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Original Article

Formulation and *In Vitro* Evaluation of Floating Microspheres of Anti Diabetic Drug

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ABSTRACT

Objective: The study intended to develop and evaluate floating microspheres containing anti-diabetic drug of Saxagliptin. **Method:** Microspheres were prepared by Ionotropic gelation method. The release microspheres were formulated using different polymers Eudragit RL, HPMC K4M, and Gum Acacia with different concentrations. The formulations were evaluated for Particle size, *in vitro* release studies, FTIR spectra, DSC studies, and micrometric properties. **Results and Discussion:** Prepared microspheres were characterized for their particle size, drug entrapment efficiencies (79.50%-96.86%), percentage Buoyancy (93.87%-98.86%).The FTIR spectra and DSC studies have shown stable properties of Saxagliptin and revealed the absence of interactions between the drug and selected polymers. The *in vitro* release studies were performed in 0.1 N HCL, which showed a release of 92.64% at the end of 24 hours in case of the best formulation. Fitting to the *in vitro* release data to Korsmeyer Peppas equation indicated that Quasi–Fickian diffusion of drug release.
Conclusion: It can be concluded that Saxagliptin floating microspheres produces prolonged and site-specific drug delivery for the treatment of Diabetes mellitus.

1. INTRODUCTION

The population of patient with chronic diseases has recently been increasing. So there is necessary of taking the drug for an extended period and/or multiple doses of same and/or different medicines simultaneously, which can lead to an increase in non-compliance. Controlled release dosage form covers a wide range of prolonged action formulations that provide continuous release of the active ingredients at a predetermined rate and for a predetermined time. Oral release dosage form has been the field of focus for three decades attributed to the fact of overweighed benefits despite its limitation of the unsuitability of drugs that are easily absorbed from the GIT and have short life are eliminated

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quickly from the blood circulation, so they require frequent dosing [1].

Floating drug delivery system is also called the hydrodynamically balanced system (HBS). Floating drugs delivery system (FDDS) possess lower bulk density than the gastric fluid exerting buoyancy in the stomach leading to slow drug release in an extended manner before it reaches absorption window. In the present formulation, dual benefits of buoyancy as well as actions achieved with an intention to maintain the study state of drug release [2].

Microsphere can be defined as "solid, approximately spherical particles ranging from 1 to 1000 µm." They are made up of polymeric, waxy, or other protective materials, i.e., biodegradable synthetic polymers and modified natural products such as starch, gum, protein, fat, and wax. The natural polymers include albumin and gelatin. The synthetic polymers include polylactic acid and polyglycolic acid [3].

Multiple unit dosage forms are dispersed in the gastrointestinal system more homogeneously than the single unit dosage forms. This enables prolonged and continuous input of the drug to the stomach and upper part of GIT and reduces absorption differences.

Microspheres are one of the multiple-unit polymeric drug delivery systems able to protect the drug from degradation, and hence these have been widely preferred for the controlled release of drugs[4].

Diabetes is one of the primary cause of death and disability in the world. Diabetes (Diabetes mellitus), a metabolic disorder has a condition in which the amount of glucose in the blood is too elevated, and it's characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, and it may present with characteristic symptoms such as thirst, polyuria, blurring of vision, weight loss, risk of cardiovascular, peripheral vascular and cerebrovascular disease, etc., which is controlled by using anti-diabetic drugs[5].

Saxagliptin is a dipeptidyl peptidase-4 (DPP-4) inhibitor antidiabetic for the treatment of type 2 diabetes. It has a molecular weight of 315.41 and a molecular formula of C18H25N3O2. It's half-life 2.5hrs. The metabolism of saxagliptin is primarily mediated by cytochrome P450 3A4/5 (CYP3A4/5). Saxagliptin is eliminated by both renal and hepatic pathways. DPP-4 inhibitors are a class of compounds that work by affecting the action of natural hormones in the body called incretins. Incretins decrease blood sugar by increasing consumption of sugar by the body, mainly through increasing insulin production in the pancreas, and by reducing the production of sugar by the liver6.

Saxagliptin floating microspheres may reduce fluctuation of drug blood concentration and provides better treatment for Diabetes Mellitus. Hence, there is a need to develop Saxagliptin floating microspheres. In the present study, we tried to create Saxagliptin floating microspheres using different three polymers.

