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FLOATING MICROSPHERES OF REPAGLINIDE: FORMULATION, OPTIMIZATION, CHARACTERIZATION AND *IN VITRO* EVALUATION

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ABSTRACT

Drugs that are easily absorbed from the gastrointestinal tract (GIT) and having short half life are eliminated quickly from blood circulation and need frequent dosing such as repaglinide. Therefore, floating microspheres of repaglinide were prepared using ethylcellulose (EC) alone and in combination with hydroxypropylmethylcellulose (HPMC) by solvent diffusion-evaporation technique. Various process variables were studied during formulation and their effect on various properties were recorded. Increase in stirring rate slightly increases the drug release and was found to be in the range of 70.2-86.3% for EC whereas 78.6-86.4% for EC: HPMC formulations. The mechanism of drug release was studied and found to follows first order kinetics. The regression of optimized formulations were found to be 0.926 (E6) and 0.955 (H9).

Key words: Repaglinide, Microspheres, *In vitro*, Entrapment efficiency, Floating, Gastrointestinal tract.

INTRODUCTION

Among the different routes of drug administrations, the most easy, flexible and convenient is the oral route for the patient. Drugs with narrow absorption window are desired to retain at the site of absorption for a longer period of time in order to obtain controlled release of the drug. Several approaches have been developed to retain the drug delivery system at the gastrointestinal tract such as sedimentation, floatation, expansion, mucoadhesion and modified shape system[1-4]. Drugs having short half life are eliminated quickly from blood circulation and require frequent dosing; therefore, controlled release formulations of such drugs have been developed to increase the bioavailability[5]. Floating drug delivery is useful for several categories of drugs, which act locally in the stomach,

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poorly soluble at an alkaline pH, having narrow window of absorption, unstable in the intestine or colonic environment and primarily absorbed in the stomach[6]. Floating devices administered in a single-unit form as hydrodynamically balanced systems are unreliable in prolonging the gastric retention time owing to their all-or-none effect and thus may cause high variability in local irritation and bioavailability due to large amount of drug delivery at a particular site of GIT[7].

The objective of the present research work was to develop multiparticulate floating drug delivery system of repaglinide with EC and HPMC as release control polymer. Repaglinide is a nonsulfonylurea oral hypoglycemic agent of meglitinide class and has rapid onset, low bioavailability (50%) and short half life (1 h)[8]. Therefore, it was chosen as drug candidate for development of floating microsphere to increase gastroretention time (GRT).

EXPERIMENTAL

Materials and methods

Repaglinide was procured as gift sample from Torrent Pharmaceuticals, Ahemdabad, India. Ethyl cellulose and hydroxypropyl methylcellulose (HPMC 5cps) were purchased from Himedia Chemicals, India. Ethanol, dichloromethane (DCM) and other solvents were purchased from SD Fine Chemicals Mumbai, India. All other chemicals used were of analytical grade.

Compatibility study

The pure drug (repaglinide) and the mixture of drug with EC and HPMC were analyzed by FTIR spectroscopy to study their compatibility. IR spectra were taken by FTIR spectrophotometer (Shimadzu 8400, Japan) instrument.

Formulation of floating microspheres

Microspheres of repaglinide were prepared by solvent diffusion-evaporation technique with slight modification[9]. EC and HPMC were dissolved in mixture of dichloromethane and ethanol (1:1). Drug and 0.1% of polyethylene glycol was dispersed and stirred for 15 min using high speed mechanical stirrer (Remi equipments, India). The above solution was then slowly poured into 80 mL of 0.46% w/v of polyvinyl alcohol used as an emulsifier and stirred for 1 hr, for complete evaporation of organic phase. Formulation design of microspheres is shown in Tables 1 and 2. The resulting microspheres were washed five times thoroughly with distilled water and dried for 1 hr at room temperature. Process variables like polymer concentration, drug: polymer ratio and stirring rate were studied during formulation.

