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Design, development and evaluation of hollow microspheres of Repaglinide

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ABSTRACT

The objective of the study was to prepare hollow microsphere as a new dosage form of floating drug delivery system with prolonged stomach retention time. Hollow microspheres containing Repaglinide were prepared by emulsion diffusion method using Eudragit S100 and Eudragit L100 as release retardant polymer. The prepared microspheres were evaluated for particle size, % drug loading, in-vitro buoyancy and in-vitro drug release, drug-excipients compatibility studies by FT-IR and surface morphology by SEM. The FT-IR studies showed compatibility of drug with the excipients used in the formulations. The particle sizes of the microspheres were ranges between192.2 ±12.2 to 321.8 ±8.3 µm. The developed microspheres showed good in-vitro buoyancy, high entrapment efficiency and drug loading with sustained release of drug for 9h. The mechanism of drug release followed Higuchi kinetic model with non-Fickian diffusion, the SEM studies showed smooth surface and shape of the hollow microspheres. The developed formulation of Repaglinide in the form of floating microsphere has a great potential for therapy of diabetes type-II in future.

Keywords: Repaglinide, floating drug delivery systems (FDDS), hollow microspheres, hypoglycaemic agent, in-vitro buoyancy

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INTRODUCTION

Drugs with short half-life are eliminated quickly from blood circulation, therefore require frequent dosing and reduce patient compliance. Oral controlled release (CR) formulations came into existence to overcome this problem. CR formulations attempt to maintain a constant plasma level of drug by controlling drug release from the formulation. A significant obstacle for these systems is the Gastric Retention Time (GRT), which is a physiological limitation. Hence, prolongation of GRT is very important for drugs with short half-life and poor bioavailability. GRT can be prolonged by increasing gastric retention of the formulation. There are several approaches used by researchers to prolong GRT, which include: (i) Mucoadhesive delivery systems, in which formulation adheres to the mucosal surface of GIT; (ii) Swellable delivery systems, which swells after administration and prevents it pass through pylorus; and (iii) density controlled delivery systems, in which formulation either sink in or float over the gastric fluid [1].

Floating systems are recommended particularly for drugs which are primarily absorbed in the stomach and for drugs acting locally on the stomach. Also, if the drug have a narrow window of absorption or is unstable at intestinal pH, floating approach can be used. Floating can be achieved by inclusion of highly porous systems, by inclusion of swellable agents or agents which generates CO_2 to create and maintain buoyancy [2].

Repaglinide, S(+)2-ethoxy-4(2((3-methyl-1-(2-(1-piperidinyl) phenyl)butyl) amino)-2-oxoethyl) benzoic acid, is a potent second generation oral hypoglycemic agent widely used in treatment of non-insulin dependent diabetic mellitus. It is a fast and short-acting meglitinide analog was chosen as the drug candidate because of very short biological half-life of 1hour, low bioavailability (50%) and poor absorption in the upper intestinal tract; hence it is indicated for the development of a dosage form with increased gastric residence time [3]. The objective of present work was to formulate prolonged-release floating microspheres of Repaglinide and to evaluate the prepared formulation for various parameters to optimize the formula.

EXPERIMENTAL SECTION

Materials

Repaglinide was provided as a gift sample from Torrent Pharmaceuticals, Eudragit S 100 and Eudragit L 100 were purchased from Evonik Degussa Ltd., Mumbai, Dicloromethane was purchased from SD fine chemicals, all other excipients are of analytical grade.

Drug–excipient compatibility study by FT-IR spectroscopy [4]

Repaglinide and different grades of Eudragits were subjected to compatibility studies. The drug and polymer were mixed physically in 1:1 ratio and FT-IR spectra were recorded on a Bruker spectrophotometer (Model-220, Germany) in the range of 4000-400 cm^{-1}.

Preparation of Hollow Microspheres

Hollow microspheres were prepared by the emulsion diffusion method established by Kawashima *et al*., [5] as per the composition given in Table 1. Weighed amount of Repaglinide was mixed with polymers (Eudragit S 100 & L 100) in different ratios (1:1, 1:2 and 1:3) in a mixture of Dichloromethane and Ethanol (1:1) at room temperature. Glycerol monostearate was used as emulsifying agent. The drug-polymer solution thus prepared was then poured into an aqueous solution of Polyvinyl Alcohol (0.75% w/v, 200 ml) maintained at constant temperature of 40°C, forming an oil-in-water (o/w) type emulsion. The resultant emulsion was stirred, employing a propeller type agitator at 300 rpm. The finely dispersed droplets of the polymer solution of drug were solidified in the aqueous phase via diffusion of the solvent. After agitating the system for 1 h, the resulting polymeric particulate systems were sieved between 500 and 1000 µm (between Sieve No. 35 and 18) and dried overnight at 40°C to produce hollow microspheres.

Table 1: Composition of hollow microsphere

Ingredients	Batch codes					
	S1	**S2**	**S3**	**L1**	**L2**	**L3**
Repaglinide (mg)	10	10	10	10	10	10
Eudragit S 100 (mg)	10	20	30	-	-	-
Eudragit L 100 (mg)	-	-	-	10	20	30
Glycerol monostearate (ml)	5	5	5	5	5	5
DCM: Ethanol mixture (1:1) (ml)	15-20	15-20	15-20	15-20	15-20	15-20
PVA (0.75% w/v)	200	200	200	200	200	200

Evaluation of prepared hollow microspheres

Surface morphology [6, 7]

Hollow microspheres were observed using a scanning electron microphotograph (SEM). The samples were coated with gold and scanned randomly and photomicrographs were taken with a scanning electron microscope.