2. MATERIALS AND METHODS

Materials:

Saxagliptin was purchased from Swapnaroop chemicals Ltd. Gum Acacia, HPMC K4M, and Eudragit RL were purchased from Hi-Media Laboratories Pvt. Ltd, all other reagents and chemicals used were for the analytical grade.

Methods:

Determination of λmax of Saxagliptin in 0.1N HCl:

A solution of Saxagliptin was scanned in UV range between 200 to 400nm (LabIndia 3000+ spectrophotometer, Japan). Saxagliptin showed maximum absorbance at 232 nm in 0.1N HCl.

PREPARATION OF THE MICROSPHERES:

Preparation of microspheres loaded Saxagliptin using Gum Acacia, HPMC K4M, Eudragit RL.

Ionotropic gelation method:

Floating microspheres were prepared by Ionotropic gelation method. Sodium alginate was dissolved insufficient amount of water with maintaining the temperature between 40-50°c. Then the required amount of polymers like Eudragit RL, Gum Acacia, and HPMC K4M was added into it. When the polymer dissolved, the drug was added into it and dispersed in the polymer solution. A 10% calcium chloride solution was prepared as a continuous phase and placed it on the calcium chloride solution by using #24gauge needle, $cacl_2$ solution acts as a crosslinking agent and causes gelation of the poured droplets leading to the formation of microspheres. The prepared floating microspheres were allowed to stand in the $cacl_2$ solution for 30 minutes for curation. After that, the microspheres were filtered by Whatman filter paper. The filtered microspheres were dried in a hot air oven at 50°C temperature and stored[7].

Table 1: Composition of Saxagliptin floating microspheres prepared using three polymers

INGREDIENTS	F1	F2	F3	F4	F5	F6	F7	F8	F9
Saxagliptin (mg)	50	50	50	50	50	50	50	50	50
Gum Acacia(mg)	100	150	200	-	-	-	-	-	-
HPMC K4M(mg)	-	-	-	100	150	200	-	-	-
Eudragit RL(mg)	-	-	-	-	-	-	100	150	200
Sodium alginate(mg)	200	200	200	200	200	200	200	200	200
Sodium bicarbonate(mg)	200	200	200	200	200	200	200	200	200
Calcium chloride solution 10% W/V	100	100	100	100	100	100	100	100	100
Water	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

Evaluation of microspheres:[8]

1) The percentage yield of Microspheres:

The prepared microspheres were collected and weighed from different formulations. The measured weight was divided by the total amount of drug and polymers which were used for the preparation of the microspheres to obtained percentage yield.

$$\%\,Yield = \frac{weight\,of\,floating\,microspheres}{weight\,of\,drug + weight\,of\,polymer} \times 100.$$

2) Drug entrapment efficiency:

To determine entrapment efficiency, 20mg accurately weighted microspheres were crushed and dissolved in 100ml 0.1 N HCL. The microspheres were kept to soak for overnight. After that, the solution was filtered through a Whatman filter. After appropriate dilution with 0.1 N HCL, the drug content was determined spectrophotometrically at 220 nm.

$$\%\ Drug\ entrapment\ efficiency = \frac{calculated\,drug\,concentration}{Theoretical\,drug\,concentration} \times 100$$

3) Percentage Buoyancy :

Microspheres were spread over a surface of a USP XXII dissolution apparatus type II filled with 900ml 0.1 Mol/lit HCl containing 0.02% tween 80. The medium is to be agitated with a paddle rotating at 50rpm for 12 hrs. the floating and the settled portion of microspheres will be recovered separately. The microspheres were dried and weighed. Buoyancy percentage was calculated as the ratio of the mass of the microspheres that remain floating and the total mass of the microspheres.

$$Percentage\ Buoyancy = \frac{weight\,of\,floating\,microspheres}{Weight\,of\,floating\,microsphers + weight\,of\,settled\,microspheres} \times 100$$

4) Microscopic examination:

Microspheres were observed under a compound microscope. It gives an indication of particle size and shape of microspheres.