Table 1: Formulation design of EC microspheres

Code	Drug : EC	ETH : DCM	PVA	Speed (rpm)
E 1	1:1	1:1	80	600
E 2	1:2	1:1	80	600
E 3	1:3	1:1	80	600
E 4	1:4	1:1	80	600
E 5	1:1	1:1	80	900
E 6	1:2	1:1	80	900
E7	1:3	1:1	80	900
E8	1:4	1:1	80	900

Table 2: Formulation design of EC and HPMC microspheres

Code	Drug : EC: HPMC	ETH : DCM	PVA	Speed (rpm)
H 1	1:1:1	1:1	80	600
H 2	1:2:1	1:1	80	600
H 3	1:3:1	1:1	80	600
H 4	1:1:2	1:1	80	600
H 5	1:1:3	1:1	80	600
H 6	1:1:1	1:1	80	900
H 7	1:2:1	1:1	80	900
H 8	1:3:1	1:1	80	900
H 9	1:1:2	1:1	80	900
H 10	1:1:3	1:1	80	900

Characterization of microspheres

Micromeritic properties

Microspheres were characterized for certain properties such as particle size, tapped density, bulk density, angle of repose and percentage porosity. Particle size is measured using an optical microscopy and mean particle size was calculated by measuring 200-300 particles with calibrated ocular micrometer. The flow characteristics are measured by angle

of repose by fixed funnel method. Tapped density was determined using tapping method. Porosity (ε)[10] was calculated by using equation:

$$\varepsilon = (1- P_\rho/ P_t) \times 100 \quad \ldots(1)$$

Morphology

The surface morphology of the microspheres was examined by scanning electron microscopy (SEM).

Floating behavior

50 mg of the microparticles were placed in SGF (pH 1.2, 100 mL) containing Tween 20 (0.02 w/v %) and stirred at 100 rpm. The buoyant microparticles were seperated from setteled particles by filtration after 12 hrs. Particles of both the types were dried and weighed. The buoyancy was calculated as follows:

$$\text{Buoyancy (\%)} = W_f / (W_f+W_S) \times 100 \quad \ldots(2)$$

Where W_f and W_s are the weights of the floating and settled microparticles, respectively[11].

Entrapment efficiency and yield

50 mg of microparticles were weighed, crushed and suspended in 10 mL of ethanol and was kept for 12 hrs to dissolve the polymeric shell for extraction of drug. After filtration and suitable dilution, repaglinide content in the filtrate was analyzed spectrophotometrically at 247 nm. The percentage drug entrapment and yield[11] were calculated as follows:

$$\text{\% Drug entrapment} = (\text{Calculated drug content/Theoretical drug content}) \times 100 \quad \ldots(3)$$

$$\text{\% Yield} = (\text{Total weight of floating microparticles/Total weight of drug and polymer}) \times 100 \quad \ldots(4)$$

In vitro drug release studies

In vitro dissolution study was carried out in a paddle type six-station dissolution test apparatus (Veego, VDA-6DR, USP Std). A weighed amount of floating microspheres equivalent to 16 mg of the drug was placed in 0.1 N HCl (1.2 pH) containing Tween 20 (0.02 w/v %) maintained at 37 ± 0.5°C and a rotation speed of 100 rpm. Perfect sink condition was maintained. 5 mL sample was withdrawn at each 30 min interval, filtered and analyzed spectrophotometrically at 247 nm. All experiments were conducted in triplicate.

Evaluation of *in vitro* release kinetics

The kinetics of drug release can be known by substituting the *in vitro* release data to different kinetic models such as zero order (% cum. drug retained *vs.* t), First order (log % cum. drug release *vs.* t), Higuchi model (% cum. drug release *vs* square root of time) and Peppas exponential equation (log % cum. drug release *vs* log time). Regression coefficient (R^2) values were calculated for the linear curves obtained by regression analysis[12,13].

RESULTS AND DISCUSSION

Formation of microspheres

The floating microspheres were prepared by solvent diffusion-evaporation technique using EC and HPMC as polymer. A suspension of polymer and drug in solvent having equal ratios of ethanol and dichloromethane forms the organic phase. This organic phase was poured into an aqueous phase containing polyvinyl alcohol. The organic solvents get rapidly distributed into the external aqueous phase; thereby the polymer precipitated around the drug particle and due to the evaporation of entrapped solvent microspheres were formed. The ratio of dichloromethane with ethanol also affects the morphology of the microspheres and the best spherical shape was obtained, when the ratio of ethanol to dichloromethane was 1:1.

Compatibility studies

Chemical stability of drug and polymers were studied by FTIR and spectra were shown in Figs. 1-3. Spectra of EC shows a sharp band at 2929 cm^{-1} and 3481 cm^{-1} associated with a CH and OH stretching vibrations, respectively.

