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Formulation and evaluation of sustained release saxagliptin microspheres by ionotropic gelation method

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Abstract: The objective of the current investigation is to reduce dosing frequency and improve patient compliance by designing and systematically evaluating sustained release microspheres of an antidiabetic agent, saxagliptin. Saxagliptin microspheres were formulated using sodium alginate as the controlled release polymer by ionotropic gelation technique. The polymer sodium alginate alone and along with different coating polymers like pectin, ethyl cellulose was used in different ratios (1:1,1:1.5, 1:2) to formulate batches F1 to F9. The resulting microspheres were evaluated for particle size, densities, flow properties, morphology, recovery yield, drug content, drug entrapment efficiency and *in vitro* drug release behavior. The formulated microspheres were discrete, spherical with relatively smooth surface, and with good flow properties. The drug entrapment efficiency obtained in the range 70.4% to 95.2%. Among different formulations, the fabricated microspheres of batch F3 had shown the optimum percent drug encapsulation of microspheres and the sustained release of the saxagliptin for about 9 h. *In vitro* study showed that drug release slowly increases as the pH of the medium is increased. Release pattern of saxagliptin from microspheres of batch F3 followed Higuchi model and zero-order release kinetic model. The value of 'n' was found to be 0.867. The data obtained thus suggest that a microparticulate system can be successfully designed for sustained delivery of saxagliptin and to improve dosage form characteristics for easy formulation.

Key words: Microencapsulation; Ionotropic gelation; Microspheres; Sodium alginate; ethyl cellulose; Pectin.

Introduction

The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body to achieve promptly and then maintain the desired concentration. That is the drug delivery system should deliver the drug at a rate dictated by the needs of the body over a specified period of treatment.[1] The design of proper dosage form regimens is an important element in accomplishing this goal. An important issue in the development of these systems is to avoid inter subject variations and to improve the absorption of poorly absorbed drug by using such systems[2].

The term "sustained release" is used to describe a dosage form formulated to retard the release of a therapeutic agent such that its appearance in to systemic circulation is delayed and or prolonged and its plasma profile is sustained in duration. The onset of pharmacological action is often delayed and duration of its therapeutic effect is often sustained. Controlled release dosage forms are designed to release drug *in vivo* according to predictable rates that can be verified by *in vitro* measurements. Controlled release technology implies a quantitative understanding of the physicochemical mechanism of drug availability to the extent that the dosage form release rate can be specified. Various designations such as smart targeted, intelligent, novel and therapeutic have been given to sustained release systems [3,4].

Saxagliptin is an antidiabetic agent which acts by inhibiting dipeptidyl peptidase-4 (DPP-4) for treating type 2 diabetes. 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 production of sugar by the liver.[5,6] In present work, a muco adhesive gastro retentive microparticulate system (microspheres) for saxagliptin were developed by ionotropic gelation technique using afore mentioned polymers. The microspheres were spherical in shape and the drug remained dispersed in the polymer matrix at amorphous state. Scanning electron microscopy(SEM), FTIR and *in vitro* dissolution studies were performed to characterize the microspheres. The method of microencapsulation is based on ion gelation technique involving alginate polymers alone and or in combination with other polymers.

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Materials and Methods

Materials

Saxagliptin and ethyl cellulose were obtained from Spectrum laboratories, Hyderabad, India. Sodium alginate, pectin, calcium chloride was procured from SD fine chemicals Ltd., Mumbai, India.

Preparation of microspheres[7, 8]

Microspheres containing saxagliptin were prepared by ionotropic gelation technique employing solution of sodium alginate alone (2-4% w/v) and sodium alginate with different coating polymers like ethyl cellulose, pectin and $CaCl_2$ as counterion in three different batches. In the first set three batches of drug loaded microspheres were prepared (F1, F2, F3). In a beaker sodium alginate (2-4%) dissolved in water using magnetic stirrer. Saxagliptin (200 mg) was dispersed in 50 ml of sodium alginate solution and the above solution was dropped using a hypodermic syringe into calcium chloride (3% w/v) solution. Microspheres formed immediately and were left into the original solution for 1 h to ensure internal gelification. Then they were filtered, washed with alcohol and dried at room temperature

In the second set two batches of drug-loaded Microspheres were prepared (F4,F5,F6) using sodium alginate and pectin as a coating polymers. To 50 ml of de ionized water, pectin (0.5 – 1% w/v) were added and stirred with the electric stirrer to form mucilage. Then sodium alginate (3% w/v) was added to form uniform mucilage. Then finely weighed quantity (200 mg) saxagliptin was added and homogenized for 5min. The resulting dispersion was dropped through syringe into 100 ml of 5% w/v aqueous calcium chloride solution and stirred at 100 rpm. After stirring for 10 minutes the formed beads were separated, washed with distilled water, air dried and finely dried 60^0C for 6 h.

