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Research Article

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Development and Evalution of Ethylcellulose Based Floating Microspheres of Atrovastatin by Novel Solvent Evaporation Matrix Erosion Method

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Abstract Floating drug delivery system is one of the novel drug delivery system. Floating drug delivery system has a bulk density less than gastric fluids and thus it remains buoyant in the stomach without affecting gastric emptying rate for a prolonged period of time. Atrovastatin is a competitive inhibitor of HMG-CoA reductase with half-life 14hr. HMG-CoA reductase catalyzes the reduction of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) to the mevalonate, which is rate-limiting step in hepatic cholesterol biosynthesis. Inhibition of the enzyme decreses *denovo* cholesterol synthesis, increasing explanation of low density lipoprotein receptors (LDL receptors) on hepocytes.This increases LDL uptake by the hepatocytes, reducing the amount of low density lipoprotin-cholesterol in the blood. Atrovastatin also reduces blood levels of triglycerides and slightly increases levels of HDL-cholesterol. The floating microsphers of Atrovastatin is being prepared by solvent evaporation method by using ethyl cellulose as a polymer and ethanol as a solvent and after the preparation of microsphere; it's evaluated for particle size, percentage yield, micromeritic properties, in vitro drug and in-vitro buoyancy release of microsphere, drug polymer compatibility, scaning electron microscopy, incorporation efficiency.

Keywords Atrovastatin, Ethyl cellulose, Floating microspheres

Introduction

The oral route is increasingly being used for the delivery of therapeutic agents because the low cost of the therapy and ease of administration lead to high levels of patient compliance. More than 50% of the drug delivery systems available in the market are oral drug delivery systems [1]. Controlled-release drug delivery systems (CRDDS) provide drug release at a predetermined, predictable, and controlled rate. Controlled-release drug delivery system is capable of achieving the benefits like maintenance of optimum therapeutic drug concentration in blood with predictable and reproducible release rates for extended time period; enhancement of activity of duration for short half-life drugs; elimination of side effects; reducing frequency of dosing and wastage of drugs; optimized therapy and better patient compliances [2-3].

Recent advances in novel drug delivery system to enhance the safety and efficacy of the drug molecule by formulating a dosage form being suitable for regulation. The high level of patient assent has been observed in taking oral dosage forms is due to the ease of administration and handling of dosage forms. There are lot of improvement has been seen in oral controlled drug delivery system in the last few years, this system has been of limited success in case of drugs with a poor absorption window throughout the GIT (Gastro Intestinal Tract). To modify the GIT time

is one of the main challenge in the development of oral controlled drug delivery systems. Gastric emptying of dosage forms is quite variable process and ability to prolong and control the emptying time is valuable asset for dosage form, which reside in stomach for a long time than conventional dosage forms. Several difficulties are look towards in designing controlled released systems for better absorption and enhanced the bioavailability [4]. Conventional oral dosage forms such as capsules, tablets provide specific drug concentration in systemic circulation without offering any control over drug delivery and also cause great fluctuations in plasma drug levels. Although single unit floating dosage form have been broadly studied, these single unit dosage form have the disadvantage of a release all or nothing emptying process while the multiple unit particulate system pass through the GIT to avoid the vagaries of gastric emptying and thus release the drug more consistently. The uniform distribution of these multiple unit dosage forms along the GIT could result in more reproducible drug absorption and reduced risk of local irritation; this gave birth to oral controlled drug delivery and led to development of Gastro-retentive floating microspheres.

Microspheres can be explained as solid, around spherical particles ranging in size from 1 to 1000 µm. The Microspheres are generally free flowing powders consisting of proteins or synthetic polymer, which are biodegradable in nature. Solid biodegradable microspheres integrate a drug dispersed or dissolved throughout particle matrix have the potential for controlled release of drug. Microspheres are small in size and therefore have large surface to volume ratios. The use of microspheres in pharmaceuticals have a number of advantages Viz., odor and taste masking, conversion of oils and other liquids to solids for facilitate, protection of drug against environment (moisture, heat, light and oxidation), separation of incompatible materials, to increase flow of powders, production of controlled release and targeted medications. The most significant physico-chemical characteristics that may be controlled in microspheres manufacture are; particle size and distribution, ratio of drug to polymer, polymer molecular weight, and various attempts have been done to retain the dosage form in the stomach as a way of increasing retention time.