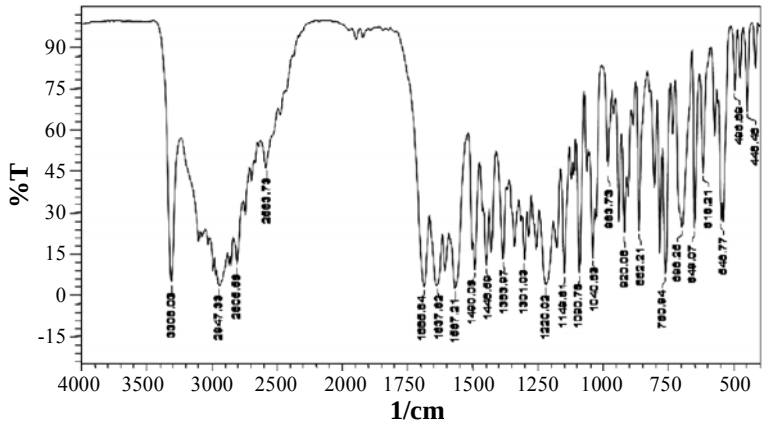

Fig. 1: IR Spectra of Repaglinide

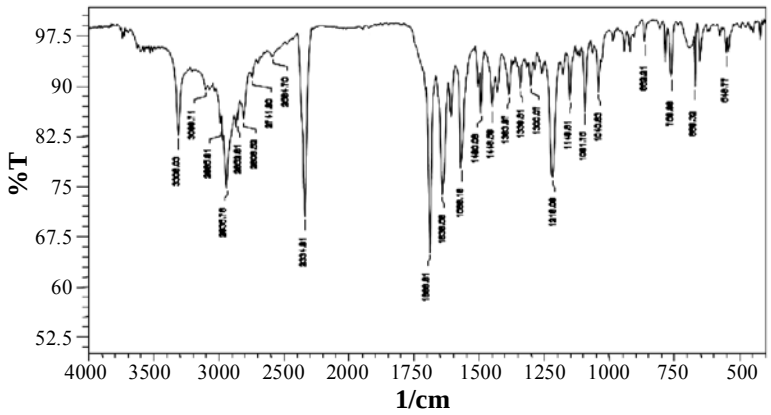

Fig. 2: IR Spectra of drug and EC

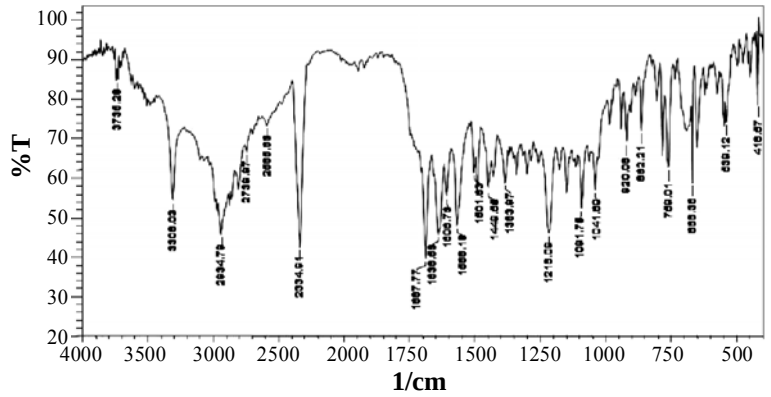

Fig. 3: IR Spectra of drug and HPMC

Spectra of HPMC show bands at 3480 cm^{-1} and 2973 cm^{-1} of OH and CH stretching vibrations, along with etheral C-O-C group stretching vibration between 1166-1000 cm^{-1}. IR spectra of mixture of both show peaks at 3308 cm^{-1} (NH stretching), 2934 cm^{-1} (CH stretching) and 1687 cm^{-1} (C=O) having no interaction, as there was no major shift in the absorption bands of drug was found. Data of IR were tabulated in Table 3.

Table 3: Comparison of IR spectra of drug and drug polymer combination

S. No.	System	N-H (cm^{-1})	C-H (cm^{-1})	C=O (cm^{-1})	CH_3 (cm^{-1})
1.	Repaglinide (RG)	3308	2947	1685	1220
2.	RG-HPMC	3308	2935	1686	1218
3.	RG-EC	3308	2934	1687	1218

The IR spectrum of formulation H9 is shown in the Fig. 4, having peaks at 1608 cm^{-1} (C=O) and 2974 cm^{-1} (CH) along with disappearance of some peaks may be due to encapsulation by polymers.

