Research Article

FORMULATION AND *IN VITRO* EVALUATION OF STOMACH SPECIFIC FLOATING MICROSPHERES OF SIMVASTATINShabeena Aqdas^{1*}, V. Uma Maheshwara Rao¹, B. Sirisha¹, P. Raj Kumar², P. Vijaya lakshmi³, M. Ajitha⁴¹CMR College of Pharmacy, Kandlakoya (V), Hyderabad, Telangana, India²Project Coordinator, Spectrum Pharma Research Solutions, Hyderabad, Telangana, India³Department of Pharmaceutics, Guru Nanak College, Hyderabad, Telangana, India⁴Center for Pharmaceutical Sciences, IST, JNTUH, Hyderabad, Telangana, India

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DOI: 10.7897/2230-8407.0511170**ABSTRACT**

The overall prevalence of dyslipidemia in India in 2013 was 10 % to 73 %. The prevalence of hyperlipidemia is more in females (33.2 %) than in males (32.4). On an average 32.8 % population is suffering from hyperlipidemia. The objective of the present study is to prepare and evaluate the stomach specific floating microspheres of simvastatin. Simvastatin microspheres were prepared by ionotropic gelation method using polymers such as sodium alginate, guar gum, pectin and carbopol. Total 12 formulations were prepared by using the above polymers. The prepared microspheres were evaluated for their appearance, solubility, physical properties (bulk density, tapped density, compressibility index, angle of repose), particle size, swelling index, SEM studies, buoyancy property, entrapment efficiency, *in-vitro* drug release and drug release kinetics. All formulations showed compliance with pharmacopoeial standards. The *in-vitro* drug release studies were conducted using USP dissolution apparatus type II (paddle) in 900 ml of 0.1 N HCl for first 2 h, next 1 h in 6.8 pH phosphate buffer and the remaining 9 h in 7.4 pH phosphate buffer. Surface morphology (SEM analysis) was conducted with F2, F7, F10 and F12 formulations. Drug polymer interaction studies (FTIR) were performed with all the polymers. The microspheres were smooth and elegant in appearance, showed no visible cracks as confirmed by SEM and FTIR studies indicated the lack of drug polymer interaction. Based on dissolution data, formulation F12 was the best formulation with a drug release of 98.96 %. The *in-vitro* release data of all microsphere formulations were plotted in various kinetic equations to understand the mechanisms and kinetics of drug release. The drug release kinetics of formulation F12 was of zero order with non-fickian mechanism according to Korsmeyer-Peppas equation.

Keywords: Simvastatin, Sodium alginate, Pectin, Guar gum, Carbopol, Ionotropic gelation.**INTRODUCTION**

Microspheres are solid spherical particles free flowing particles consisting of proteins or synthetic polymers which are biodegradable in nature ranging in size from 1-1000 μm .¹ Stomach Specific FDDS have a bulk density less than gastric fluids and so remain buoyant in the stomach without affecting the gastric emptying rate for a prolonged period of time. While the system is floating on the gastric contents, the drug is released slowly at the desired rate from the system. After release of drug, the residual system is emptied from the stomach. This result in an increased GRT and a better control of fluctuations in plasma drug concentration.² Floating drug delivery systems (FDDS) first described by Davis (1968), 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 systems float over the gastric contents, the drug is released slowly at the desired rate, which results in increased gastroretention time and reduces fluctuations in plasma drug concentration.³ Simvastatin is an antihyperlipidemic which belongs to the category of statin and inhibits the enzyme HMG-CoA reductase. HMG-CoA reductase catalyzes conversion of HMG-CoA to mevalonate, which is the rate limiting step in de novo cholesterol synthesis. Competitive inhibition of HMG CoA reductase by simvastatin decreases hepatocyte cholesterol synthesis. The associated reduction in intracellular cholesterol concentration induces LDL-receptor expression on hepatocyte cell surface, which results in an increased extraction of LDL-C from the blood and decreased circulating LDL-C concentration. Simvastatin also have beneficial effects on other lipid

