upto 10ml and analyzed by UV-Visible spectroscopy (Shimadzu 1800, Japan) at 209nm using phosphate buffer pH 6.8 as blank. Dissolution tests were performed in triplicate.[21,22,23]

Dissolution kinetics
The dissolution profile of optimizied formulation was subjected to various models such as Zero order Kinetics (percentage drug release against time),First order kinetics (log percentage drug unreleased against time),Higuchi (percentage drug released against square root of time), Korsemeyer –Peppas(log percent drug released against log of time)and to assess the kinetics of the drug release from prepared Nateglinide loaded microsponges.[24]

Stability Studies
Stability study of optimized formulation was carried out to point out any chemical changes made in the formulation after storing it at elevated temperature and humidity conditions. Chemical and physical stability of optimized Nateglinide loaded microsponge formulation was assessed at $40^0c\pm2^0c$/ 75% ±5% RH as per ICH Guidelines. The powder of microsponge formulation equivalent to 50 mg of Nateglinide was packed with aluminium strip and stored for three months. Sample was analysed after three months for drug content and in-vitro dissolution profile.[25]

Porosity
Porosity of microsponges was calculated by using following equation.

RESULTS AND DISCUSSION

In the present work, FT-IR spectra of Nateglinide and physical mixture of drug and polymer were examined. In FT-IR spectra of Nateglinide the major peaks at 1739 cm $^{-1}$which indicates that **C=O** stretching vibration .2939 cm $^{-1}$ which indicates that **C=H** group is present ,3314 cm $^{-1}$ indicates **N-H** bond is present ,1445 cm $^{-1}$ indicates **– CH_3** group is present. All these peaks were present in physical mixture of drug and polymer. This is an indication of no drug-polymer interaction and hence it can be said that the polymer is compatible with the active pharmaceutical ingredient. The FT-IR spectra of drug and drug with polymer are shown in Figure. 1 and 2. Particle size analysis showed that the mean perimeter was found to be in the range of 6.67 to 39.25 µm. Mean particle size of formulations 1:1 to 13:1 is given in table 2. The SEM Micrograph of the microsponges at 20X and 1µm showed the porous surface of microsponge and it is shown in Figure. 3 and 4. DSC thermograph of Nateglinide is shown in Figure. 5 which shows melting endotherm at138.62^0c i.e. melting point and crystalline state of drug. DSC thermograph of microsponge (11:1) formulation is shown in Figure. 6. Thermograph showed melting endotherm at 148.17^0c indicates that drug was embedded in matrix of polymer used. Loading efficiency of microsponges was increased with increase in concentration of drug in drug: polymer ratio. Maximum loading efficiency was found for 11:1 drug: polymer ratio. Results obtained from calculation are shown in table 2.Maximum production yield was found for batch containing 11:1 drug: polymer ratio. The porosity of microsponges was decreased with increase in concentration of drug in drug: polymer ratio. Maximum porosity i.e. 75.51% was found for 1:1 ratio of drug: polymer and minimum porosity i.e. 34.52% was found for 13:1 ratio of drug: polymer. Results obtained from calculation are shown in table 2.Release study has shown that drug release from the microsponges was in controlled manner as compared to pure drug and it was increased with increase in concentration of drug in drug: polymer ratio upto certain limit i.e. 11:1 ratio of drug: polymer. Drug release for 13:1 ratio of drug: polymer was found to be fairly similar to that of 11:1 ratio of drug: polymer. So it can be postulated that there was not considerable change in release pattern after increasing concentration of drug in drug: polymer ratio. Hence 11:1 proportion of drug: polymer should be an ideal and optimized ratio for considering drug release. The pure drug filled in capsules has shown 31.80 % drug release within 1 hr and 91.45% drug

release within first 5 hours and marketed formulation shows 96.09 % release which indicated that microsponges has better controlled drug release i.e. 97.60% upto 12 hours was found for formulation containing 11:1 ratio of drug: polymer.

Comparison of dissolution profile of different microsponge formulations shown in figure 7 and Comparison of dissolution profile of optimized formulation (11:1) with that of plain drug and marketed preparation is shown in figure 8.

