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Research Article

Formulation and In-vitro Evaluation of Repaglinide Floating Microspheres

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Abstract

Repaglinide is an antidiabetic drug in the class of medications known as meglitinides, and was invented in 1983. Repaglinide is an oral medication used in addition to diet and exercise for blood sugar control in type 2 diabetes mellitus.The mechanism of action of repaglinide involves promoting insulin release from β-islet cells of the pancreas; like other antidiabetic drugs, a main side effect concern is hypoglycemia. Inthepresent work, gastro retentive floating microspheres of Repaglinide using sodium alginate along with Locust bean gum Chitosan gellan gum as copolymers were formulated to deliver Repaglinide via oral route. The results of this investigation indicate thationic cross linking technique Ionotropic gelation method can be successfully employed to fabricate Repaglinide microspheres. The technique providescharacteristic advantage overconventional microsphere method, which involvesan all-aqueous system, avoids residual solvents in microspheres. Other methods utilize largervolume of organic solvents,which are costly and hazardous because of the possible explosion,air pollution, toxicity and difficult to remove traces of organic solvent completely.FT-IR spectraof the physical mixture revealed that the drug is compatible with the polymers and copolymers used. Increase in the polymer concentration led to increase in% Yield, % Drug entrapment efficiency, Particle size, % swelling and % Mucoadhesion.The *invitro* drug release decreased with increase in the polymer and copolymer concentration.Analysis ofdrug release mechanism showed that the drug release fromthe formulations followed non-Fickian diffusion and the best fit model was found tobe zero order. Based on the results of evaluation tests formulation coded F3 was concluded as best formulation.

Keywords: Repaglinide, Locust bean gum, Chitosan, gellan gum and floating microspheres.

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Contents

1. Introduction

Floating drug delivery systems (FDDS) or hydro-dynamically balanced systems have a bulk density lower than gastric fluids and therefore remain floating in the stomach without affecting the gastric-emptying rate for a prolonged period. Microspheres can be defined as solid, approximately spherical particles ranging in size from 1 to 1000 micro meter. The Microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers, which are biodegradable in nature [1]. Solid biodegradable microspheres incorporating a drug dispersed or dissolved throughout particle matrix have the potential for controlled release of drugs. Microspheres are small in size and therefore have large surface to volume ratios [2].

Repaglinide is an antidiabetic drug in the class of medications known as meglitinides, and was invented in 1983. Repaglinide is an oral medication used in addition to diet and exercise for blood sugar control in type 2 diabetes mellitus.The mechanism of action of repaglinide involves promoting insulin release from β-islet cells of the pancreas; like other antidiabetic drugs, a main side effect concern is hypoglycemia. In the present work, gastro retentive floating microspheres of Repaglinide using sodium alginate along with Locust bean gum Chitosan gellan gum as Copolymers were formulated to deliver Repaglinide via oral route. The results of this investigation indicate thationic cross linking technique Ionotropic gelation method can be successfully employed to fabricate Repaglinide microspheres.

2. Materials and Methods

Materials

Repaglinide (Natco labs), Gellan gum, Locust bean gum (SD fine Chemicals. Ltd, Mumbai), chitason, Sodium bicarbonate, Sodium alginate, Calcium chloride (Merck specialities Pvt.Ltd)

Preparation of Repaglinide Floating Microspheres

Batches of microspheres were prepared by ionotropic gelation method which involved reaction between sodium alginate and polycationic ions like calcium to produce a hydrogel network of calcium alginate. Sodium alginate and the mucoadhesive polymer were dispersed in purified water (10 ml) to form a homogeneous polymer mixture. The API, Repaglinide (100 mg) were added to the polymer premix and mixed thoroughly with a stirrer to form a viscous dispersion. The resulting dispersion was then added through a 22G needle into calcium chloride (4% w/v) solution. The addition was done with continuous stirring at 200rpm. The added droplets were retained in the calcium chloride solution for 30 minutes to complete the curing reaction and to produce rigid spherical microspheres. The microspheres were collected by decantation, and the product thus separated was washed repeatedly with purified water to remove excess calcium impurity deposited on the surface of microspheres and then air-dried. [3]