Particle size [8]

Particle size analysis of drug-loaded microspheres was performed by optical microscopy using a compound microscope (Erma, Tokyo, Japan). A small amount of dry microspheres was suspended in n-hexane (10 mL). The suspension was ultra-sonicated for 5 seconds. A small drop of suspension thus obtained was placed on a clean glass slide. The slide containing microspheres was mounted on the stage of the microscope and 300 particles were measured using a calibrated ocular micrometer. The average particle size was determined by using the Edmondson's equation $D_{mean} = \Sigma nd/\Sigma n$, where n= number of microspheres observed and d= mean size range. The process was repeated 3 times for each batch prepared.

***In-vitro* Buoyancy [9, 10]**
300 milligrams of the microspheres were placed in 900 ml of Simulated Gastric Fluid (SGF) IP. The mixture was stirred at 100 rpm in a dissolution apparatus for 8 h. After 8 h, the layer of buoyant microspheres was pipetted and separated by filtration. Particles in the sinking particulate layer were separated by filtration. Particles of both types were dried in desiccators until constant weight. Both the fractions of microspheres were weighed and buoyancy was determined by the weight ratio of floating particles to the sum of floating and sinking particles.

% Buoyancy = $[W_f / W_f + W_s)] \times 100$

Where W_f and W_s are the weights of the floating and settled microspheres respectively and all the determinations were made in triplicate.

Drug loading and Drug entrapment efficiency [11]
50 mg of dried microspheres containing repaglinide were dissolved in 10 ml of Simulated Gastric Fluid (SGF) followed by sonication for 30 minutes to dissolve the polymer and to extract the drug. The dissolved drug amount was measured spectrophotometrically with a UV detector (UV-160A, Shimadzu, Japan) at 243 nm. Drug content of microspheres was calculated according to following equations:

$$\% \ Drug \ loading = \frac{Weight \ of \ drug \ in \ microspheres}{Weight \ of \ microspheres \ recovered} \ \mathbf{X\ 100}$$

$$\% \ Drug \ entrapment \ efficiency = \frac{Actual \ amount \ of \ Drug \ in \ Microspheres}{Theoretical \ amount \ of \ Drug \ in \ Microspheres} \ \mathbf{X\ 100}$$

***In-vitro* drug release [12, 13]**
The *in-vitro* release rate of repaglinide of from microspheres was determined in a USP XXIII paddle type dissolution apparatus (Labindia dissolution test apparatus USP). A weighed amount of microspheres equivalent to 100 mg of drug was filled into a hard gelatin capsule (#3) and placed in the dissolution apparatus. 900 ml of Simulated Gastric Fluid (SGF) was used as dissolution medium. The dissolution medium was maintained at 37 ± 1 °C at a rotation speed of 100 rpm. Perfect sink conditions prevailed during the drug release study. Ten milliliter (10 ml) samples were withdrawn at each 1 h interval and filtered. The initial volume of the dissolution medium was maintained by replacing 10 ml of fresh dissolution medium after each withdrawal. Samples were analyzed using UV spectrophotometer (Shimadzu UV-1800, Japan) at 243 nm against appropriate blank.

Release kinetics [14-16]
In order to understand the kinetics and mechanism of drug release, the dissolution data of the optimized formulation was fitted with various kinetic equations like zero order (cumulative% release vs. time),first order (log% drug remaining vs. time), Higuchi's model (cumulative% drug release vs. square root of time), Peppas plot (log of cumulative% drug release vs. log time). Moreover, R^2 (coefficient of correlation) values were calculated for the linear curve obtained by regression analysis of the above plots [17].

The equations of different release kinetics are as follows:

Zero order equation:	$Q = Q_0 - K_0 t$;
First order equation:	$\ln Q = \ln Q_0 - K_1 t$;
Higuchi equation:	$Q = K_2 t^{1/2}$;
Korsmeyer-Peppas equation:	$Q/Q_0 = Kt^n$

In the equations, K_0 to K_2were release rate constants, Q/Q_0 was fraction of drug released at time t, K was constant and n was diffusion constant that indicates general operating release mechanism [18].

It is known that the Peppas model is widely used to confirm whether the release mechanism is Fickian diffusion and non-Fickian diffusion. The 'n' (release exponent of Korsmeyer-Peppas model) value could be used to characterize different release mechanisms[19]. The interpretation of n values was done in the following manner:
n<0.5 (0.45) - quasi-Fickian Diffusion
n=0.5 (0.45) - Diffusion mechanism
0.5<n<1 - Anomalous (non-Fickian) Diffusion - both diffusion and relaxation (erosion)

n=1 (0.89) - Case 2 transport (zero order release)
n>1 (0.89) - Super Case 2 transport (relaxation)

RESULTS AND DISCUSSION

Drug-excipient compatibility study

FT-IR spectra of repaglide is shown in (Fig 1) and the following characteristics peaks were obtained at 3307.18 cm^{-1} assigned to N-H stretching vibration, 1685.60 cm^{-1} corresponding to the carbonyl group, 2937.57 cm^{-1} for -CH stretching, 1603$cm^{-1}$for C=C and 1447 cm^{-1} for CH deformation. The above characteristics peaks for are also retained in the physical mixture of the drug and excipients used in the formulation (Fig 2) indicating the absence of interaction.