Objectives of Formulation of Floating Microsphere

The purpose of this research was to formulate and evaluate Floating microspheres of Atorvastatin calcium. As the bioavailability of Atorvastatin Calcium is 13% to 14% owing to major hepatic first pass metabolism. It has an elimination half-life of 13hr and has an absorption zone from the upper intestinal tract. Efficacy of the administered dose may get diminished due to the incomplete drug release from the device above the absorption zone. Atorvastatin Calcium requires twice a day drug dosage in order to maintain adequate plasma concentration. Because of poor bioavailability and rather high first pass metabolism, it is necessary to develop floating preparation with extended clinical effect [5-12].

- To Prepare and characterize novel Floating Microspheres for gastro retentive drug delivery of drug name
- To encapsulate drug name to achieve a uniform concentration of the drug at the absorption site and to reduce the frequency of administration.
- To increase the poor oral bioavailability of the drug.
- To study various formulation variables those ultimately affect the release of the drug.

Materials & methods

Preparation of Floating Microspheres of Atrovastatin

Floating Microspheres were prepared by novel solvent evaporation matrix erosion method. 20% ethyl cellulose solution was prepared by dissolving required quantity of ethyl cellulose in a mixture of acetone and methanol (1:1). 5 ml of this solution was used in the preparation of each batch of microspheres as specified in Table 1. Required quantity of PEG and/or Atorvastatin, as per the formula given in Table 1 was dissolved separately in 5 ml of methanol. The above two solutions were mixed and stirred thoroughly to form a homogeneous solution. This mixture was dispersed in 50 ml of liquid paraffin, and stirred with the help of a mechanical stirrer (Remi, India,

approx. 500 rpm) for about 6 h at room temperature to form rigid spherical spheres. Microspheres were filtered using Whatman filter paper, washed with 15 ml of petroleum ether thrice and dried at 40 °C overnight [13-14].

Table 1: Batch specifications of different batches of microspheres prepared using polymer and with their blends

Formulation code	Drug (mg)	Polymers		Stirring Speed (RPM)	Stirring time (hrs)
		EC (%)	PEG 4000 (g)		
F1	40	5	0. 375	500	6
F2	40	10	0. 375	500	6
F3	40	15	0. 375	500	6
F4	40	20	0. 375	500	6
F5	40	25	0. 375	500	6
F6	40	30	0.375	500	6
F7	40	25	0.375	1000	6
F8	40	25	0.375	1500	6
F9	40	25	0.375	2000	6
F10	40	25	0.375	2500	6
F11	40	25	0.375	1500	4
F12	40	25	0.375	1500	8
F13	40	25	0.375	1500	10

EC: Ethyl cellulose; **PEG**: Polyethylene glycol

FT-IR study

Fourier transform infrared Spectroscopy of different compounds were performed for identification of that particular compound. FTIR Spectroscopy of Atorvastatin Calcium and mixture of drug and polymer was done using KBr pellets. FTIR Spectroscopy can also be used to investigate and predict any physicochemical interactions between different components [15].

Evaluation of Floating Microspheres

Percentage yield:

The prepared microspheres were dried, collected and weighed. The measured weight was divided by the total amount of all non-volatile components which were used for the preparation of the microspheres.

% Yield = (Actual weight of product / Total weight of drug and polymer) x 100

Determination of drug loading and encapsulation efficiency:

100mg of microspheres were weighed and kept overnight in 0.1N HCl to extract Atorvastatin. Suitable dilutions of the filtrate were prepared and the quantity of Atorvastatin was estimated at 246nm using UV–Vis spectrophotometer (Shimadzu 1800).The percentage of drug loading can be estimated by using the following formula:

$$L = Q_m / W_m \times 100$$

Where L is the percentage loading of microspheres,

Q_m is the quantity of drug in g present in W_m of microspheres

W_m is the weight of microspheres in g.

The percentage of encapsulation was determined by using the following formula:

$$E = Q_p / Q_t \times 100$$

Where E is the % of encapsulation of microspheres

Qp is the quantity of drug encapsulated in microspheres (g)

Qt is the total quantity of drug utilized for encapsulation process (g) [16-17].

Particle size analysis

The prepared microspheres were evaluated for particle size and size distribution. The size was measured using an optical microscope. From each batch floating microspheres were spread on a clean slide and the mean particle size was calculated by measuring 100 particles with the help of a calibrated ocular micrometer [18].

Micromeritics

The microspheres were characterized by their micromeritic properties, such as particle size, bulk density, tapped density, compressibility index and flow properties.

Bulk Density

In this method floating microspheres were transferred to a measuring cylinder and is tapped manually till a constant volume is obtained .This volume is bulk volume and it includes True volume of the powder and void space among the microspheres.

