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Der Pharmacia Lettre, 2011: 3 (4) 299-316
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Development, *In vitro* and *In vivo* Evaluation of Novel Floating Hollow Microspheres of Rosiglitazone Maleate

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ABSTRACT

The objective of the present work was to formulate floating hollow microspheres of Rosiglitazone Maleate (RSM), which is soluble and shows better absorption in gastric pH. Microspheres were prepared by modified Quasi-emulsion diffusion technique using ethyl cellulose, eudragit S100, polyethylene oxide and Hydroxyproply methyl cellulose (HPMC K15M) as polymers. The formulations were evaluated for micromeritic properties, in vitro, in vivo buoyancy, % yield, entrapment efficiency, in vitro and in vivo release studies. They were characterized by FT-IR and DSC. FT-IR and DSC studies indicated that there was no interaction between the drug and polymers. SEM photographs showed the outer surface of microspheres was smooth and dense where as internal surface was porous which helped to prolong floating to increase residence time in stomach. The results showed that floating microspheres could be successfully prepared with better yield (more than 54.5 ±1.2 %), high encapsulation efficiency (53±2.2 %) and narrow size distribution (223±2.7-446±5.2 μm). All the formulations floated for more than 8 h. Results showed larger the particle size, longer was the floating time. In vivo evaluation in albino rabbit confirmed floating capability microspheres for more than 8 h. In vitro drug release studies showed controlled release of rosiglitazone maleate for over 12 h. The release behaviour best fitted mostly in peppas and zero order equations. In vivo evaluation of blood glucose levels in albino rats showed that floating microspheres of rosiglitazone maleate had better glycemic control than conventional dosage form. From the results it can be concluded that gastric floating hollow microspheres can be successfully used for the delivery of rosiglitazone maleate to control blood glucose level.

Key words: Hollow microspheres, Rosiglitazone Maleate, Ethyl cellulose, *In vivo*, modified Quasi-emulsion diffusion technique.

INTRODUCTION

Rosiglitazone maleate (RSM) is an oral hypoglycaemic agent, which improves glycemic control by improving insulin sensitivity. It is effective only in the presence of insulin. It decreases insulin resistance at peripheral sites and in the liver. This results in insulin-dependent glucose

disposal and reduced hepatic glucose output. The half-life of rosiglitazone maleate is 3-4 h and it reaches a peak plasma concentration after 1 h. It is highly soluble in 0.1mol/l HCl (11.803 mg/ml) and its solubility decreases with increasing pH over the physiological range. Rosiglitazone is a highly selective and potent agonist for the peroxisome proliferator-activated receptor-gamma (PPAR γ) (1). In humans, PPAR receptors are found in key target tissues for insulin action such as adipose tissue, skeletal muscle, and liver. Activation of PPAR γ nuclear receptors regulates the transcription of insulin-responsive genes involved in the control of glucose production, transport, and utilization. In addition, PPAR γ-responsive genes also participate in the regulation of fatty acid metabolism (2,3).

Many oral controlled drug delivery systems have been developed to improve the bioavailability. For oral solid drug delivery systems, drug absorption is unsatisfactory and highly variable between the individual despite excellent *in vitro* release patterns (4). It is necessary to optimize both the residence time of the system within the gastrointestinal tract and the release rate of the drug from the system. Various attempts have been made to prolong the residence time of the dosage forms within the stomach. The prolongation of the gastric residence time of delivery devices could be achieved by mucoadhesive or bioadhesive systems, high density systems, magnetic systems, superporous hydrogels, raft forming systems, low density systems floating hollow microspheres and floating ion exchange resins. One of the approach is bio adhesive system that 'stick' dosage forms to the mucin-epithelial cell surface, providing longer transit time due to adhesion of the device to the gastric wall. Such adhesion may cause problems, such as irritation to the mucosa if an over dose of the drug occur locally. Another suggestion is floating dosage forms. These have a specific density that is lower than that of the gastric fluids; they remain buoyant in the stomach content. Most of the floating systems are dominated by single unit dosage forms (Hydrodynamically balanced systems). The main drawback of this system is high variability of the GI transit time, due to its all or nothing emptying process. Hence, a multiple unit floating system which can be distributed widely throughout the GI tract, providing a possibility of achieving a longer and more reliable release of drugs has been sought (5,6).

The principle of buoyant preparation offers a simple and practical approach to achieve increased gastric residence time for the dosage form and sustained drug release. The device prepared with the present technique is a polymeric microsphere with a round cavity. This microsphere was also called "microballoon" due to its characteristic internal hollow structure and excellent *in vitro* buoyancy. Hollow microspheres are in strict sense, spherical empty particles without core. These microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers, ideally having a size less than 200 micrometer. The advantages of hollow microspheres includes improvement in patient compliance by decreasing dosing frequency, enhanced bioavailability despite first pass effect because fluctuations in plasma drug concentration is avoided, a desirable plasma drug concentration is maintained by continuous drug release, better therapeutic effect of short half-life and site-specific drug delivery to stomach drugs can be achieved, gastric retention time is increased because of buoyancy, drug releases in controlled manner for prolonged period, can be achieved, enhanced absorption of drugs which solubilise only in stomach, superior to single unit floating dosage forms as such microspheres releases drug uniformly and there is no risk of dose dumping, avoidance of gastric irritation, because of sustained release effect, floatability and uniform release of drug through multiparticulate system. 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 (7,8).

The gastroretentive drug delivery systems may be highly useful for delivery of many different kind drugs. These would provide the best results for that drugs act locally in the stomach or absorbed primarily in the stomach. For many drugs that are absorbed mainly from the proximal part of small intestine, controlled release in the stomach would results in improved bioavailability (9).

The objective of the present work is to formulate hollow microspheres. The hollow microspheres were prepared by modified quasi-emulsion solvent diffusion method. Buoyant properties and efficiency of drug entrapment within microspheres were optimized. In the present study, the drug release from hollow microspheres containing rosiglitazone maleate was investigated. Although a number of articles are published for usage of rosiglitazone maleate as hollow microspheres, our literature search showed that no previous papers were published using the polymers employed in the present study. In this study we have modified the method of preparation to develop a novel method in terms of stirrer speed, temperature and addition of polymeric solution to get better percentage yield.

MATERIALS AND METHODS

Rosiglitazone maleate was gift sample from "Matrix Lab Hyderabad" as a model drug, HPMC K15M, Eudragit L100, Polyethylene oxide, Aldrich Mumbai; Ethyl cellulose was obtained from Aldrich Germany. Tween 80 Lobachem, India. All other reagents were analytical grade. Ethical clearance for the handling of experimental animals was obtained from the institutional animal ethical committee (IAEC) of JSS College of pharmacy, Mysore constituted for the purpose.

Preparation of hollow microspheres

Floating microspheres with a central hollow cavity were prepared by using a modified Quasi-emulsion diffusion technique (figure 1). Weighed quantities of RSM, Ethyl cellulose, polyethylene oxide and hydroxy propylmethyl cellulose (HPMC K15M) were dissolved in a mixture of ethanol and dichloromethane (1:1 solvent ratio) at room temperature in a magnetic stirrer at 50 rpm for 50 min. This solvent was poured drop wise into 100 mL distilled water containing 2 mL of Tween 80 maintained at a temperature of 50 ± 2 °C. The resultant solution was stirred with a pitched-blade-type impeller type agitator at 1100 rpm for 3 h to allow the volatile solvent to evaporate. This resulted in the formation of microspheres. Different ratios of polymers were used to prepare the microspheres. The various formulations are tabulated in Table 1.

