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FORMULATION AND *IN-VITRO* EVALUATION OF REPAGLINIDE-LOADED MICROSPHERES

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Abstract:

The current study was conducted using floating repaglinide microspheres. The polymers HPMC 100 and ethyl cellulose were used. Micromeritic characteristics were tested on floating microspheres. A mean particle size of 381.55±2.54 to 493.24±2.43 micrometers was determined. For floating microsphere formulations F1 through F10, the yield ranged from 84.05±0.39 to 97.48±0.57. To increase the duration of a drug; stomach residence time, floating microspheres were prepared. In vitro, buoyancy ranged from 70.42±1.36 to 95.81±2.11 for formulations F1 through F10, respectively. The range of the entrapment efficiency for formulations F1 through F10 was 81.62±1.72 to 95.62±2.07. Formulations made using HPMC100 were shown to contain up to 90% of the medication, according to the dissolving data. For up to 12 hours, the F5 formulation delayed the maximal drug release.

Keywords: *Repaglinide, Microspheres, Micromeritics, buoyancy, drug release.*

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INTRODUCTION:

Oral dosage forms capable of having prolonged retention in the stomach to extend drug delivery for a longer time have been receiving much attention nowadays. Gastric residence time (GRT) is one of the important factors affecting the bioavailability of drugs in pharmaceutical dosage forms. Variable and short gastric emptying time can result in incomplete drug release from the delivery system above the absorption zone (stomach and upper part of small intestine), thereby, abolishing the effectiveness of the administered dose [1-2]. Drug bioavailability can be sufficiently increased by prolonging GRT through gastroretentive dosage form such as a floating drug delivery system (FDDS). Floating drug delivery remains buoyant over gastric and intestinal fluid owing to their lower density than aqueous medium. In floating dosage form both single and multiple systems have been developed [3-4]. Multiple unit dosage forms can be an attractive alternative to single unit forms as they have been shown to reduce inter and intra variabilities in drug absorption as well as to lower the probability of accumulation of dose [5].

Gastrointestinal tract targeting dosage forms are prepared to release the drug at the gastrointestinal site. Several types of gastrointestinal target dosage forms including intragastric floating systems, high-density systems, mucoadhesive systems that adhere to the gastric mucosal surface to extend GRT, magnetic systems, and unfoldable extendible or swellable systems have been developed [6].

A floating drug delivery system is useful for several categories of drugs that act locally in the stomach, are poorly soluble in alkaline pH, have a narrow window of absorption, are unstable in the intestine or colonic environment, and are primarily absorbed in the stomach [7]. Drugs having solubility in an acidic medium and higher absorption in the upper part of the intestine can be used to deliver through a floating system [8].

Repaglinide is an oral hypoglycemic agent and the first member of the meglitinide class, used to treat type-2 diabetes mellitus. It blocks the ATP-dependent potassium channel to stimulate the release of insulin by binding to specific sites on pancreatic β-cells. Repaglinide requires frequent dosing before meals due to its short half-life and there are side effects such as skeletal muscle pain, headache, and git effects [9]. Microspheres encapsulated with anti-diabetic drugs, increase the effectiveness and release of the drug in a controlled manner from the polymeric membrane and thereby, maintain its concentration for a longer duration. Due to short-lasting action, fast clearance, enzymatic stability, and absorption throughout git make repaglinide a suitable target for developing gastroretentive dosage form [10].

MATERIALS & INSTRUMENTS:

Repaglinide was purchased from Hetero. Pvt Ltd.Ethyl Cellulose, Eudragit S100 was purchased from SD Fine Chemicals Ltd. Ethanol, Dichloromethane, Tween 80, Hydrochloric acid was purchased from Merk Pvt Limited, Mumbai.

METHODOLOGY:

Drug and Excipient Compatibility studies:

To ascertain whether the pure drug and excipients were compatible, FTIR spectra obtained on a Bruker FTIR Germany (Alpha T) were utilized. Over a yellow ZnSe crystal, the solid powder sample was put. The spectra were recorded at wavelengths ranging from 4000 to 400 cm-1 [11].

