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Formulation Development and Evaluation of Gastroretentive Delivery System (Microspheres) Using Natural Polymer

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ABSTRACT

Microspheres are novel drug delivery approach to control release of pharmacologically active ingredient as per patient need. Natural polymers like fenugreek mucilage are cheap, biodegradable and have been proved safe for pharmaceutical formulation. Higher loading efficiency was observed for all the formulations and also the drug release was observed for the period of 12 hours. Thus, the simvastatin microsphere using fenugreek mucilage showed promising results in retarding the drug release. It can be concluded from whole study that due to the formation of polymeric network system or other active moiety can be easily entrapped with the matrix and hydration and swelling of natural polymer controlled their release pattern

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INTRODUCTION

Microspheres are defined as "Monolithic sphere or therapeutic agent distributed throughout the matrix either as a molecular dispersion of particles" (or) can be defined as the structure made up of the continuous phase of one or more miscible polymers in which drug particles are dispersed at the molecular or macroscopic level. Microspheres are small spherical particles, with diameters in the micrometer range (typically 1 µm to 1000 µm). Microspheres are sometimes referred to as microparticles which are made of various polymers. Biodegradable synthetic polymers and modified natural products such as starches, gums, proteins, fats, and waxes are used. The natural polymers include albumin and gelatin, the synthetic polymer includees polylactic acid and polyglycolic acid. The solvents used to dissolve the polymeric materials are chosen according to the polymer and drug solubility, stabilities, process safety and economic considerations *(Chein, 1992).* Microspheres for oral use have been employed to sustain the drug release and to reduce or eliminate gastrointestinal tract irritation. In addition, multi particulate delivery systems spread out more uniformly in the gastrointestinal tract. This results in more reproducible drug absorption and reduces local irritation when compared to single-unit dosage forms such as no disintegrating, polymeric matrix tablets. Unwanted intestinal retention of the polymeric material, which may occur with matrix tablets on chronic dosing, can also be avoided *(Mathew et al., 2008).*

Therapeutic action *(Sipai Altaf et al., 2012; Hardenia, et al., 2011; Anandea et al., 2008).*Adhesion can be defined as sticking of a drug to the membrane by using the sticking property of the water-soluble polymers. Adhesion of drug delivery device to the mucosal membrane such as buccal, ocular, rectal, nasal etc. can be termed as bio-adhesion. These kinds of microspheres exhibit a prolonged residence time at the site of application and cause intimate contact with the absorption site.

In floating types, the bulk density is less than the gastric fluid and so remains buoyant in the stomach without affecting the gastric emptying rate. The drug is released slowly at the desired rate if the system is floating on gastric content and increases gastric residence and increases fluctuation in plasma concentration. Moreover, it also reduces the chances of striking and dose dumping. One another way it produces a prolonged therapeutic effect and therefore reduces dosing frequencies *(Dutta et al., 2001; Kawashima et al., 1991).*

The ability to incorporate reasonably high concentrations of the drug. Stability of the preparation after synthesis with a clinically acceptable shelf life. Controlled particle size and dispersibility in aqueous vehicles for injection. Release of active reagent with a good control over a wide time scale.

Biocompatibility with a controllable biodegradability. Susceptibility to chemical modification. *(Mohan et al., 2014; SreeGiri et al., 2014)*

MATERIAL & METHOD

Formulation Development

Extraction and Isolation of Fenugreek mucilage

The clean, dry fenugreek seeds were grounded under mild conditions using a laboratory mixer. The grounded seeds were then sieved to remove the germ which possesses the lowest hardness. The remaining part was soaked overnight in water, to allow the gum to swell. The swelled gum was separated from the other components of the seeds via filtration through a muslin cloth.

The separated gum was either used as it is, *i.e.* in the viscous form or precipitated using commercial ethyl alcohol, dried and finally grinded to fine powder (*Ragheb et al., 2015).*

Isolated Gum Dry Gum

Figure 1: Extraction and Isolation of Fenugreek mucilage

Evaluation of mucilage

Determination of percentage yield

Percentage yield of mucilage was determined using this formula.