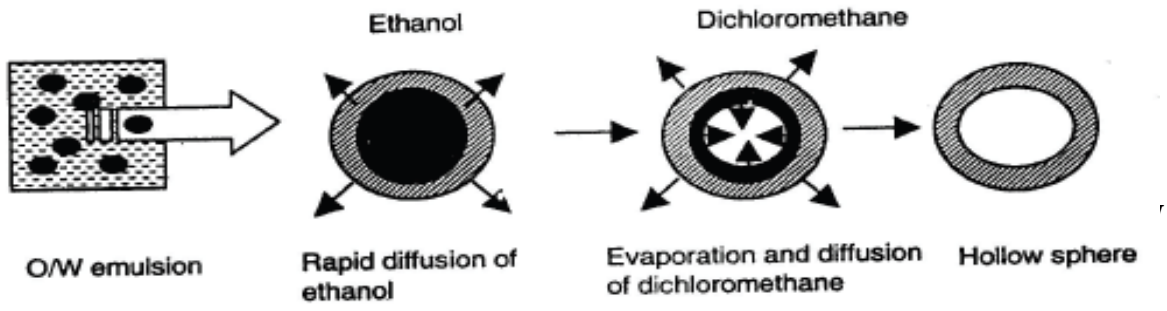


Table 1: formulation chart of RSM hollow microspheres

INGREDIENTS	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
Ethyl cellulose (gm)	1	1	2	1	1	1	2	1	2	1	-
Polyethylene oxide (gm)	-	1	1	2	-	-	-	-	-	-	-
HPMC K15M (gm)	-	-	-	-	1	2	1	-	-	-	-
Eudragit L100 (gm)	-	-	-	-	-	-	-	1	1	2	1
Solvent ratio * (ml)	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1
Drug (gm)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Tween 80(ml)	2	2	2	2	2	2	2	2	2	2	2

****** ethanol and dichloromethane of 30 ml each.*

% Drug entrapment efficiency and yield of floating microspheres

Floating microspheres equivalent to 4 mg of drug was dissolved in 10 mL ethanol. The samples were assayed for drug content using UV spectrophotometer at 228 nm after suitable dilution. No interference was found due to the other floating microspheres components at 228 nm. The percentage drug entrapment efficiency and yield were calculated as follows (10,11).

$$\% \text{ Drug entrapment efficiency} = \frac{\text{Calculated drug concentration}}{\text{Theoretical drug concentration}} \times 100 \quad (1)$$

$$\% \text{ Yield} = \frac{\text{Total weight of floating microspheres}}{\text{Total weight of drug and polymer}} \times 100 \quad (2)$$

Drug-Excipients Compatibility Studies:

Fourier Transform Infrared Spectroscopy

The Fourier transform infrared (FT-IR) spectra of samples were obtained using FT-IR spectrophotometer (Shimadzu, 8400 S, Japan). About 2–3 mg of samples was mixed with dried potassium bromide of equal weight and compressed to form a KBr disc. The samples were scanned from 400 to 4,000 cm^{-1}wave number.

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) experiments were carried out to characterize the physical state of RSM in microspheres as well as to find out the presence of any interaction among drug and the excipients. Rosiglitazone, Ethyl cellulose, polyethylene oxide and HPMC K15M samples were put in aluminum pan and hermetically sealed. The heating rate was 10°C/min; nitrogen served as purged gas and the system was cooled down by liquid nitrogen. The differential thermal analyzer (Pyris Diamond TG/DTA PerkinElmer; Singapore) was used for this purpose.

Surface Morphology

The surface morphology of the microspheres was examined by scanning electron microscopy (SEM; JSM-5200, Jeol, Japan) operated at 15 kVon samples gold-sputtered for 120 s at 10 mA, under argon at low pressure.

Sphericity of the microsphere

To determine the sphericity, the tracings of prepared microspheres (magnification 45x) were taken on a black paper using camera lucida, (Model -Prism type, Rolex, India). Circulatory factor (S) was calculated using,

$$S = \frac{p^2}{12.56 \times A} \quad (3)$$

Where A is area (cm^2) and, P is the perimeter of the circular tracing (10).

***Micromeritic properties of microsphere*:**
The microspheres were characterised by their micromeritic properties, such as particle size, tapped bulk density, compressibility index and angle of repose (values useful in prediction of flowability).

Particle size
The particle size of the microspheres was measured using an optical microscopic method and the mean particle size was calculated by measuring 425 particles with the help of a calibrated ocular micrometer with stage micrometer (12).

Angle of repose
Angle of repose (θ) of all the formulations was determined by using a fixed funnel method, which measures the resistance to particle flow and calculated as follows.

$$\text{Tan } \theta = \frac{2H}{D} \qquad (4)$$

Where H is height and D is the diameter microspheres heap of the pile, which is formed on a graph paper after making the microspheres flow from the glass funnel.

Tapped bulk density
The tapping method was used to determine the tapped density of the microspheres using tapped density testing apparatus (Electro lab tapped density tester ETD-1020) and percent compressibility index as follow:

$$\text{Tapped density} = \frac{\text{Mass of microspheres}}{\text{Volume of microspheres after tapping}} \qquad (5)$$

$$\% \text{ Compressibility index} = \left[1 - \frac{V}{V_0}\right] \times 100 \qquad (6)$$

Where V_0 and V are the volumes of the sample before and after the standard tapping (13).

Floating Characteristics:
In vitro buoyancy of microspheres
The floatation study was carried out to ascertain the floating behaviour of the microspheres prepared with various polymer combinations. Floating behaviour of hollow microspheres was studied using a USP dissolution test apparatus II by spreading the microspheres (100 mg) on 900 mL of 0.1 N HCl containing 0.02 % v/v tween 80 as surfactant. The medium was agitated with a paddle rotating at 100 rpm and maintained at 37° ± 0.5 °C for 12 h. Both the floating and the settled portions of microspheres were collected separately. The microspheres were dried and weighed. The percentage of floating microspheres was calculated using the following equation (14).

$$\% \text{ floating capability} = \frac{\text{Weight of floating microspheres}}{\text{Initial weight of floating microspheres}} \times 100 \qquad (7)$$

In vivo floating behaviour

Barium sulphate loaded microspheres were prepared by adopting the procedure as described before except for using barium sulphate instead of drug. Healthy rabbit weighing approximately 2.3 Kg was used to assess *in vivo* floating behaviour. Ethical clearance for the handling of experimental animals was obtained from the institutional animal ethical committee (IAEC) of JSS College of pharmacy, Mysore constituted for the purpose. The animal was fasted for 12 h and the first X-ray photographed to ensure absence of radio opaque material in the stomach. The rabbit were made to swallow barium sulphate loaded microspheres with 30 ml of water. During the experiment rabbit were not allowed to eat but water was provided. At predetermined time intervals the radiograph of abdomen was taken using an X-ray machine (15).

In vitro drug release study

The release rate of RSM from microspheres was determined using USP dissolution testing apparatus II (basket type). The dissolution test was performed using 900 mL of 0.1 N HCl, at 37± 0.5 ^{o}C and 50 rpm. Microspheres equivalent to 4 mg RSM were used for the test. Aliquots (5mL) were withdrawn at hourly interval for 12 h, sample was replaced by its equivalent volume of fresh dissolution medium to maintain the sink condition. The samples were analyzed at 228 nm using shimadzu UV-1700 UV spectrophotometer. The release kinetics was fitted into various models using PCP dissolution v2.08 software.