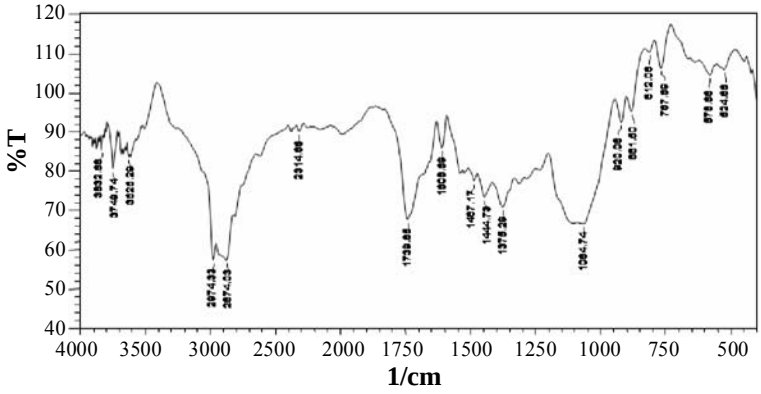

Fig. 4: IR Spectrum of H 9 Formulation

Micromeritic properties

Particle size was in the range of 164 ± 15 to 311 ± 19 µm for all the formulations. The mean particle size significantly increased with increasing EC concentration but as the stirring speed was increased from 600 to 900, particle size decreases. The tapped and bulk density values were ranged from 0.43 to 0.57 g/cm^3 and 0.90 to 1.78 g/cm^3, respectively. As the value of angle of repose was found to be < 40º, which indicates good flowability and formulation to be non-aggregated. The porosity of all the formulations was in the range of 43.4 to 72.4%. The results of micromeritic properties were shown in Table 4.

Table 4: Micromeritic properties of microspheres

Code	Mean particle size (µm)	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Angle of repose	Porosity (%)
E 1	210 ± 10	0.90 ± 0.21	0.45 ± 0.01	28.1 ± 2º	50.0 ± 1
E 2	243 ± 13	0.91 ± 0.16	0.46 ± 0.03	32.2 ± 4º	49.5 ± 5
E 3	279 ± 35	0.98 ± 0.11	0.45 ± 0.04	36.7 ± 3º	54.0 ± 4
E 4	311 ± 19	1.10 ± 0.04	0.43 ± 0.06	38.4 ± 2º	60.9 ± 6
E 5	187 ± 13	0.90 ± 0.15	0.48 ± 0.01	29.6 ± 5º	53.3 ± 2

Cont…

Code	Mean particle size (µm)	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Angle of repose	Porosity (%)
E 6	200 ± 16	0.92 ± 0.23	0.52 ± 0.09	31.2 ± 6º	43.4 ± 7
E 7	234 ± 24	0.99 ± 0.14	0.56 ± 0.04	34.9 ± 4º	43.4 ± 1
E 8	276 ± 28	1.32 ± 0.01	0.49 ± 0.05	37.8 ± 4º	62.8 ± 2
H 1	164 ± 15	1.45 ± 0.13	0.48 ± 0.06	30.2 ± 5º	66.8 ± 6
H 2	173 ± 09	1.63 ± 0.08	0.52 ± 0.08	32.2 ± 1º	68.0 ± 5
H 3	184 ± 12	1.75 ± 0.27	0.54 ± 0.04	38.5 ± 2º	69.1 ± 3
H 4	170 ± 14	1.78 ± 0.11	0.49 ± 0.02	40.3 ± 4º	72.4 ± 8
H 5	187 ± 13	1.68 ± 0.16	0.53 ± 0.09	33.0 ± 2º	68.5 ± 2
H 6	172 ± 10	0.98 ± 0.18	0.42 ± 0.07	31.7 ± 3º	57.1 ± 9
H 7	198 ± 15	1.05 ± 0.23	0.45 ± 0.06	35.6 ± 3º	55.0 ± 7
H 8	234 ± 25	1.22 ± 0.21	0.50 ± 0.07	38.4 ± 5º	59.1 ± 2
H 9	267 ± 35	1.43 ± 0.14	0.54 ± 0.09	37.4 ± 6º	62.2 ± 1
H 10	244 ± 27	1.24 ± 0.26	0.49 ± 0.06	40.1 ± 4º	60.4 ± 3

Values are average of three readings ± standard deviation

Morphology

EC and EC-HPMC based microspheres were spherical in appearance as shown in Fig. 5. SEM photographs reveal pores on the surface as well as hollow microspheres interior, which makes the microspheres float on the simulated GIT fluid. The outer surface of the microspheres was smooth and dense.