Bulk Density = mass of microspheres/ bulk volume

Tapped Density

In this method floating microspheres were transferred to a measuring cylinder & tapped for 100 times. After tapping volume of microspheres was visually examined .The ratio of mass of microspheres to volume of microspheres after tapping gives tapped density of floating microspheres.

Tapped density = mass of microspheres / volume of microspheres after tapping

Carr's (compressibility) index

Compressibility index (C.I.) or Carr's index value of micro particles was computed according to the following equation

% compressibility = (Tapped density – Bulk density X 100)/ Tapped density

The value given below 15% indicates a powder with usually give rise to good flow characteristics, whereas above 25% indicate poor flow ability**.** [100]

Percent compressibility index was also determined by using formula,

$$\% \text{ compressibility} = 1 - V/V_0 \times 100$$

Here V & V_0 are the volumes of the sample after and before the standard tapping respectively.

Haussners ratio

Hausners ratio of microspheres was determined by comparing tapped density to bulk density using the equation,

Haussners ratio=bulk density /tapped density

Values less than 1.25 indicate good flow (= 20% Carr), whereas greater than 1.25 indicates poor flow (= 33% Carr) [19].

Angle of repose

Angle of repose (α) of the microspheres, which measures the resistance of particle flow, was determined by a fixed funnel method. The height of the funnel was adjusted in such a way that the tip of the funnel just touches the heap of

the blends. Accurately weighed microspheres were allowed to pass through the funnel freely on the surface [20]. The height and radius of the powder was measured and angle of repose was calculated using the following equation,

$$\alpha = \tan^{-1} h/r$$

Scanning electron microscopy

The sample for the scanning electron microscopy (SEM) analysis was prepared by sprinkling the microspheres one side of double adhesive stub. The stub was then coated with gold using Jeol JFC 1100 sputter coater. The SEM analysis of the microspheres was carried out by using Jeol JSM 5300, Japan. The microspheres were viewed at an accelerating voltage of 15kV [21].

Test for buoyancy

The microspheres (200 mg) were transferred to a series of six 500 ml beakers containing 400 ml of simulated gastric fluid without enzymes maintained at 37 °C. The content of the beakers was stirred at 100rpm by magnetic pellet. At different time intervals (2, 4, 6, 8, 10, 12h) floating and non-floating microspheres were separated, dried at 45 °C until a constant weight is obtained.

Floatation time: The time required for 50% (w/w) of the microsphere to float was noted as floatation time. It was determined by the method described under buoyancy but sampling was done at 5, 10, 15 and 20min to determine flotation time.

***In vitro* release studies**

The drug release rate from the floating microspheres was determined using USP XXIII basket type dissolution apparatus. Weighed amount of floating microspheres was filled into a capsule and placed in the basket. 0.1N HCl (pH 2) was used as dissolution medium and maintained at 37 ± 2 °C at a rotation speed of 60 rpm. Volume of dissolution medium was kept 900 ml. 5 ml sample was withdrawn at predetermined time. First sample was withdrawn at 30 min. interval and next five samples were withdrawn at 1 h interval. The samples were analyzed spectrophotometrically at 246 nm to determine the concentration of drug present. The initial volume of the dissolution fluid was maintained by adding 5 ml of fresh dissolution fluid after each withdrawn. All experiments were conducted in triplicate [22-23].

Results and Discussion

In the current research, floating Microspheres loaded with Atrovastatin were developed and evaluated.