Differential Scanning Calorimetry:

DSC-Hitachi is used to determine the melting point and enthalpy of pure drugs and optimized formulations. The powder is put into aluminum pans, sealed using a crimping machine, and given nitrogen gas. The gas regulating unit keeps the air dry. The graph is plotted once the DSC Spectra are recorded.

Preparation of Floating Microspheres

The solvent evaporation process was used to make the floating microspheres. Excipients and the required polymer were taken in various proportions, as stated in Table 3. Ethanol and dichloromethane (1:1) were used to dissolve the medication and excipients. Using a syringe, the drug and polymer solution were gradually injected into 100 ml of water that contained 5% V/V Tween 80. For one hour, the preparation was swirled at 300 rpm. Filtered and left to dry at room temperature for an entire night, the acquired floating microspheres [12].

Table 1: Preparation of floating Microspheres

Formulation code	Drug (mg)	Ethylcellulose (mg)	Eudragit S 100 (mg)	Sodium Bicarbonate (mg)	DCM (ml)	Ethanol (ml)
F1	100.0	50.0	-	50.0	5.0	5.0
F2	100.0	100.0	-	50.0	5.0	5.0
F3	100.0	150.0	-	50.0	5.0	5.0
F4	100.0	200.0	-	50.0	5.0	5.0
F5	100.0	250.0	-	50.0	5.0	5.0
F6	100.0	-	50.0	50.0	5.0	5.0
F7	100.0	-	100.0	50.0	5.0	5.0
F8	100.0	-	150.0	50.0	5.0	5.0
F9	100.0	-	200.0	50.0	5.0	5.0
F10	100.0	-	250.0	50.0	5.0	5.0

Characterization of Microspheres:

Percentage Yield:

After drying, the manufactured floating microspheres for each formulation were weighed. After then, the formula below was used to compute the % yield.

$$\% \text{ Yield: } \frac{\text{Actual Weight of dried microspheres}}{\text{Total Weight of drug and excipients}} \text{ X } 100$$

Drug entrapment efficiency:

The amount of drug inside the microspheres may be approximately determined by crushing them. The powder was added to a 100 ml volumetric flask, dissolved in 10 ml of methanol, and then the volume was increased by 0.1N HCl to 100 ml [13]. kept for approximately an hour to sonicate. Whatmann filter paper was used to filter the solution following the correct dilution, and the absorbance was then measured spectrophotometrically at the suitable wavelength.

$$\% \text{ Drug Entrapment Efficacy : } \frac{\text{Experimental Drug content}}{\text{Theoratical Drug content}} \text{ X } 100$$

Micromeritic properties

Angle of repose:

To find it, the funnel method was applied [14]. A vertically liftable funnel was used to pour the mixture through until the cone reached its highest point (h).

$$\theta = \tan^{-1} h/r$$

Bulk Density:

To determine the apparent bulk density (b), the powder combination was poured into a graduated cylinder. We computed the powder's weight (M) and bulk volume (Vb).

$$\rho_b = M / V_b$$

Tapped density:

For a predetermined amount of time (100 tappings), a known mass of blend (M) was carried in the measurement cylinder [15]. The mix's weight and the minimal volume (Vt) it took up in the cylinder were also measured.

$$\rho_t = M / V_t$$

Compressibility Index or Carr's Index:
Compressibility is the most straightforward method for measuring the ease with which a material may be made to flow.

$$\text{Carr's Index} = \rho_b - \rho_t / \rho_b * 100$$

Hausner Ratio (H):
The Hausner's ratio is a lagging indicator of powder flow simplicity. This formula is used to calculate it.:

$$\text{Hausner's ratio (H)} = \rho t/\rho b$$

here ρ_t = tapped density, ρ_b = bulk density

In-vitro Buoyancy:
Two milligrams of floating microspheres were dissolved in 100 milliliters of 0.1 N hydrochloric acid solution (pH 1.2) to simulate stomach fluid at 37°C. After 12 hours, the layer of buoyant microspheres (Wf) and sinking microspheres (Ws) were pipetted and separated by filtration after the mixture had been stirred with a paddle at 50 revolutions per minute [16]. The microspheres of both kinds were dried for a whole day at 40°C.

$$\text{Buoyancy (\%)} = \frac{W_f}{W_f + W_s} x100$$

Where Wf and Ws = the masses of the floating and settled microspheres, respectively.

