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FORMULATION, CHARACTERIZATION AND EVALUATION OF MICROCAPSULE OF SAXAGLIPTIN

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ABSTRACT

The present study was carried out to develop Saxagliptin drug delivery System in the form of Microcapsule dosage form of Saxagliptin by using EC and HPMC and thereafter formulating the formulation. From the study it is observed that formulation act as prolonged dosage form. As the stirring speed increased the size of Microcapsule decreases and increases the released rate drug. The prepared microcapsule of saxagliptin also gave good percent yield, drug entrapment and In- vitro release. The Microcapsules of F3 batch were found to be satisfactory in terms of percent yield, percent drug entrapment and In-vitro release. Prepared Microcapsule were evaluated for measurement of mean particle size determination of zeta potential, percentage yield, drug entrapment, shape and surface characterization of Microcapsules by scanning electron microscopy (SEM), Floating time, In-vitro release studies, and drug release kinetic data analysis. The mean size of the Microcapsules was determined by photo correlation spectroscopy (PCS) on a submicron particle size analyzer (Horiba Instruments) at a scattering angle of 90°. A sample (0.5mg) of the Microcapsules suspended in 5 ml of distilled water was used for the measurement. The results of measurement of mean particle size found: 128.4nm. The zeta potential of the drug-loaded Microcapsules was measured on a zeta sizer (Malvern Instruments) by determining the electrophoretic mobility in a micro electrophoresis flow cell. All the samples were measured in water at 25°C in triplicate. Results of zeta potential of optimized formulation F3 found: -35.69 mV. The maximum Percentage Yield, Drug Entrapment, Percentage Buoyancy and floating lag time was found to be formulation F3 in floating Microcapsule. The optimized formulation of both batches subjected to further studies.

1. INTRODUCTION

Medication activity can be enhanced by growing new medication conveyance framework, for example, the Microcapsule sedate conveyance framework. These frameworks stay in close contact with the ingestion tissue, the mucous layer, discharging the medication at the activity site prompting a bioavailability increment and both nearby and foundational impacts (Carvalho et al., 2010). The oral course of medication organization constitutes the most helpful and favored methods for sedate conveyance to foundational dissemination of body. However oral organization of the greater part of the medications in traditional measurements frames has here and now restrictions because of their failure to limit and confine the framework at gastro- intestinal tract.

Microcapsules constitute an essential piece of these particulate medication conveyance frameworks by uprightness of their little size and productive bearer limit. Microcapsules are the bearer connected medication conveyance framework in which molecule estimate is ranges from 1-1000 µm extend in distance across having a center of medication and completely external layers of polymer as covering material. Be that as it may, the accomplishment of these Microcapsules is restricted because of their short habitation time at site of assimilation. It would, in this way be worthwhile to have implies for giving a private contact of the medication conveyance framework with the engrossing layer. Microcapsules have focal points like proficient retention and upgraded bioavailability of the medications because of a high surface to volume proportion, a substantially more cozy contact with the bodily fluid layer and particular focusing of medications to the ingestion site (Parmar et al., 2010).

Microcapsules incorporate micro particles and microcapsules (having a center of medication) of 1-1000 µm in distance across and comprising either totally of a floating polymer or having an external covering of it, individually. Microcapsules, as a rule, can possibly be utilized for focused and controlled discharge sedate conveyance; however coupling of floating properties to Microcapsules has extra preferences e.g. effective assimilation and bioavailability of the medications because of high surface to volume proportion, a considerably more personal contact with the mucous layer, particular focusing of medications to the ingestion site.

Diabetes mellitus is a combination of heterogeneous disorders commonly presenting with episodes of hyperglycemia and glucose intolerance, as a result of lack of insulin, defective insulin action, or both (Sicree et al., 2006). Such complications arise due to derangements in the regulatory systems for storage and mobilization of metabolic fuels, including the

catabolism and anabolism of carbohydrates, lipids and proteins emanating from defective insulin secretion, insulin action, or both (Shillitoe, 1988; Votey and Peters, 2004).

Saxagliptin, sold under the brand name Onglyza, is an oral hypoglycemic of the dipeptidyl peptidase-4 inhibitor class. Early development was solely by Bristol- Myers Squibb; in 2007 AstraZeneca joined with Bristol-Myers Squibb to co-develop the final compound and collaborate on the marketing of the drug.