The present study of dissolution was analysed by kinetics model software. The R^2 values for various release models are 0.9692 for Zero order, 0.9056 for First order, 0.9144 for Higuchi, 0.9916 for Korsemeyer-Peppas. The results showed that the optimized batch followed korsmeyer- peppas model kinetics. The R^2 value of korsmeyer- peppas model was found close to one. The Drug Release Kinetics for best fitting optimized batch was calculated and it is shown in table 3. The release exponent n = 0.8 indicates that it is following non -Fickian release (anomalous), this means that drug release followed controlled release mechanism. Optimized formulation was subjected to stability studies as per ICH guidelines. Parameters such as drug content and in-vitro drug release were measured before and after 3 months of stability. Results of stability studies are shown in table 4. Physical appearance of optimized formulation was unaffected or did not show any significant change. Results of stability studies has shown that there is no significant change in above mentioned parameters upto 3 month period of given temperature and humidity conditions during stability studies. Thus, it can be proved from the stability studies that the prepared formulation was stable up to 3 month period.

Table 2: Evaluation of Microsponges.

Parameter	Formulation						
	1:1	**3:1**	**5:1**	**7:1**	**9:1**	**11:1**	**13:1**
Mean perimeter (µm) ± SD	16.86± 0.036	39.25± 0.020	6.67± 0.037	7.12± 0.040	29.35± 0.0407	14.25± 0.023	24.59± 0.044
Loading efficiency% ± SD	80.75± 0.026	85.28± 0.022	90.20± 0.022	9474± 0.020	96.67± 0.035	98.53± 0.036	97.03± 0.085
Production Yield %	28	52	78.33	75	56.8	80.83	70
Porosity % ± SD	75.51± 0.021	72.36± 0.026	65.83± 0.017	62.63± 0.021	59.94± 0.015	49.95± 0.017	34.52± 0.014

Table 3: Drug release kinetics for optimized batch.

Sr. No.	Model Fitting	R^2 Value	N
1.	Korsmeyer- peppas	0.9916	0.8223

Table 4: Stability study of optimized formulation.

Sr. No.	Stability Parameters	Before Stability Testing	After Stability Testing
			1 Month
1.	Drug Content (%) ± SD	98.91 ± 0.026	98.90 ± 0.024
2.	In-vitro drug release study (%) ± SD	97.60 ± 0.031	97.54 ± 0.028

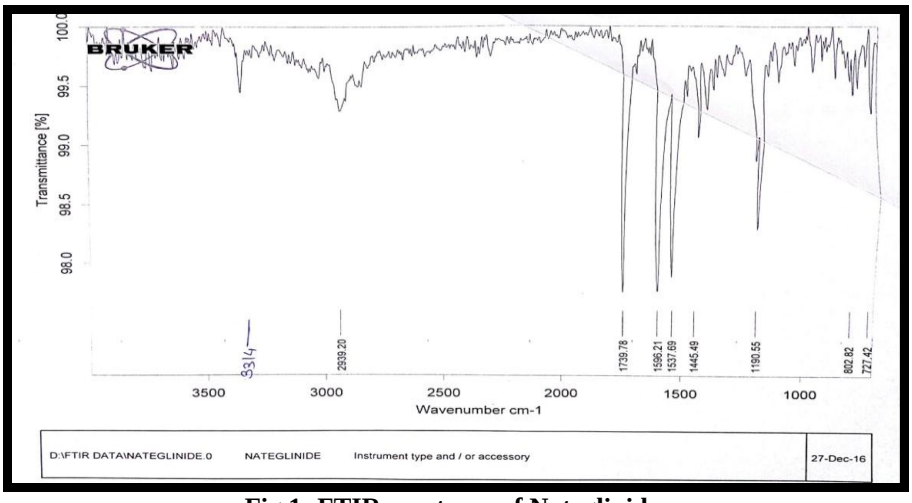

Fig 1: FTIR spectrum of Nateglinide.

