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FORMULATION AND EVALUATION OF GASTRORETENTIVE FLOATING MICROSPHERES OF ATORVASTATIN

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ABSTRACT:

The development of gastroretentive drug delivery systems is a promising approach to enhance the bioavailability and therapeutic efficacy of drugs with narrow absorption windows in the upper gastrointestinal tract. Atorvastatin, a lipid-lowering agent, exhibits optimal absorption in the upper part of the gastrointestinal tract. Thus, formulating gastroretentive floating microspheres of atorvastatin can potentially improve its bioavailability and therapeutic outcomes. This study focuses on the formulation, characterization, and evaluation of gastroretentive floating microspheres of atorvastatin to achieve prolonged gastric retention and controlled drug release. Floating microspheres of atorvastatin were prepared using the emulsion solvent evaporation method. Ethyl cellulose was employed as the polymer to form the microspheres due to its biocompatibility and ability to control drug release. Various formulations were developed by varying the polymer concentration and the drug-to-polymer ratio. The prepared microspheres were evaluated for their particle size, morphology, entrapment efficiency, buoyancy, and in vitro drug release. In conclusion, the formulated gastroretentive floating microspheres of atorvastatin exhibited excellent buoyancy, high entrapment efficiency, and sustained drug release characteristics. These properties make them a promising approach for enhancing the bioavailability and therapeutic efficacy of atorvastatin. The gastroretentive floating microspheres can potentially provide a convenient and effective means of delivering atorvastatin, reducing dosing frequency and improving patient compliance. Further clinical studies are warranted to establish the efficacy and safety of this novel drug delivery system in human subjects.

Keyword: Buoyancy, Drug release, Entrapment efficiency, Bioavailability, Therapeutic efficacy.

INTRODUCTION

Hyperlipidemia is a condition excess of fatty substances called lipids, largely cholesterol and triglycerides, in the blood. It is also called hyperlipoproteinemia because these fatty substances travel in the blood attached to proteins. This is the only way that these fatty substances can remain dissolved while in

circulation. The most recent cholesterol management guidelines (the third report of the adult treatment panel APT III), which are issued by the national cholesterol education program (NCEP) in june 2010, redefine the levels at which blood cholesterol should be treated. These new evidence-based recommendations are departure from the NCEP's previous guidelines (ATP II) in several ways. American heart association defined hyperlipidemia is a high level of fats in the blood. These fats, called lipids include cholesterol and triglycerides.

Floating microspheres are gastroretentive drug delivery systems based on a non-effervescent approach. Hollow microspheres, microballoons or floating microparticles are terms used synonymously for floating microspheres. Floating microspheres are, in a strict sense, spherical empty particles without a core. These are free-flowing particles, with size ranging from 1 to 1000 µm have developed non-effervescent hollow polycarbonate microspheres by using an emulsion solvent evaporation method. This gastrointestinal transit-controlled preparation is designed to float on gastric juice with a specific density of less than one. This property results in delayed transit through the stomach. The drug is released slowly at desired rate, resulting in increased gastric retention with reduced fluctuations in plasma drug concentration. The objective of the present review is to focus on the method of preparation, and the various parameters affecting the performance and characterization of floating microspheres. The present review is a source of detailed information about the various aspects of floating microspheres.

Applications of floating microspheres:

1. Floating microspheres can be used as carriers for drugs with so-called absorption windows, these substances, for example antiviral, antifungal and antibiotic agents (sulphonamides, quinolones, penicillins, cephalosporins, aminoglycosides and tetracyclines) are taken up only from very specific sites of the GI mucosa.

2. Hollow microspheres of non-steroidal anti-inflammatory drugs are very effective for controlled release, and reduce the major side effect of gastric irritation. For example, floating microspheres of indomethacin are quite beneficial for rheumatic patients.

3. Floating microspheres are especially effective in the delivery of sparingly soluble and insoluble drugs.

4. Hollow microspheres can greatly improve the pharmacotherapy of the stomach through local drug release.

Advantages of floating microspheres

1. Bioavailability enhances, despite first pass effect, because fluctuations in plasma drug concentration are avoided, and a desirable plasma drug concentration is maintained by continuous drug release.

2. Superior to single-unit floating dosage forms, as such microspheres release drugs uniformly and there is no risk of dose dumping.

3. Enhanced absorption of drugs that solubilise only in stomach.

4. Site-specific drug delivery to the stomach can be achieved.

5. Avoidance of gastric irritation, due to sustained release effect.

6. Better therapeutic effect of short half-life drugs can be achieved.

MATERIALS AND METHODS

Preparation of Floating microspheres of Atorvastatin

Microspheres loaded with Atorvastatin were prepared using solvent diffusion-evaporation method using HPMC and EC. Drug and polymer in proportion of 1:3, 1:3.5 and 1:4 were dissolved in 1:1 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 microspheres were collected by decantation, while the non-floating microspheres were discarded. The microspheres were dried overnight at 40±2°C and stored in desicator.

Table 1 Formulations of the Floating Microspheres Prepared

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

Preparation of mucoadhesive microsphere of Atorvastatin

Chitosan microspheres were prepared by ionotropic gelation method.

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

Preparation II: The drug and sodium alginate was dissolved in 100 ml of water.