Floating systems are low density systems that have sufficient buoyancy to float over the gastric contents and remain in the stomach for a prolonged period. While the system floats over the gastric contents, the drug is released slowly at the desired rate, which results in increased gastro-retention time and reduces fluctuation.

Advantages of floating drug delivery

Enhanced Bioavailability

Enhanced First-Pass Biotransformation

Targeted therapy for local ailments in the upper GIT

Reduced fluctuations of Drug concentration

Reduced counter-activity of the Body

Improved Receptor activation selectivity

Minimized adverse activity at the Colon

2. MATERIALS AND METHODS

2.1. Materials: saxagliptin was purchased from SD fine, Dichloromethane, ethanol, ethyl cellulose, PVA and acetic acid were purchased from hi fine

2.2. Methods

2.2.1. Characterization of drug

Physiochemical properties of saxagliptin

a) Physical evaluation

It refers to the evaluation by sensory characters, appearance, of the drug, etc. Physical evaluation of drug is given in table 1.

Table 1: Physical evaluation of drug.

S. No.	Sensory characters	Result
1.	Colour	White to light yellow crystalline powder
2.	Odor	Odorless
3.	Taste	Tasteless

Solubility: Solubility of the drug was determined by taking some quantity of drug (about 1-2 mg) in the test tube separately and added the 5 ml of the solvent (water, ethanol, methanol, 0.1N HCL, 0.1N NaOH, Chloroform) Shake vigorously and kept for some time. Note the solubility of the drug in various solvents (at room temperature). Solubility of the drug is given in table 2.

Table 2: Solubility of saxagliptin.

Solvent used	Saxagliptin
Distilled Water	Sparingly Soluble
0.1 N Hydrochloric acid	Soluble
Ethanol	Freely Soluble
Methanol	Freely Soluble
Ethyl acetate	Slightly Soluble
0.1 N NaOH	Sparingly Soluble

Melting point

It is one of the parameters to judge the purity of drugs. In case of pure chemicals, melting points are very sharp and constant. Since the drugs contain the mixed chemicals, they are described with certain range of melting point.

Procedure for determine melting point

A small quantity of powder was placed into a fusion tube. That tube was placed in the melting point determining apparatus (Chemline CL-725) containing castor oil. The temperature of the castor oil was gradual increased automatically and read the temperature at which powder started to melt and the temperature when all the powder gets melted.

Table 3: Melting point of saxagliptin.

S. No.	Melting Point of Saxagliptin	Average Melting Point of Saxagliptin
1.	103-106°C	103-106°C
2.	103-107°C	
3.	103-106°C	

Determination of pH (1 w/v solution in water)

Procedure

About 100mg of the Powder was taken and dissolved in 10ml of distilled water with sonication and filtered. The pH of the filtrate was checked with standard glass electrode.

Table 4: pH of the solution.

S. No.	pH of the solution	Average pH of the solution
1.	6.68	6.683±0.0057
2.	6.69	
3.	6.68	

Identification test

FTIR Spectroscopy

Infra- red spectrum is an important record which gives sufficient information about the structure of a compound. This technique provides a spectrum containing a large number of absorption band from which a wealth of information can be derived about the structure of an organic compound. The region from 0.8 µ to 2.5 µ is called Near Infra-red and that from 15 µ to 200 µ is called Far infra-red region Identification of Saxagliptin was done by FTIR Spectroscopy with respect to marker compound. Saxagliptin was obtained as White to off white powder. It was identified from the result of IR spectrum as per specification.

Sample of pure saxagliptin

The IR spectrum of sample drug shows the peak values which are characteristics of the drug and the graph were shown in figure no.

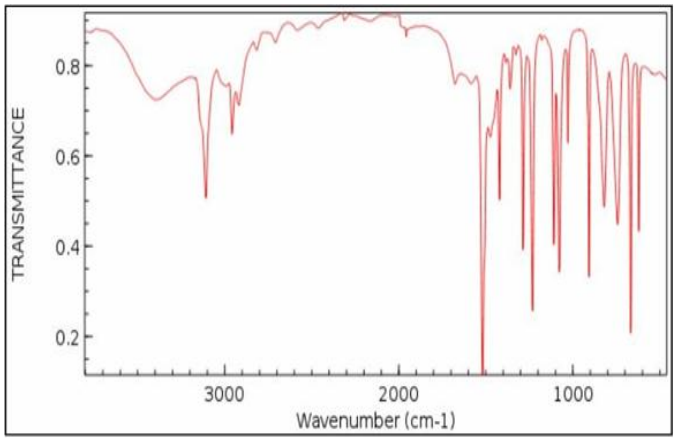

Figure 1: FT-IR Spectrum of Pure Drug (Saxagliptin).