parameters such as increase in high-density lipoprotein cholesterol (HDL-C) concentration and decreases in triglyceride concentration. Simvastatin however is practically insoluble in water and freely soluble in chloroform, methanol and ethanol. It is poorly absorbed from the gastrointestinal (GI) tract. It has a half-life of 3 h, protein binding of 95 % and bioavailability of 5 %. Simvastatin has an absorption window in upper G.I tract and as a result, displays low bioavailability. Simvastatin is difficult to formulate into sustained release dosage forms, because on arrival of colon or even before, its absorption is diminished or non-existent. In the present study, efforts were made to increase the residence time of simvastatin at or above the absorption window through preparation of gastro retentive capsule considering the fact that it is stable under gastric condition.⁴

MATERIALS AND METHODS**Materials**

Simvastatin and pectin was obtained from Spectrum Lab, Hyderabad, Telangana, India. Sodium alginate, Guar gum, Carbopol and were obtained as a gift sample from S.D Chemicals Pvt. Ltd, Mumbai, Maharashtra, India. Calcium chloride was purchased from S. D. Fine Chem. Ltd., Mumbai, Maharashtra, India. All solvents used were of analytical grades and were used as obtained.

Methods

Drug-Excipient Interaction Studies

This type of interaction was studied with the help of Shimadzu FTIR spectrophotometer, in which KBR pellet method used to determine the interactions.

Formulation

Total 12 batches of formulations were planned accordingly such as F1, F2 and F3 (sodium alginate in 0.5 %, 1 % and 1.5 %) F4, F5 and F6 (alginate and guar gum in 0.25 %, 0.5 % and 0.75 %) F7, F8 and F9 (alginate and pectin in 0.25 %, 0.5 % and 0.75 %) F10, F11 and F12 (alginate and carbopol in 0.25 %, 0.5 % and 0.75 %) as reported in Table 1. Accurate amount of drug and polymer were weighed. The polymer solution was prepared with water until it forms a clear solution. Then the drug (simvastatin) was added to the above polymer solution and stirred well to form a clear solution containing polymer and drug. In order to prevent the air bubbles, the solution was kept for sonication till the bubbles disappear. The resulting solution was taken in a syringe with needle no.26 through which the solution was injected into a petri plate containing calcium chloride solution. The microspheres thus obtained were filtered by decantation using whatmann filter paper. The filtered spheres were dried in the oven at 60° C for 6 h.⁵

Evaluation Studies

Flow Properties

Bulk Density

It is the ratio of total mass of powder to the bulk volume of the powder. It is measured by pouring weighed microspheres of about 10 g into a measuring cylinder and bulk volume is noted.

$$\text{Bulk density} = \text{Weight of powder} / \text{Bulk volume}$$

Tapped Density

It is the ratio of total mass of powder to the tapped volume of powder. 10 g of microspheres were taken into a measuring cylinder. The cylinder is tapped 100 times from a height of 2 inches until a constant volume is obtained.

$$\text{Tapped density} = \text{Weight of powder} / \text{Tapped volume}$$

Hausner's Ratio

Hausner's ratio is the ratio of tapped density to bulk density.

Angle of Repose

The frictional forces in a loose powder can be measured by the angle of repose, θ .

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

Where h=height of the heap r=radius of the heap

It is determined by pouring the microspheres a conical on a level, flat surface, measured the included angle with the horizontal.⁶

Particle Size

Particle size analysis of drug-loaded microspheres was performed by optical microscopy (Olympus Model Sxz-12). Few microspheres were suspended in purified water (10 ml). The sample was mounted on a clean glass slide and placed on mechanical stage of the microscope. The eye piece of microscope fitted with a micrometer and the size of spheres was determined.⁷

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 dessicators. The samples were mounted on a specimen studies using double sided adhesive tape and gold-palladium alloy of 120Å. The sputtering was done for nearly 3 minutes and uniform coating on the samples was obtained to enable good quality SEM images.⁸