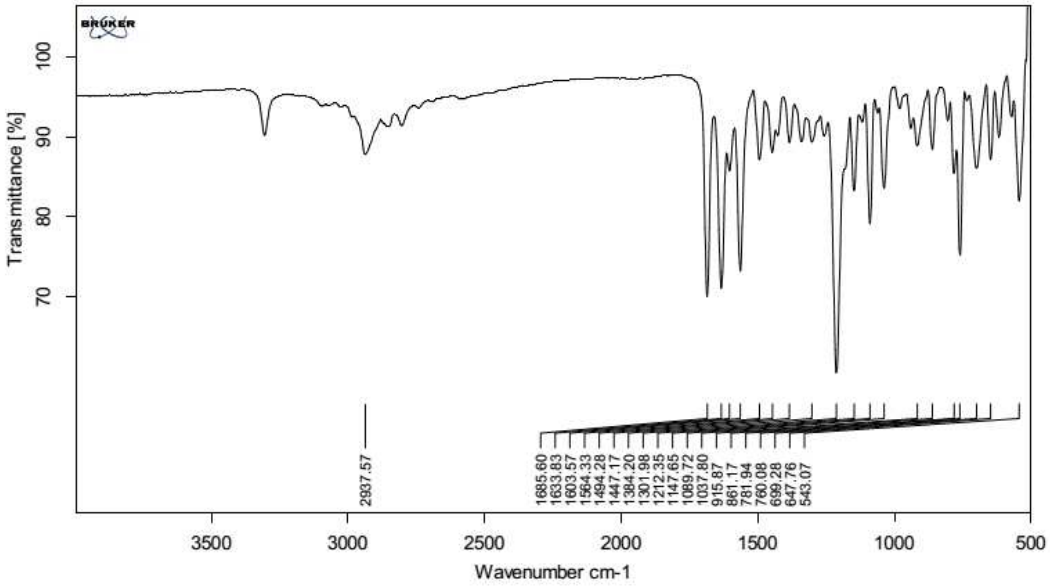

Fig 1: FT-IR spectra of Repaglinide

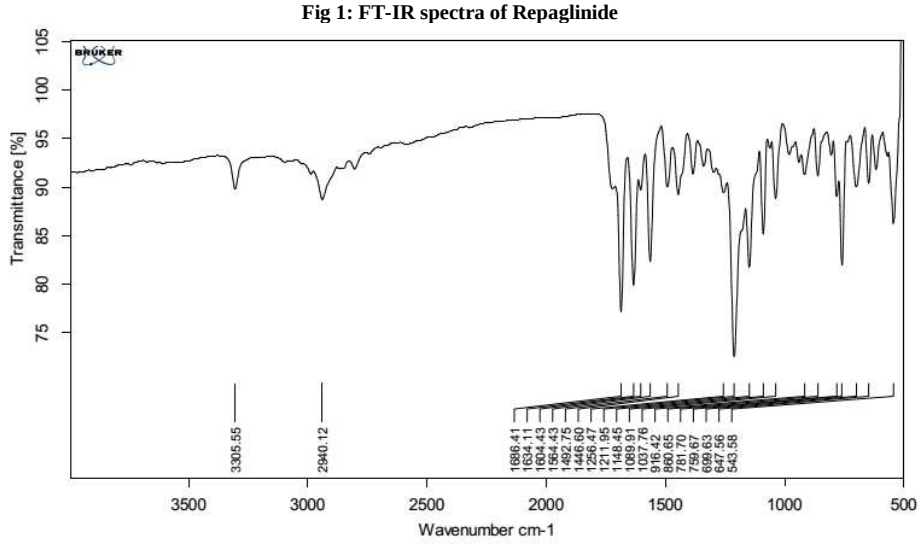

Fig 2: FT-IR spectra of 1:1 physical mixture of Repaglinide and excipients used in the formulations

Surface morphology

Surface morphology of the hollow microspheres was examined by scanning electron microscopy. The surface of the prepared hollow microsphere was found to be rough and porous, presumably because of rapid evaporation of

volatile solvent from the polymeric matrix. The core of micro balloons were found to be hollow, therefore they will float over the gastric content. The SEM images of formulations were shown in Fig 3.