Loss on drying: The moisture in a solid can be expressed on a wet weight or dry weight basis. On a wet weight basis, the water content of a material is calculated as a percentage of the weight of the wet solid. The term loss on drying is an expression of moisture content on a wet weight basis.

Procedure

Loss on drying is directly measured by IR moisture balance. Firstly, calibrated the instrument by knob then taken 5 gm sample (powder) and set the temp at 100°C to 105°C for 15 minutes and constant reading set the knob and check % moisture.

Table 5: Loss of drying of drug sample.

S. No.	Initial weight	Final weight after 15 minutes	% loss of drying	Avg. % loss of drying
1.	1gm	0.998 gm	0.2%	0.366±0.152
2.	1gm	0.995 gm	0.5%	
3.	1gm	0.996 gm	0.4%	

Determination of λ max of saxagliptin

The λmax of Saxagliptin was determined by running the spectrum of drug solution in double beam ultraviolet spectrophotometer.

Procedure

Accurately weighed 10 mg of drug was dissolved in 10 ml of 0.1 N Hcl solution in 10 ml of volumetric flask. The resulted solution 1000µg/ml and dilution to make it to a concentration range of 5-25µg/ml. The spectrum of this solution was run in 200-400 nm range in U.V. spectrophotometer (Labindia-3000+). The spectrum peak point graph of absorbance of Saxagliptin versus wave length was shown in figure:

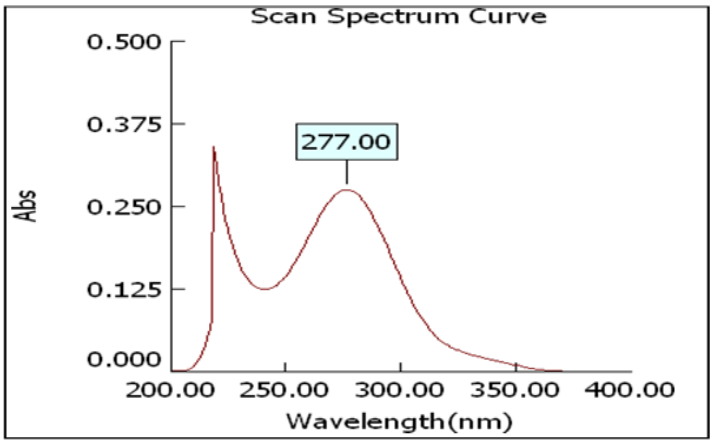

Figure 2: Wavelength maxima of Saxagliptin in 0.1 N Hcl at 277nm.

Calibration curve of Saxagliptin at λ max 277nm Observation table:

Table 6: Calibration curve of saxagliptin.

S. No.	Conc. (µg/ml)	Absorbance
1	5	0.139±0.002

2	10	0.275±0.003
3	15	0.395±0.001
4	20	0.524±0.002
5	25	0.645±0.001

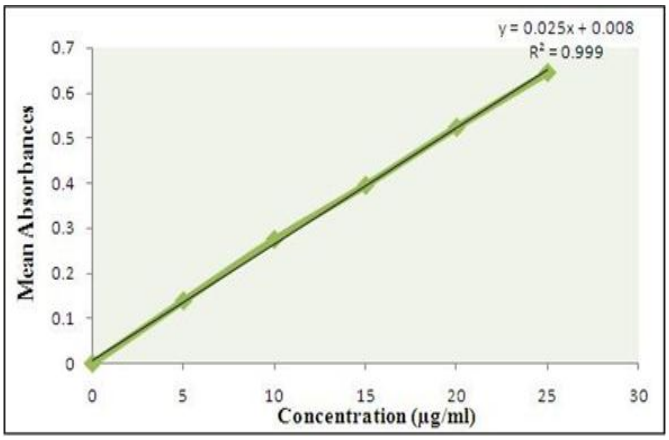

Figure 3: Calibration curve of Saxagliptin in 0.1 N Hcl at 225nm.