In third set, two batches of Microspheres were prepared (F7, F8, F9) using sodium alginate and ethyl cellulose as a coating polymer. To 50 ml aqueous sodium alginate solution, weighed quantity (200 mg) saxgliptin was dispersed uniformly. Bubble free dispersion was dropped through a syringe into 100 ml of ethyl cellulose solution containing 5% w/v calcium chloride (ethyl cellulose dissolved in 10ml of 5% w/v acetic acid). Stirred at 100 rpm. After stirring for 30 minutes, the coated beads were separated by filtration, washed with distilled water, air dried and finally dried 60^0C for 6 h.

Evaluation parameters of microspheres [9,10]:

Measurement of Micromeritic Properties of microspheres:[11,12]

a. Granulometric Study: Granulometric study was conducted to determine the particle size distribution pattern. For this study sieve analysis was carried out on mechanical sieve shaker, using different meshes (#12, #16, #22, #30) of American Society of Testing Materials (ASTM). The size distribution of microspheres is reported.

Angle of repose: The flow properties were investigated by measuring the angle of repose of drug-loaded microspheres using fixed-base cone method to assess the flowability. In this method, a funnel was secured with its tip at a 1cm height (H) above the graph paper that was placed on a flat horizontal surface. Microspheres were carefully poured through the funnel until the apex of the conical pile just touched the tip of the funnel. Measure the height of heap pile (H) and the radius of the base (r) with ruler. The angle of repose was determined by using the equation, and reported in table no.1.

$$\tan \theta = H/R \quad \text{or} \quad \theta = \tan^{-1} H/R$$

Where, θ = angle of repose,
R = radius of the base of pile
H = height of pile.

Bulk and tapped density: The bulk and tapped densities were measured in a 10-mL graduated measuring cylinder to measure packability of the microspheres. The sample contained in the measuring cylinder was tapped mechanically by means of constant velocity rotating cam with change in its initial bulk density to a final tapped density when it has attained its most stable form. Each experiment was carried out in triplicate. The bulk and tapped density can be determined and reported in table no.1.

Particle size analysis: Particle size analysis of drug-loaded Microspheres was performed by optical microscopy (Oslympus Model Szx-12). A small amount of microspheres was suspended in purified water (10ml). Mount the sample on a clean glass slide and placed it on mechanical stage of the microscope. The eye piece of microscope fitted with a micrometer by which the size of the beads could be determined. The process was repeated for each batch of prepared Microspheres and mean particle size can be reported in figure no.1.

b. Surface study:

The surface morphological details of the Microspheres were determined by using a scanning electron microscope (SEM) model JSM, 35CF JEOL, Japan. The samples were dried thoroughly in vacuum dessicator before mounting on brass specimen studies. The samples were mounted on a specimen studies using double sided adhesive tape, and gold-palladium alloy was coated on the sample using spatter coating unit (Model E5100 Polar on, UK) in an argon ambient of 8-10 pascal with plasma voltage about 2Kv and discharge current about 20 mA. The sputtering was done for nearly 3 minutes to obtain uniform coating on the samples to enable good quality SEM images. The SEM operated at low accelerating voltage of about 15KV with load current of about 80mA. The condenser lens position was maintained between 4.4 – 5.1. The objective lens aperture has a diameter of 240 microns and the working distance WD = 39mm.

c. Loose-Surface Crystal Study:

In this study accurately weighed 25 mg of microspheres (#16) were suspended in the phosphate buffer pH 6.8 and was shaken vigorously for 5min. The drug leached out from the surface of the microspheres was analyzed at 243nm wavelength spectrophotometrically. Results are reported.

d. Swelling Properties:

The swelling properties of prepared microspheres were determined in acidic buffer pH 1.2. Thirty dried beads were placed in a beaker to which 200 mL of buffer solution and then stirred with a magnetic stirrer at a speed 50 rpm. After 1h interval, the equilibrium swollen beads were observed and measured under optical microscope. The magnitude of swelling was presented by the ratio of the mean diameter of swollen beads to the mean diameter of the dried beads before the test.

e. Drug Entrapment Efficiency (DEE):

Drug entrapment efficiency of microspheres was performed by accurately weighed 50 mg of microspheres were suspended in 100 mL of phosphate buffer pH 6.8±0.1. The resulting solution was kept for 24 hours. Next day it was stirred for 15 min and subjected for filtration. After suitable dilution, saxagliptin content in the filtrate was analyzed spectrophotometrically at 210 nm using Shimadzu 1201 UV-visible spectrophotometer. The obtained absorbance was plotted on the standard curve to get the exact concentration of the entrapped drug. Calculating this concentration with dilution factor we get the percentage of actual drug encapsulated in Microspheres.