Evaluation of Repaglinide Floating Microspheres [4]

Angle of repose: The angle of repose of powders was determined by the funnel method. Accurately weighed powders were taken in a funnel. The height of the funnel was adjusted in such a way that the tip of the funnel just touched the apex of the heap of the powders. The powders were allowed to pass through the funnel freely onto the surface. The diameter and height of the powder cone was measured and angle of repose was calculated by using the given formula. The results were tabulated in Table 3.

$$\tan\theta = \frac{h}{r}$$

Where,

h = height of the powder cone

r= radius of the powder cone

Bulk density and tapped density:

A quantity of 10gms of powder from each formula was introduced into a 10 ml measuring cylinder. After the initial volume was observed, the cylinder was tapped continuously until no further change in volume was observed. Then bulk density (BD) and tapped density (TD) were calculated by using the given formula and the results were tabulated in Table 3.

$$BD = \frac{\text{Weight of the powder}}{\text{Initial volume}}$$

$$TD = \frac{\text{Weight of the powder}}{\text{Tapped volume}}$$

Carr's index:

The Compressibility of the powder blend was determined by Carr's compressibility index. It is indirectly related to the relative flow rate, cohesiveness and particle size. It is a simple test to evaluate the bulk density and tapped density of a powder and the rate at which it is packed. The formula for carr's Index is given below and the results were tabulated in Table 3.

$$\text{Carr's index}\,(\%) = \frac{TD - BD}{TD} \times 100$$

Hausner's ratio:

The Hausner's ratio is a number that is correlated to the flowability of a powder or granular material. It is calculated by using the given formula. The results were tabulated in Table 3.

$$\text{Hausner's ratio} = \frac{TD}{BD}$$

Percentage yield:

The percentage of production yield was calculated from the weight of dried microsphe-res recovered from each batch and the sum of the initial weight of starting materials.

The % yield was calculated using the following formula:

$$\% \text{ Yield} = \frac{\text{Practical mass (Microspheres)}}{\text{Theoretical mass (Polymer + Drug)}} \times 100$$

Drug entrapment efficiency:

Microspheres equivalent to 100 mg of the drug Repaglinide were taken for evaluation. The amount of drug entrapped was estimated by crushing the microspheres. The powder was transferred to a 100 ml volumetric flask and dissolved in 10ml of methanol and the volume was made up using simulated gastric fluid pH 1.2. After 24 hours the solution was filtered through Whatmann filter paper and the absorbance was measured after suitable dilution spectrophotometrically at 245 nm. The amount of drug entrapped in the microspheres was calculated by the following formula,

$$\% \text{ Drug Entrapment Efficiency} = \frac{\text{Experimental Drug Content}}{\text{Theoretical Drug Content}} \times 100$$

Particle size analysis:

Samples of the microparticles were analyzed for particle size by optical microscope. The instrument was calibrated and found that 1unit of eyepiece micrometer was equal to 12.5µm. Nearly about 100 Microparticles sizes were calculated under 45 x magnifications[5].

The average particle size was determined by using the Edm ondson's equation:

$$D_{mean} = \frac{nd}{n}$$

Where,

n – Number of microspheres observed

d – Mean size range

Swelling study:

Firstly we were prepared 0.1N HCl maintained its pH -1.2. Then we were taken a petridish and transfer the specific amount of floating microspheres, added a 0.1 N HCl. The floating microspheres were taken out from the HCl and soak the extra water with the help of tissue paper. Then weight them and it was calculated by using the following formula[6].

$$\text{Swelling Index} = \frac{\text{Mass of swollen microspheres- Mass of dried microspheres}}{\text{Mass of dried microspheres}} \times 100$$