***In vitro* drug release study:**
Using a USP type I (basket) dissolution testing apparatus from Lab India, the dissolving research of floating microspheres was carried out over 12 hours. The dissolution medium, 900ml of 0.1N HCl, was stirred at 100 RPM at 37 ºC + 0.5 ºC. 5 ml samples were taken at the necessary intervals to calculate the medication release [17]. By using UV Spectrophotometry at the appropriate wavelength, the samples were examined.

Ex-Vivo Mucoadhesion Study
The microspheres' mucoadhesive qualities are tested on the mucosa of a goat's digestive system using phosphate buffer, per the monograph. Slides are immediately hung on the arm of a USP pill-dissolving test device at 37°C with the required support after weighed microspheres have been placed to moist, washed tissue samples. We measure the weight of microspheres that leak off at different times.
The following equation calculates the mucoadhesion percentage [18].

$$\text{\% Mucoadhesion} = \frac{Wa - W1}{Wa} \text{ X } 100$$

where Wa is the mass of the applied microspheres and W1 is the mass of the removed microspheres

Drug Release Kinetics:
A. Zero Order

$$\% R = kt$$

This is true for dosage forms such as Matrix tablets carrying low soluble drug concentrations, coated forms, osmotic systems, and transdermal methods [19].

B. First Order

$$\text{Log (fraction unreleased)} = kt/2.303$$

The model can be applied to the analysis of hydrolysis kinetics and release profiles of pharmacological dosage forms, including those containing chemicals that dissolve in water in porous media.

C. Matrix (Higuchi Matrix)

$$\% R = kt\ 0.5$$

The matrix tablets used in this model include a medication that is water soluble and is dispersed in a homogeneous swellable polymer matrix.

D. Peppas Korsmeyer Equation

When more than one sort of release phenomenon may be present or when the release mechanism is well-known, this model is frequently used [20]. Different release mechanisms could be characterized by the 'n' values as:

$$\% R = kt\ n$$

$$\log \% R = \log k + n \log t$$

RESULTS & DISCUSSION:

Drug- Excipient Compatibility studies:

The drug's peak is caused by the alcohol group, terminal CH3 group, secondary amine, ketonic group, and C=O stretching in COOH and CONH. In a drug and polymer mixture, the primary peaks of the drug's infrared spectra were unchanged, and it was seen that the main peaks of Repaglinide were present.