The drug entrapment efficiency was determined using following relationship: -

$$\%DEE = \frac{\text{Actual Drug Content}}{\text{Theoretical Drug Content}} \times 100$$

f. *In-vitro* Dissolution Studies [13,14]:

The *in vitro* drug release studies were performed using Dissolution Apparatus USP using simulated gastric fluid (pH 1.2 buffer) for nine hours. An accurately weighed amount of drug loaded mucoadhesive microspheres equivalent to 50 mg of saxagliptin, was added to 900 mL of dissolution medium and the release of saxagliptin, from mucoadhesive microspheres was investigated at about 100 rpm at temperature 37 °C ± 0.5 °C. During dissolution 5 mL aliquot was withdrawn at different time intervals of 1 to 9 h and same was replaced with equal volume of fresh medium. The withdrawn samples were filtered through Whatmann filter paper no.42 and absorbance was measured at 210 nm using UV-Visible Spectrophotometer. Cumulative percent drug released was found out at each time interval and graph was plotted between cumulative % drug released and time in hours. Treatment of drug release data with different kinetic equations. Analysis of drug release from microspheres was performed with a flexible model that can identify the contribution to overall kinetics, mechanism of drug release and the dissolution data obtained for optimized formulation was treated with the different release kinetic equations.[15].

Results and Discussion

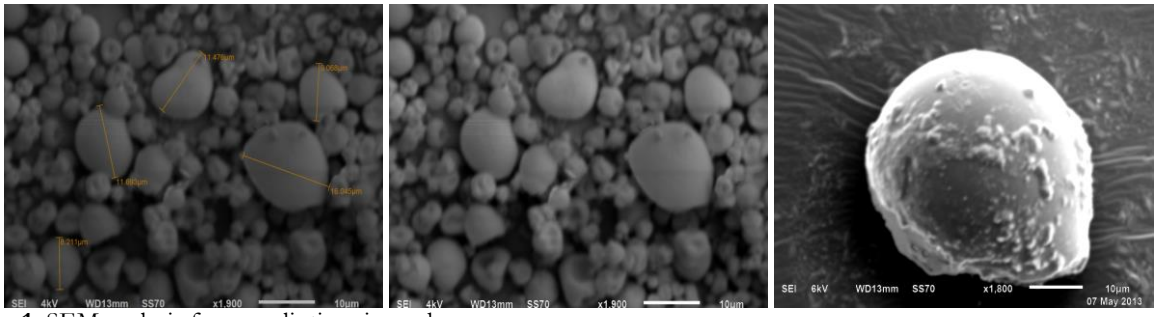


Fig.1: SEM analysis for saxagliptin microspheres.

Table 1:

Batch no.	Angle of Repose (°)	Bulk Density (g/ml)	Tapped Density (g/ml)	Carr's Index	Hausner's ratio	Mean diameter (µm)	% yield	drug entrapment efficiency (%)
F1	31.49±1.18	0.70±0.04	0.79±0.16	12.48	1.31	841.11±43.5	91.11	70.405
F2	30.38±0.542	0.61s±0.07	0.72±0.02	19.14	1.19	767±38.02	89.92	77.248
F3	28.49±3.65	0.776±0.09	0.86±0.07	11.46	1.11	747.91±32.8	95.65	85.317
F4	25.50±1.81	0.726±0.01	0.76±0.05	5.78	1.05	845.9±42.69	90.23	90.945
F5	25.54±0.90	0.725±0.03	0.77±0.04	6.62	1.06	873.59±20.75	91.67	94.702
F6	25.67±0.467	0.719±0.02	0.81±0.02	13.35	1.13	942.85±24.65	90.76	89.921
F7	24.13±0.40	0.807±0.07	0.78±0.06	9.54	1.09	974.2±28.72	88.90	95.270
F8	27.84±1.81	0.808±0.05	0.86±0.01	9.93	1.06	889.01±20.52	94.10	86.378
F9	26.56±0.597	0.774±0.03	0.84±0.02	9.04	1.09	928.57±7.43	93.16	88.050

Standard deviation (SD) n=3; (∑ SD=standard deviation, n=3).