Determination of swelling index

The swelling index is the volume in ml occupied by 1g of drug; including any adhering mucilage after it has been swollen in an aqueous liquid for 4h .The swelling index of Fenugreek mucilage powder, was determined according to the (BP, 2001). One gram of mucilage powder was taken in a 25 ml ground glass stoppered cylinder graduated over a height of 120 to 130 mm in 0.5 divisions. To this 25 ml of water was added and this was shaken vigorously every 10 m for 1h and then allowed to stand for 24 h. The volume occupied by mucilage was measured. The Swelling index was calculated from the mean of three determinations.

Swelling Index % (SI) = (W2 – W1/W1) x 100

W1= Initial Volume in ml

W2= Final Volume in ml

Loss on drying of isolated gum

The moisture in a solid can be expressed on a wet weight or dry wet basis. On a wet weight basis, the water content of a material is calculated as a percentage of the weight of the weight 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 (Labgo Infrared Moisture Balance). Firstly calibrated the instrument by knob then taken 5.000 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.

Preparation of mucoadhesive microsphere

Chitosan microspheres were prepared by ionotropic gelation method.

Preparation I: Chitosan stock solution (1% w/v) was prepared by dissolving chitosan in acetic acid (1% v/v) at room temperature.

Preparation II: The drug and sodium alginate was dissolved in 10 ml of water.

Preparation III: 1% Sodium tripolyphosphate solution was prepared.

Preparation IV: Solution of preparation I was slowly added in preparation III with continuous starring on magnetic stirrer.

Preparation II was added in preparation IV through a disposable syringe needle into a gently agitating. The dropping rate and falling distance were kept constant. The solution was magnetically stirred for half an hour followed by filtration and rinsing with distilled water. Gel like beads were obtained which was air dried for twenty four hours followed by oven drying for six hours at 40˚C.

Table 1: Different formulations of simvastatin

Sr. No	Formulation Code	Simvastatin (mg)	Chitosan (mg)	Sod. Alginate (mg)	Fenu greek (mg)
1.	F1	50	25	-	25
2.	F2	50	50	-	25
3.	F3	50	25	25	-
4.	F4	50	50	25	-
5.	F5	50	-	25	25
6.	F6	50	-	50	25

Evaluation of microspheres

Percentage Yield

The prepared microspheres with a size range of 200-300nm 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 microspheres *(Rajinikanth et al., 2008).*

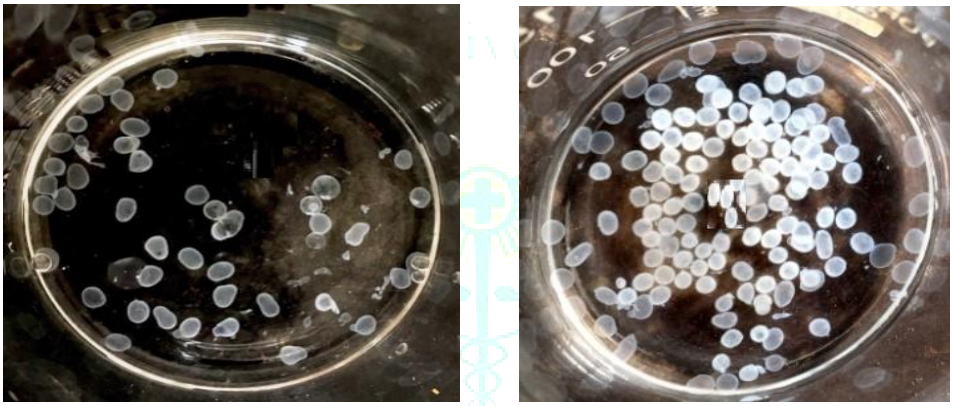

Figure 2: Formulated microsphere

Drug Entrapment

The various formulations of the mucoadhasive microspheres were subjected for drug content. 10 mg of mucoadhasive microspheres from all batches were accurately weighed and crushed. The powder of microspheres were dissolved in 10 ml 1.2 pH Buffer and centrifuge at 1000 rpm. This supernatant solution is than filtered through Whatman filter paper No. 44. After filtration, from this solution 0.1 ml was taken out and diluted up to 10 ml with 1.2 pH Buffer. The percentage drug entrapment was calculated using calibration curve method.

Measurement of mean particle size

The mean size of the microspheres 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 microspheres suspended in 5 ml of distilled water was used for the measurement *(Kamel et al., 2001).*

Determination of zeta potential

The zeta potential of the drug-loaded microspheres was measured on a zeta sizer (Malvern Zetasizer) by determining the electrophoretic mobility in a micro electrophoresis flow cell. All the samples were measured in water at 25°C in triplicate *(Kamel et al., 2001*).