In vivo evaluation

In vivo evaluation studies of the F3 and pure drug were carried out on normal healthy male albino rats selected with average body weight of about 300-350 gm. They were housed individually in polypropylene cages, maintained under standard conditions (12 h light and 12-h dark cycle; 27±2°C; 50±10% relative humidity); the animals were fed with standard rat pellet diet and water with glucose. Ethical clearance for the handling of experimental animals was obtained from the institutional animal ethical committee (IAEC) constituted for the purpose. Non-insulin dependent diabetes mellitus (NIDDM) was induced in overnight fasted animals by a single intraperitoneal injection of Alloxan. It was administered intra peritoneally at the dose of 120 mg/kg for all group animals except the group I animals, which were served as control. The blood glucose level was determined after 72 h of Alloxan administration. The blood glucose level was determined using Glucometer. The animals having blood glucose level more than 187 mg/dl were chosen for the experiment. All the animals showed hyperglycemia after 72 h of Alloxan administration. Only the rats found with permanent NIDDM were used for *in vivo* evaluation studies. For the control (group I & II), the fasting was done overnight and water with glucose was allowed. For group 3 and group 4, pure drug and hollow microspheres were administered orally with oral gauss in the morning following overnight fasting. No food and liquid except water with glucose were given to the animals during the experiment. After collection of zero-hour blood sample, F3 was administered orally through oral gauss. Blood sample was taken by pricking from the tail vein of the rat at every 1 h interval. Plasma glucose levels were determined using one touch ACCU-Chek Active® (16).

Accelerated stability studies

Drug decomposition or degradation occurs during storage, because of chemical alteration of the active ingredients or due to product instability, leading to lower concentration of the drug in the dosage form, hence the stability of pharmaceutical preparation need to be evaluated. The objective of stability studies is to predict the shelf life of a product by accelerating the rate of decomposition, preferably by increasing the temperature and relative humidity (RH) conditions. Stability studies were carried out at 40±2 °C and 75± 5% relative humidity for 90 days.

RESULTS AND DISCUSSION

The hollow microspheres were prepared by modified Quasi-emulsion diffusion technique. In this process, the diffusion of ethanol precedes the evaporation of dichloromethane from the droplet in to the aqueous medium drastically reducing the solubility of polymer in the droplet and forming a gel like film on the surface. The mechanically strong solidified film produced at the surface of droplet with further depletion of ethanol prevented rupture and shrinkage of microspheres during the evaporation of dichloromethane from the droplets. The cavity produced by gas phase was gradually filled with water due to the reduced pressure inside the droplet that was caused by evaporation of dichloromethane. The hollow microsphere was prepared by removing water from the cavity of the microsphere with air drying. The presence of tween 80 prevents aggregation of droplets, as it acts as an emulsifying agent. It was assumed that it gets adsorbed at the interface between droplets and the aqueous medium. It was observed that sudden addition of polymers solution in to the water containing Tween 80 leads to the formation of large polymer precipitates, which causes decrease the percentage of microspheres yield.

The efficiency of drug entrapment into hollow microspheres could be ascribed to the distribution coefficient between water and dichloromethane under the preparation conditions.

The sphericity of the prepared microspheres was confirmed and the calculated values were nearer to 1. The sphericity factors calculated for the microspheres are presented in Table 2 and shown in Figure 2.

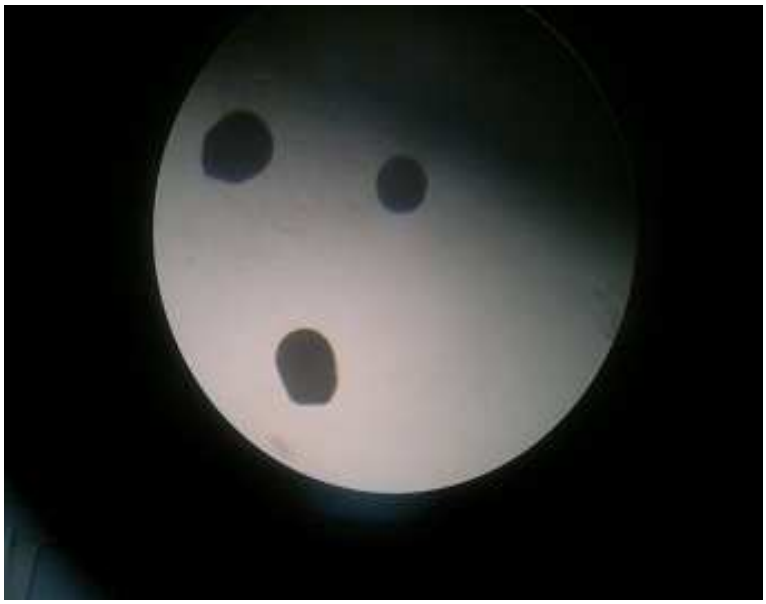


Fig 2: image using camera lucida showing sphericity of hollow microspheres at 10X magnification.

The sphericity factors for all formulations were in the range 1.01±0.2 to 1.29±0.6 and the sphericity values of best formulations F3, F7 and F9 were 1.05±0.2, 1.07±0.1 and 1.16±0.1 respectively. The sphericity value nearer to 1 indicates that the prepared formulations were spherical in nature. A similar sphericity factor calculated for indomethacin pellets was reported by Desay *et al* (17). The particle size range between 223±2.6 to 446±5.2 and the mean particle size of the F3 microspheres was 312± 4.1. The microspheres prepared with ethyl cellulose, HPMC and eudragit showed higher particle size as compared with ethyl cellulose and polyethylene oxide combination ($p<0.05$). The mean particle size of the microspheres

significantly increased with increase in polymer concentration was reported by Kamila *et al* (18). Larger the particle were produced due to the rapid polymer precipitation, leading to hardening and avoiding further particle size reduction during solvent evaporation. Another possibility is that, by rapidly removing the solvent, the inward shrinking of the polymer could be avoided: this can be achieved by slowly removing the solvent (13). Particle size distribution curve for F3 is shown in Figure 3 and the average particle size of the formulations was shown in the Table 2.

Table 2: characterization or micromeritic properties of hollow microspheres

Formulation Code	Mean size (µm) *	Angle of repose	%Compressibility Index *	Tapped density (gm/cm^3) *	Sphericity
F1	257±5.7	25±0.7	20.8±1.1	0.201±1.03	1.01±0.2
F2	306±2.3	28±1.2	16.2±1.6	0.197±1.2	1.03±0.7
F3	312±4.1	28±0.9	13.7±1.1	0.225±0.9	1.05±0.2
F4	308±3.7	28±2.1	18.6±2.0	0.166±1.3	1.15±1.3
F5	223±2.6	24±1.4	23.9±0.9	0.138±0.7	1.24±0.9
F6	334±3.4	29±1.1	26.1±1.5	0.210±1.3	1.08±0.5
F7	446±5.2	28±2.0	21.9±1.1	0.141±1.1	1.07±0.1
F8	347±4.1	28±1.5	25.8±1.3	0.228±1.12	1.14±0.9
F9	393±1.9	27±1.1	18.8±2.4	0.154±1.3	1.16±0.1
F10	377±2.8	26±2.3	21.7±1.7	0.174±1.0	1.29±0.6
F11	302±1.7	26±1.9	20.8±0.7	0.281±0.9	1.15±0.4