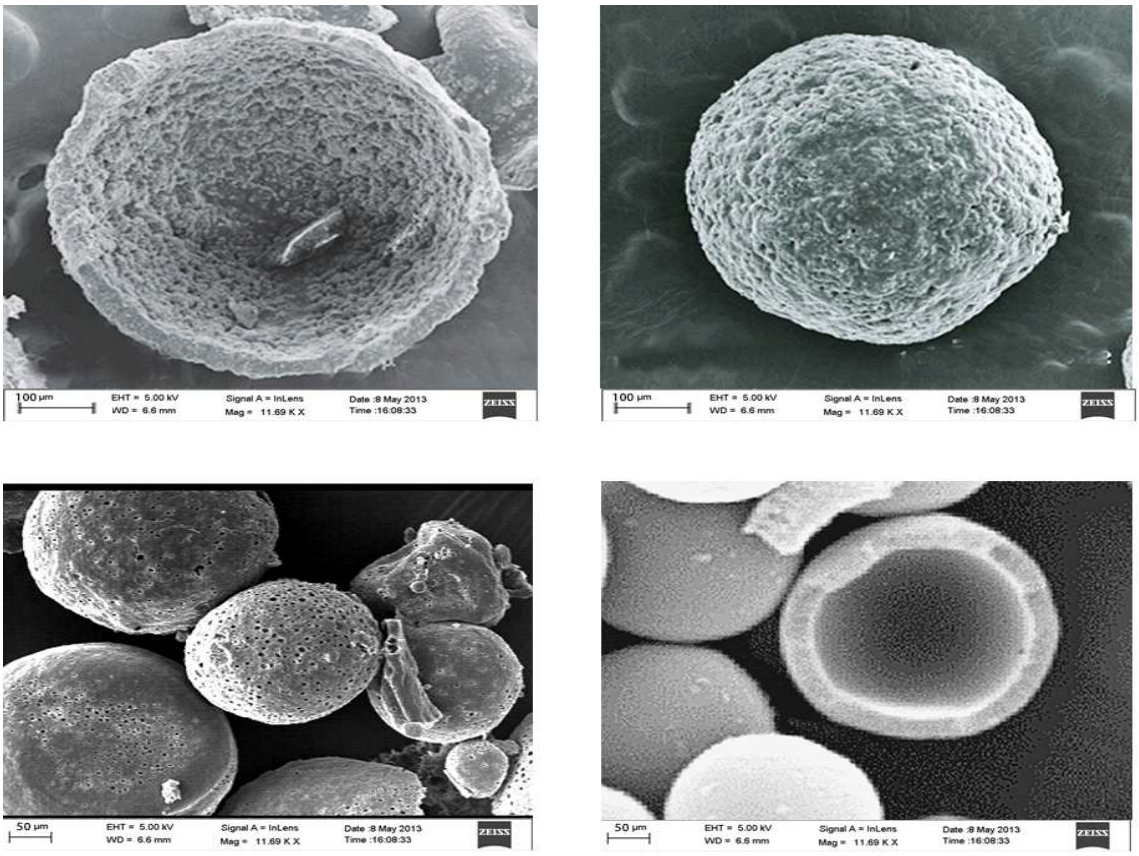

Fig 3: Scanning electron photomicrographs of prepared hollow microspheres

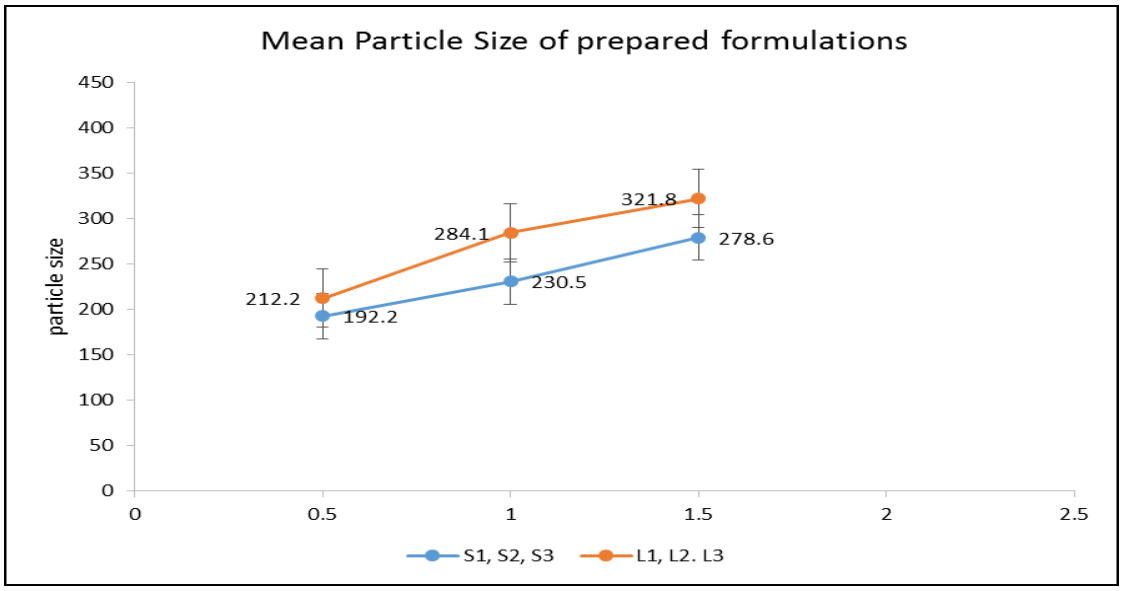

Fig 4: Comparison of Mean Particle size of hollow microspheres of different formulations

Particle size

Results of particle size analysis are shown in Fig 4. The average particle size was found to be in the range of 192.2 ± 12.2 to 321.8 ± 8.3µm. The mean particle size was significantly varied according to concentration of polymer used for the preparation of microspheres. The particle size of the hollow microspheres produced with Eudragit S 100 (Formulation S1, S2, and S3) as polymer is smaller with respect to use of polymer Eudragit L 100 (Formulation L1, L2 and L3) in the formulations.

Entrapment efficiency and drug loading

The results of entrapment efficiency and drug loading are shown in Table 2 and Fig 5. The higher percent of drug loading and entrapment efficiency was obtained in the formulations varies from 53.85 ± 0.45 to 67.80 ± 0.56 and 79.32 ± 1.02 to 86.44 ± 1.14 respectively. Formulation L3 has shown the highest percentage of drug loading, most probably because it large particle size. On the other hand, formulation S1 showed the least percentage of drugs loading, as it was having the smallest particle size, which means it, was having the largest overall active surface area and therefore maximum drug loss from the surface during washing of microspheres [20].

Table 2: Entrapment efficiency and drug loading of all formulations

Batch No.	% yield*	Mean Particle Size ± SD* (µm)	Drug entrapment efficiency ± SD*(%)	Drug loading ± SD*(%)
S1	90 .05± 0.30	192.2 ± 12.2	79.32± 1.02	53.85± 0.45
S2	87.1 ± 28	230.5± 15.4	84.01± 0.98	60.21± 0.89
S3	92.34 ± 28	278.6± 7.5	86.44± 1.14	64.85± 0.86
L1	91.09 ± 0.87	212.2± 10.3	67.98± 1.21	55.34± 1.7
L2	92.66 ± 0.22	284.1± 14.1	77.51± 1.01	61.76± 0.99
L3	90.10 ± 34	321.8± 8.3	79.54±1.6	67.80± 0.56