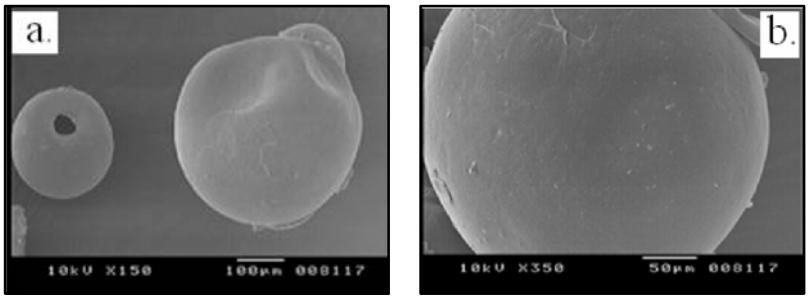

Fig. 5: SEM (a) EC & HPMC based microspheres and (b) EC microspheres

Percentage buoyancy, percentage yield and entrapment efficiency

It has been observed that more than 70% microspheres remained floating at the end of 12 hrs and such floating time is considered as good performance. Tween 20 (0.02 w/v %), was added to SGF, to reduce the surface tension. Percent yield increases from 70.5 to 83.7% with increased EC concentration in formulations prepared with 600 rpm speed, whereas it decreased for microspheres prepared by 900 rpm. Thus, increase in stirring speed decreases percent yield. A formulation with high content of HPMC shows better yield and best is for H8 (87.2%). Results are tabulated in Table 5. The poor water solubility of the drug helps in good entrapment within the polymeric microparticles. The entrapment efficiency increases with increase in polymer concentration in all the formulations. Entrapment is in the range of 51.2 to 76.7 and 60.5 to 74.3 for EC & EC: HPMC microspheres.

Table 5: Percent buoyancy, entrapment and yield of formulations

Code	Buoyancy (%)	Entrapment (%)	Yield (%)	*In vitro* drug release (%)
E 1	70.6 ± 2.1	53.2 ± 1.4	70.5 ± 0.6	84.5 ± 0.2
E 2	74.5 ± 3.0	68.4 ± 1.8	78.3 ± 1.1	79.5 ± 1.2
E 3	80.9 ± 1.4	74.2 ± 0.2	81.3 ± 2.0	75.2 ± 0.5
E 4	85.2 ± 3.4	76.7 ± 2.5	83.7 ± 0.2	70.2 ± 2.1
E 5	72.9 ± 1.5	51.2 ± 0.4	68.5 ± 0.6	86.3 ± 24
E 6	79.4 ± 2.4	65.0 ± 2.2	76.1 ± 1.5	84.6 ± 0.8
E 7	81.0 ± 0.1	72.3 ± 0.1	79.8 ± 0.6	78.6 ± 2.6
E 8	82.3 ± 1.2	75.4 ± 1.2	81.0 ± 1.2	74.1 ± 3.1
H 1	74.3 ± 1.8	66.5 ± 0.3	70.0 ± 0.5	85.0 ± 0.7
H 2	75.9 ± 0.8	70.4 ± 0.8	77.1 ± 1.4	82.0 ± 2.4
H 3	79.2 ± 0.5	74.3 ± 0.5	80.5 ± 2.4	78.6 ± 1.6
H 4	78.8 ± 1.5	71.5 ± 1.5	78.5 ± 2.5	81.4 ± 0.1
H 5	80.3 ± 2.9	69.1 ± 3.5	84.3 ± 0.2	80.1 ± 3.2
H 6	70.2 ± 1.6	60.5 ± 0.1	74.3 ± 1.1	86.4 ± 3.1