Preparation III: 1% calcium chloride 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 intoa 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 2 Formulations of the mucoadhasive microspheres

Sr. No	Formulation Code	Atorvastatin (mg)	Chitosan (mg)	Sod. Alginate (mg)
1.	**F4**	50	50	50
2.	**F5**	50	50	75
3.	**F6**	50	50	100

RESULTS AND DISCUSSION

A) Physical evaluation

Table 3 List of Sensory characters

S. No.	Characters	Result
1.	Colour	White to off-white powder
2.	Odor	Odorless
3.	Taste	Tasteless

B) Determination of pH (1 % w/v solution in water) Procedure:

Table 5: pH of the Atorvastatin

S. No.	pH of the solution	Average pH of the solution
1.	7.61	7.61
2.	7.61	
3.	7.5	

C) Identification Test

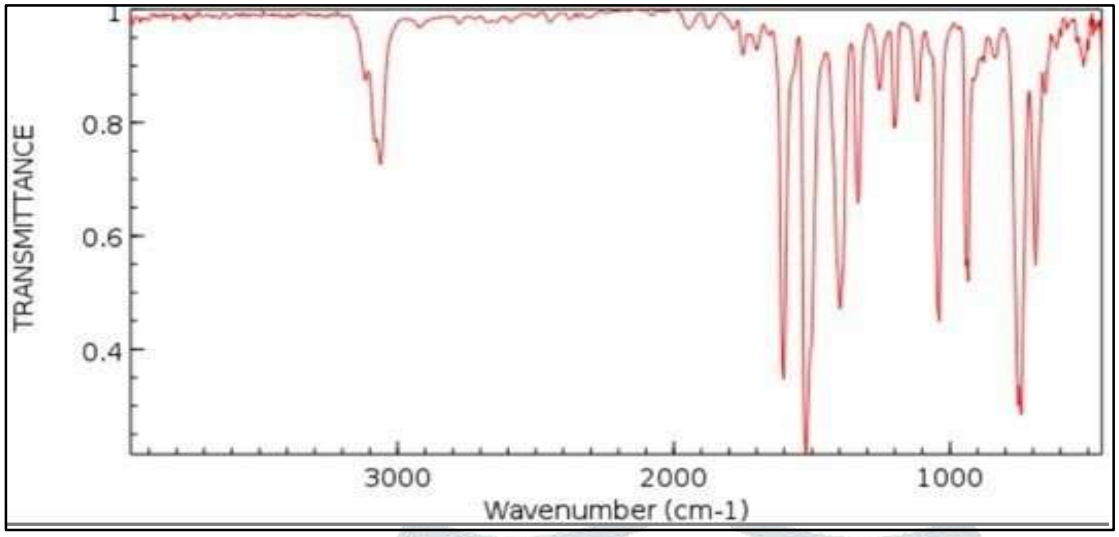

Figure 1 : FT-IR Spectrum of Drug and excipients

D) Loss on drying

Table 6: Loss of drying of drug sample

S. No.	Initial weight	Final weight after 15 minutes	% loss of drying	Avg. % loss of drying
1.	1gm 1gm	9.89 gm	1.1 %	1.1 %
2.		9.89 gm	1.1 %	1.1%

E) Tapped density:

Table 7: Tapped density of Atorvastatin

S. No.	Bulk mass	Tapped volume	Tapped density	Avg. tapped density
1.	1 gm	2.8 ml	0.357 g/cm^3	0.357 g/cm^3
2.	1 gm	2.8 ml	0.357 g/cm^3	
3.	1 gm	2.7 ml	0.370g/cm^3	

F) Compressibility index (Carr's index):

Table 8: C.I. of Atorvastatin

S. No.	Bulk density	Tapped density	C.I.
1.	0.333 g/cm^3	0.357 g/cm^3	6.722

G) Hausner ratio:

Table 9: Hausner ration of Atorvastatin

S. No.	Bulk density	Tapped density	Hausner ratio
1.	0.333 g/cm^3	0.357 g/cm^3	1.072

H) Determination of λ max of Atorvastatin:

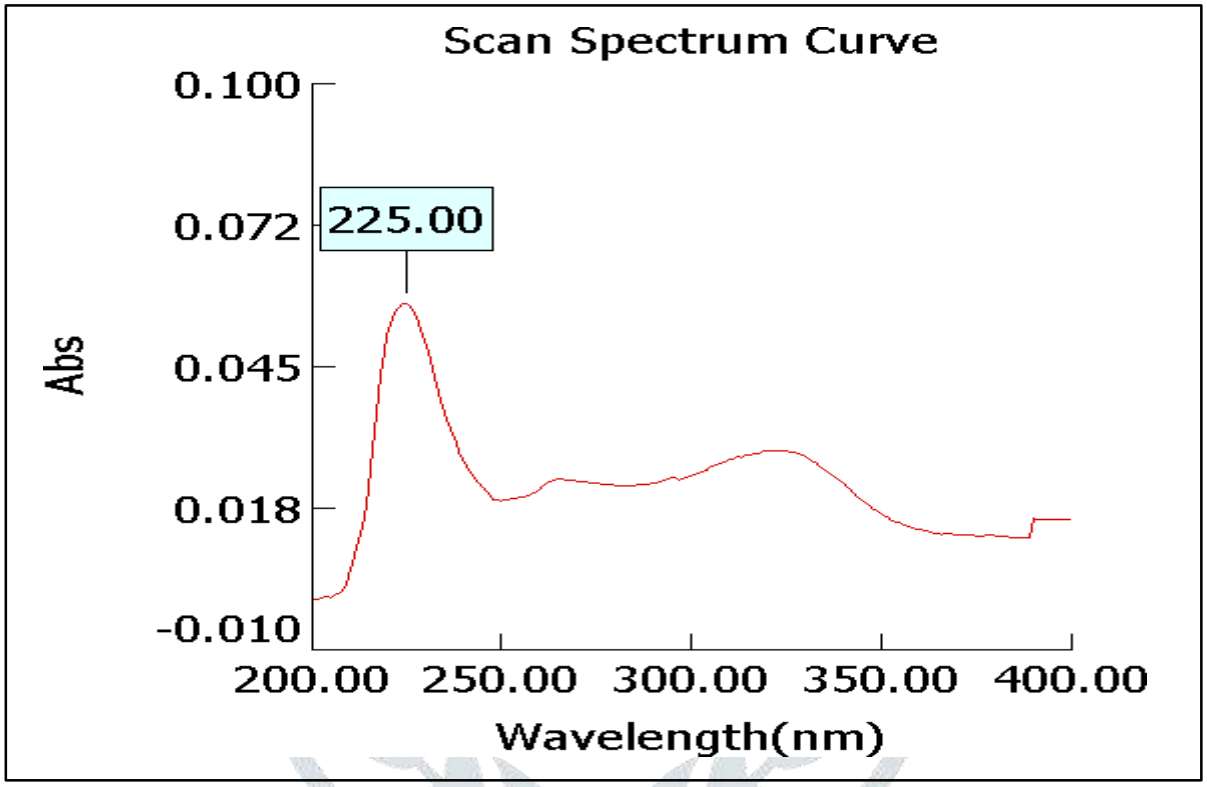

Figure 2: Wavelength maxima of Atorvastatin in 0.1 N Hcl at 225 nm

I) Calibration curve of Atorvastatin at λ max 225nm Observation table:

Table 10: Calibration curve of Atorvastatin

S. No.	Conc. (µg/ml)	Absorbance
1	10	0.117
2	20	0.213
3	30	0.318
4	40	0.398
5	50	0.497

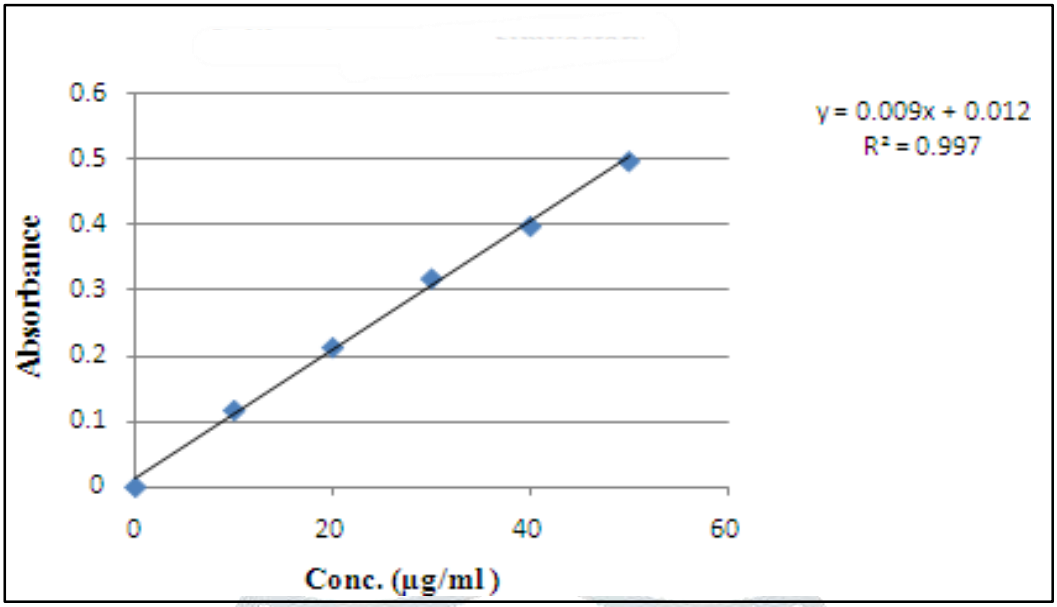

Figure 3: Calibration curve of Atorvastatin in 0.1 N Hcl at 225nm

Evaluation of Atorvastatin 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 82.56 – 93.58%.

Table 11 Percentage Yield for Different Formulation

Formulation	Percentage Yield
F_1	82.56
F_2	83.56
F_3	89.98
F_4	85.56
F_5	88.85
F_6	93.58

Drug Entrapment

The drug entrapment efficacies of different formulations were in range of 78.05- 83.25% w/w. 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 Atorvastatin microspheres.

Table 12 Drug Entrapment for different formulation

Formulation	Drug entrapment (% w/w) of prepared microsphere
F_1	78.05
F_2	79.98
F_3	82.56
F_4	80.12
F_5	81.14
F_6	83.25

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

Percentage Buoyancy and floating lag time of floating microsphere

Table 13 Percentage Buoyancy and floating lag time of floating microsphere

Formulation	Floating Lag Time	Percentage Buoyancy
F_1	45 Sec	65.56
F_2	35 Sec	70.25
F_3	30 Sec	78.98

Particle size analysis

The mean size of the microspheres 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 microspheres 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 and mucoadhesive microsphere was found 150.9 nm and 256.0 nm respectively.