**mean ±SD, n = 3*

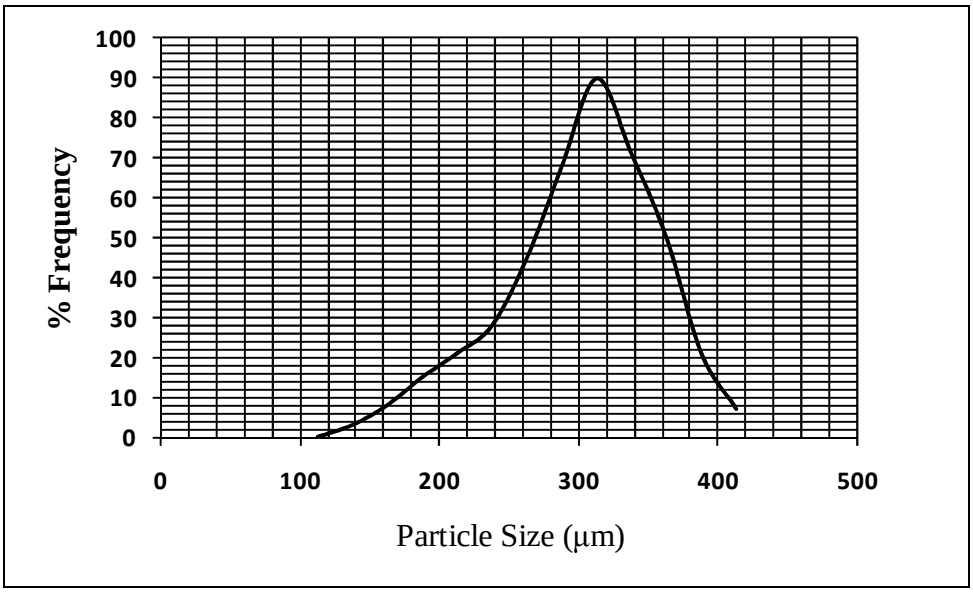


Fig 3: particle size distribution curve for F3 (MEAN±S.D, N=3)

Percentage yield and encapsulation efficiency

During the process of microencapsulation, the mechanical variables cause loss of final product and hence process yield may not be 100 %. Hollow microspheres were weighed after drying and the percentage yield was calculated. The test for drug content was carried out to ascertain that the drug is uniformly loaded in the formulation. Relatively high encapsulation efficiency was observed for all microsphere formulations. The encapsulation efficiency ranged between 53±2.2 % and 89±1.9 %. It was found that the encapsulation efficiency increased with increasing amount of polymers in the hollow microspheres ($p<0.05$). Formulation F3, F7, F9 showed the relatively higher encapsulation efficiency as these formulations composed of high concentration of polymer. When 1:10 & 1.20 (w/w) drug/polymer concentrations were used the quality of hollow microspheres formed was poor. These were irregularly shaped, not free flowing, and presented with lots of indentation. Hollow microspheres were only formed when the polymer concentration was increased to ratios of 1:30 and above (w/w) with respect to the drug concentration. As the amount of ethyl cellulose was increased the formulation specifications were also better. During the process of microencapsulation, the mechanical variables caused loss of final product and hence process yield was not 100%. Among all formulations, F3, F7 and F9 showed maximum percentage yield and drug loading. The results obtained are given in Table 3.

Table 3: percentage yield, encapsulation efficiency and floating capability of hollow microspheres of RSM

Formulation Code	% Yield ± SD*	(%) Floating capability *	Encapsulation efficiency (%)
F1	56.5±0.2	83±1.2	53±2.2
F2	69.2±2.1	72±1.4	72±2.1
F3	94.3±0.9	82±1.9	89±1.9
F4	88.3±1.8	76±0.9	69±1.4
F5	58.6±1.3	78±0.7	58±1.3
F6	54.5±1.2	70±1.1	56±2.2
F7	72.3±1.4	80±1.0	71±1.4
F8	78.1±2.2	84±1.2	74±2.1
F9	85.4±1.7	82±0.4	89±1.3
F10	67.6±2.1	76±1.3	65±2.3
F11	79.0±0.9	65±0.9	88±1.7

**mean ± SD, n = 3*

Drug-Excipients Compatibility Studies

FT-IR analysis

Rosiglitazone maleate pure drug and the F3 subjected for FT-IR spectroscopic analysis for compatibility studies and to ascertain whether there is any interaction between the drug and the polymers used. The IR spectra of rosiglitazone maleate and drug-loaded hollow microspheres were found to be identical. The characteristic IR absorption peaks of rosiglitazone maleate at C=N (1608 cm^{-1}), Ar-C-H (3030 cm^{-1}), aliphatic C-H (2980 cm^{-1}) C=O stretching at 1703 cm^{-1} NH (3220 cm^{-1}) C-O (1246 cm^{-1}), C-S (773 cm^{-1}) & C-N(1260 cm^{-1}) were present in drug-loaded hollow microspheres. The FT-IR spectra of the pure drug and formulation F3 indicated that characteristics peaks of rosiglitazone maleate were not altered without any change in their position after successful entrapment in the hollow microspheres, indicating no chemical interactions between the drug and carriers used. FT-IR spectra of the hollow microspheres showed all the rosiglitazone maleate characteristics absorption bands suggesting the absence of interactions between the drug and the other components of the formulations. These results indicate the method used to prepare hollow microspheres does not affect the physicochemical properties of the systems (18).

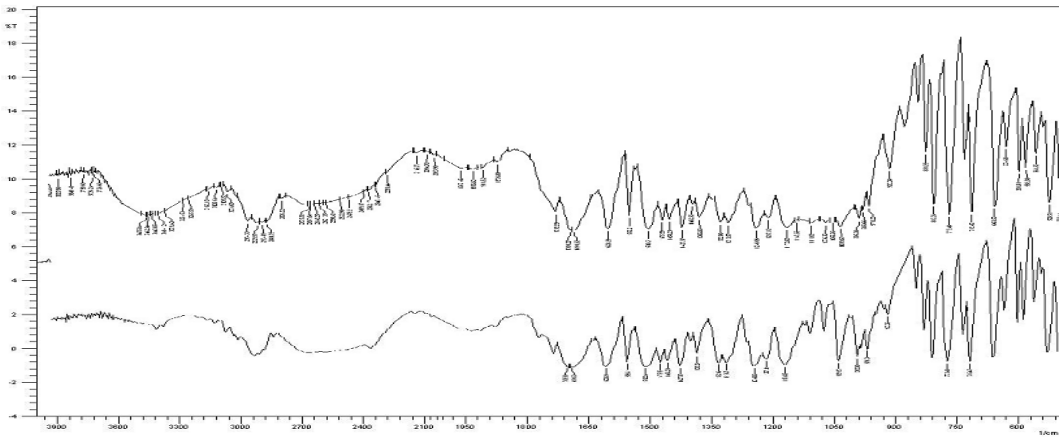


Fig 4: FT-IR spectra of pure drug and formulation

Differential Scanning Calorimetry (DSC)

DSC is very useful in the investigation of the thermal properties of hollow microspheres, providing both qualitative and quantitative information about the physicochemical state of drug inside the hollow microspheres. There is no detectable endotherm if the drug is present in a molecular dispersion or solid solution state in the polymeric hollow microspheres loaded with drug. In the present investigation, DSC thermograms of pure drug, drug loaded hollow microspheres (formulation F3, F7 & F9) were taken as shown in Figures 3-5. The thermal properties of the drug and the mixture of the drug and polymers are of important interest since this can help to ascertain the crystalline and amorphous status of the entrapped drug in the polymers to assess the interaction among different components of the formulation during the fabrication process (18).

The DSC thermogram of pure rosiglitazone Maleate showed a sharp melting endothermic at temperature 153.96^{o}C. This melting endotherm was also observed for rosiglitazone Maleate - loaded hollow microspheres (F3, F7 & F9) at 67.24^{o}C, 150.9^{o}C & 152.7^{o}C indicating absence of drug and polymer interactions showed a relatively flat thermal profile indicative of the amorphous nature of the polymer.