5) Fourier-transform infrared spectroscopy (FT-IR)

Drug-polymer interactions were studied by FTIR spectroscopy. Pure drug and excipients were subjected to FT-IR studies. The samples were intimately mixed with dry powder potassium bromide. The powder mixture was taken in a diffused reflectance samples and the spectra recorded by scanning in the wavelength of 500-4000 cm^{-1} in an FT-IR spectrophotometer. The samples analyzed by FT-IR include.

a) Pure drug
b) Physical mixture of drug + Eudragit RL
c) Physical mixture of drug+ HPMC K4M
d) A physical blend of drug+ Gum acacia
e) Optimized formulation

6) DSC Study

Differential scanning calorimetry was used to measure enthalpy changes due to changes in the physical and chemical properties of the material as a function of temperature.

7) In-vitro Drug Release study:

A USP basket apparatus was used to study in-vitro drug release studies were carried out for all batches in USP type I dissolution test apparatus at 100 rpm, and the dissolution medium was 900ml of 0.1N HCL solution. Microspheres containing 100mg of the drug was used for dissolution study. 5ml of the aliquot was withdrawn at predetermined intervals. Significant dilution was made with 0.1 N HCL solution and filter the solution and analyzed for the drug content spectrophotometrically at 220 nm against a suitable blank. An equal volume of the dissolution medium was replaced in the vessel after each withdrawal to maintain sink condition.

3. RESULTS AND DISCUSSION

The standard plot of Saxagliptin in 0.1N HCl:

Standard solutions (2-12 µg/ml) of Saxagliptin were prepared in 0.1N HCl, and absorbance was measured at 232 nm using UV- Spectrophotometry. The usual plot of Saxagliptin is as shown in (Figure 1). The correlation coefficient obtained was 0.998, and the equation of the regression line was y =0.097x-0.048 was used to calculate the concentration of unknown samples of *in vitro* studies.

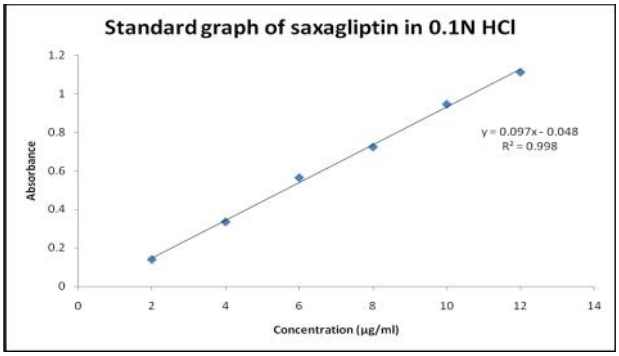

Fig 1: Standard plot of Saxagliptin in 0.1 N HCl

Evaluation of Drug Polymer Interaction by FTIR:

Drug excipients compatibility was confirmed by FTIR spectroscopy. The pure drug showed the

Characteristic peaks are similar to literature values shown in Table 2, which indicates the purity of the drug. All the absorption peaks of Saxagliptin were retained in the physical mixtures of drug with various polymers (HPMC K4M, Eudrait RL, and Gum acacia) and optimized formulation F4. The spectra of material combinations and optimized formulation F4 did not show any shift of vibration bands of Saxagliptin can be observed in Figure 2. It indicates that there was no chemical interaction between the drug and the selected excipients.