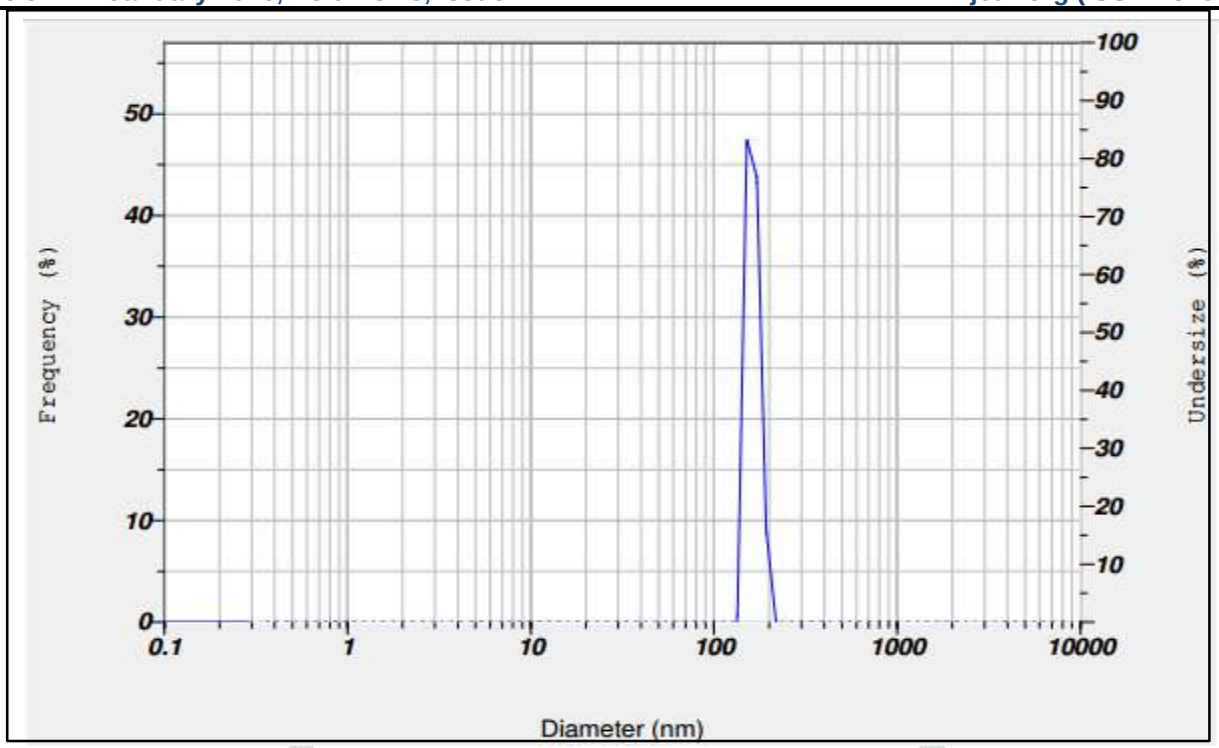

Figure 4: Particle size data of floating microsphere

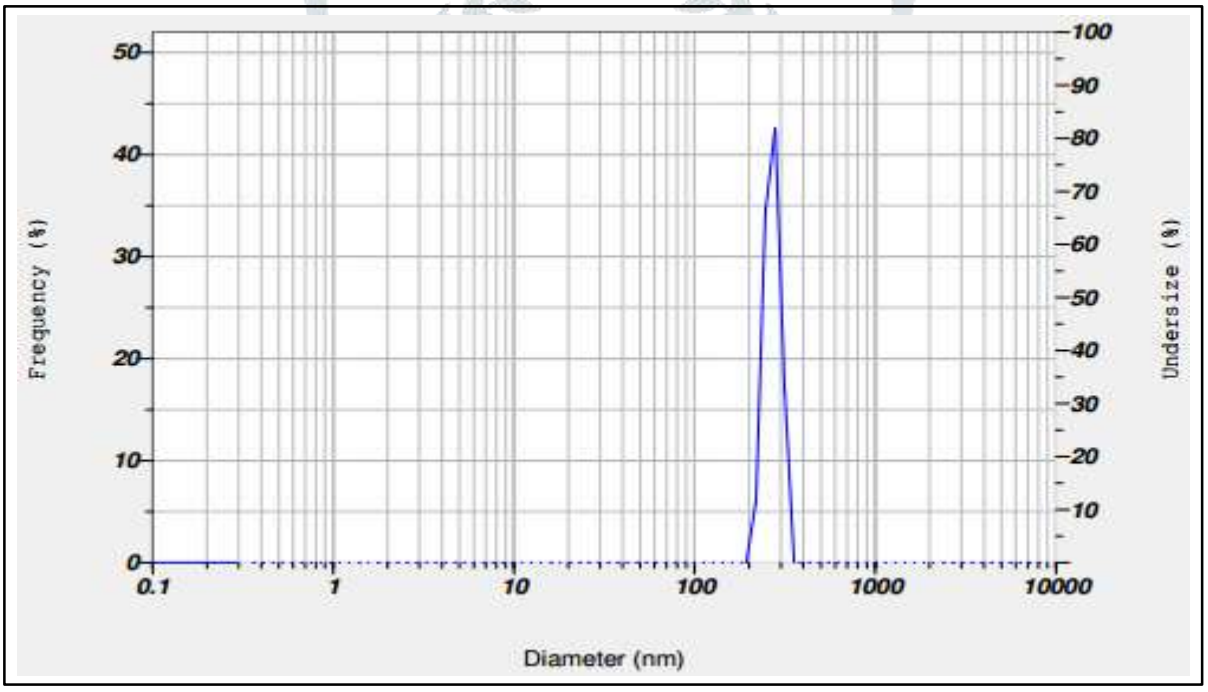

Figure 5: Particle size data of mucoadhesive microsphere

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 F3 of floating and mucoadhesive microsphere was found -10.7 mVand

-33.6 mV respectively.