None of the peaks of RSM could be detected in the thermogram of drug loaded hollow microspheres thus neglecting the possibilities of the presence of crystalline drug in the formulation. A peak, detected at 67.24^{o} C, 150.9^{o} C & 152.7^{o} C, confirmed molecular dispersion of RSM in the formulation which is characterized by a single T_m that shift between those of pure drug and polymer as a function of drug to polymer ratio in the mixture.

Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) revealed the discrete, spherical shaped spheres with rough surface and presence of holes /hollow cavity due to the collapse of the wall of the microspheres during in situ drying process. Thus the rate of solvent removal from the embryonic microspheres exerts an influence on the morphology of the end product. Porous structure was observed on the surface of microspheres shell due to the rapid diffusion of the solvent, there is a possibility of rupture of some microspheres. Microspheres floated more than 12 h because of presence of hollow cavity. SEM photographs were shown in figure 8

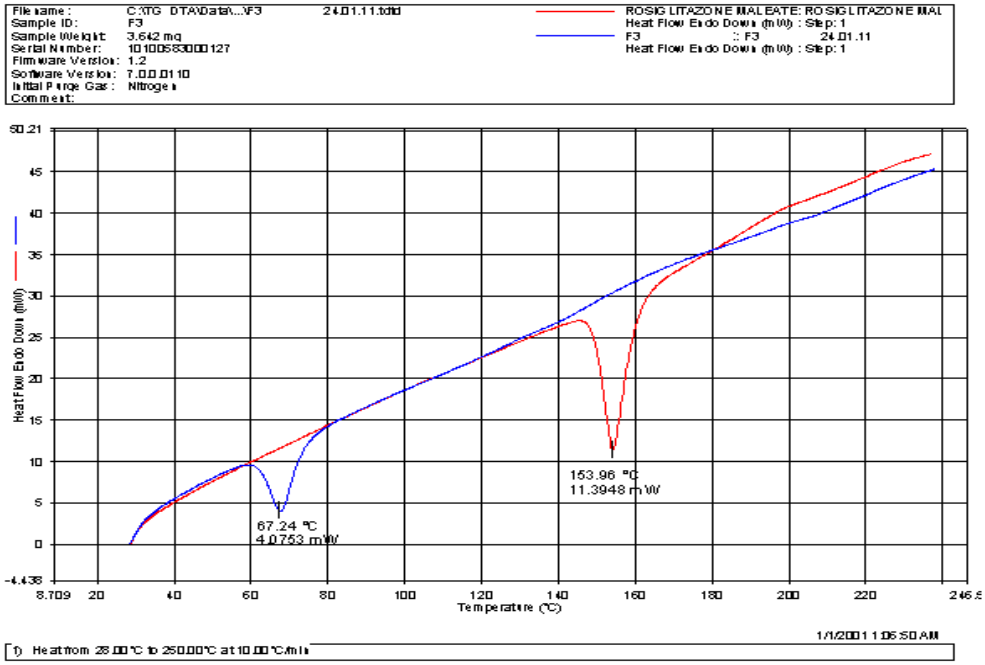


Fig 5: DSC curves of pure RSM and RSM loaded hollow microspheres F3

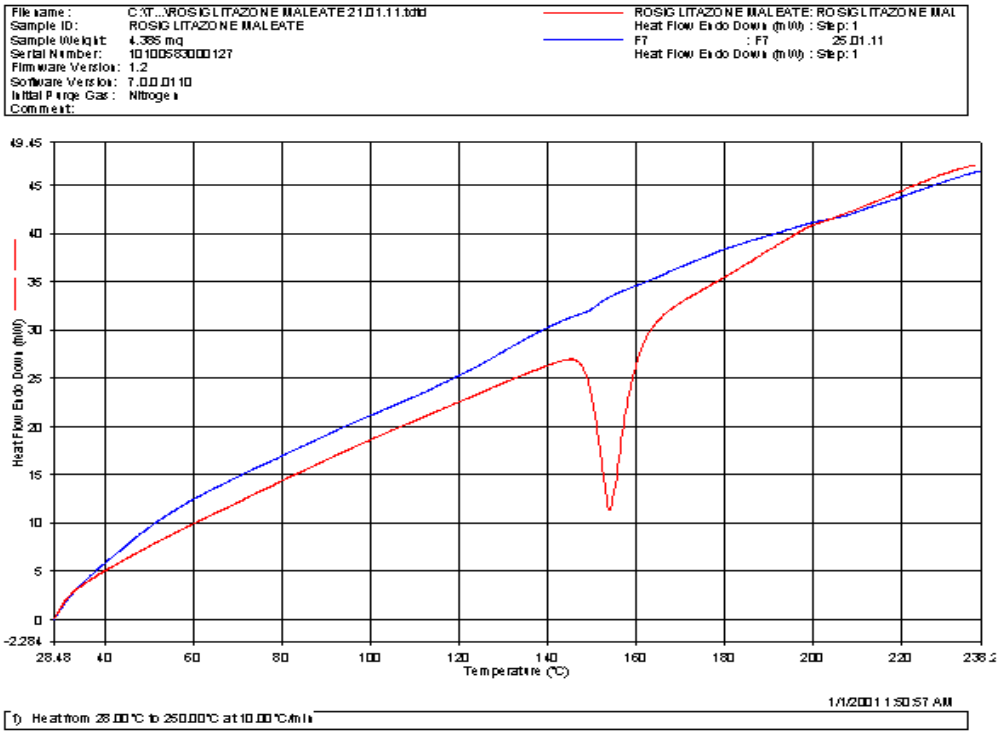


Fig 6: DSC curves of pure RSM and RSM loaded hollow microspheres F7

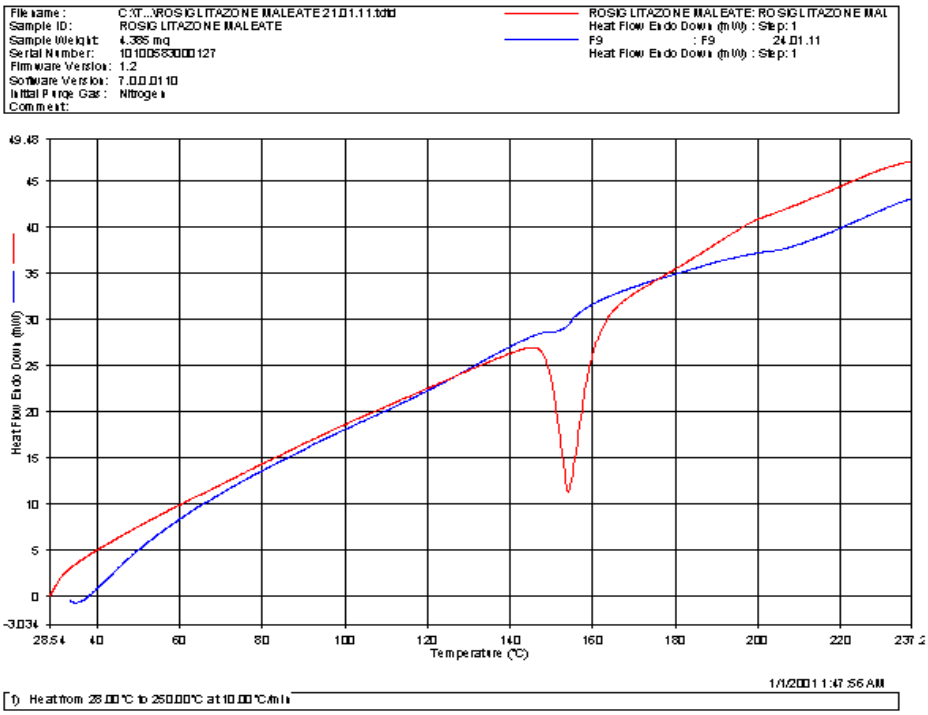


Fig 7: DSC curves of pure RSM and RSM loaded hollow microspheres F9

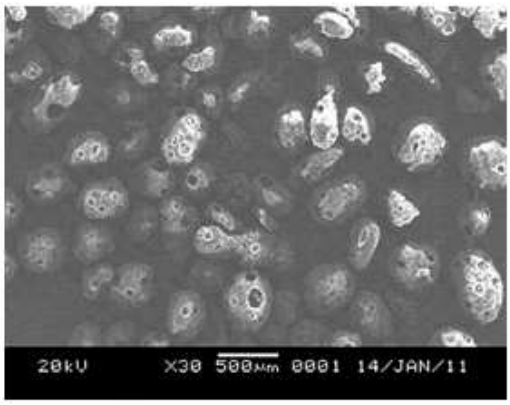


Figure- 8(a)