Swelling Properties

The swelling properties of prepared microspheres were determined in acidic buffer pH 1.2. 100 mg spheres were placed in a beaker 200 ml buffer solution and then stirred with a magnetic stirrer at a speed 50 rpm. After 1 h, the equilibrium swollen spheres were observed and measured under optical microscope. Swelling index is calculated as

$$(\text{Mass of swollen microspheres} - \text{mass of dry microspheres}) / \text{mass of dried microspheres} \times 100\%$$

Floating Time (Buoyancy)

50 mg of prepared microspheres were placed in 100 ml of simulated gastric fluid (pH 1.2) containing 0.2 % tween 20. The mixture was stirred at 100 rpm on a magnetic stirrer. After 4 h of interval, the layer of buoyant microspheres were pipetted and separated by filtration. Particles in the sinking condition were also separated by filtration. Both the particles were dried in dessicators and weighed. The buoyancy was determined using the formula,

$$\% \text{ buoyancy} = W_f / W_f + W_s \times 100$$

Drug Entrapment Efficiency (DEE)

Drug entrapment efficiency of microspheres was performed by suspending accurately weighed 50 mg of microspheres in 100 ml of phosphate buffer pH 7.4 $\pm$ 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, simvastatin content in the filtrate was analyzed spectrophotometrically at 243 nm using Shimadzu 1201 UV-visible spectrophotometer.¹⁰ The drug entrapment efficiency was determined using following relationship:-

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

In-Vitro Dissolution Studies

Hard gelatin capsules were filled with microspheres equivalent to 100 mg of simvastatin. USP II rotating basket apparatus was used for the study. Dissolution fluid consists of 900 ml of simulated gastrointestinal fluids of increasing pH namely pH 1.2 (2 h), pH 6.8 (1 h) and pH 7.4 (up to 9 h) maintained temperature at 37°C $\pm$ 0.5°C and the basket was rotated at a constant speed of 50 rpm. Aliquots of samples were withdrawn after predetermined periods of time and the same volume of fresh medium was added immediately to the test medium. The samples withdrawn were filtered through a 0.45 μ m membrane filter. The drug content was determined in the filtrate after appropriate dilution and analyzed at 243 nm spectrophotometrically.¹¹

Table 1: Composition of the Formulations

Formulation Code	Simvastatin (mg)	Sodium alginate (%) (w/v)	Guar gum (%) (w/v)	Pectin (%) (w/v)	Carbopol (%) (w/v)	Calcium Chloride (%) (w/v)
F1	500	0.5 %	-	-	-	3 %
F2	500	1 %	-	-	-	5 %
F3	500	1.5 %	-	-	-	7 %
F4	500	0.25 %	0.25 %	-	-	5 %
F5	500	0.5 %	0.5 %	-	-	5 %
F6	500	0.75 %	0.75 %	-	-	5 %
F7	500	0.25 %	-	0.25 %	-	5 %
F8	500	0.5 %	-	0.5 %	-	5 %
F9	500	0.75 %	-	0.75 %	-	5 %
F10	500	0.25 %	-	-	0.25 %	5 %
F11	500	0.5 %	-	-	0.5 %	5 %
F12	500	0.75 %	-	-	0.75 %	5 %

Table 2: Pre Compression parameters of Batches F1 – F12

Formulation Code	Bulk Density	Tapped Density	Hausner's Ratio	Carr's Index	Angle of Repose
F1	0.532	0.618	1.161	13.91	26.56
F2	0.539	0.641	1.189	15.91	29.68
F3	0.536	0.645	1.203	16.89	28.36
F4	0.554	0.625	1.126	11.21	30.96
F5	0.538	0.622	1.156	13.50	26.56
F6	0.553	0.633	1.144	12.63	31.14
F7	0.537	0.643	1.197	13.99	30.96
F8	0.554	0.632	1.140	12.34	31.38
F9	0.534	0.626	1.172	14.69	29.38
F10	0.541	0.655	1.207	17.40	27.50
F11	0.554	0.625	1.128	11.36	31.21
F12	0.538	0.644	1.197	16.96	28.50