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

From the formulated batches of microspheres, formulations (F4) 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 *(Kamelet al.,2001).*

In-vitro Release Studies

7.4.6.1 *In vitro* drug release in simulated gastric fluid

The prepared microsphere was evaluated for *in vitro* drug release. The drug release studies were carried out using USP XXII paddle type Dissolution test apparatus. The dissolution study was carried out in 900 ml dissolution medium which was stirred at 100 rpm maintained at 37±0.2°C.

A weighed quantity of formulation (100 mg) was spread over the surface of dissolution media (900 ml) at 37±0.2°C. Samples were withdrawn at different time interval and compensated with same amount of fresh dissolution medium. Volume of sample withdrawn was made up to 10ml by continuous media. The samples withdrawn were assayed

spectrophotometrically at 232.0 nm for simvastatin and using UV visible spectrophotometer. The release of simvastatin was calculated with the help of Standard curve of simvastatin.

Drug release kinetic data analysis

Several kinetic models have been proposed to describe the release characteristics of a drug from matrix. The following three equations are commonly used, because of their simplicity and applicability. Equation 1, the zero-order model equation (Plotted as cumulative percentage of drug released vs time); Equation 2, Higuchi's squareroot equation (Plotted as cumulative percentage of drug released vs square root of time); and Equation 3, the Korsemeyer-Peppas equation (Plotted as Log cumulative percentage of drug released vs Log time).

To study the release kinetics of simvastatin from the Mucoadhasive microspheres the release data was fitted to these four equations

Zero order equation: When a graph of the cumulative percentage of the drug released from the matrix against time is plotted, zero order release is linear in such a plot, indicating that the release rate is independent of concentration.

$Q_t = k_0.t$

Where Q_t is the percentage of drug released at time t and k_0 is the release rate constant;

First order equation

$In (100-Q_t) = In\ 100- k_I.t$

Where k_I is the release rate constant;

Higuchi's equation

$Q_t = k_H.t^{1/2}$

Where K_H is the Higuchi release rate constant

Korsemeyer-Peppas

The curves plotted may have different slopes, and hence it becomes difficult to exactly pin-point which curve follows perfect zero order release kinetics. Therefore, to confirm the kinetics of drug release, data were also analyzed using Korsemeyer's equation.

$$Q_t/Q_\infty = k_{KP}.t^n$$

Where Q_t/ Q_∞ is the fraction of drug released at time t, k_{KP}a constant compromising the structural and geometric characteristics of the device and n is the release exponent.

The slope of the linear curve gives the 'n' value. Peppas stated that the above equation could adequately describe the release of solutes from slabs, spheres, cylinders and discs, regardless of the release mechanism. The value of 'n' gives an indication of the release mechanism. When n = 1, the release rate is independent of time (typical zero order release / case II transport); n = 0.5 for Fickian release (diffusion/ case I transport); and when 0.5 < n < 1, anomalous (non-Fickian or coupled diffusion/ relaxation) are implicated. Lastly, when n > 1.0 super case II transport is apparent. 'n' is the slope value of log M_t/M_∞ versus log time curve.

Stability studies for optimized formulation

The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light and to establish a retest period for the drug substance or a shelf life for drug product and recommended storage conditions. In general, a drug substance should be evaluated under storage condition (with appropriate tolerances) that test its thermal stability and if applicable, its sensitivity to moisture. Three types of storage conditions are used i.e. long term, Accelerated and where appropriate, Intermediate.

Accelerated Testing, are the studies designed to increase the rate of chemical degradation or physical change of a drug substance or drug product by using exaggerated storage conditions as part of the formal stability studies.

The optimized formulation F4 was taken and accelerated stability study was performed by taking suitable quantity of microspheres. The microspheres were placed in air-tight glass container at 40±2°C/75±5% RH. At suitable sampling interval the samples were withdrawn and evaluated for various parameters.

Sampling Intervals

Storage conditions	Sampling intervals
Real time storage 30°C/75% RH	0, 3, 6, 9, 12, 18, 24, 36, 48, 60, months
Accelerated 40°C/75% RH	0, 1, 3, 6 months

RESULTS AND DISCUSSION

The percentage yield of mucilage was found to be 61%.