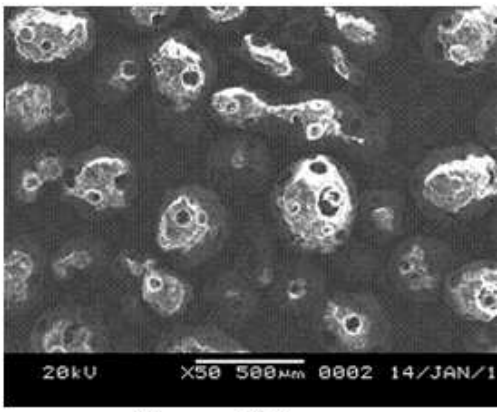


Figure- 8(b)

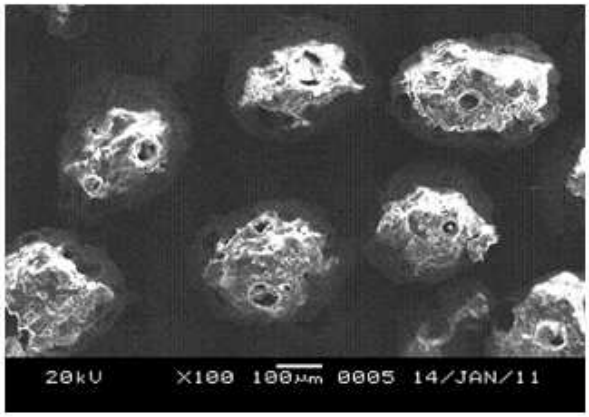


Figure-8(c)

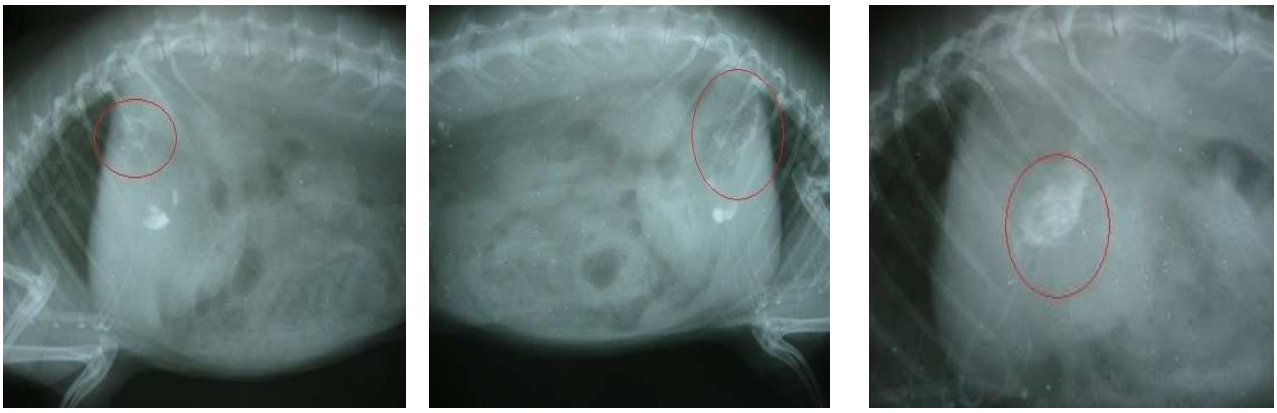
Fig 8: SEM pictures of hollow microspheres formulations (A) F9, (B) F7 AND (C) F3.

In vitro buoyancy

To assess the floating properties, the microspheres were placed in 0.1 N HCl containing 0.02 % v/v tween 80 as surfactant to simulate gastric condition. Use of 0.02 % v/v tween 80 was to account for the wetting effect of natural surface active agents, such as phospholipids in the gastrointestinal tract. Despite the solution being stirred for more than 8 h, the hollow microspheres still floated indicating that microspheres exhibit excellent buoyancy effect. The density of values of hollow microspheres (< 1.000 g/cm^3) was less than that of the gastric fluid (< 1.004 g/cm^3) further supporting floating nature. The *in vitro* floating test was conducted on the microspheres (13). In all showed excellent floating capability about 70 ± 1.1 %. All the formulation showed buoyancy of more than 8 h. Ethyl cellulose played an important role as concentration of ethyl cellulose increased buoyancy increased. The floating behaviour was controlled by the increasing the concentration of ethyl cellulose (7 cps) in different formulations and getting a better floatability as compared to other combination. Among all formulations, F1, F3, F7 and F9 showed maximum percentage floating ability.

***In vivo* buoyancy**

The *in vivo* floating behavior of ethyl cellulose, polyethylene oxide (2:1) as in formulation (F3) hollow microspheres loaded with barium sulphate was investigated by radiographic images (X-ray photographs) of rabbit's stomach at specific periods. The amount of X-ray opaque material in these hollow was sufficient to ensure visibility by X-ray but at same time the amount of barium sulphate (100 mg) was low enough to enable the hollow microspheres to float. The hollow microspheres did not adhere to the gastric mucous and floating on the gastric fluid for about more the 12 h. This was evident by the X-ray photographs taken at 4 h, 8 h & 12 h. It is shown in fig 9 (A, B and C respectively).



(a) at 4 h **(b) at 8 h** **(c) at 12 h**

Fig 9: X-RAY photographs showing floating ability of hollow microspheres.

In vitro drug release

Solubility of RSM depends on pH. RSM is a drug easily absorbed in the stomach. Maximum absorption may be expected with increasing solubility in acid environment. Hence the floating form was developed. It was assumed that better solubility of RSM in an acidic environment of the stomach may result in a greater amount of the drug absorbed and its greater concentration in plasma. It is known that microspheres constitute multiple-unit dosage forms which have many advantages as compared to tablets. They spread more evenly in the stomach which leads to a decreased risk of high local concentration and of adverse effects. Moreover, these forms are

characterized by a high reproducibility of release due to a relatively large surface and a short diffusion way of the drug. The *in vitro* release profiles of formulations are presented in Fig 10 and 11. Due their floating nature, the microspheres were forcibly immersed into the dissolution medium to avoid adherence to the surface of the jar, thus leading to nonparticipation in the dissolution process. The drug release was extended to 12 h. The F3, F4 and F11 showed initial burst release. This is attributed to the release of drug from the surface of microspheres as the drug might have migrated to the surface along with water during the drying process or presence of uncovered drug crystals on the surface of the microspheres. After 1 h, drug released slowly. In case of other formulations burst release was not observed probably due to drug was sufficiently encapsulated in the shell (13). *In vitro* dissolution studies of RSM from floating hollow microspheres were carried out for all formulations in pH 1.2 hydrochloric acid buffer for 12 h using electrolab dissolution test apparatus II. It was found that formulations F1, F2, F3, F4, F5, F6, F7, F8, F9, and F10 & F11 showed 43.0% - 80.76% of release at 8 hour and 75.3% - 99 % of release at 12 h ($p<0.05$). Microspheres prepared with ethyl cellulose and HPMC, (F5, F6 and F7) showed less release compared to other combination. This was probably due to gelation property of HPMC, which forms gel matrix after contact with dissolution medium.