The linear regression analysis was done on Absorbance data points. The results are as follow for standard curve

Slope = 0.025

The intercept = 0.008

The correlation coefficient (r2) = 0.999

Formulation development

Preparation of floating microcapsule of saxagliptin

Floating Microcapsules loaded with Saxagliptin were prepared using solvent diffusion-evaporation method using HPMC and EC in different ratio like 1:1, 1:1.5, 1:2 w/w. Drug and polymer in proportion of drug and polymers were dissolved in 1:2 mixture of solvent system of ethanol and dichloromethane. This clear solution was poured slowly in a thin stream into the aqueous solution of 1% polyvinyl alcohol. The emulsion was continuously stirred for 3 h at a speed of 500 rpm at 27±2°C. The floating Microcapsules were collected by decantation, while the non- floating Microcapsules were discarded. The Microcapsules were dried overnight at 40±2°C and stored in desicator.

Table 6: Formulations of the floating Microcapsules prepared.

Sr. No	Formulation Code	Saxagliptin (mg)	HPMC (mg)	EC (mg)
1.	F1	50	50	50
2.	F2	50	50	75
3.	F3	50	50	100

4.	F4	50	100	50
5.	F5	50	100	75
6.	F6	50	100	100

Evaluation of microcapsules

Percentage yield

The prepared Microcapsules with a size range of 1µm to 1000µm were collected and weighed from different formulations. The measured weight was divided by the total amount of all non-volatile components which were used for the preparation of the Microcapsules.

$$\% \text{ Yield} = \frac{\text{Actual weight of product}}{\text{Total weight of drug and polymer}}$$

Drug entrapment

The various formulations of the Floating Microcapsules were subjected for drug content. 10 mg of Floating Microcapsules from all batches were accurately weighed and crushed. The powder of Microcapsules were dissolved in 10 ml 0.1 N HCl and centrifuge at 1000 rpm. This supernatant solution is than filtered through whatmann filter paper No. 44. After filtration, from this solution 0.1 ml was taken out and diluted up to 10 ml with 0.1 N HCl.

The percentage drug entrapment was calculated using calibration curve method.

Floating behavior: Ten milligrams of the floating Microcapsules were placed in 0.1 N HCl (100 mL). The mixture was stirred at 100 rpm in a magnetic stirrer. After 10 h, the layer of buoyant Microcapsule 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 a constant weight was obtained. Both the fractions of Microcapsules were weighed and buoyancy was determined by the weight ratio of floating particles to the sum of floating and sinking particles.

Measurement of mean particle size

The mean size of the Microcapsules was determined by Photo Correlation Spectroscopy (PCS) on a submicron particle size analyzer (Malvern Instruments) at a scattering angle of 90°. A sample (0.5mg) of the Microcapsules suspended in 5 ml of distilled water was used for the measurement.

Determination of zeta potential

The zeta potential of the drug-loaded Microcapsules was measured on a zeta sizer (Malvern Instruments) by determining the electrophoretic mobility in a micro electrophoresis flow cell. All the samples were measured in water at 25°C in triplicate.

Shape and Surface Characterization of Microcapsules by Scanning Electron Microscopy (SEM)

From the formulated batches of Microcapsules, formulations (F3) which showed an appropriate balance between the percentage releases were examined for surface morphology and shape using scanning electron microscope Jeol Japan 6000. Sample was fixed on carbon tape and fine gold sputtering was applied in a high vacuum evaporator. The acceleration voltage was set at 10KV during scanning. Microphotographs were taken on different magnification and higher magnification (200X) was used for surface morphology.

In-vitro release studies

The drug release rate from Floating Microcapsules was carried out using the USP type II (Electro Lab.) dissolution paddle assembly. A weighed amount of Floating Microcapsules equivalent to 100 mg drug were dispersed in 900 ml of 0.1 N HCI (pH=1.2) maintained at 37 ± 0.5°C and stirred at 50 rpm. One ml sample was withdrawn at predetermined intervals and filtered and equal volume of dissolution medium was replaced in the vessel after each withdrawal to maintain sink condition. The collected samples analyzed spectrophotometrically at 277nm to determine the concentration of drug present in the dissolution medium.