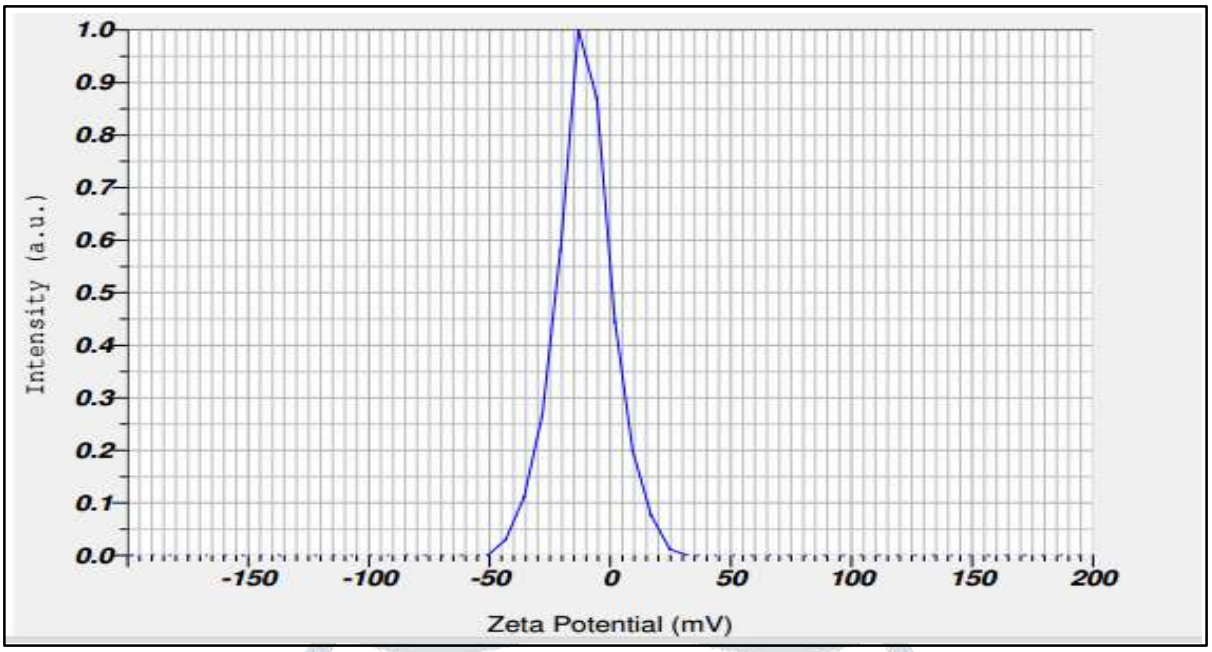

Figure 6: Zeta potential data of floating microsphere

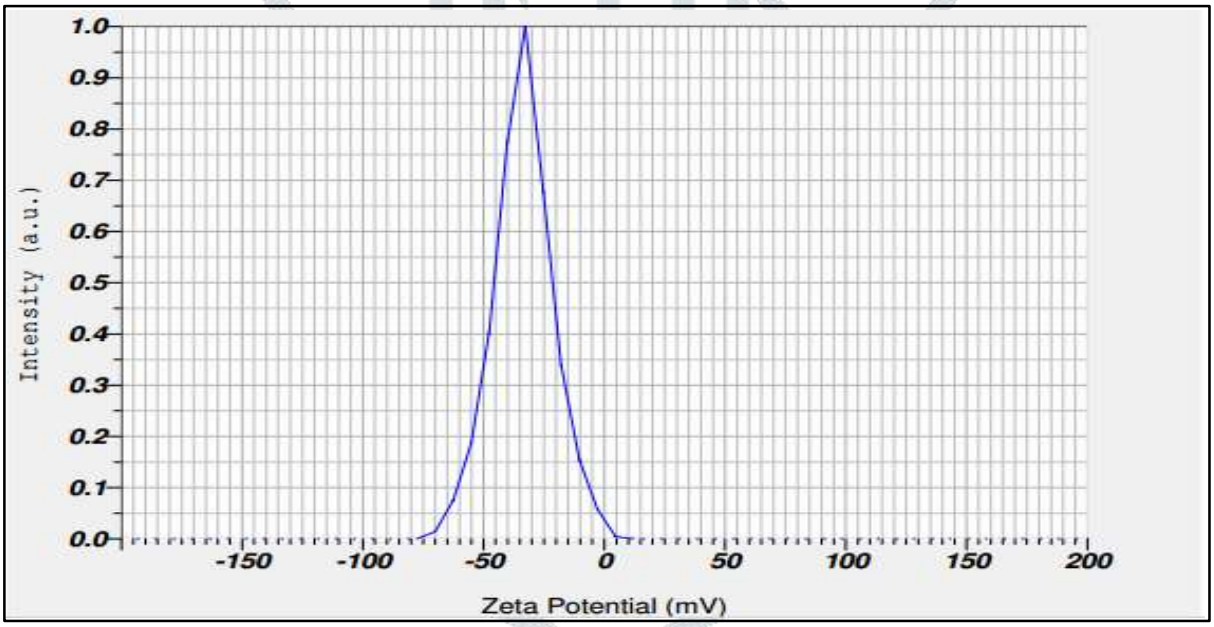

Figure 7: Zeta potential data of mucoadhesive microsphere

Scanning Electronic Microscopy

Shape and surface characteristic of Atorvastatin microspheres examine by Scanning Electronic Microscopy analysis. Surface morphology of formulation examines at two different magnification 55X which illustrate the smooth surface of floating Microspheres.

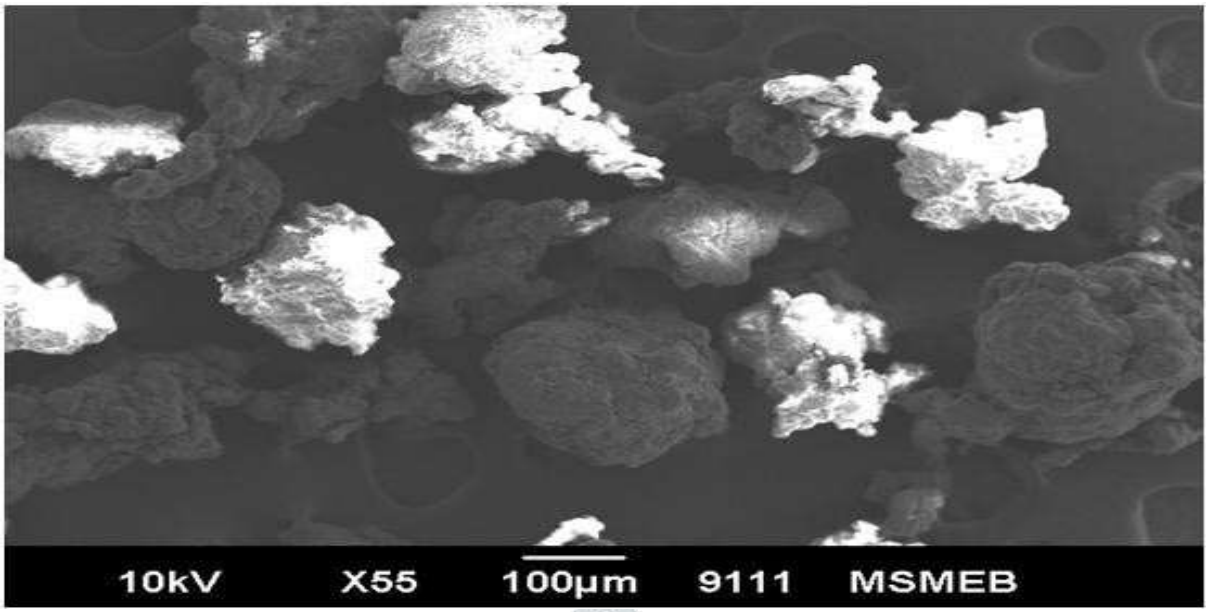

Figure 8: Scanning Electronic Microscopic image of optimized floating formulation F-3