F1 and F11showed more than 99 % drug release at the end of 12 h, which may be due to low polymer concentration and smaller the particle size with large surface area were produced and hence drug release.

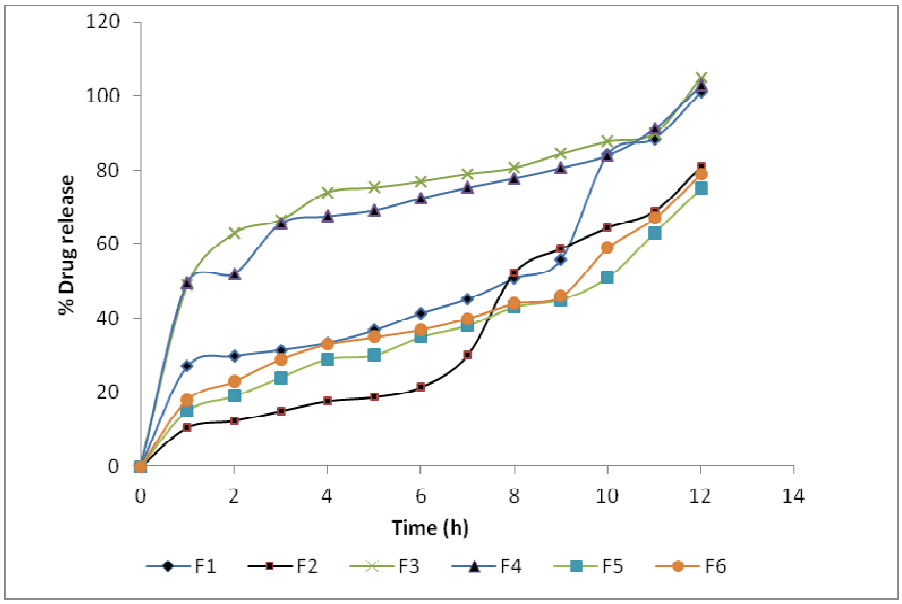


Fig 10: *In vitro* drug release profile of hollow microsphere formulations of F1-F6 (MEAN ± SD, N = 3)

The combination of water soluble polymer and a controls release polymer in the formulation F3 produced maximum sustain release. The *in vitro* release showed the maximum release of more than 101 % in 12 h**.** Among the formulated hollow microspheres, those prepared from blend of polymer showed better release. In 6 to 8 hours, 43 % to 80 % of drug was released in 0.1N HCl buffer. It was observed that as the concentration of ethyl cellulose increased, the % cumulative release of RSM increased.

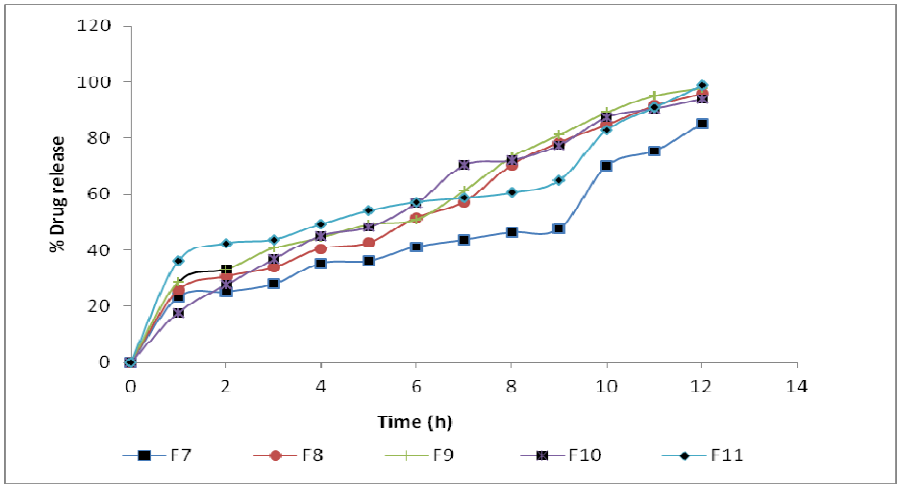


Fig 11: *In vitro* drug release profile of hollow microsphere formulations of F7-F11 (MEAN ± SD, N = 3)

Table 4: Data of various parameters of model fitting of RSM-hollow microsphere

Formulation Code	Zero Order		Matrix		Peppas		Hix Crow		n	Model Fitting
	R	K	R	K	R	K	R	K		
F1	0.9119	7.4852	0.9001	20.6745	0.8655	21.0374	0.8852	0.0360	0.4652	Zero order
F2	0.9580	6.0171	0.8414	16.6972	0.9167	6.6850	0.9235	-0.0273	0.8953	Zero order
F3	0.7415	-0.0726	0.8856	30.6762	0.9870	51.2435	0.7237	-0.0566	0.2323	Peppas
F4	0.7537	-0.0680	0.9137	29.3030	0.9756	47.5736	0.7993	-0.0529	0.2457	Peppas
F5	0.7379	-0.0819	0.9087	31.1171	0.9424	49.6149	0.7415	-0.0726	0.2638	Peppas
F6	0.9329	7.7974	0.8810	22.2338	0.8661	19.7600	0.7093	-0.0540	0.5262	Zero order
F7	0.9356	6.7487	0.9269	19.4473	0.9191	18.1946	0.9272	-0.0313	0.5134	Zero order
F8	0.9659	8.5748	0.9621	24.7344	0.9585	20.6041	0.9674	-0.0467	0.5763	Hix crow
F9	0.9440	9.1599	0.9690	25.4820	0.9576	24.1214	9588	-0.0488	0.5161	Matrix
F10	0.9645	8.8042	0.9769	25.4800	0.9963	17.3033	0.9885	-0.0475	0.6874	Peppas
F11	0.7606	9.1837	0.9214	24.8622	0.8861	32.9603	0.7806	-0.0490	0.3348	Matrix

Mathematical model fitting of obtained drug release data

The *in vitro* release studies data was fitted into various mathematical models to determine the best-fit model. The results indicated that, the best-fit model was found to be the data is given in Table 4. As per the literature reviews we can knew that low water soluble drug the self-erosion of the matrix will be the principal model. Zero order kinetics was followed by the following formulation F1, F2, F7 & F6 as in the case of hollow microspheres with low soluble drug following the profile release the same amount of drug by unit of time and it is the ideal method of drug release in order to achieve a pharmacological action. Peppas model was followed by the following formulation F3, F4, F5& F10 as in the case of hollow microspheres shows diffusion from a controlled release polymeric system that release occurs in one-dimensional way and that the system width-thickness or length-thickness relation in release. This model is generally used to analyze the release polymeric dosage forms or more than one type of release phenomena could be involved. The formulation F3, F4 & F5 shows the fickian diffusion and only the F10 shows anomalous transport. Matrix (Higuchi) was followed by the following formulation F9 & F11 as in the case hollow microspheres drug release from spherical homogeneous matrix system & planar or spherical system having granular matrix. The drug concentration in the matrix is lower than its solubility & the release occurs through pores in the matrix. Hixon Crowel was followed by the following formulation F8 as in the case hollow microspheres shape factors for cubic or spherical particles should be kept constant if the particles dissolve in an equal manner by all side.