Evaluation of mucoadhesive property

The mucoadhesive property of microspheres was evaluated by an in vitro adhesion testing method known as wash-off method. Freshly excised pieces of goat stomach mucous were mounted on to glass slides with cotton thread. About 20 microspheres were spread on to each prepared glass slide and immediately thereafter the slides were hung to USP II tablet disintegration test, when the test apparatus was operated, the sample is subjected to slow up and down movement in simulated gastric fluid pH 1.2 at 37^0C contained in a 1-litre vessel of the apparatus. At an interval of 1 hour up to 8 hours the machine is stopped and number of microspheres still adhering to mucosal surface was counted [7].

$$\% \text{ Mucoadhesion} = \frac{\text{Number of microspheres adhered}}{\text{Number of microspheres applied}} \times 100$$

In- vitro buoyancy

The floatation studies were carried out to ascertain the floating behaviour of the prepared formulations. Beaker method was initially used to have an idea of the floatation behaviour of the proposed dosage form. 50 mg of floating microparticles were placed in each of four 50 ml beakers containing 20 ml of 0.1N HCl containing 0.02% tween 80. The beakers were shaken in a biological shaker at 37ºC ± 0.5ºC at 40 RPM. Floating microspheres were collected at 4,8 and 12 hrs and dried till constant weight was obtained. The percentage of floating microspheres was calculated by the following Equation[8].

$$\% \text{ Buoyancy} = \frac{\text{Weight of floating microspheres}}{\text{Total initial weight of floating microsphere}} \times 100$$

In vitro drug release study:

The dissolution studies were performed in a fully calibrated eight station dissolution test apparatus (37 ± 0.5^0C, 50 rpm) using the USP type – I rotating basket method in simulated gastric fluid pH 1.2 (900ml). A quantity of accurately weighed microspheres equivalent to 100mg Repaglinide each formulation was employed in all dissolution studies. Aliquots of sample were withdrawn at predetermined intervals of time and analyzed for drug release by measuring the absorbance at 245 nm. At the same time the volume withdrawn at each time intervals were replenished immediately with the same volume of fresh pre-warmed simulated gastric fluid pH 1.2 maintaining sink conditions throughout the experiment[9].

In-Vitro Drug Release Kinetics

The release data obtained was fitted into various mathema tical models.The parameters 'n' and time component 'k', the release rate constant and 'R', the regression coefficient were determined by Korsmeyer-Peppas equation to understand the release mechanism[10].

Table 1: Composition of different formulations of Repaglinide microspheres

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Repaglinide (mg)	100	100	100	100	100	100	100	100	100
Sodium alginate (mg)	150	150	150	150	150	150	150	150	150
Locust bean gum (mg)	100	150	200	-	-	-	-	-	-
Chitosan (mg)	-	-	-	100	150	200	-	-	-
Gellan gum (mg)	-	-	-	-	-	-	100	150	200
Sodium bicarbonate (mg)	20	20	20	20	20	20	20	20	20
Calcium chloride (%)	5	5	5	5	5	5	5	5	5
Water (ml)	10	10	10	10	10	10	10	10	10

3. Results and discussion

Table 2: Standard curve data of Repaglinide

Concentration (µg /ml)	Absorbance
1	0.178
2	0.336
3	0.506
4	0.682
5	0.825

Table 2 shows the calibration curve data of Repaglinide in simulated gastric fluid pH 1.2 at 245nm with a regression values in simulated gastric fluid pH 1.2. The curve was found to be linear in the concentration range of 1-5µg/ml.