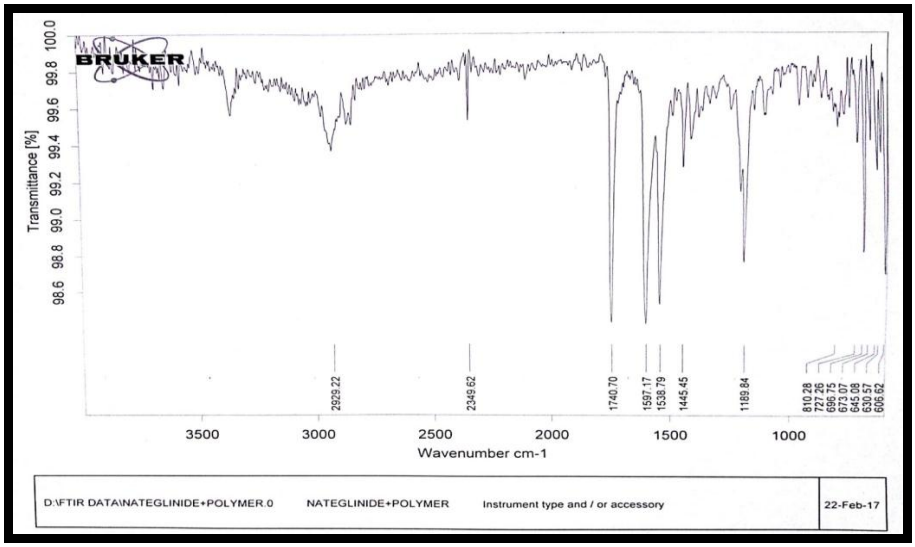

Fig 2: FTIR spectrum of Nateglinide and polymer's physical mixture.

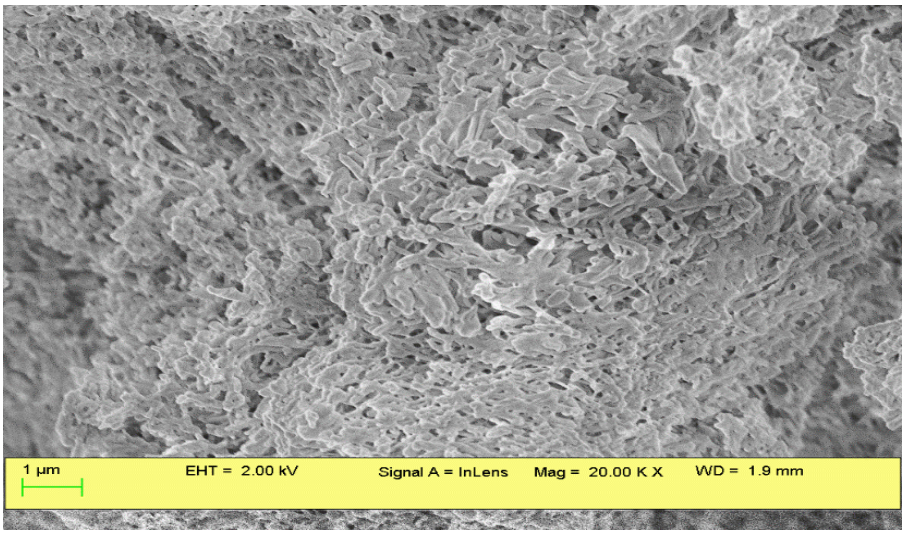

Fig 3: SEM photograph of microsponge formulations.

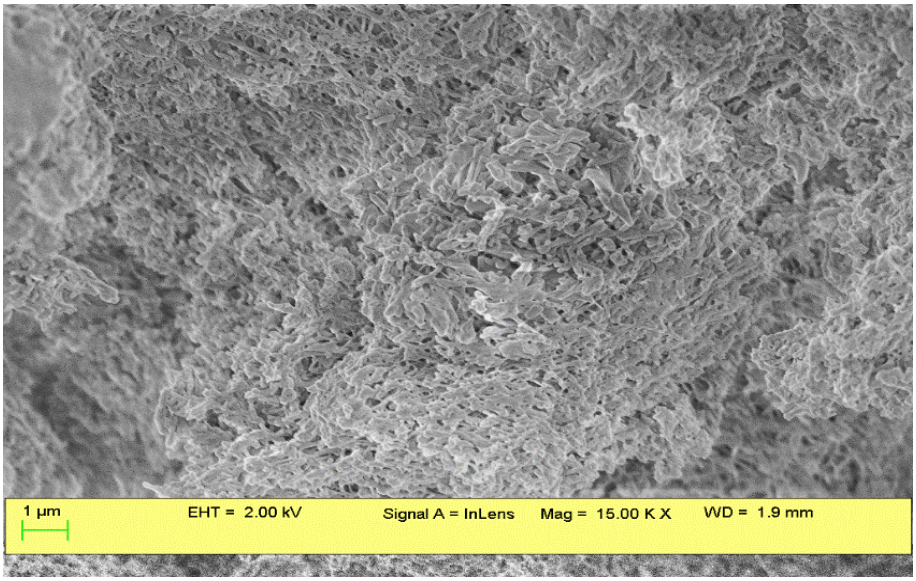

Fig 4: SEM photograph of microsponge formulations.