RESULT AND DISCUSSION

Evaluation of saxagliptin microcapsules

Percentage yield

Percentage yield of different formulation was determined by weighing the Microcapsules after drying. The percentage yield of different formulation was in range of 76.65±0.32–85.45±0.56%. The maximum Percentage Yield was found in formulation F3, 85.45±0.56 as compare to all formulation.

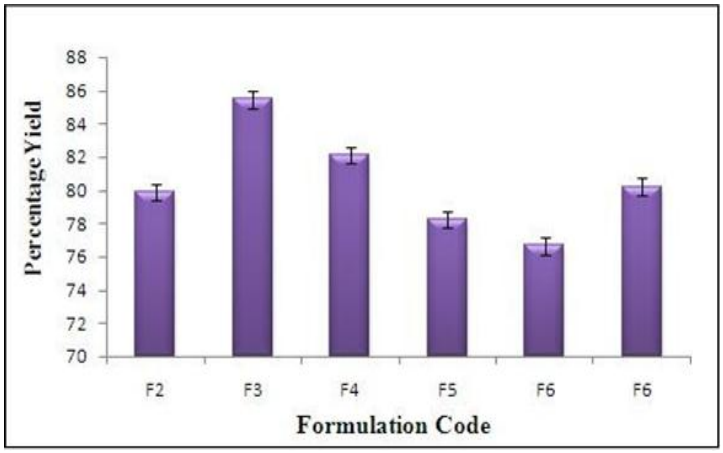

Figure 4: Percentage yield for different formulation.

Drug entrapment

The drug entrapment efficacies of different formulations were in range of 63.23±0.65-76.56±0.65% w/w.

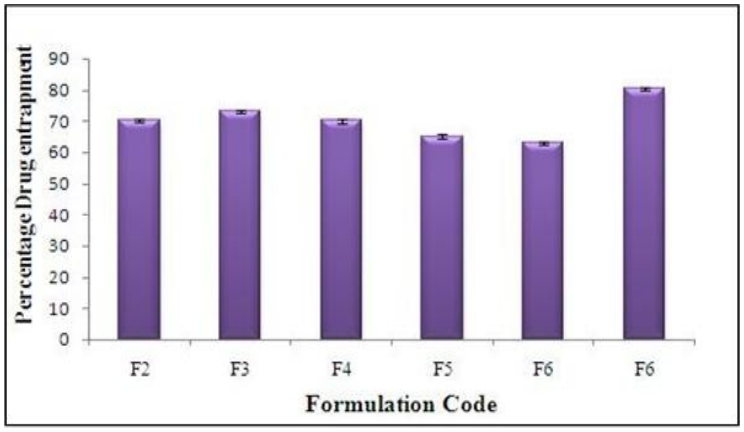

Figure 5: Drug entrapment for different formulation.

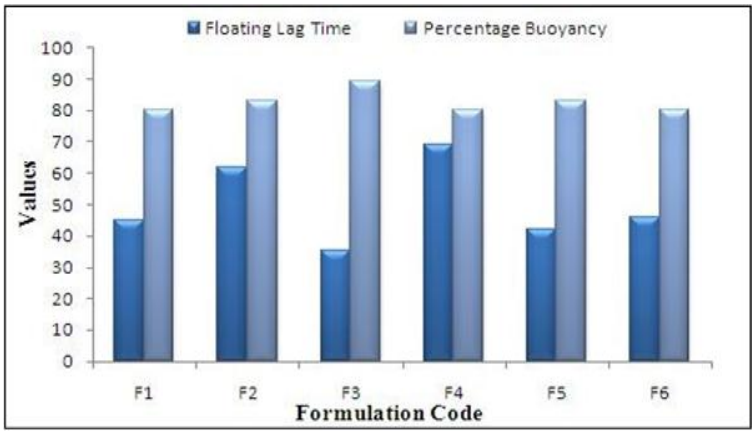

Figure 6: Floating Lag Time and Percentage buoyancy for different formulation.

The maximum Percentage Yield, Drug Entrapment, Percentage Buoyancy and floating lag time was found to be formulation F3 in floating Microcapsule. The optimized formulation of both batches subjected to further studies.