**SD= Standard deviation (n= 3)*

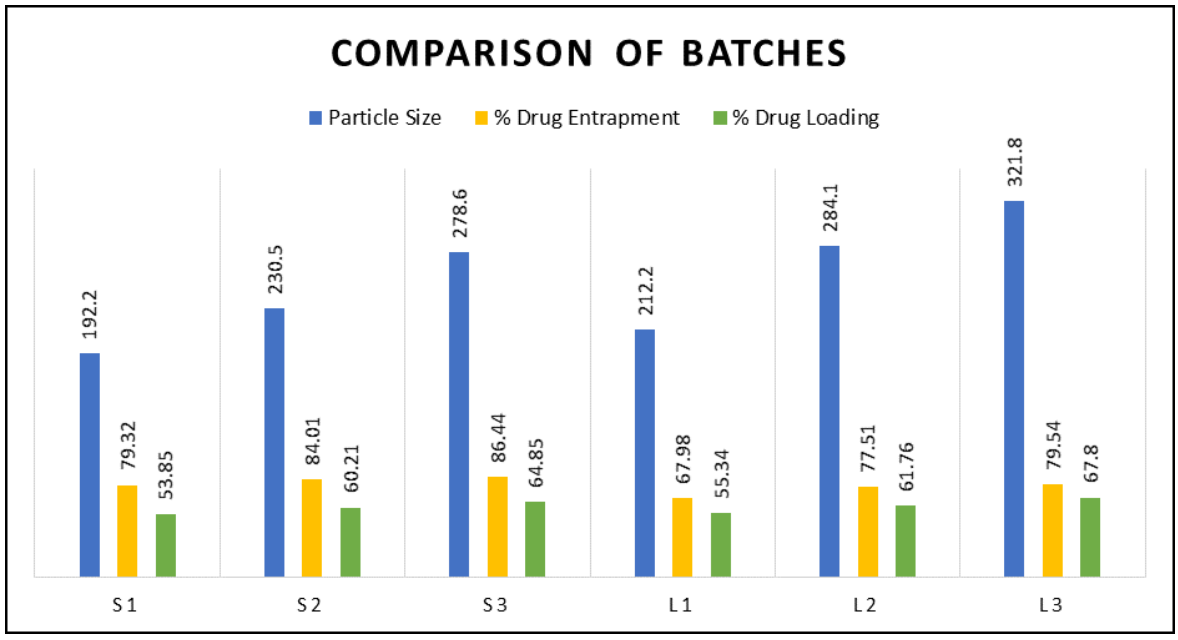

Fig 5: Comparative diagram of prepared formulations with respect to mean particle size, % drug entrapment, and % drug loading

Buoyancy

The in vitro buoyancy of the formulations was tested in Simulated Gastric Fluid (SGF), the percentage buoyancy was calculated and shown in Table 3 and Figure 6. The entire formulations batch showed more than 90% buoyancy at initial 1 h, as the time was progressed the percentage buoyancy was decreased to a minimum buoyancy of 13.1 ± 2.3 % in formulation F3 and maximum buoyancy of 30.1 ± 2.3 with that of formulation S3 after 10 h. thus this result suggest that the grade of eudragits polymers has a significant role in the buoyancy of the prepared hollow microspheres.

Table 3: Results of buoyancy test of prepared batches

Time (Hrs.)	Percentage buoyancy of prepared batches (%) ± SD*					
	S1	S2	S3	L1	L2	L3
0	100	100	100	100	100	100
1	94.1± 1.2	96.4±1.2	92.3±2.2	96.4 ± 2.0	92.1 ± 2.1	91.4 ± 1.7
2	88± 2.1	89.2±0.4	84.4±1.2	89.5 ± 1.8	85.2 ± 1.8	82.3 ± 2.2
3	81.4± 1.3	80.3±1.3	75.6±1.7	82.4 ± 3.3	72.2 ± 1.7	76.6 ± 3.0
4	76.3± 0.6	75.3±2.1	67.6±1.6	78.6 ± 2.7	67.3 ± 2.1	68.5 ± 2.6
5	69.5± 1.4	68.8±2.1	60.3±1.8	71.9 ± 3.2	62.7 ± 1.8	55.6 ± 2.8
6	58.7±1.3	61.5±1.6	53.4±1.4	64.7 ± 2.7	51.4 ± 2.0	40.9 ± 2.7
7	49.4±2.0	55.4±0.9	48.6±1.3	57.7 ± 2.6	42.6 ± 1.9	33.7 ± 2.3
8	40.9±1.7	44.7±1.3	31.8±1.3	48.4 ± 2.8	30.7 ± 2.7	27.7± 1.9
9	31.1± 1.2	34.6±2.2	24.6±0.7	35.2 ± 2.2	24.2 ± 1.8	20.8 ± 2.1
10	23±1.2	24.6±1.7	17.5±1.1	30.1 ± 2.3	16.1 ± 1.7	13.1 ± 2.3