FTIR studies:

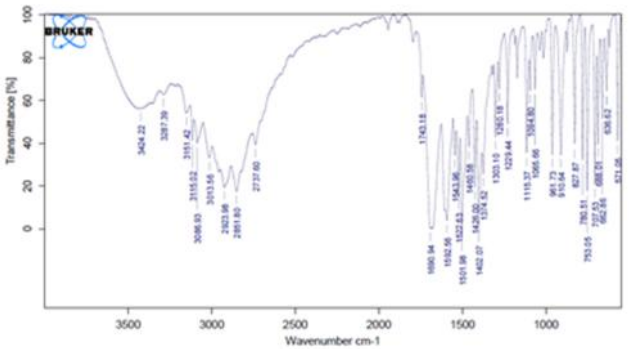

Fig 1: FTIR Spectrum of pure drug

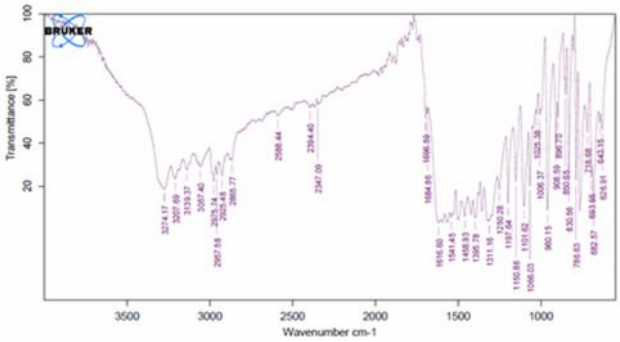

Fig 2: FTIR spectrum of optimized formulation

Drug polymer compatibility studies were carried out using Fourier Transform Infrared spectroscopy to establish any possible interaction of Drug with the polymers used in the formulation. The FT-IR spectra of the formulations were compared with the FTIR spectra of the pure drug and no interactions were found.

Table 3 : Micromeritic properties of the prepared formulations

Formulations	Bulk Density (gm/cm^3)	Tap Density (gm/cm^3)	Carr's Index (%)	Hausner's ratio	Angle Of Repose(Θ)
F1	0.53	0.52	12.09	1.09	24.13
F2	0.51	0.53	13.46	1.11	23.08
F3	0.52	0.54	15.82	1.05	25.63
F4	0.53	0.55	16.34	1.11	23.14
F5	0.51	0.54	14.67	1.06	24.57
F6	0.52	0.56	13.15	1.11	22.94
F7	0.53	0.52	12.97	1.06	24.15
F8	0.51	0.53	13.06	1.08	24.08
F9	0.51	0.51	14.13	1.06	23.13

The values for angle of repose were found in the range of 22°-26°. Bulk densities and tapped densities of various formulations were found to be in the range of 0.51 to 0.53 (gm/cc) and 0.51 to 0.56 (gm/cc) respectively. Carr's index of the prepared blends fall in the range of 12.09% to 16.34%. The Hausner's ratio fall in range of 1.05 to 1.11. From the result it was concluded that the powder blends had good flow properties and these can be used for tablet manufacture.

Table 4: Percentage yield and percentage drug entrapment efficiency of the prepared microspheres

Formulation code	% yield	Drug Content (mg)	% Drug entrapment efficiency
F1	97.37	97.03	97.16
F2	95.71	97.52	97.52
F3	97.28	98.67	98.34
F4	97.64	97.28	97.38
F5	95.19	97.36	98.45
F6	97.52	98.14	97.46
F7	96.08	97.16	97.12
F8	95.06	97.17	98.18
F9	97.12	97.06	97.05

Percentage Yield: It was observed that as the polymer ratio in the formulation increases, the product yield also increases. The low percentage yield in some formulations may be due to blocking of needle and wastage of the drug-polymer solution, adhesion of polymer solution to the magnetic bead and microspheres lost during the washing process. The percentage yield was found to be in the range of 97.37 to 97.28 for microspheres containing sodium alginate along with Locust bean gum as copolymer, 97.64 to 97.52% for microspheres containing sodium alginate along with Chitosan as copolymer and 96.08 to 97.12% for microspheres containing sodium alginate along with Gellan gum as copolymer.