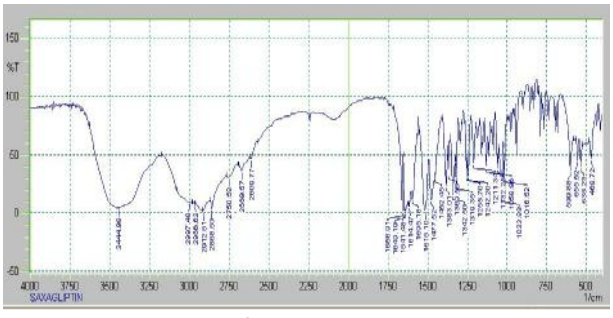

Fig 2: FT-IR spectroscopy of saxagliptin

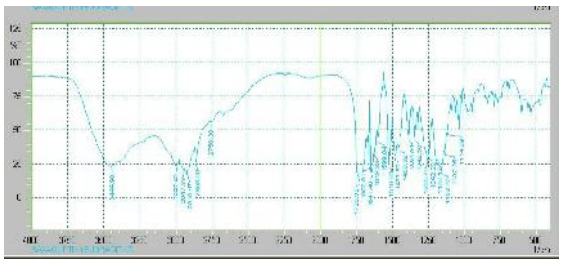

Fig 3: FT-IR spectroscopy of saxagliptin + HPMC K4M

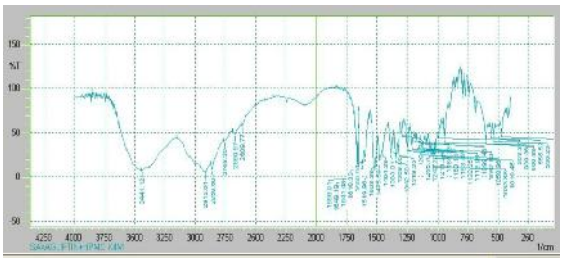

Fig 4: FT-IR spectroscopy of saxagliptin + Eudragit RL

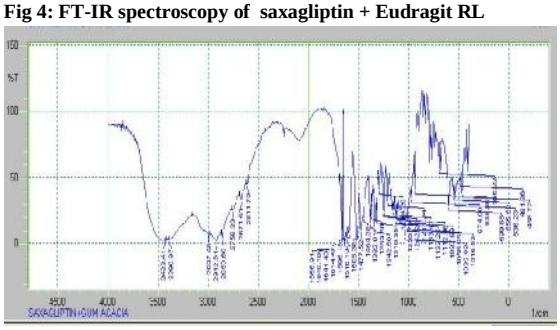

Fig 5: FT-IR spectroscopy of saxagliptin + Gum acacia

Table 2: Functional group and their characteristic peak values of Saxagliptin and optimized formulation (F4) obtained by FTIR studies[9]

SL NO	Functional group	Literature frequency(cm^{-1})	Pure drug (cm^{-1})	Optimized formulation (F4)
1	N-H stretching	3450.12	3444.98	3441.12
2	C-H stretching	2925.32	2912.61	2912.61
3	OH stretching	3304	3301	3301
4	C-N stretching	1618.67	1614.47	1618.33
5	C-O stretching	1260.45	1255.70	1255.70

Characterization by DSC :

In order to find out drug and excipients compatibility, DSC analyses were also performed. Pure Saxagliptin displayed a sharp endothermic peak at 93.68 ^{0}C. The DSC curve of formulation F4 demonstrated an endothermic peak at 109.28^{0}C. DSC results did not show any major interactions.

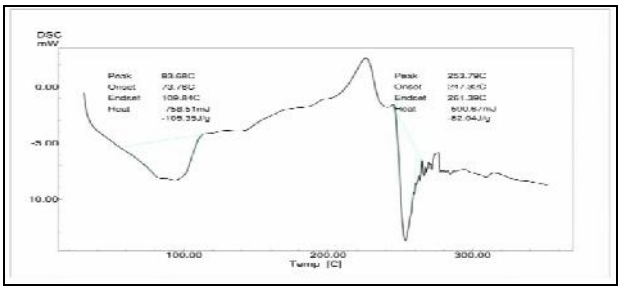

Fig 6: DSC Thermogram of the pure drug Saxagliptin

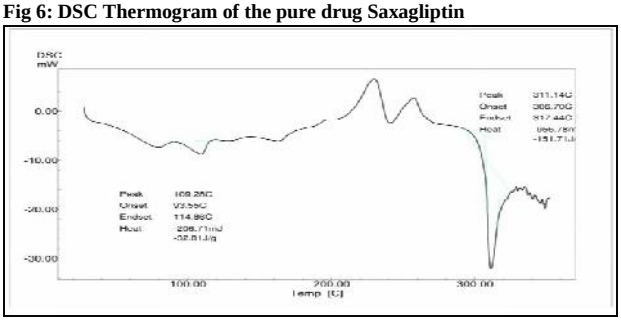

Fig 7: DSC Thermogram of optimized formulation F4

The dose selected for the formulation was 5 mg as per dosage regimen of Saxagliptin. To get a good yield, I have calculated for multiplication of treatments. Totally nine formulations were prepared using different polymers (Eudragit RL, HPMC K4M, and Gum Acacia) in different ratios like (1:1, 1:2 and 1:3) as shown in methodology section (Table 1).