**SD= Standard deviation (n= 3)*

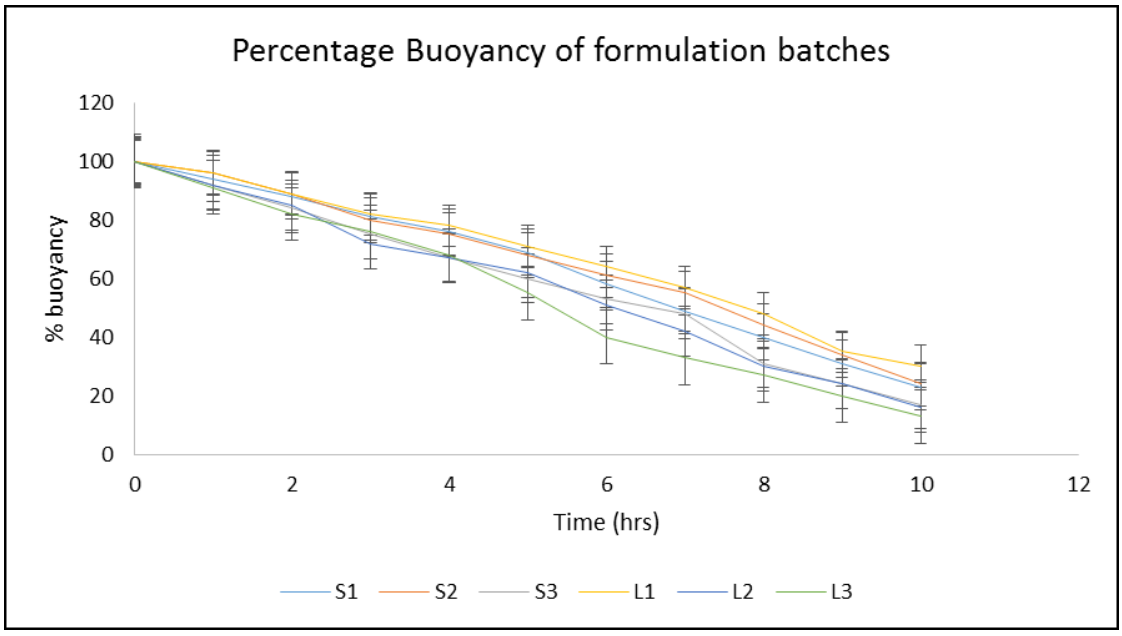

Fig 6: Percent buoyancy of prepared hollow microspheres

***In-vitro* drug release**

The *in-vitro* drug release of all formulations was shown in Table 4 and Figure 7. The S1, formulation showed 98.8% at 6h, S2 and S3 formulations showed 99.2%, 73.8% of drug release at 9h respectively where as the L1 formulation 99.7% at 7h, L2 and L3 formulations showed 90.2% and 68.8% at 9h respectively. From the data, S2 was clearly showing the best release profile after 9 hrs. No burst release of drug was observed in any of the formulations, which suggest that Repaglinide molecules were entrapped over the hollow cavity of the microspheres. With increase in polymer concentration in the formulation the percentage drug release was decreased. The concentration of polymer increased the density of polymer matrix, which resulted in increase of diffusion path length; this decreased the overall release of drug from polymer matrix. Another probable reason is, drug release form microsphere is significantly affected by the size of microspheres. Increase in polymer concentration leads to increase in size of microspheres thus drug release from microspheres having low drug to polymer ratio found to significantly decrease. This result was in agreement with the earlier studies performed by Dey *et al.*, 2011 [21] Analyzing the data obtained from all the evaluator tests, formulation S2 was finally selected as optimized formulation, hence it was chosen for further release kinetic studies for better understanding of kinetics and mechanism of drug release from the formulation.

Table 4: *In-vitro* drug release from the prepared formulations

TIME (h)	% Cumulative Drug Release from formulations (%)					
	S1	S2	S3	L1	L2	L3
0	0	0	0	0	0	0
0.5	28.3	19.5	8.8	8.2	6.9	4.6
1	40.2	25.5	11.4	13.2	11.1	8.8
1.5	56.4	34.2	18.5	21.5	22.1	16.4
2	60.4	43.8	24.5	32.4	30.1	24.5
3	68.8	55.6	31.4	44.3	41.7	32.7
4	75.9	63.1	40.6	64.7	49.0	38.2
5	89.1	71.1	48.7	75.9	57.8	45.2
6	98.8	77	54.8	87.5	66.7	51.8
7		86.7	64.6	99.7	75.5	60.3
8		95.9	70.3		86.3	64.9
9		99.2	73.8		90.2	68.8

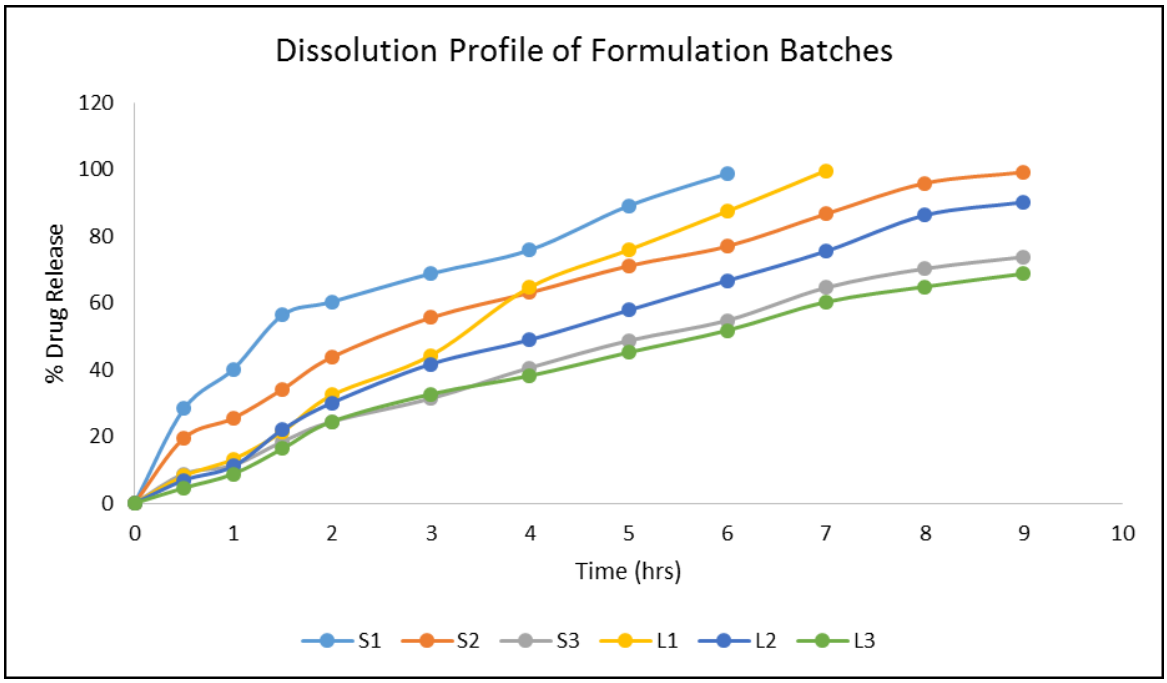

Fig 7: Comparison of in-vitro drug release from prepared formulations

Release Kinetics

The data obtained for the in vitro release of the optimized formulation (S2) were fitted into equations for the zero order, first order, and Higuchi release models. The interpretation of data was based on the value of a resulting regression coefficient. The results are shown here Table 5 and Fig 8-11. The *in-vitro* drug release showed the highest regression coefficient values for Higuchi's model (r2=0.9937) followed by Zero-order (r2=0.9518), indicating the release of drug from matrix as a square root of time dependent process and n value of Korsmeyer-Peppas model was 0.933 confirmed non-Fickian diffusion mechanism.