Drug Entrapment Efficiency

Percentage Drug entrapment efficiency of Repaglinide ranged from 97.16 to 98.34% for microspheres containing sodium alginate along with Locust bean gum as copolymer, 97.38 to 97.46 % for microspheres containing sodium alginate along with Chitosan as copolymer and 97.12 to 97.05 % for microspheres containing sodium alginate along with Gellan gum as copolymer. The drug entrapment efficiency of the prepared microspheres increased progressively with an increase in proportion of the respective polymers. Increase in the polymer concentration increases the viscosity of the dispersed phase. The particle size increases exponentially with viscosity. The higher viscosity of the polymer solution at the highest polymer concentration would be expected to decrease the diffusion of the drug into the external phase which would result in higher entrapment efficiency.

Average particle size

The mean size increased with increasing polymer concentration which is due to a significant increase in the viscosity, thus leading to an increased droplet size and finally a higher microspheres size. Microspheres containing sodium alginate along with Locust bean gum as copolymer had a size range of 601µm, 613µm, 598µm, microspheres containing sodium alginate along with Chitosan as copolymer exhibited a size range between 689µm, 645µm, 632µm and microspheres containing sodium alginate along with Gellan gum as copolymer had a size range of 613µm, 689µm, 703µm. The particle size data is presented in Tables 5 to 13 and displayed in Figures. The effect of drug to polymer ratio on particle size is displayed in Figure. The particle size as well as % drug entrapment efficiency of the microspheres increased with increase in the polymer concentration.

Table 5: Particle size data of F1

PARTICLE SIZE RANGE (µm)	MIDPOINT SIZE RANGE (d)	FREQUENCY (n)	AVERAGE PARTICLE SIZE (µm)
200-300	250	11	601µm
300-400	350	22	
400-500	450	23	
500-600	550	25	
600-700	650	19	
		∑n=100	

Table 6: Particle size data of F2

PARTICLE SIZE RANGE (µm)	MIDPOINT SIZE RANGE (d)	FREQUENCY (n)	AVERAGE PARTICLE SIZE (µm)
300-400	350	21	613µm
400-500	450	25	
500-600	550	15	
600-700	650	13	
700-800	750	11	
800-900	850	15	
		∑n=100	

Table 7: Particle size data of F3

PARTICLE SIZE RANGE (µm)	MIDPOINT SIZE RANGE (d)	FREQUENCY (n)	AVERAGE PARTICLE SIZE (µm)
400-500	450	20	598µm
500-600	550	21	
600-700	650	18	
700-800	750	19	
800-900	850	20	
		∑n=100	

Table 8: Particle size data of F4

PARTICLE SIZE RANGE (µm)	MIDPOINT SIZE RANGE (d)	FREQUENCY (n)	AVERAGE PARTICLE SIZE (µm)
500-600	550	15	689µm
600-700	650	19	
700-800	750	16	
800-900	850	22	
900-1000	950	28	
		∑n=100	

Table 9: Particle size data of F5

PARTICLE SIZE RANGE (µm)	MIDPOINT SIZE RANGE (d)	FREQUENCY (n)	AVERAGE PARTICLE SIZE (µm)
200-300	250	26	645µm
300-400	350	28	
400-500	450	13	
500-600	550	18	
600-700	650	15	
		∑n=100	

Table 10: Particle size data of F6

PARTICLE SIZE RANGE (µm)	MIDPOINT SIZE RANGE (d)	FREQUENCY (n)	AVERAGE PARTICLE SIZE (µm)
300-400	350	21	632µm
400-500	450	23	
500-600	550	16	

600-700	650	19	
700-800	750	21	
800-900	850	7	
		∑n=100	

Table 11: Particle size data of F7

PARTICLE SIZE RANGE (µm)	MIDPOINT SIZE RANGE (d)	FREQUENCY (n)	AVERAGE PARTICLE SIZE (µm)
400-500	450	12	613µm
500-600	550	16	
600-700	650	19	
700-800	750	16	
800-900	850	22	
900-1000	950	15	
		∑n=100	