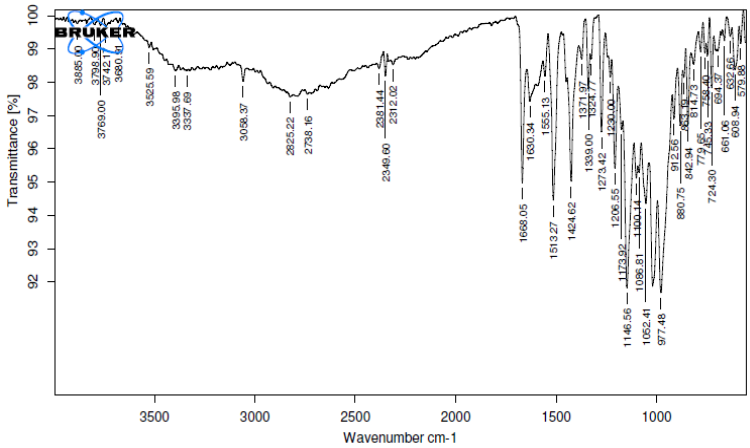


Fig.2: FTIR spectrum of optimised formulation F3

Table 2: In vitro release kinetic data of drug loaded microsphere

Formulation code	Cumulative Percent Drug Release (%CDR)									
	0 h	1 h	2 h	3 h	4 h	5 h	6 h	7 h	8 h	9 h
F1	0	40.69±1.02	50.26±1.77	56.16±2.37	67.9±1.85	83.52±3.87	101.24±1.73	--	--	--
F2	0	33.49±2.09	39.79±0.81	54.09±1.59	61.43±0.80	63.86±3.44	88.30±0.43	100.4±1.35	--	--
F3	0	27.36±3.44	32.81±1.73	37.93±0.77	42.12±2.47	61.79±3.67	62.89±2.11	68.17±8.44	88.93±1.08	100±1.42
F4	0	66.17±1.62	83.65±0.75	91.63±1.80	99.4±1.54	--	--	--	--	--
F5	0	51.72±0.63	58.83±2.41	70.89±2.83	83.46±3.49	100.13±0.43	--	--	--	--
F6	0	52.67±3.32	61.47±3.68	66.52±1.22	77.78±1.22	87.64±1.67	92.54±5.67	--	--	--
F7	0	25.79±4.38	54.98±3.14	73.97±6.07	98.22±1.47	--	--	--	--	--
F8	0	36.83±1.88	36.78±3.49	54.25±4.03	77.98±2.74	89.19±3.01		--	--	--
F9	0	27.74±5.17	48.63±5.39	62.98±6.56	76.89±4.75	86.17±3.80	92.54±5.67	--	--	--

(∑ SD=standard deviation, n=3)

Table 3: Model fitting data of the release profile for saxaglptin using five different models

Formulation	Mathematical Models (Kinetics)				
	Zero order	First order	Higuchi matrix	Koresmeyer peppas 'n'value	Hixson crowell
F1	0.869	0.713	0.930	1.537	0.932
F2	0.905	0.710	0.927	1.437	0.925
F3	0.953	0.705	0.935	1.284	0.931
F4	0.914	0.642	0.924	1.972	0.891
F5	0.800	0.753	0.872	1.694	0.915
F6	0.789	0.836	0.925	1.467	0.856
F7	0.904	0.946	0.932	2.275	0.934
F8	0.888	0.791	0.912	0.922	0.914
F9	0.947	0.725	0.923	1.746	0.880