Table 5: correlation coefficient and kinetic equation for different models of the optimized batch (S2)

Models	Coefficient of correlation (r^2)	Equation
Zero-order	0.9518	y = 10.127x + 16.302
First order	0.8419	y = -0.1841x + 2.1396
Higuchi's model	0.9937	y = 34.297x - 4.7338
Korsmeyer-Peppas model	0.4914	y = 0.933x + 1.1668

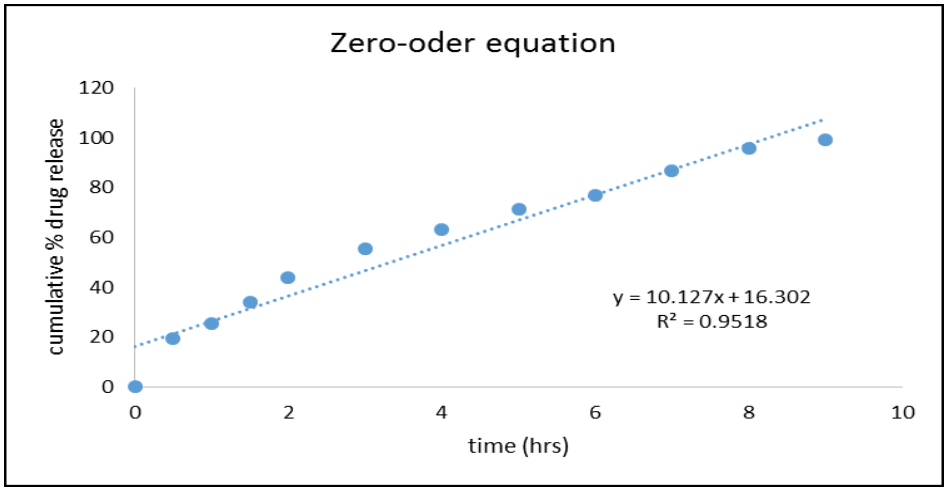

Fig 8: Zero-order Release kinetics for formulation S2

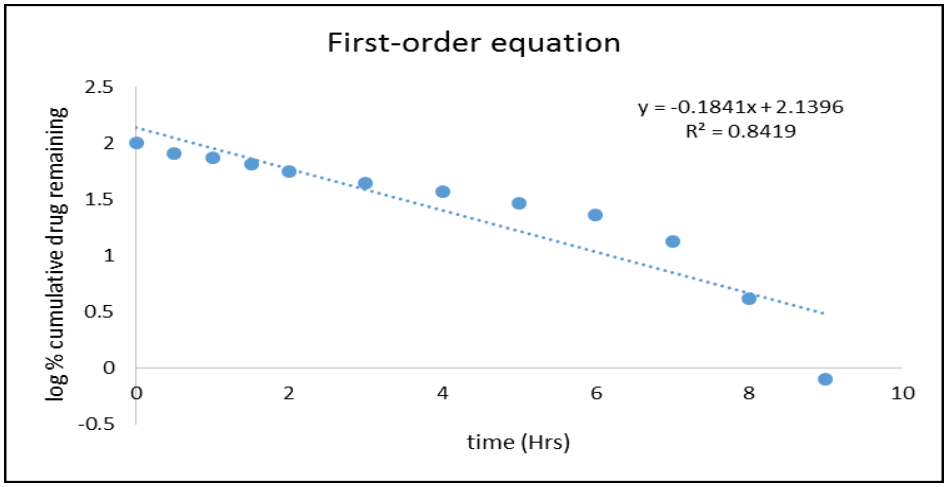

Fig 9: First-order Release kinetics for formulation S2

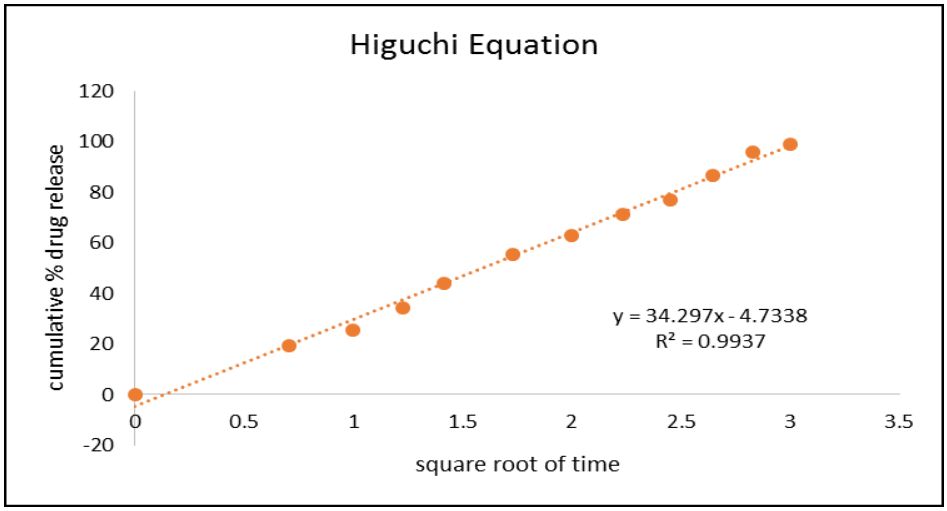

Fig 10: Higuchi Model Release kinetics for formulation S2

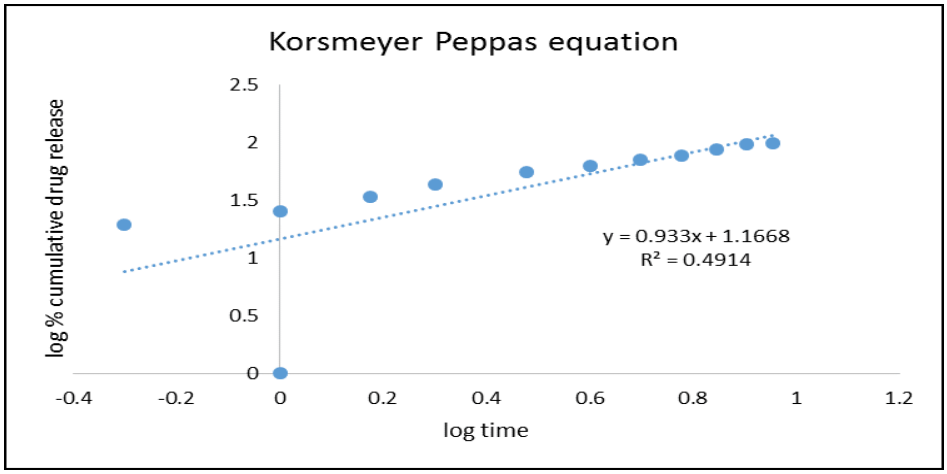

Fig 11: Korsmeyer Peppas Model Release kinetics for formulation S2

CONCLUSION

The floating hollow microsphere of Repaglinide was successfully prepared by emulsion diffusion method. The FT-IR studies showed compatibility of drug with the excipients used in the formulations. The particle sizes of the microspheres were ranges between192.2 ± 12.2 to 321.8 ± 8.3µm. The developed microspheres showed good *in-vitro* buoyancy, high entrapment efficiency and drug loading with sustained release of drug for 9h. The mechanism of drug release followed Higuchi kinetic model with non-Fickian diffusion, the SEM studies showed smooth surface and shape of the hollow microspheres. The developed formulation of Repaglinide in the form of floating microsphere has a great potential for therapy of diabetes type-II in future.

REFERENCES

[1] Prasanth VV, Chakraborthy AM, Mathew ST, Mathapan R. *Int J Pharm Biomed Res*, **2011**, 2 (2): 332-338
[2] Maghsoodi M, Hemati E, Qadermazi B, Yari Z. *Advanced Pharmaceutical Bulletin*, **2011**, 1(2): 55-61
[3] http://www.drugbank.ca/drugs/DB00912
[4] Ahmed AB, Nath LK. *Int J Pharm Pharm Sci*, **2014**, 6(2): 468-475.