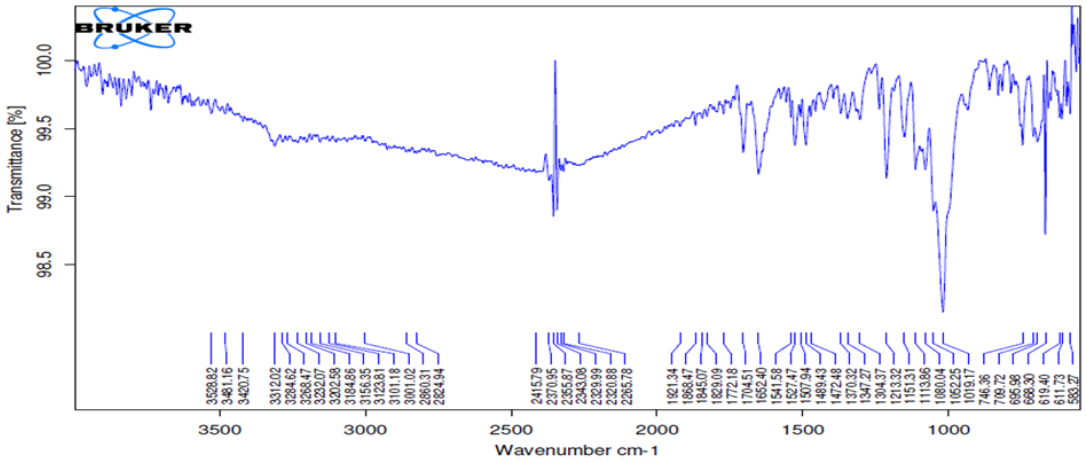

Figure 1: FTIR of Repaglinide

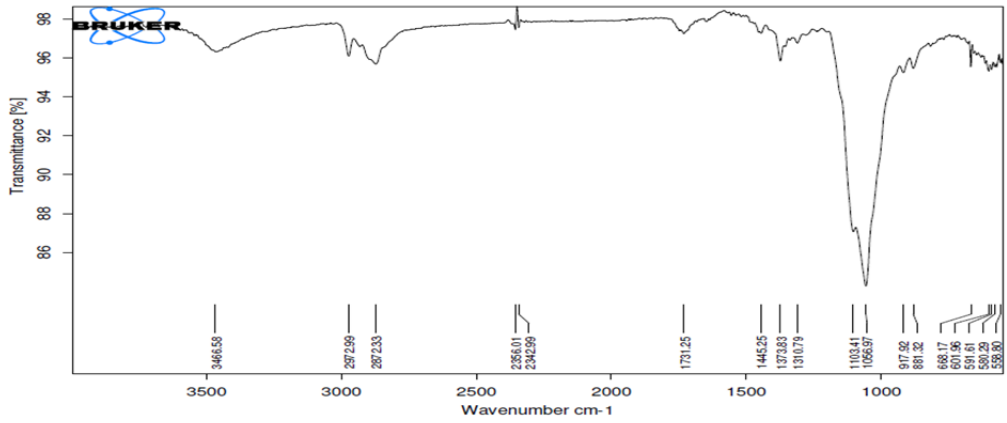

Figure 2: FTIR of Optimized formula

DSC (Differential Scanning Calorimetry) Studies:

RPG's thermograms revealed a distinct endothermic peak at 131.3°C. This suggests the presence of pure repaglinide in its crystalline form. For the pure powders, the drug-lipid physical combination had two endothermic peaks at roughly the same temperatures. Both Repaglinide and EC are still in the crystalline state, and no interaction was observed, however there was a tiny widening of the Repaglinide peak that may indicate a modest affinity between the two. While the HPMC melting peak seemed somewhat displaced, the Repaglinide melting

peak completely vanished within its melting temperature range. This observation implies the transformation of the crystalline form of Repaglinide into its amorphous form.