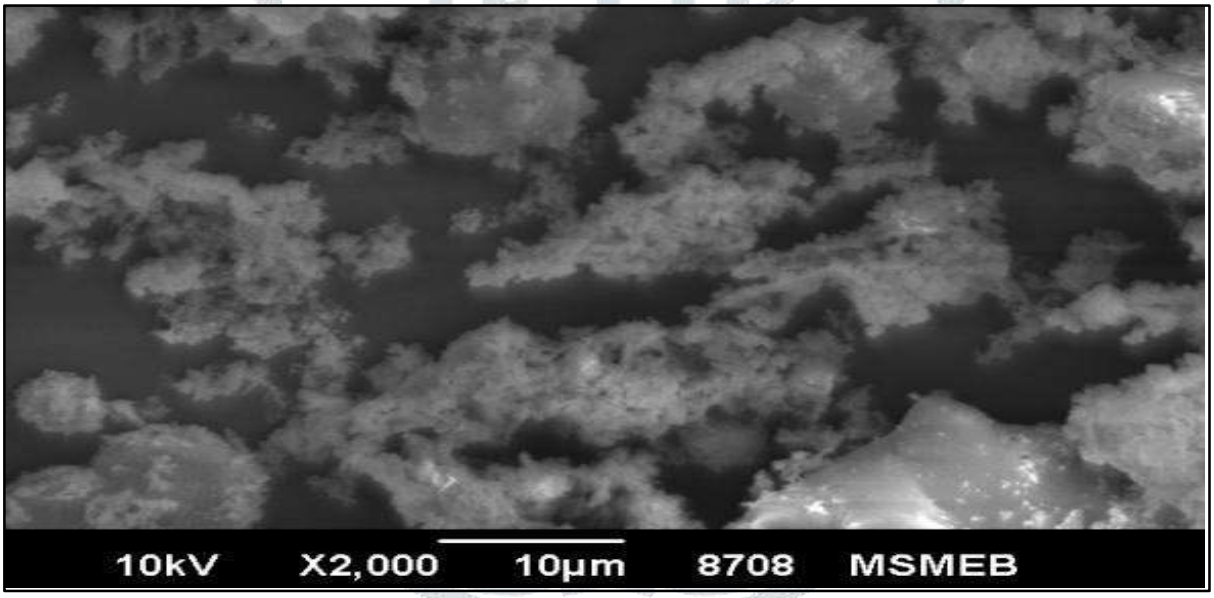

Figure 9: Scanning Electronic Microscopic image of optimized mucoadhesive floating formulation F-3

In-Vitro drug release study

In vitro drug release study of Atorvastatin loaded Microsphere Comparative release study of all formulation F1-F6

Table 14 Comparative Release Study data of formulation F1-F6

Time	% of Drug Release

(hr)	F1	F2	F3	F4	F5	F6
0.5	36.55	30.62	28.99	33.60	30.51	22.19
1	55.61	51.32	36.69	55.61	52.31	41.90
2	69.99	65.63	42.59	73.30	68.81	51.69
4	85.59	83.41	50.17	88.99	80.60	74.66
6	98.71	95.60	65.58	99.28	85.52	87.71
8	-	98.19	78.92	-	98.96	92.63
10	-	-	92.61	-	-	98.84
12	-	-	98.60	-	-	99.93

Graph of release study of formulation F1-F6

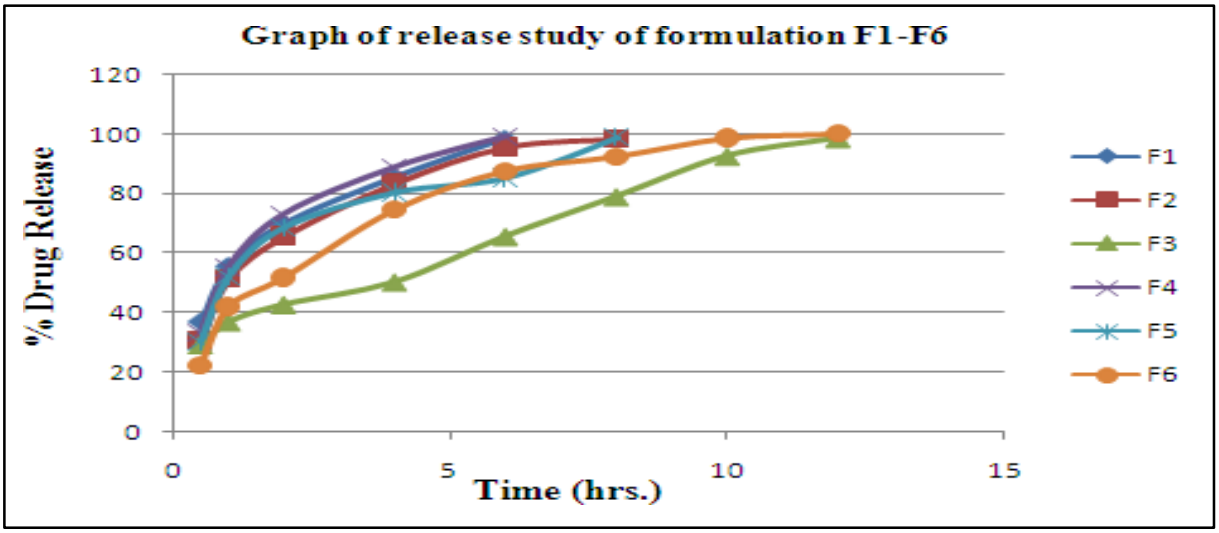

Figure 10: Graph of release study of formulation F1-F6

Table 15 Release Kinetics of optimized formulation of floating microsphere 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	28.980	1.462	71.020	1.851
1	1.000	0.000	36.700	1.565	63.300	1.801
2	1.414	0.301	42.560	1.629	57.440	1.759
4	2.000	0.602	50.140	1.700	49.860	1.698
6	2.449	0.778	65.520	1.816	34.480	1.538
8	2.828	0.903	78.890	1.897	21.110	1.324
10	3.162	1.000	92.560	1.966	7.440	0.872
12	3.464	1.079	98.540	1.994	1.460	0.164