Particle size analysis

The mean size of the Microcapsules was determined by photo correlation spectroscopy (PCS) on a submicron particle size analyzer (Malvern Instruments) at a scattering angle of 90°. A sample (0.5mg) of the Microcapsules suspended in 5 ml of distilled water was used for the measurement. The results of measurement of mean particle size of optimized formulation F3 of floating Microcapsule was found to be 128.4nm.

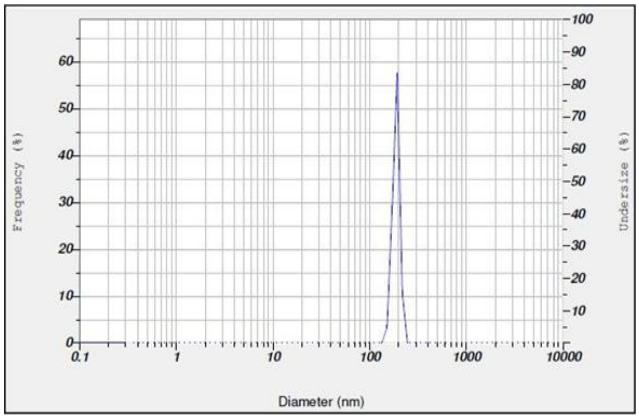

Figure 7: Particle size data of optimized Microcapsule formulation F3.

Zeta potential

The zeta potential of the drug-loaded Microcapsules was measured on a zeta sizer (Malvern Instruments) by determining the electrophoretic mobility in a micro electrophoresis flow cell. All the samples were measured in water at 25°C in triplicate. Results of zeta pot ential of optimized formulation F4 of floating Microcapsule was found -35.69 mV.

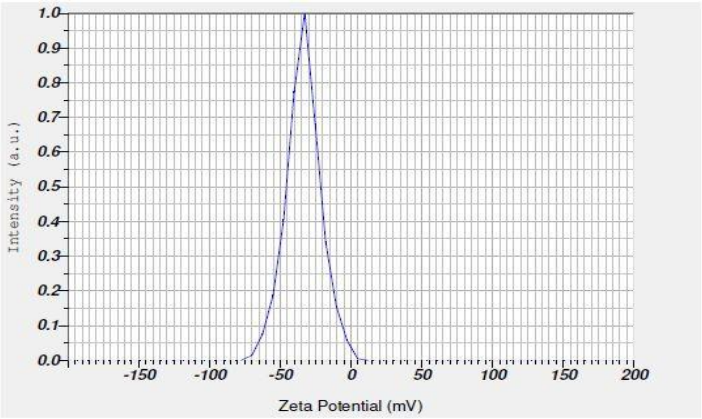

Figure 8: Zeta potential data of floating Microcapsule F3.

In-Vitro drug release study

In vitro drug release study of Saxagliptin loaded Microcapsule Comparative release study of all formulation F1-F6.

Table 7: Release Study data of formulation F1-F6.

Time	% of Drug Release					
(hr)	F1	F2	F3	F4	F5	F6
0.5	36.45	35.45	26.65	15.65	13.24	13.25
1	45.65	40.25	45.65	22.12	23.56	20.23
2	69.89	63.12	55.32	31.48	29.89	26.65
4	79.98	74.65	60.36	42.23	40.12	33.65
6	95.65	88.98	68.89	54.45	51.15	45.65
8	-	96.32	72.32	65.85	60.12	56.45
10	-	-	88.95	70.23	68.89	69.98
12	-	-	98.89	78.89	75.45	73.12

Graph of release study of formulation F1-F6

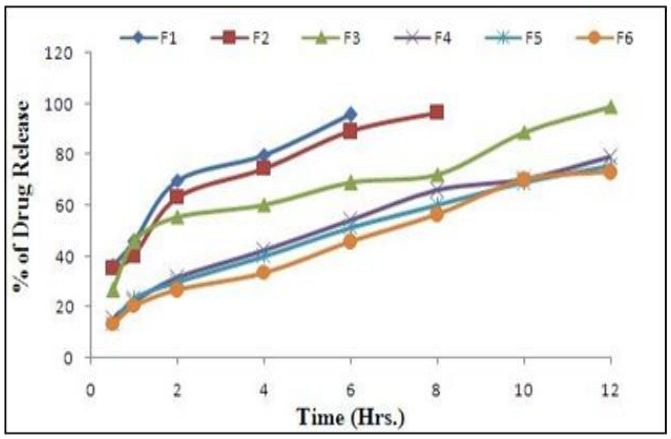

Figure 9: Graph of release study of formulation F1-F6.