In vivo evaluation

In vivo evaluation of the hollow microspheres were carried out in healthy male albino rat by measuring blood glucose level after oral administration with F3 microspheres equivalent to the dose of the drug, 4-mg/kg body weight in comparison with administration of pure drug at same dose. The antihyperglycemic effect of formulation and pure drug in diabetic rats time intervals is represented in Figure 12. When rosiglitazone maleate filled in capsule was given orally, the blood glucose level started to decrease from the second hour. After the sixth hour, blood glucose level reached to almost normal level but after the seventh hour blood glucose level started to increase again. On the contrary, the selected formulation (F3) of RSM blood glucose level started to decrease from the third hour and this decrease continued up to the ninth hour until blood glucose reached to normal level. This was maintained up to the 13th hour and blood glucose was found to be 88.9 mg/dL. The lowering of blood glucose level was slower, as expected; in case of RSM hollow microspheres than pure RSM due to its higher dissolution rate of pure drug in gastric fluid of the rats. (18).

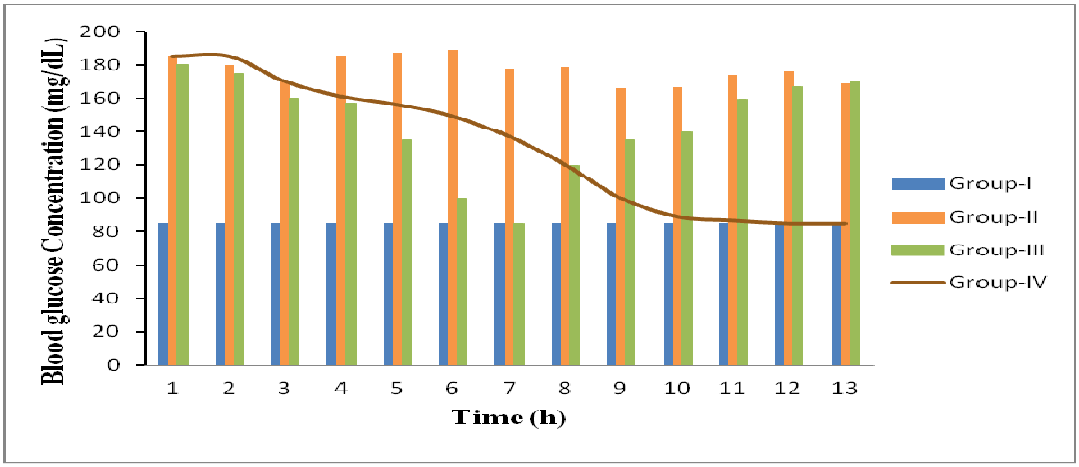


Fig 12: Comparison of *in vivo* plasma glucose level in alloxan-induced diabetic albino rat following oral administration of pure drug (group III) and rosiglitazone hollow microsphere F3 (group IV) with plasma glucose level of normal rat (group I) and alloxan- induced diabetic rat without drug (group II) (mean±SD, N = 3)

Table 5: stability study for drug content of RSM-hollow microspheres

Stability condition	Sampling (days)	Drug content (%)
40 ºC/75% RH	0	89.98± 2.4
	7	89.90 ± 1.9
	15	89.88± 2.1
	30	89.43 ± 3.0
	60	89.31± 2.4
	90	89.27± 1.8

***mean ± SD, n = 3**

Accelerated Stability studies

The objective of stability studies is to predict the shelf life of a product by accelerating the rate of decomposition, preferably by increasing the temperature and RH. The RSM containing selected formulation F3 was subjected to stability studies carried out by storing at 40ºC/75% RH for 3

months (climatic zone IV condition for accelerated testing) to assess their stability . These samples were analyzed and checked for changes in physical appearance and drug content at regular intervals. The obtained data is presented in Table 5. From the table, it is clear that the formulation did not undergo any chemical changes/interaction during the study period.

CONCLUSION

Hollow microspheres of rosiglitazone maleate having a spherical structure were prepared successfully in a single step using modified quasi emulsion solvent diffusion technique. The microspheres of the batch (F3) exhibited 89±1.9 % drug entrapment efficiency, mean particle size of 312±4.1µm and 80% release in 8 h and 82% floating ability. *In vivo* floatation behaviour confirmed by taking the X-ray by at 4 h, 8 h & 12 h. The *in vitro* release showed the F3 formulation was maintain up to the 12^{th} h and blood glucose was found to be 88.9 mg/dL. Results of the stability studies showed that there were no significant changes in the drug content and physical appearance. It may be concluded that dosage form can control the release, avoid dose dumping, and extend the duration of action of a drug with prolonged floating time.

Acknowledgement
The authors are highly thankful to Dr. H.G. Shivakumar, Principal, JSS College of Pharmacy, Mysore. The authors are grateful to Strides Arco Lab Pvt. Ltd India for providing the necessary facility for obtaining DSC of the samples.

REFERNCES

[1] Cox PJ, Ryan DA, Hollis FJ, Harris AM, Miller AK, Vousden M, *Drug Metab. Dispos*., **2000**, 28, 772–780.
[2] Wagstaff AJ, Goa KL, *Drugs*, **2002**, 62, 1805–3187.
[3] Idris I, Gray S, Donnelly R, *Diabetologia*, **2003**, 46, 288–290.
[4] NK.Jain, Progress in Controlled and Novel Drug Delivery System, CBS Publisher; New Delhi, **2004**,1,76.
[5] SP.Vyas, RK .Khar, Controlled drug delivery: concepts and advances, Vallabh Prakashan; Delhi, **2002**, 1, 156-157.
[6] Rao MRP, Borate SG, Thanki KC, Ranpise AA, Parikh GN, *Drug Dev. Ind. Pharm*., **2009**, 35, 7, 834-842.
[7] Gangadharappa HV, Pramod Kumar TM, Shivakumar HG, *Ind. J. Pharm. Res. Edu*., **2007**, 41, 295-305.
[8] Chaurasia H, Jaina K, Prajapatis K,Chaurasia D, Gupta R, Arya R, *Ind. Pharm.*, **2007**, 6, 65, 101-103.
[9] Iannuccelli V, Coppi G, Bernabei MT, Cameroni R, *Int. J. Pharm*., **1998**, 174, 47–54.
[10] Perumal D, Dangor CM, Alcock RS, Hurbons N, Moopanar KR, *J. Microencap*., **1996**, 16, 475-487.
[11] Kahatani AA, Sherigara BS, *J. Mater. Sci. Mater. Med*., **2009**, 20, 1437-1445.
[12] Yuveraj S, Tanwar SN, Garima Rani Ojha , *Rev. Bras. Cienc. Farm*, **2007**,43, 4, 40- 47.
[13] Kumaresh SS, Anandrao RK, Tejraj MA, *Drug Dev. Ind. Pharm*., **2001**, 27, 6, 507–515.
[14] Martin A, Bustamante P, Chum AHC, Physical Pharmacy, BI Waverly; New Delhi, Pvt.Ltd, **1996**, 423-452.
[15] Joseph NJ, Lakshmi S, Jayakrishnan A, *Acta Pharm*., **2005**, 55, 277-285.
[16] Whitehead L, Fell JT, Collet JH, Sharma HL, Smith AM, *J. Control Release*., **1998**, 55, 3-12.
[17] Deasy PB, Law MF, *Int. J. Pharm*., **1997**, 148, 201-209.

[18] Madan MK, Nita M, Lakshmi KG, Bijan KG, *AAPS Pharm. Sci. Tech*., **2009**, 10, 3, 887-899.