Table 12: Particle size data of F8

PARTICLE SIZE RANGE (µm)	MIDPOINT SIZE RANGE (d)	FREQUENCY (n)	AVERAGE PARTICLE SIZE (µm)
500-600	550	31	689µm
600-700	650	16	
700-800	750	19	
800-900	850	15	
900-1000	950	19	
		∑n=100	

Table 13: Particle size data of F9

PARTICLE SIZE RANGE (µm)	MIDPOINT SIZE RANGE (d)	FREQUENCY (n)	AVERAGE PARTICLE SIZE (µm)
400-500	450	17	703µm
500-600	550	19	
600-700	650	19	
700-800	750	25	
800-900	850	20	
		∑n=100	

Table 14: Percentage swelling of the prepared microspheres

S.No.	Formulation Code	Initial (Wt)	Final (Wt)	Percentage Swelling
1	F1	100	117.3	17.3
2	F2	100	116.5	16.5
3	F3	100	118.6	18.6
4	F4	100	113.4	13.4
5	F5	100	114.3	14.3
6	F6	100	116.4	16.4
7	F7	100	114.5	14.5
8	F8	100	116.7	16.7
9	F9	100	117.8	17.8

The swelling ratio is expressed as the percentage of water in the hydrogel at any instant during swelling. Swellability is an important characteristic as it affects mucoadhesion as well as drug release profiles of polymeric drug delivery systems. Swellability is an indicative parameter for rapid availability of drug solution for diffusion with greater flux.

In-Vitro Drug Release Studies

Dissolution studies of all the formulations were carried out using dissolution apparatus USP type I. The dissolution studies were conducted by using dissolution media, pH 1.2. The results of the in-vitro dissolution studies of formulations F1 to F3, F4 to F6 and F7 to F9 are shown in figures.

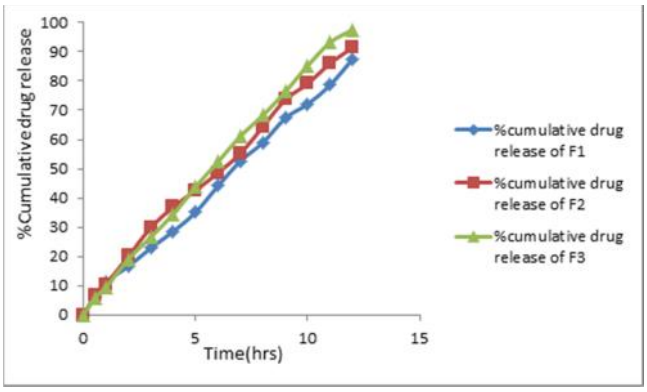

Fig 3: Comparison of *In-Vitro* drug release profile of Repaglinide microspheres containing sodium alginate along with Locust bean gum as copolymer

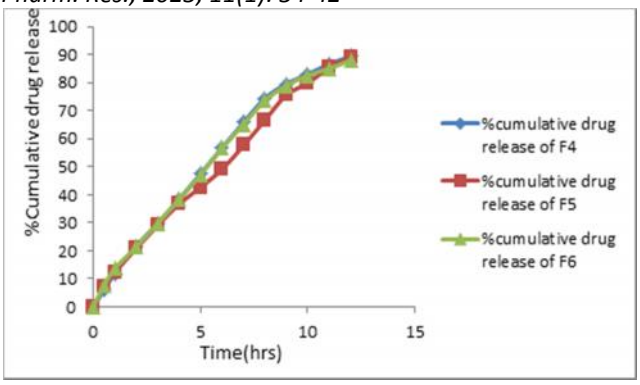

Fig 4: Comparison of *In-Vitro* drug release profile of Repaglinide microspheres containing sodium alginate along with chitosan as copolymer