Cont…

Code	Buoyancy (%)	Entrapment (%)	Yield (%)	*In vitro* drug release (%)
H 7	74.7 ± 1.2	65.4 ± 3.1	79.1 ± 3.2	84.2 ± 1.5
H 8	80.9 ± 1.5	69.3 ± 1.4	87.2 ± 0.2	79.1 ± 0.2
H 9	83.8 ± 3.4	72.5 ± 0.5	86.5 ± 0.1	83 ± 2.4
H 10	86.2 ± 0.4	70.5 ± 1.9	85.3 ± 1.0	81.1 ± 1.2

Values are average of three readings ± standard deviation

In vitro drug release study

In vitro release studies were carried out in 0.1 N HCl (pH 1.2). Release of drug shows no burst effect in any of the formulation, which indicates homogeneous drug distribution. An increase in polymer concentration in both the batches decreases the release rate. At higher polymer concentration, the increased density of the polymer matrix results in increased diffusional path length, which results in decrease percent drug release from the matrix. Also at low polymer concentration, smaller particles with larger surface area were produced resulting in increased percent drug release. More than 70% of the drug was released from all the floating microspheres after 12 hrs.

In EC formulations prepared by 600 and 900 rpm speed, the drug release decreases by 84.5 to 70.2% from E1 (1:1) to E4 (1:4) and 86.3 to 74.1% from E5 (1:1) to E8 (1:4) on increasing ethylcellulose ratios, respectively. The release of same ratio of polymeric preparations (E2 and E6) show increase in release rate from 79.5 to 84.6% on increasing speed of rotation from 600 to 900 rpm. Thus, rate of drug release was found to increase with increase in stirring rate.

Formulations prepared by EC: HPMC polymer shows increase in drug release in the initial hours as compared to EC formulations, which may be due to hydrophilic nature of HPMC. The release rate was decreased from 85 to 78.6 (H1-H3) and 86.4 to 79.1 (H 6-H8) with increasing EC ratios. The release rate of H4 and H5 increases due to increase concentration of HPMC polymer as compared to H3. Similarly, release rate of H9 and H10 is increased as compared to H8 formulation. On the basis of the favorable values of buoyancy, drug content, particle size, yield and drug release E6 and H9 were selected as optimized formulations. The *in vitro* release of repaglinide from both type of formulations were shown in Fig. 6 and 7.