Table 3: Mean Diameter and Entrapment Efficiency of Microspheres

S. No	Formulation Code	Mean Diameter (μm)	Entrapment Efficiency (%)
1.	F1	820.14	68.82
2.	F2	784.25	77.82
3.	F3	768.50	85.30
4.	F4	830.20	58.91
5.	F5	770.16	64.11
6.	F6	776.15	79.14
7.	F7	860.13	79.28
8.	F8	891.56	92.80
9.	F9	970.62	86.56
10.	F10	981.17	88.05
11.	F11	906.70	90.05
12.	F12	930.15	95.34

Table 4: Floating Time of Microspheres

Formulation	Floating lag time (seconds)	Total floating time
F1	27 $\pm$ 1	>12 h
F2	34 $\pm$ 2	>12 h
F3	41 $\pm$ 4	>12 h
F4	19 $\pm$ 1	>12 h
F5	26 $\pm$ 6	>12 h
F6	42 $\pm$ 3	>12 h
F7	30 $\pm$ 2	>12 h
F8	27 $\pm$ 4	>12 h
F9	21 $\pm$ 1	>12 h
F10	19 $\pm$ 5	>12 h
F11	15 $\pm$ 2	>12 h
F12	22 $\pm$ 4	>12 h

Table 5: Swelling Behavior of Microspheres in 0.1N HCl

Formulation Code	Mean Diameters of Microspheres at various time intervals		
	0 h (μm)	1 h (μm)	2 h (μm)
F1	522.15	638.60	662.30
F2	494.25	606.15	623.40
F3	478.50	587.50	603.20
F4	520.14	648.12	652.30
F5	474.56	601.12	613.30
F6	468.56	577.50	603.17
F7	560.15	684.05	610.40
F8	590.55	605.65	631.30
F9	670.60	696.40	729.10
F10	682.15	703.20	726.15
F11	608.60	632.40	658.70
F12	620.15	640.20	662.70

Table 6: % Cumulative drug release of formulations F1-F6

S. No.	Time (h)	F1	F2	F3	F4	F5	F6
1.	1	22.48	28.46	18.80	23.1	27.56	21.5
2.	2	48.30	44.12	31.32	47.29	43.1	31.3
3.	3	66.31	57.71	38.12	65.31	52.64	37.11
4.	4	73.54	67.2	49.93	72.54	62.2	49.77
5.	5	87.34	77.3	62.91	86.34	76.3	61.81
6.	6	99.01	90.01	75.30	94.01	80.61	74.3
7.	7	-	-	-	-	-	97.4
8.	8	-	-	-	-	-	-
9.	9	-	-	-	-	-	-
10.	10	-	-	-	-	-	-
11.	11	-	-	-	-	-	-
12.	12	-	-	-	-	-	-

Table 7: % Cumulative drug release of formulations F7-F12

S. No	Time (h)	F7	F8	F9	F10	F11	F12
1.	1	29.27	19.56	16.73	8.78	7.26	9.58
2.	2	45.97	27.36	21.54	18.56	10.25	11.34
3.	3	57.63	35.13	35.77	31.31	19.56	12.45
4.	4	64.54	51.92	49.79	39.12	27.27	21.67
5.	5	85.10	65.54	57.77	54.76	46.96	32.41
6.	6	97.13	75.35	69.37	76.32	58.65	49.90
7.	7	-	88.14	72.25	85.12	69.44	52.83
8.	8	-	98.09	87.11	98.74	75.42	65.56
9.	9	-	-	98.78	-	82.14	77.30
10.	10	-	-	-	-	99.89	84.13
11.	11	-	-	-	-	-	90.98
12.	12	-	-	-	-	-	98.96

Figure 1: SEM photograph of F12 Formulation

Figure 2: *In-Vitro* Dissolution Profile For Batch F1– F3

Figure 3: *In-Vitro* Dissolution Profile For Batch F4– F6

Figure 4: *In-Vitro* Dissolution Profile For Batch F7– F9

Figure 5: In-Vitro Dissolution Profile For Batch F10– F12

Mathematical Modeling of Drug Release Profile

To analyze the mechanism of drug release from the tablets, the results of *in vitro* release data were plotted in various kinetic models like zero order, first order, Higuchi model and Korsmeyer- peppas.