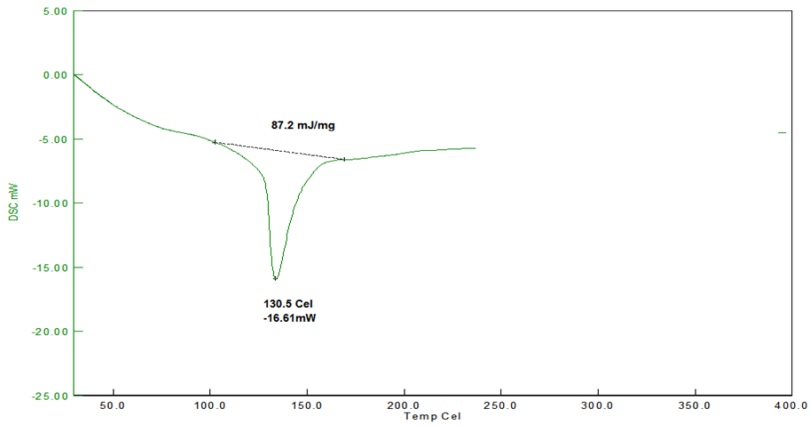

Figure 3: Repaglinide DSC Spectrum

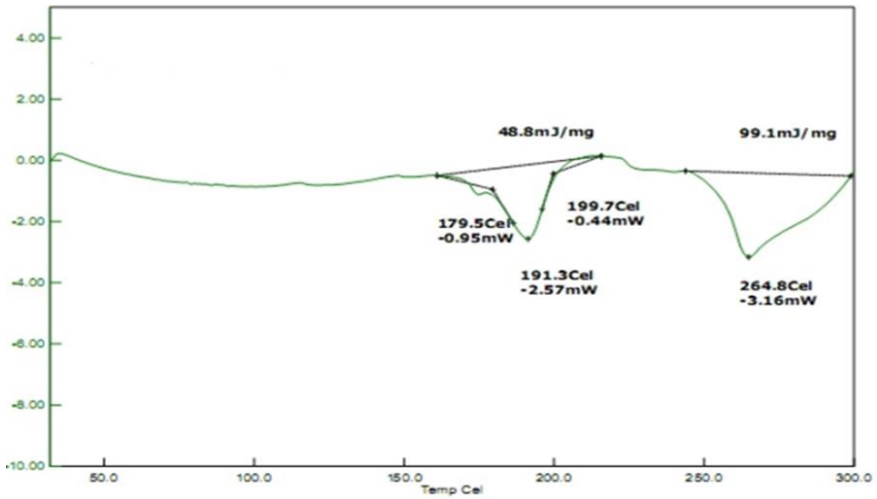

Figure 4: Optimized Repaglinide loaded microspheres

SEM studies:

The spherical nature of the microspheres is evident from the photographs. Figure 5 confirms that F6 microspheres with flat surfaces have a spherical shape. The last perfect formulation F6 can achieve the required in vitro release profile at pH 7.4 thanks to its good spherical shape and particle size of 130.57±4.52 µm. In an acidic environment, its drug release is the lowest (3.25%), while its encapsulation efficiency is much higher (84.92±2.26%).

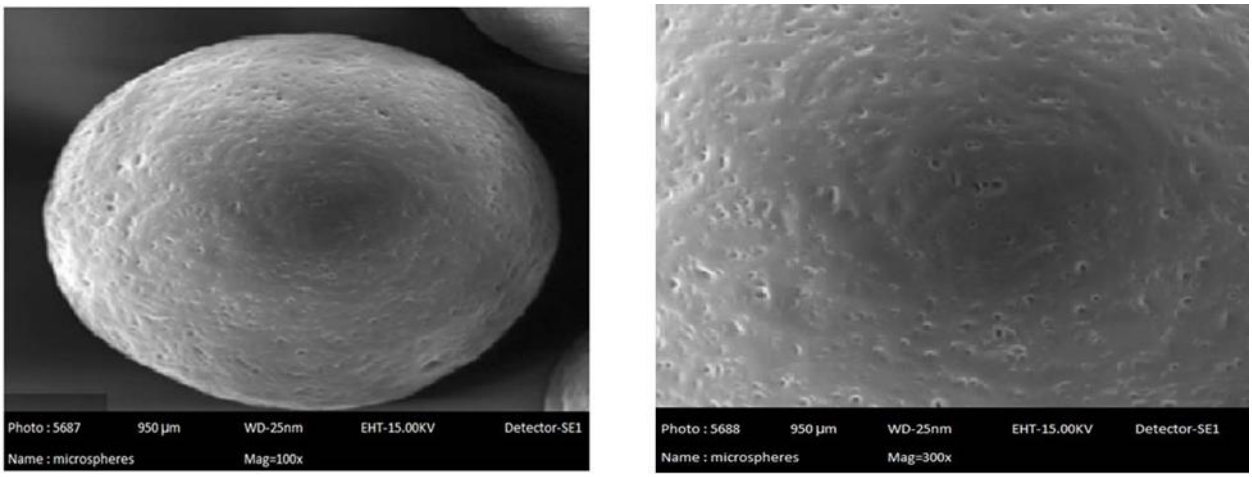

Figure 5: SEM of optimized floating microspheres

Characterization of microspheres:

Micromeritic properties were tested on floating microspheres, and all parameters were within limits.