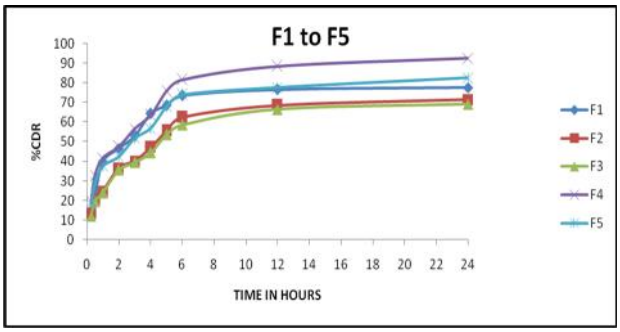

Fig 8: *In* Vitro drug release profile of F1 to F5 formulations

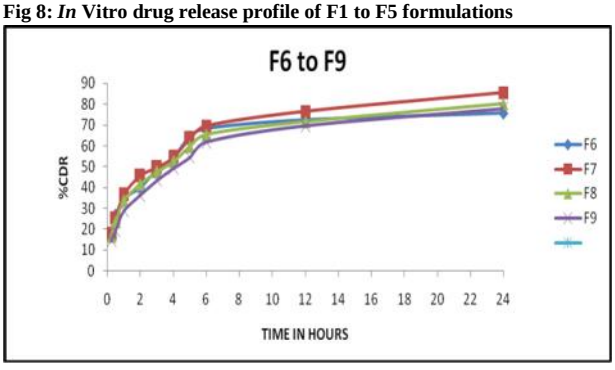

Fig 9: *In vitro* drug release profile of F6 to F9 Formulations

The prepared drug-loaded floating microspheres were studied to know percentage yield, percentage drug entrapment efficiency, and percentage buoyancy. The percentage yield was found to be in the range of 93.16% to 95.71% for microspheres containing sodium alginate along with Gum Acacia as polymer, 94.69% to 98.92% for microspheres containing sodium alginate along with HPMC

K4M as polymer and 96.71% to 98.18% for microspheres containing sodium alginate along with Eudragit RL as a polymer. It indicates that all nine formulations were floated sufficiently for 24 hours in the selected dissolution medium i.e., 0.1 N hydrochloric acid solution. Hence, the developed formulations may float in the stomach during oral administration.

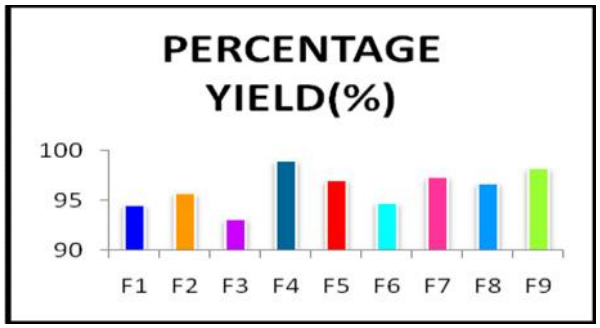

Fig 10: Percentage yield of microspheres from F1 to F9

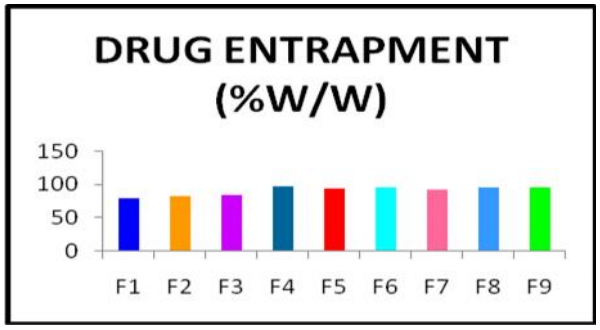

Fig 11: Percentage drug entrapment efficiency of microspheres from F1 to F9

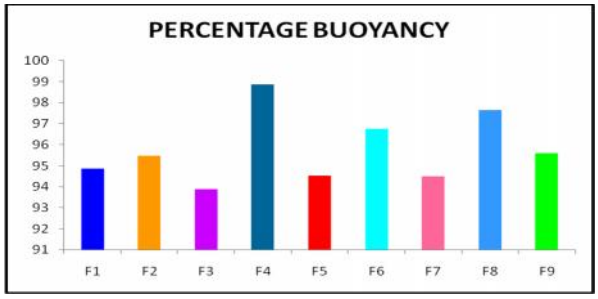

Fig 12: Percentage Buoyancy of formulation from F1 to F9

***In vitro* drug release profile**

Further to optimize, developed floating microspheres of Saxagliptin were subjected to *in-vitro* drug release studies. The release of the drug (Saxagliptin) from the microspheres varied according to the different ratio of the polymer. As a concentration of Eudragit RL, Gum Acacia and HPMC K4M were increased the release of the drug decreased. F4 batch showed maximum drug release (92.64 %) at the end of 24 hours because of HPMC K4M. Thus, HPMC K4M has proved to be an efficient carrier for oral drug delivery of Saxagliptin.