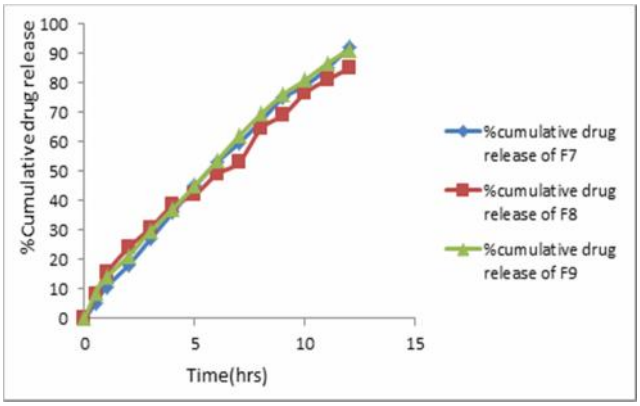

Fig 5: Comparison of *In-Vitro* drug release profile of Repaglinide microspheres containing sodium alginate along with gellan gum as copolymer

Application of Release Rate Kinetics to Dissolution Data:

Various models were tested for explaining the kinetics of drug release. To analyze the mechanism of the drug release rate kinetics of the dosage form, the obtained data were fitted into zero-order, first order, Higuchi, and Korsmeyer-Peppas release model.

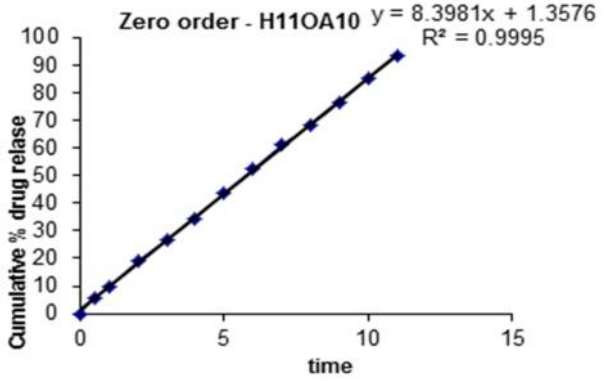

Fig 6 : Zero order release kinetics graph

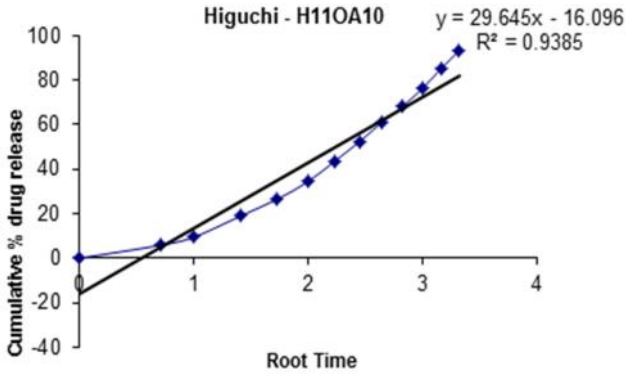

Fig 7 : Higuchi release kinetics graph

4. Conclusion

In the present work, gastro retentive floating microspheres of repaglinide using sodium alginate along with locust bean gum chitosan gellan gum as copolymers were formulated to deliver repaglinide via oral route. The results of this investigation indicate that ionic cross linking technique ionotropic gelation method can be successfully employed to fabricate repaglinide micospheres. Increase in the polymer concentration led to increase in % Yield, % Drug entrapment efficiency, Particle size, % swelling and % Mucoadhesion. The *invitro* drug release decreased with increase in the polymer and copolymer concentration. Analysis of drug release mechanism showed that the drug release from the formulations followed non-Fickian diffusion and the best fit model was found to be zero order. Based on the results of evaluation tests formulation coded F3 was concluded as best formulation.

Acknowledgement

I am thankful to the Principal, Dr. K. Harinadha Baba, Narayana Pharmacy College, for providing facilities to carry out the research work.

5. References

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