Table 2: Micromeritic property of floating microspheres of Repaglinide

Formulation code	Mean particle size	Bulk density (gm. /cm^3)	Tapped density (gm. /cm^3)	Hausners ratio	Carrr's index	Angle of repose
F1	320.15±3.62	0.35±0.03	0.45±0.01	1.12±0.01	18.15±0.03	27.04±1.93
F2	265.82±4.95	0.41±0.05	0.47±0.01	1.14±0.02	12.76±0.02	26.02±1.80
F3	240.31±6.02	0.40±0.01	0.48±0.02	1.2±0.01	16.66±0.03	26.56±1.43
F4	265.89±9.83	0.35±0.02	0.40±0.05	1.14±0.05	12.5±0.06	27.72±1.89
F5	130.57±4.52	0.40±0.07	0.47±0.04	1.17±0.03	14.8±0.04	30.88±2.78
F6	235.67±6.31	0.44±0.01	0.50±0.02	1.13±0.02	12±0.05	26.56±1.68
F7	256.31±7.25	0.32±0.06	0.39±0.08	1.21±0.04	11.13±0.01	28.49±1.71
F8	250.74±6.31	0.39±0.01	0.45±0.02	1.15±0.06	13.33±0.04	26.80±1.68
F9	167.94±5.04	0.41±0.05	0.48±0.07	1.17±0.01	14.5±0.08	27.11±1.59
F10	324.05±9.86	0.37±0.07	0.44±0.06	1.18±0.02	15.90±0.5	28.04±1.08

Every value is shown as mean ± standard deviation (n=3)

***In-vitro* buoyancy:**

According to table 7.3, formulations F1 through F10 had in vitro buoyancy values ranging from 70.42±1.36 to 95.81±2.11 correspondingly. The formulation with the highest in-vitro buoyancy, F5, was 95.81±2.11. Additionally, the results demonstrated a trend for floating time to increase with particle size.

***In Vitro* drug release:**

The dissolution results made it clear that formulations made with HPMC might not contain even 90% of the medication. Therefore, their formulations were not taken into account. More than 90% of the medication was found in formulations made with ethyl cellulose. The F5 formulation delayed the maximum release of the medication for up to 24 hours. The in vitro release data (not revealed) at pH 7.4 shows how insulin

breaks down when trypsin is present and protease inhibitors are not used. Without trypsin in the dissolution medium, microsphere formulations showed enhanced human insulin release at pH 7.4.

Table 3: *In Vitro* drug release of all formulations

Time (h)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	15.2	19.83	29.6	21.85	28.6	24.6	23.1	28.84	23.4	27
4	31.6	28.05	33.5	35.46	37.2	37.2	32.6	35.71	32.49	35.9
6	43.2	39.51	40.5	41.27	46.3	40.88	43.89	45.82	40.4	44.9
8	55.4	43.8	55.6	49.86	55.6	44.26	49	54.16	46.51	52.51
10	62.2	50.96	62.1	56.38	62.8	51.39	57.4	65.78	54.8	61.36
12	69.8	65.12	73.4	61.05	69.2	57.73	68.54	73.2	63.21	69.46
14	74.6	70.1	81.43	67.84	73.1	69.89	74.43	81.74	71.47	78.35
16	83.2	77.9	90.1	78.24	79.6	78.15	81.2	88.1	80.48	85.36
18	87.6	85.6	99.6	82.43	87.2	82.25	88.9	97.54	86.2	88.43
20	93.2	93.2		92.59	99.23	90.38	95.2		90.2	91.08