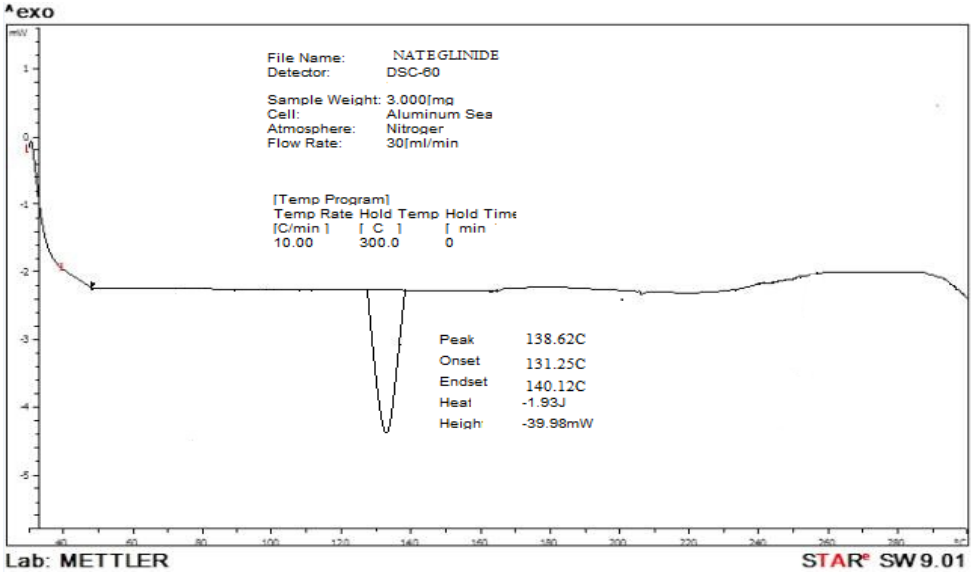

Fig. 5: DSC Thermograph of Nateglinide.

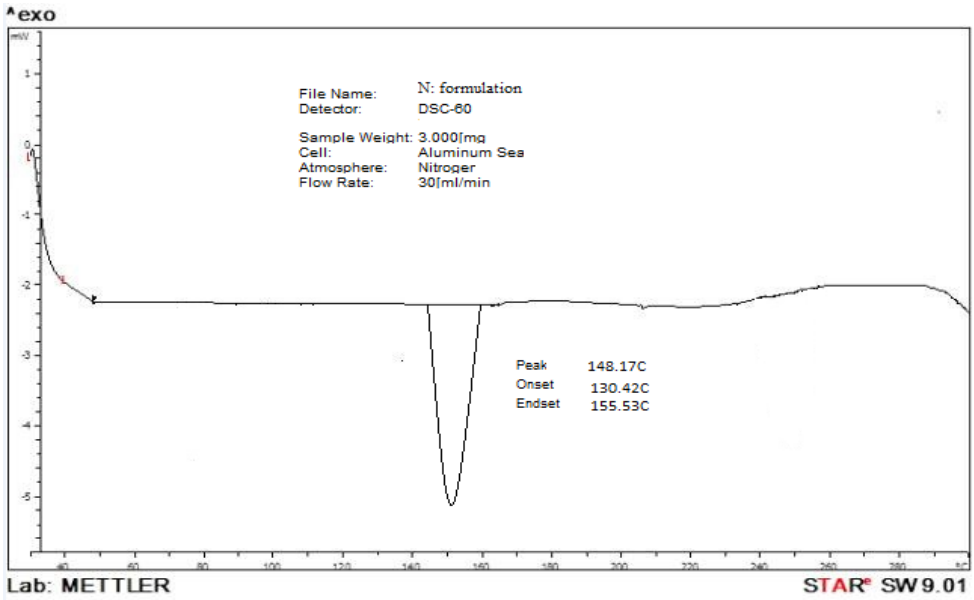

Fig 6: DSC Thermograph of MS (11:1) Formulation.