Physio-chemical characterization of mucilage

The separated mucilage was evaluated for swelling index, loss on drying, density, compressibility index and angle of repose.

Table 2: Physio-chemical characterization of mucilage

S. No	Characterization	Results
1.	Appearance	Mucilaginous
2.	Color	Brown
3.	State	Solid

The swelling index of isolated mucilage was found to be 62.32 ±0.637%.

The loss on drying of isolated mucilage was found to be 74.066±0.0498%.

Evaluation of simvastatin microspheres

Percentage Yield

Percentage yield of different formulation was determined by weighing the Microspheres after drying. The percentage yield of different formulation was in range of 61.25±0.23–78.85±0.65%

Table 3: Percentage Yield of Different Formulation

Formulation Code	Percentage Yield*
F1	65.58±0.45
F2	70.23±0.32
F3	73.36±0.45
F4	78.85±0.65
F5	61.25±0.23
F6	69.98±0.74

***Average of three determination (n=3)**

Drug Entrapment

The drug entrapment of different formulations was in range of 78.05- 83.25% w/w.

Table 4: Drug Entrapment for Different Formulation

Formulation	Drug entrapment (% w/w) of prepared microsphere*
F1	75.65±0.23
F2	72.23±0.45
F3	69.98±0.65
F4	82.21±0.21
F5	70.12±0.36
F6	65.45±0.74

***Average of three determination (n=3)**

This is due to the permeation characteristics of HPMC that could facilitate the diffusion of part of entrapped drug to surrounding medium during preparation of simvastatin microspheres.

The maximum Percentage Yield, Drug Entrapment was found to be formulation F4. The optimized formulation of batches subjected to further studies.

Particle size analysis

The mean size of the microspheres was determined by photo correlation spectroscopy (PCS) on a submicron particle size analyzer (Malvern zetasizer) at a scattering angle of 90°. A sample (0.5mg) of the microspheres suspended in 5 ml of distilled water was used for the measurement. The results of measurement of mean particle size of optimized formulation F4 microsphere were found 205.5 nm respectively.

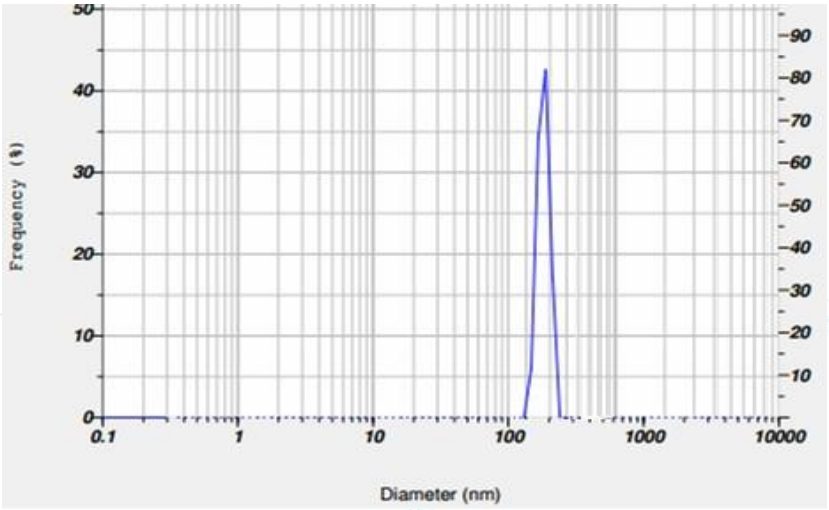

Figure 3: Particle size data of mucoadhesive microsphere

Zeta Potential

The zeta potential of the drug-loaded microspheres 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 F4 microsphere were found -35.6 mV respectively.

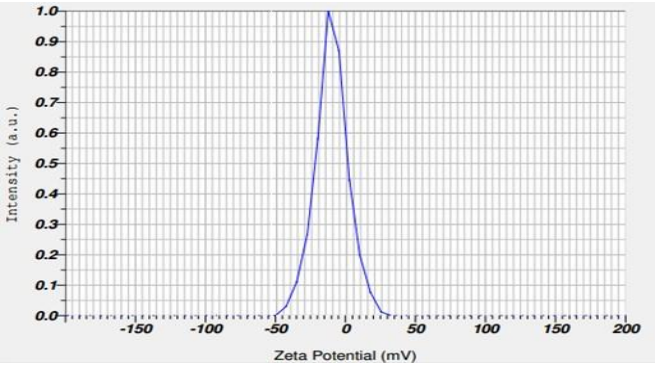

Figure 4: Zeta potential data of mucoadhesive microsphere

Scanning Electronic Microscopy

Shape and surface characteristic of Simvastatin microspheres exam in by Scanning Electronic Microscopy analysis. Surface morphology of formulation examines at two different magnifications 55X which illustrate the smooth surface of mucoadhesive Microspheres.