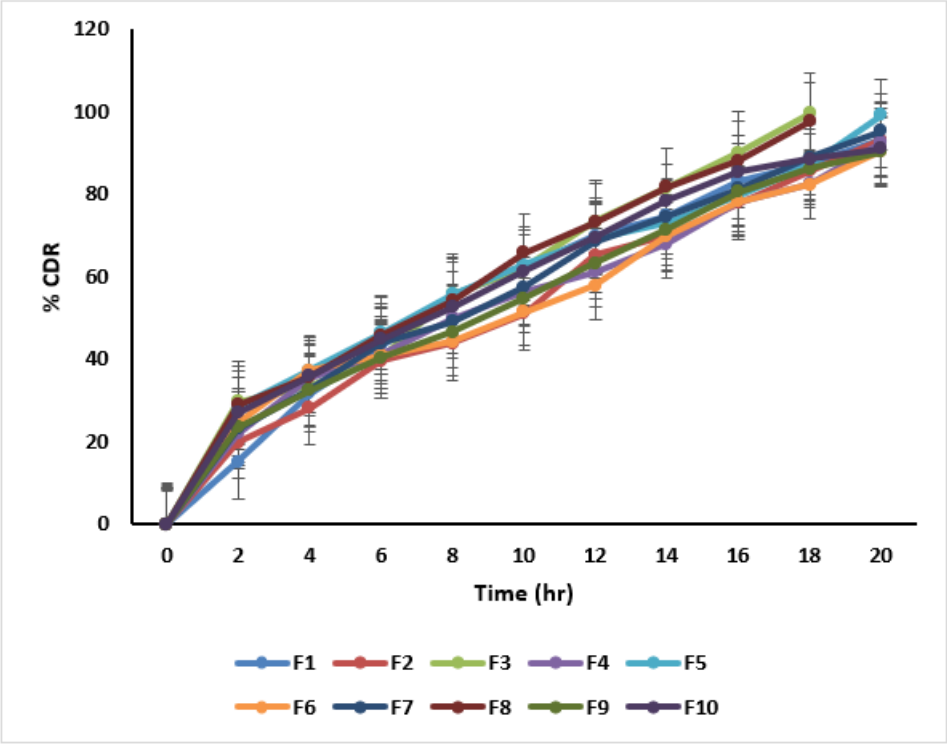

Figure 6: *In Vitro* drug release of all formulations

Ex-vivo Mucoadhesion study:

Table 4: In-vitro Mucoadhesion test

Formulation Code	After 1st hr	After 2nd hr	After 3rd hr	After 4th hr
F5	93	91	83	78

Microsphere adhesion to the tissue was measured at 30-minute intervals, 1-hour intervals, and 4-hour intervals. Following analysis, the optimised formulation demonstrated mucoadhesion of over 75%.