Table 7: Release Kinetics of optimized formulation of Microcapsule F-3.

Time (h)	Square Root of Time(h)$^{1/2}$	Log Time	Cumulative % Drug Release	Log Cumulative % Drug Released	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.5	0.707	-0.301	26.65	1.426	73.35	1.865
1	1	0	45.65	1.659	54.35	1.735
2	1.414	0.301	55.32	1.743	44.68	1.650
4	2	0.602	60.36	1.781	39.64	1.598
6	2.449	0.778	68.89	1.838	31.11	1.493
8	2.828	0.903	72.32	1.859	27.68	1.442
10	3.162	1	88.95	1.949	11.05	1.043
12	3.464	1.079	98.89	1.995	1.11	0.045

Zero order release kinetics graph of optimized formulations

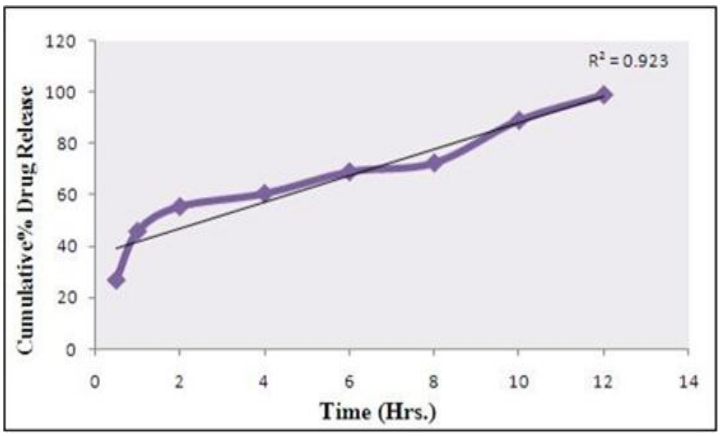

Figure 10: Zero order release kinetics graph of optimized formulations.

First order release kinetics graph of optimized formulations

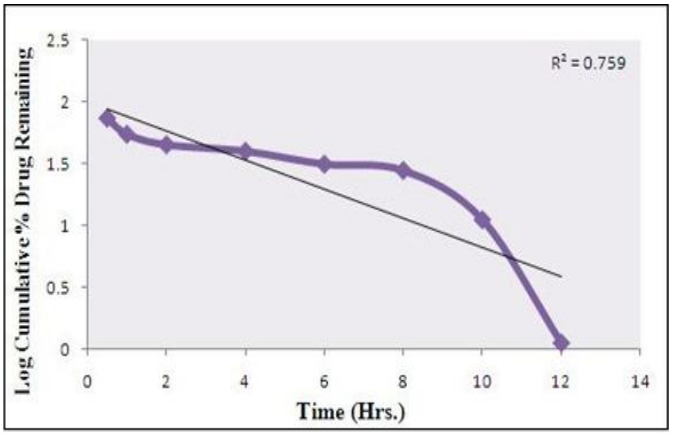

Figure 11: First order release kinetics graph of optimized formulations.

Table 8: Comparative study of regression coefficient for selection of optimized Formulation F-3.

Release Kinetics	Zero order	First order
R^2	0.923	0.759

The In vitro drug release data of the optimized formulation was subjected to goodness of fit test by linear regression analysis according to zero order, first order kinetic equation, in order to determine the mechanism of drug release. When the regression coefficient values were compared, it was observed that an 'r' value of Microcapsule was maximum zero order i.e 0.923 hence indicating drug releases from formulations was found to follow zero order for floating Microcapsule.

CONCLUSION

The present study was carried out to develop Saxagliptin drug delivery System in the form of Microcapsule dosage form of Saxagliptin by using EC and HPMC and thereafter formulating the formulation. From the study it is observed that formulation act as prolonged dosage form. As the stirring speed increased the size of Microcapsule decreases and increases the released rate drug. The prepared microcapsule of saxagliptin also gave good percent yield, drug entrapment and In- vitro release. The Microcapsules of F3 batch were found to be satisfactory in terms of percent yield, percent drug entrapment and In-vitro release.

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