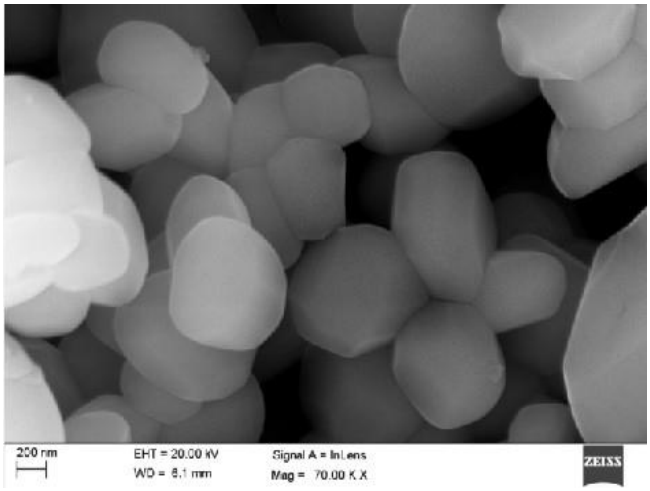

Figure 5: Scanning Electronic Microscopy image of optimized formulation F-4

Table 5: Cumulative % drug release of simvastatin from optimized formulation of microsphere

S. No.	Time (hrs)	% Cumulative Drug Release*
1	0.5	14.56±0.36
2	1	22.36±0.25
3	2	36.65±0.24
4	3	48.89±0.36
5	4	52.23±0.56
6	6	66.69±0.47
7	8	73.32±0.65
8	10	84.89±0.78
9	12	94.23±0.32

***Average of three determination (n=3)**

Table 6: *In Vitro* Drug Release Data for Coated formulation

S. No.	Time (H)	Square Root of Time	Log Time	Cumulative* Percentage Drug Release±SD	Log Cumulative Percentage Drug Release	Cumulative Percent Drug Remaining	Log cumulative Percent Drug Remaining
1	0.5	0.707	-0.301	14.56	1.163	85.44	1.932
2	1	1.000	0.000	22.36	1.349	77.64	1.890
3	2	1.414	0.301	36.65	1.564	63.35	1.802
4	3	1.732	0.477	48.89	1.689	51.11	1.709
5	4	2.000	0.602	52.23	1.718	47.77	1.679
6	6	2.449	0.778	66.69	1.824	33.31	1.523
7	8	2.828	0.903	73.32	1.865	26.68	1.426
8	10	3.162	1.000	84.89	1.929	15.11	1.179
9	12	3.464	1.079	94.23	1.974	5.77	0.761

* Average of three determinations (n=3)

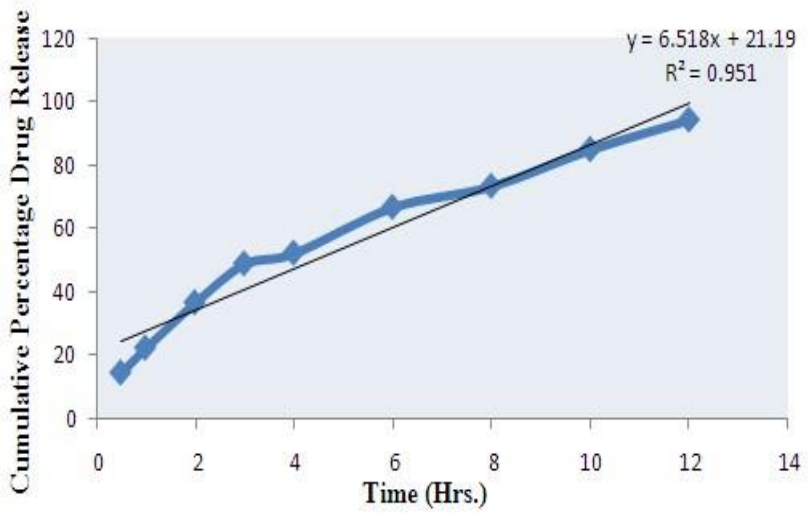

Figure 7: Cumulative Percent Drug Released Vs Time (Zero Order Plots)