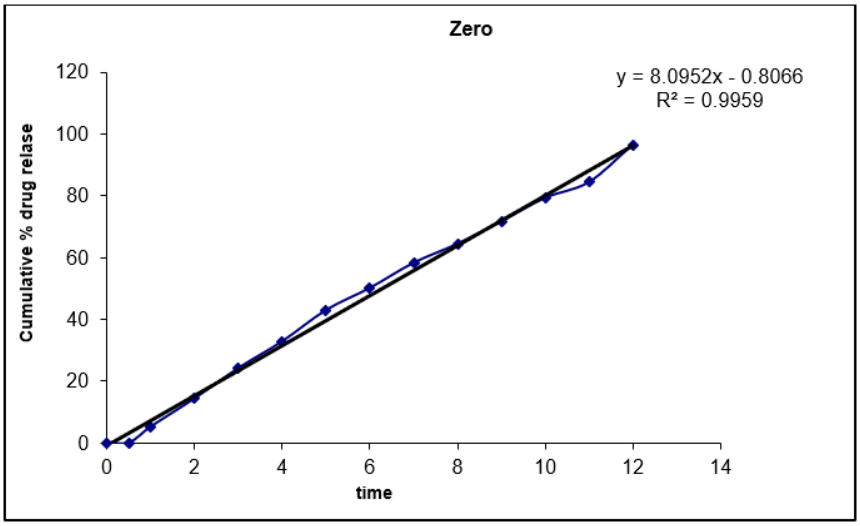

Figure 7: Zero order release kinetics

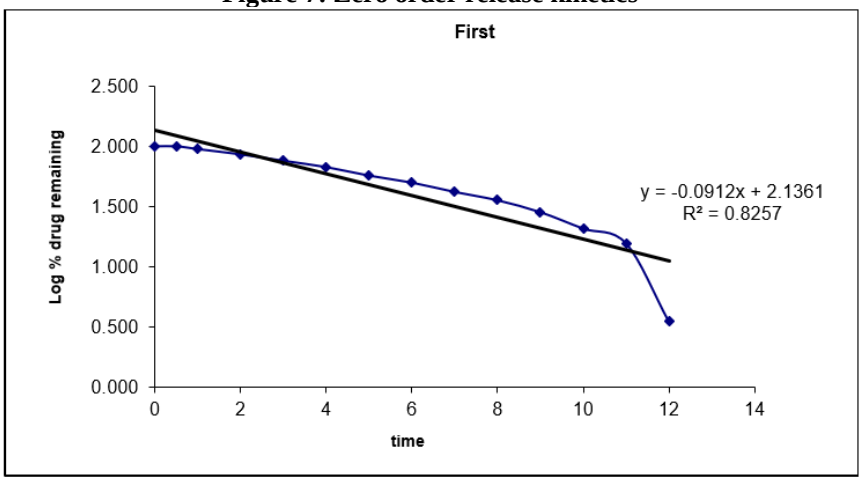

Figure 8: First order release kinetics

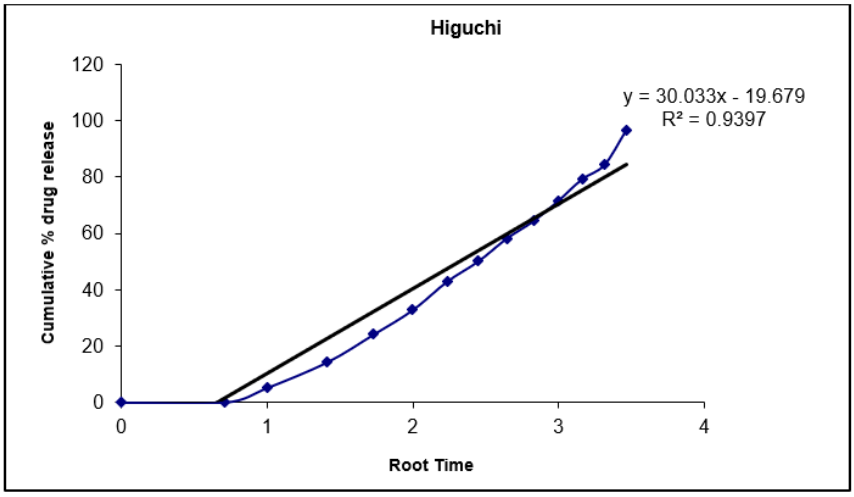

Figure 9: Higuchi release kinetics

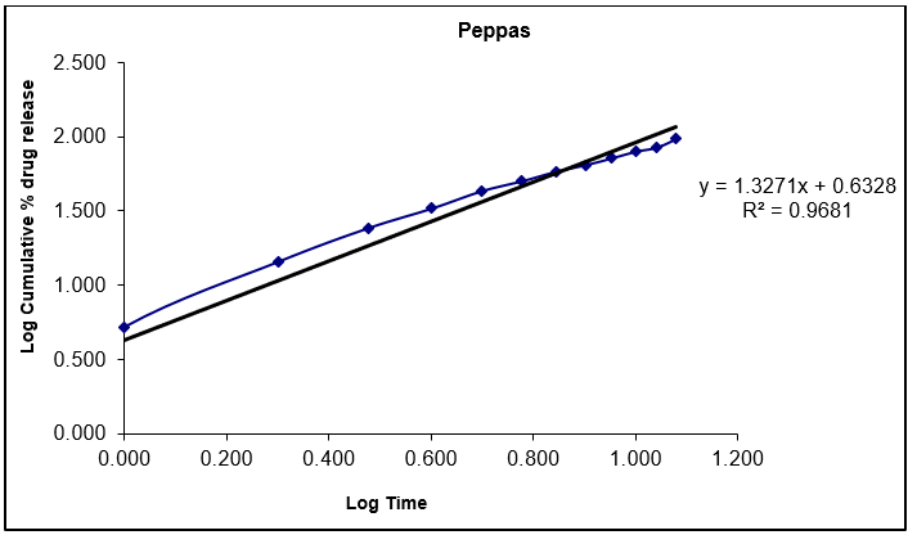

Figure 10: Peppas release kinetics

The optimised formula clearly adhered to zero order release kinetics, as demonstrated by the release kinetics data.

SUMMARY AND CONCLUSION:

The current study was conducted using floating repaglinide microspheres. Ethyl cellulose and HPMC 100 were the polymers utilized. The in vitro buoyancy of formulations F1 through F10 varied from 70.42±1.36 to 95.81±2.11, correspondingly. The dissolving results demonstrated that formulations containing HPMC100 contained up to 90% of the drug. The aforementioned formulations were thus not considered. More than 90% of the drug was found to be present in formulations produced with ethyl cellulose. The maximum release of the medication was postponed for up to 12 hours by the F5 formulation. It was therefore considered to be an optimised formulation. In terms of drug release kinetics, the optimized formulation was kept. The kinetics of Korsmeyer Peppas release were noted.

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