RESULTS AND DISCUSSION

The IR spectras of the drug and polymer combinations were compared with the spectra of pure drug and individual polymer spectras. The results were satisfactory with their characteristic absorption bands; the principle peaks obtained for the combinations were almost similar to that of the pure drug. Drug Excipient Compatibility Studies reveals that. There was no significant difference or interaction between the drugs polymers. Micromeritic properties like bulk density, tapped density, hausner's ratio and angle of repose were in acceptable range indicating flow property excellent to good. In the granulometric study, as observed from Table 6, 42.46 % to 79.50 % of microspheres were retained in #20 sieve; which proves the uniformity in the size of microspheres. It was observed that by decreasing the concentration of sodium alginate and CaCl_2 solution, spherical spheres were not formed. However, increase in the concentration of sodium alginate and calcium chloride solution makes the particles more spherical and uniform sized spheres were obtained. Particle size of drug-loaded microspheres was performed by optical microscopy. The size of the spheres was obtained in the range of 1 to 1000 μm . The mean diameter of the particles was found to decrease by increasing in the concentration of calcium chloride solution. From the SEM photographs, it has been observed that microspheres prepared with sodium alginate were more spherical in shape, exhibited uniformity and rough surface. On the lag time basis, formulation F4, F10 and F11 are showing lower lag time when compared to all other formulation lag time. The swelling behavior of the prepared microspheres was studied in pH 1.2 up to 2 h. Increasing concentration of calcium chloride in the counter ion solution, produced spheres with higher levels of Ca_2^+ ions reducing the swelling of the spheres, consequently increasing the concentration cross-linking polymers decreased the swelling properties of the spheres in acidic medium. The entrapment

efficiency was in the range of 92.28 to 95.34 % in the formulation F12 with sodium alginate and carbopol. The *in-vitro* release data shows the % cumulative drug release at 12th hour for batch F7 to F12 was in the range 93 % to 99 % w/w. Whereas batches F4, F5 and F6 were in the range of 81 to 88 % w/w and even the same drug release were observed in F1, F2 and F3 batches. The formulated microspheres showed increased drug release in pH 6.8 phosphate buffer and trend is continued in pH 7.4 phosphate buffer, may be because due to cross-linking takes place only between carboxylate residue of GG-blocks and Ca_2^+ ions forms a tight-gel network structure. The toughness of the network structure, subsequent disintegration and dissolution of alginate particles taking place through ion-exchange between the bound Ca^{+2} ions and Na^+ ions present in dissolution medium leading to extended release to some extent. Thus, the release of simvastatin from the microspheres appeared to be significantly increased with an increase in initial alginate concentration but not with calcium ions. Different kinetic models were applied to the optimized formulation and observed that F12 followed zero order kinetics with non-fickian mechanism according to Korsmeyer-Peppas equation. The best linearity was found in Korsmeyer-peppas model (where $n = 0.583$ is the release exponent). Applicability of data indicates Non-Fickian diffusion (or) Anomalous Transport as mechanism of drug release from the microspheres.

CONCLUSION

Drug release of optimized formulation F12 formulated with Carbopol and Sodium alginate as the polymers has sustained release up to 12 hours. Our study has suggested that microencapsulation by ionotropic gelation technique is inexpensive compared with other techniques and also advantages to prevent the drug related adverse effects of conventional dosage forms and maintain the sustained drug release over an extended period of time.

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