To understand the release pattern of the drug from the floating microspheres, *in vitro* release data obtained was processed and plotted as zero, first-order, Higuchi and Korsmeyer - Peppas model. The kinetic data of all formulations F-1 to F-9 could be best expressed by the zero-order equation as the plots showed the highest linearity (R^2: 0.6495 to 0.7810) then first-order release kinetics (R^2: 0.7765 to 0.8970). The "n" values obtained from Korsmeyer Peppas plots range from (0.3750 to 0.6145) indicate that mechanism of release of formulations F1, F3, F4, F8, and F9 was Quasi-Fickian diffusion, whereas the mechanism of drug release of formulations F2, F6 and F7 was Anomalous (non-fickian) diffusion. The device of drug release from the formulation F5 was found to be fickian diffusion

Table 3: Kinetic Model Fitting For Optimised Formulation (F4)

Formulation code	Zero-order R^2	First-order R^2	Higuchi R^2	Peppas R^2	Slope N
F1	0.7152	0.7952	0.8194	0.9219	0.4712
F2	0.6542	0.8472	0.7951	0.8921	0.5761
F3	0.6954	0.7945	0.7826	0.9128	0.3958
F4	0.7810	0.8970	0.8360	0.9430	0.3750
F5	0.7130	0.8146	0.7989	0.9247	0.5468
F6	0.6531	0.8256	0.6951	0.8246	0.6145
F7	0.6847	0.7765	0.7542	0.8541	0.5834
F8	0.6495	0.7841	0.7756	0.9157	0.4952
F9	0.7419	0.8624	0.7921	0.7989	0.4146

Table 4: Correlation coefficients of different mathematical models for Formulations F1 to F9

Time in hrs	Log time	SQRT	%CDR	Log %CDR	%CRR	%Log CRR
0.25	-0.6020	0.5	19.15	1.2821	80.85	1.9076
0.5	-0.3010	0.7071	32.69	1.5144	67.31	1.8280
1	0	1	41.58	1.6188	58.42	1.7665
2	0.3010	1.4142	47.59	1.6775	52.41	1.7194
3	0.4771	1.7320	56.51	1.7521	43.49	1.6383
4	0.6020	2	63.54	1.8030	36.46	1.5618
5	0.6989	2.2360	75.65	1.8788	24.35	1.3864
6	0.7781	2.4494	81.64	1.9119	18.36	1.2638
12	1.0791	3.4641	88.64	1.9476	11.36	1.0553
24	1.3802	4.8989	92.64	1.9667	7.36	0.8668

4. CONCLUSION

Floating Microspheres of Saxagliptin were prepared by using polymers like Eudragit RL, HPMC K4M, Gum acacia for sustain the drug release.

The FT-IR study concludes that there was no chemical interaction between drug and polymer. Hence the formulations were prepared with Eudragit RL, HPMC K4M, Gum Acacia. Formulation F4 provided a convenient dosage form for achieving best performance regarding in vitro release study, percentage yield, percentage drug entrapment efficiency, and percentage Buoyancy. The microspheres were evaluated for the Particle size, percentage yield, percentage drug entrapment efficiency, percentage Buoyancy &*in vitro* drug release. From the results, it was found that the formulations showed particle size in the micrometer range, good percentage yield, excellent entrapment efficiency, and slow release of the drug.

The release profile of various formulations was fitted to Zero order, First order, Higuchi & Korsemeyer-Peppas to ascertain the kinetic modeling of the drug release. In vitro release studies of F4 formulation (with HPMC K4M) confirmed the excellent controlled release of drug and release follows Korsemeyer Peppas Quasi- Fickian diffusion.

Therefore the present investigation showed promising results of Saxagliptin microspheres of formulation F4 which are prepared with HPMC K4M and proved as the best formulation.

5. ACKNOWLEDGMENT

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