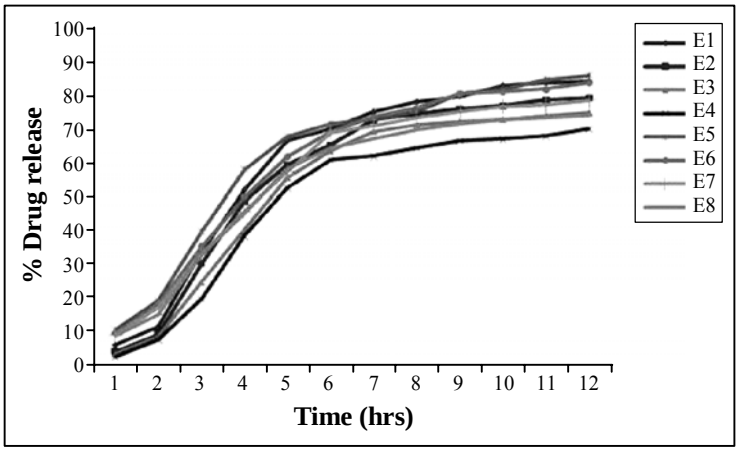

Fig. 6: *In-vitro* release of repaglinide from EC microspheres

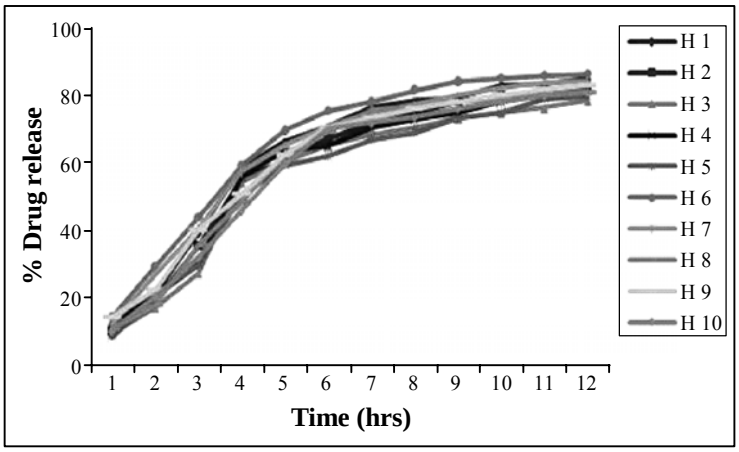

Fig. 7: *In-vitro* release of repaglinide from EC: HPMC microspheres

In-vitro release kinetics study

The data obtained from *in vitro* release was fitted to zero order, first order, Higuchi and Peppas models. It was observed that R^2 is highest for first order kinetics, which shows concentration dependent drug release, followed by Higuchi and Peppas model. The highest linearity for first order was found to be 0.955 (E5) and 0.967 (H5) in both type of formulations. First order plot of both the formulations were shown in Fig. 8 and 9. To explain the release mechanism, the Peppas equation has been applied and good linearity (0.912-0.947) has been observed. The value of n is calculated from the slope of the curve and $n \leq 0.89$, which shows coupling of diffusion and erosion mechanism indicating the

release by two processes. Results of kinetics studies were tabulated in Tables 6 and 7. From these results it can be concluded that drug release follows first order, diffusion and erosion mechanism.

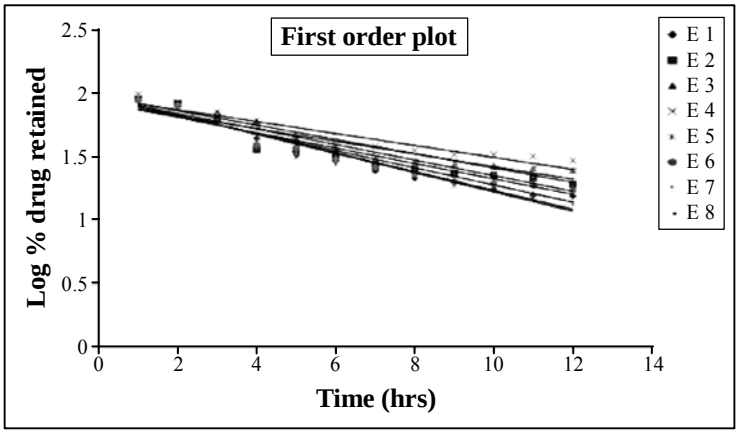

Fig. 8: First order plot of repaglinide from EC formulations (E1-E8)

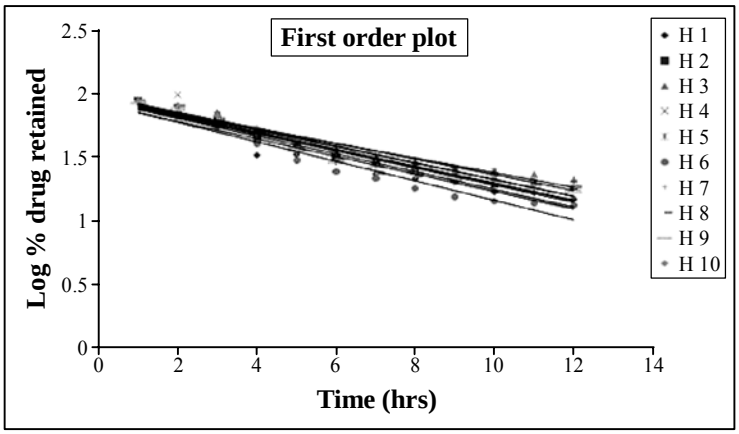

Fig. 9: First order plot of repaglinide from EC: HPMC formulations (H1-H10)

Table 6: Release kinetics of optimized formulations

Code	Zero order	First order	Higuchi model	Peppas model	
	R^2	R^2	R^2	R^2	n
E6	0.845	0.926	0.937	0.932	0.88
H9	0.855	0.955	0.937	0.944	0.74

CONCLUSION

The present study was an attempt to formulate a floating microspheres of repaglinide, in order to improve its gastric residence time and bioavailability. The designed system shows excellent floating capacity and suitable drug release pattern and it could possibly be advantageous in terms of increased bioavailability of repaglinide. The major advantages of the system are ease of preparation, good buoyancy, high entrapment efficiency and sustained release over several hours. The *in vitro* drug release was best fitted to the first order followed by Higuchi model. The n value obtained from Korsmeyer and Peppas model reveals the release mechanism followed non-Fickian transport.

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