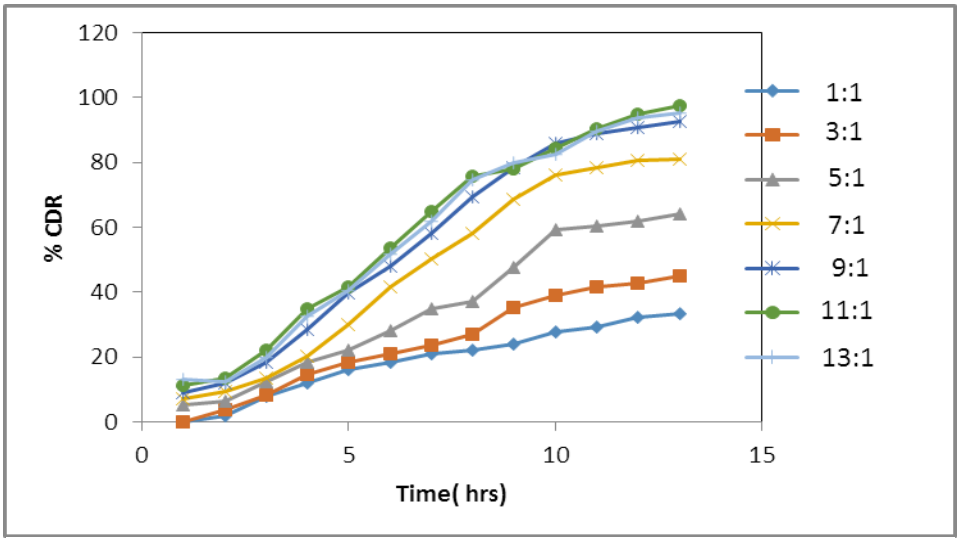

Fig 7: Comparative in-vitro drug release profile of different ratios of microsponges.

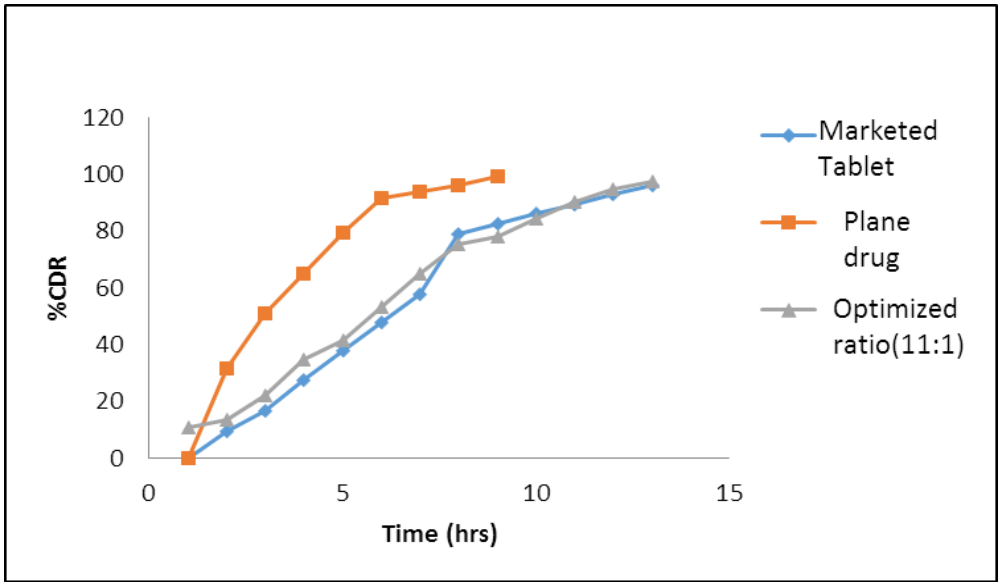

Fig 8: Comparative in-vitro drug release profile of Plane Drug, Marketed tablet and Optimized ratio of microsponges.

CONCLUSION

From all the results obtained after various evaluations it can be considered that the microsponges can be formulated using quasi emulsion solvent diffusion technique. Thus, this study presents a new approach for the preparation of modified microsponges with extended release behaviour over a prolonged duration of time which may reduced dose related side effects. The prepared microsponges exhibited characteristics of an ideal delivery system.

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