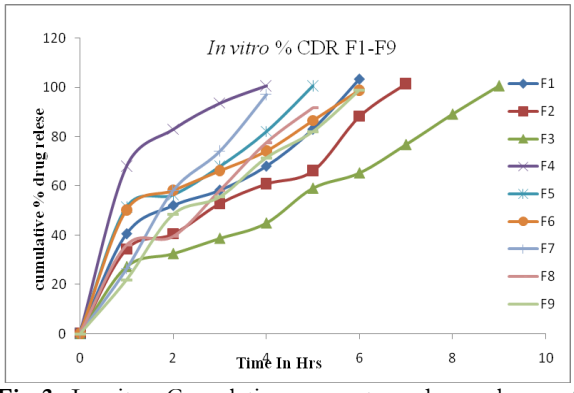


Fig.3: In vitro Cumulative percentage drug release of formulations F1 to F9

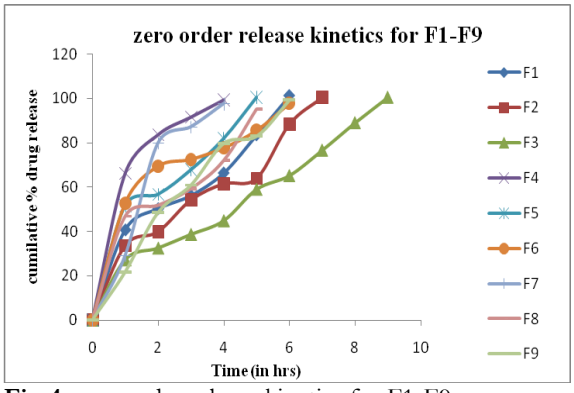
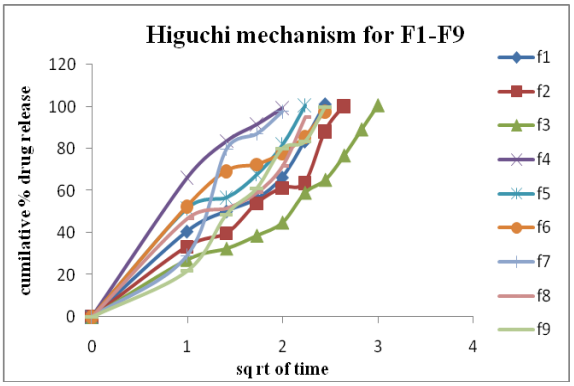


Fig.4: zero order release kinetics for F1-F9



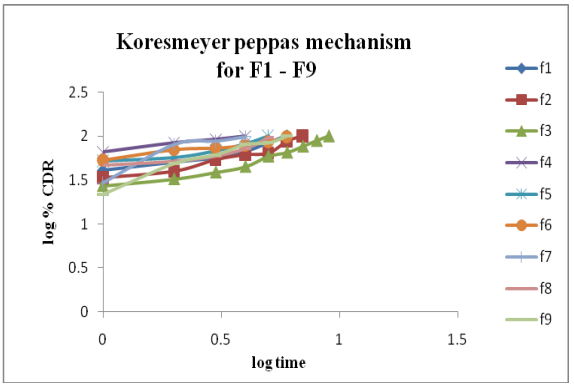


Fig.5: Higuchi & Koresmeyer peppas mechanism for F1-F9

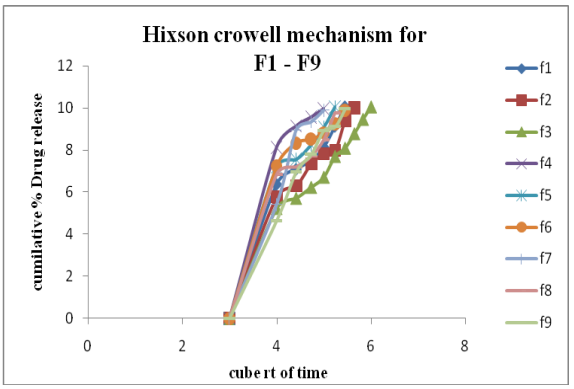


Fig.6: Hixson Crowell mechanism for F1-F9

Conclusion

Preformulation studies like pH, melting point, solubility and UV analysis of saxagliptin were complied with BP standards. The FTIR spectras revealed that, there was no interaction between polymers and saxagliptin. Oral controlled release of saxgliptin can be achieved by ionotropic gelation techniques by using polymers like sodium alginate, pectin, ethyl cellulose respectively. Surface smoothness of the saxagliptin microspheres was increased by increasing the polymer concentration, which was confirmed by SEM. As the drug to polymer ratio was increased, the mean particle size of saxagliptin microspheres was also increased. Saxagliptin microspheres with normal frequency distribution were obtained. Entrapment efficiency increases with increase in the polymer concentration. From the results, it can be inferred that there was a proper distribution of saxagliptin in the microspheres and the deviation was within the acceptable limits. The study also indicated that the amount of drug release decreases with an increase in the polymer concentration. The in vitro studies of Saxagliptin microspheres showed prolonged and sustained release of drug. The co-efficient of determination indicated that the release data was best fitted with zero order kinetics. Higuchi equation explains the diffusion sustained release mechanism. The diffusion exponent 'n' values of Korsemeyer-Peppas model was found to be in the range of 1 to 2 for the Saxagliptin microspheres prepared with Sodium alginate, pectin and Ethyl cellulose.

From the study, it is evident that promising controlled release microspheres of Saxagliptin may be developed by ionotropic gelation method and solvent evaporation techniques by using polymers like Sodium alginate, Pectin and Ethyl cellulose respectively. Our study suggested that microspheres prepared by ionotropic gelation technique is inexpensive when compared with other technique and also advantageous to prevent the drug and dose related side effects. The entire process is feasible in industrial scale and requires pilot study. The formulated microspheres are dispensed by filling them in capsules.

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