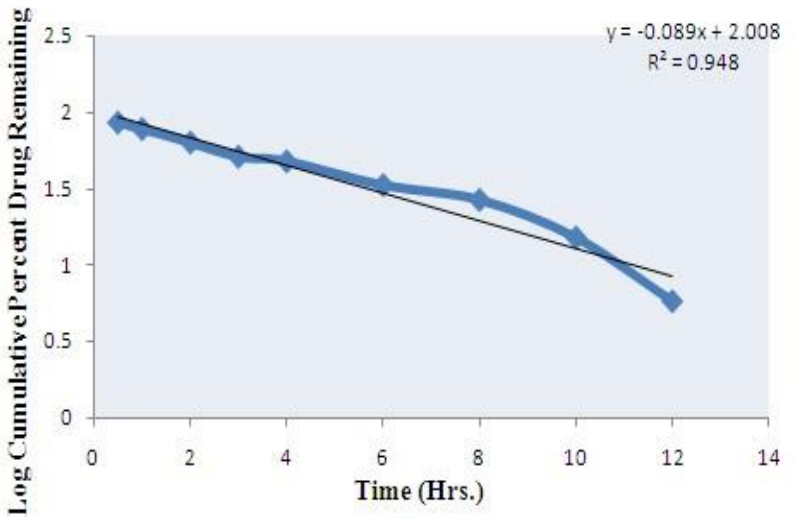

Figure 7: Log Cumulative Percent Drug Remaining Vs Time (First Order Plots)

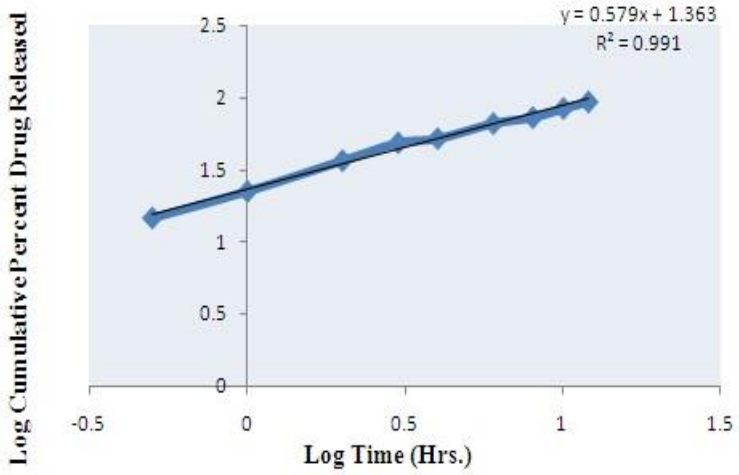

Figure 8: Log Cumulative Percent Drug Released Vs Log Time (Peppas Plots)

Regression Analysis Data of mucoadhasive microspheres Formulation

Formulation	Zero order	First order	Pappas plot
F3	y = 6.518x+21.19	y = -0.089x + 2.008	y = 0.579x –1.363
	R^2 = 0.951	R^2 = 0.948	R^2 = 0.991

Stability studies

Stability studies were carried out with optimized formulation which was stored for a period of 45 days at 4±1°C, RT and 40±1°C. The particle size of formulation was determined by optical microscopy using a calibrated ocular micrometer. The particle size of the microsphere was found to increase at RT, which may be attributed to the aggregation of microsphere at higher temperature. At 45±2°C the microsphere aggregate i.e. these microsphere were unstable at higher temperature like 45±2°C. Percent efficiency of mucoadhesive Microspheres also decrease at higher temperature Like 45±2°C.

CONCLUSION:

Microspheres are novel drug delivery approach to control release of pharmacologically active ingredient as per patient need. Natural polymers like fenugreek mucilage are cheap, biodegradable and have been proved safe for pharmaceutical formulation. Higher loading efficiency was observed for all the formulations and also the drug release was observed for the period of 12 hours. Thus, the simvastatin microsphere using fenugreek mucilage showed promising results in retarding the drug release. It can be concluded from whole study that due to the formation of polymeric network system or other active moiety can be easily entrapped with the matrix and hydration and swelling of natural polymer controlled their release pattern

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