[5] Kawashima Y, Niwa T, Takeuchi H, Hino T, Itoh Y. *J Pharm Sci*, **1992**, 81: 135–140.
[6] Chordiya MA, Gangurde H, Senthilkumaran K, Kothari LP. *Int J Pharm Investig*, **2011**, 1(2): 105–111.
[7] Nappinnai M, Kishore VS. *Indian J* Pharm Sci, **2007**, 69(4): 511-514.
[8] Chetan P, Bakliwal S, Pawar S, Rane B, Gujrathi N. *International Journal of Pharmaceutical Science and Research*, **2011**; 2(10): 2669-2674.
[9] SatoY, Kawashima Y, Takeuchi H, Yamamoto H. *Eur. J. Pharm. Biopharm*, 55: 297–304.
[10] Gupta AK, Garg SK, Pal SK, Saxena M, Sharma A. *Journal of Drug Delivery & Therapeutics*, **2011**, 1(1): 71-77
[11] Vimal K. Yadav, Brajesh Kumar, S.K. Prajapati, Kausar shafaat. *International Journal of Drug Delivery*, **2011**, 3: 357-370.
[12] Shukla R, Dwivedi N. *World Journal of Pharmacy and Pharmaceutical Sciences*, **2012**, 1(2), 772-785.
[13] Wang J, Cui F, Shi K, Yang L, Wang S, Zhang L. *Asian J Pharm Sci.* **2008**, 3 (4): 151-157.
[14] Yao H, Huijuan Y, Zhu J, Yu J, Zhang L. *Int. J. Pharm*, **2012**, 422: 211– 219.
[15] Zhang C, Xu M, Tao X, Tang J, Liu Z, Zhang Y, Lin X, Hea H, Tang X. *Int. J. Pharm*, **2012**, 430: 141– 150.
[16] Gupta A, Nagar M, Sharma P. *Int J Pharm life Sci*, **2012**, 3(2): 1437-1440.
[17] Rathnam G, Balasubramani P, Saravanakumar A. *International Journal of Pharmaceutical Science and Research*, **2011**, 2(4): 940-947
[18] Sanjivani A, Swapnila S, Shalaka D, Uddhav B, Jagdish S. *International Journal of Pharma and Bio Sciences*, **2011**, 2(3):344-358.
[19] Ebrahimi HA, Javadzadeh Y, Jalali MB, Hamidi M. *Res Pharm Sci*, **2012**, 7(5):1225.
[20] Rathnam G, Balasubramani P, Saravanakumar A. *Int J Pharm Sci Rev Res*, **2011**, 2(4), 940-947.

[21] Dey S, Kumar D, Kumar DS, Sreenivas SA., Rahul V. *International Journal of Pharma and Bio Sciences*, **2011**, 2(1):147-156