Table 16 Release Kinetics of optimized formulation of mucoadhesive microsphere 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	22.21	1.347	77.790	1.891
1	1.000	0.000	41.88	1.622	58.120	1.764
2	1.414	0.301	51.7	1.713	48.300	1.684
4	2.000	0.602	74.63	1.873	25.370	1.404
6	2.449	0.778	87.69	1.943	12.310	1.090
8	2.828	0.903	92.56	1.966	7.440	0.872
10	3.162	1.000	98.78	1.995	1.220	0.086
12	3.464	1.079	99.89	2.000	0.110	-0.959

Zero order release kinetics graph of optimized formulations:

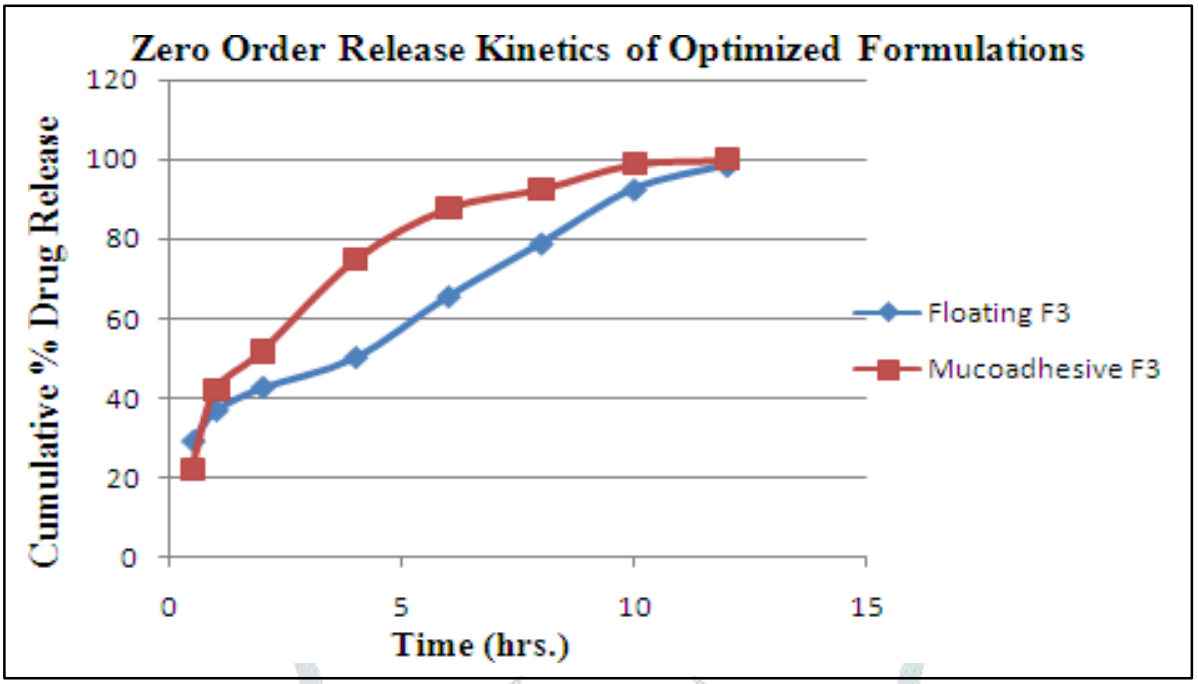

Figure 11: Zero order release kinetics graph of optimized formulations

First order release kinetics graph of optimized formulations:

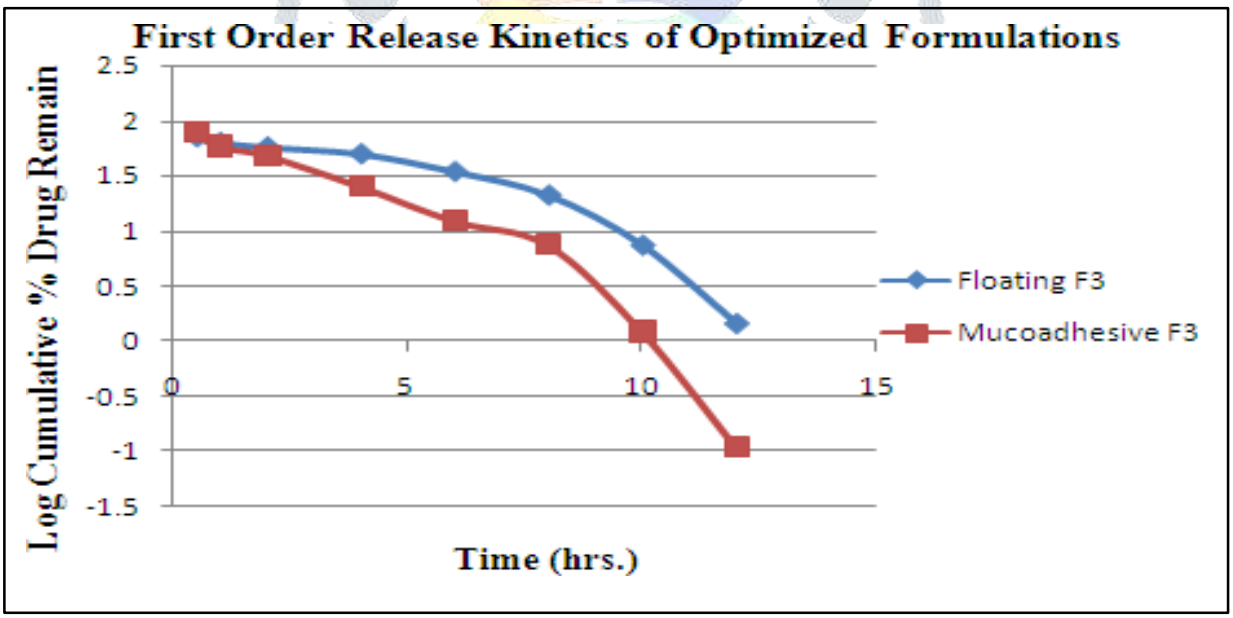

Figure 12: First order release kinetics graph of optimized formulations

Table 17: Comparative study of regression coefficient for selection of optimized Formulation F-3

Release Kinetics		Zero order	First order
R^2	Floating Microsphere	0.990	0.857
	Mucoadhesive Microsphere	0.859	0.910

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 'r' values of floating microsphere was maximum zero order i.e 0.990 in floating microsphere and first order 0.910 for mucoadhesive microsphere hence indicating drug release from formulations was found to follow zero order for floating and first order for mucoadhesive microsphere.

Stability studies of final formulation

According to ICH guidelines, 3 months accelerated stability study at 40±2°c and 75±5% RH optimized formulations (F3) was carried out. It showed negligible change over time for parameters like appearance, drug content, dissolution and assayetc., No significant difference observed in the drug content between initial and formulations stored at 40±2°c & 75±5% RH for 3 months.

SUMMARY & CONCLUSION

Drug absorption in the gastrointestinal tract is a highly variable process. Microspheres are promises to be a potential approach for gastric retention enhances the bioavailability and controlled delivery of various therapeutic agents. Significant attempts have been made worldwide to explore these systems according to patient requirements, both in terms of therapeutic efficacy and compliance. Floating and mucoadhesive Microspheres as gastro retentive dosage forms precisely control the release rate of target drug to a specific site and facilitate an enormous impact on health care. These systems also provide tremendous opportunities in the designing of new controlled and delayed release oral formulations, thus extending the frontier of futuristic pharmaceutical development. Furthermore, recent innovations in pharmaceutical investigation will surely provide real prospects for establishment of novel and effective means in the development of these promising drug delivery systems. The preliminary study showed that Atorvastatin is White to off-white and Odorless powder. It is freely soluble in ethanol and methanol, sparingly soluble in distilled water and 0.1 N NaOH, slightly soluble in 0.1 N hcl. The melting point was in the range of 136-138 °C which is compliance with the standard value of 138^0C as per Indian Pharmacopoeia. Identification of Atorvastatin was performed by UV/VIS Spectroscopy. The 10 µg/ml solutions of Atorvastatin was scanned

in the range of 200-400nm to determine the wavelength of maximum absorption for drug. The λ_{max} of atorvastatin was foundto be 225.00nm. From the respective stock solution (1mg/ml) different concentration of 10, 20, 30, 40 and 50 µg/ml atorvastatin was prepared and scanned in UV region. Their absorbances were noted at ʌ max 225.0 nm and calibration curve was plotted as absorbance vs concentration and their linearity range was determined. From the FT-IR data of the physical mixture it is clear that functionalities of drug have remained unchanged including intensities of the peak. This suggests that duringthe process drug and cholesterol has not reacted with the drug to give rise to reactant products. So, there is no interaction between them which is in favor to proceed for formulation of vesicular drug delivery system. Preformulation studies reported that the formulation of mucoadhesive microspheres of Atorvastatin can be prepared with appropriate methods. This thesis deals with the investigations carried out on the preparation and characterization of floating and mucoadhesive microspheres containing Atorvastatin with increase its oral bioavailability. Both Floating and Mucoadhesive microspheres containing Atorvastatin were prepared using emulsion solvent diffusion technique. Total six formulations were prepared using varying amount of EC and HPMC and Chitosan and Sod. Alginate. The prepared Microspheres were further evaluated for Particle size analysis, Drug entrapment, floating behavior, Percentage yield, Shape and surface characterization by Scanning electron microscopy and *In-vitro* Release Studies. The mean particle size of atorvastatin loaded optimized formulation F3 of floating and mucoadhesive microsphere was found -10.7 mV and -33.6 mV respectively. Percentage yield of different formulation was determined by weighing the Microspheres after drying. The percentage yield of different formulation was in range of 82.56 – 93.58%. The drug entrapment efficacies of different formulations were in range of 78.05- 83.25% w/w. 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 atorvastatin microspheres. The maximum Percentage Yield, Drug Entrapment, Percentage Buoyancy and floating lag time was found to be formulation F3 in both type of gastro retentive microspheres floating and mucoadhesive. The optimized formulation of both batches subjected to further studies. 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 ‘r’ values of floating microsphere was maximum zero order i.e 0.990 in floating microsphere and first order 0.910 for mucoadhesive microsphere hence indicating drug release from formulations was found to follow zero order for floating and first order for mucoadhesive microsphere. The floating and mucoadhesive microspheres of atorvastatin were successfully prepared by solvent evaporation technique and confirmed that it is a best method for preparing atorvastatin loaded microspheres from its higher percentage yield. The formulation F-3 of mucoadhesive microsphere showed better release rate compare to floating microsphere. Higher percentage of loading was also obtained in mucoadhesive microsphere. The prepared microspheres had good spherical geometry with smooth as evidenced by the scanning electron microscopy. The in vitro dissolution studies showed that atorvastatin microspheres formulation F3 showed better sustained effect over a period of 12 hours than floating formulations.

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