FT-IR Studies

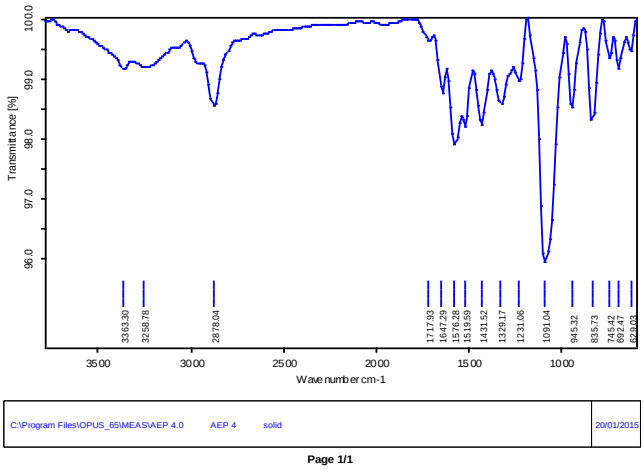

***Figure 1:** FTIR of pure drug*

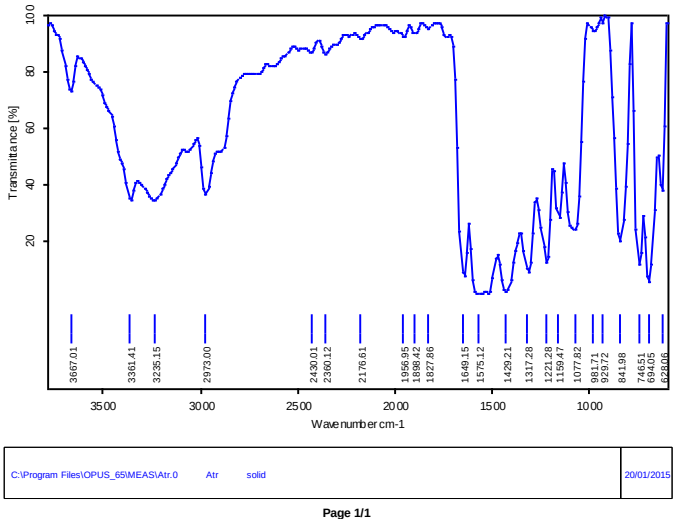

Figure 2: *FTIR of pure drug and Polymer mixture*

FTIR Spectroscopy of Atorvastatin Calcium and mixture of drug and polymer was done using KBr pellets, the spectrum indicating there is no chemical interaction between the drug molecule and polymers used.

Percentage Yield

For different formulation percentage yield was calculated by weighing the microsphere after drying. The percentage yield of floating microsphere was in range of 50.34 – 99.40 (Table 2). The increase in concentration of ethylcellulose increased the percentage yield.

Particle Size Analysis

The mean particle size of floating microspheres was in the range of 72.89 – 498.67 µm. From the above particle size result formulation code F12 having least particle size that was 72.89. It was found that increase of mean particle size with increase in polymer concentration was observed, which may be due to the fact that the increase in polymer concentration leads to a significant increase in the viscosity in a fixed volume of solvent, thus leading to an increase of the emulsion droplet size and finally a higher microsphere size. With increase in the speed of stirring, the median size of microparticles is found to be slightly higher at low stirring speed compared to that obtained at high stirring speed. Higher speed would disperse the globule into finer size thus leading to smaller microparticles. This is because smaller emulsion droplets were produced through stronger shear forces and increased turbulence. Similarly with increase of stirring time particle size of microsphere also reduce.

Percentage Drug Entrapment

The drug entrapment efficacies of the prepared microspheres were in the range of 34.54 – 97.77. The increase in concentration of EC had increased the percentage yield and percentage drug entrapment of the drug and the results are shown in Figure no. 7.5 Presence of higher quantity of ethyl cellulose is enabling better shell wall formation and retention of drug in microparticles. The stirring speed and speed affects drug entrapment efficiency of microparticles. The lower entrapment efficiency at low agitation speed may be due to inadequately stirring of droplets, which leads to increased chances of coalescing. At higher agitation speed, it was visually observed that most of the globules were forced towards the wall of the flask due to high rotation and it ultimately lowers the contact time between polymer and drug molecules. But at 1500 rpm, no sticking of polymeric material around the walls of flask was visually observed which may leads to improved drug: polymer contact time resulting highest drug entrapment efficiency.

Buoyancy Character of Microspheres

Buoyancy of the microspheres was measured by dividing the weight of floating microspheres by the total weight of floating and settled down microspheres. Various batches showed different behaviour to this property. Following table shows results obtained for different formulation at different time intervals for 8hrs.

The purpose of preparing floating microspheres was to extend the GRT of the drug. The microspheres containing EC showed good floating ability for more than 10 hrs due to insolubility of EC polymer in SGF (pH 1.2).

Table 2: Percentage Yield, Particle Size, % Drug entrapment, % Buoyancy

S. No.	Formulation code	% Yield	Particle Size (µm)	% Drug Entrapment	% Buoyancy (8 hrs)
1	F4	78.56	208.56	78.45	65.12
2	F5	84.87	396.90	84.86	85.89
3	F6	91.80	498.67	87.10	84.98
4	F7	95.09	210.67	90.44	85.17
5	F8	98.34	128.34	95.10	90.95
6	F9	95.10	113.54	91.23	81.72
7	F10	92.66	95.55	84.40	82.39
8	F11	82.32	181.09	92.66	75.23
9	F12	99.40	72.89	97.77	90.82

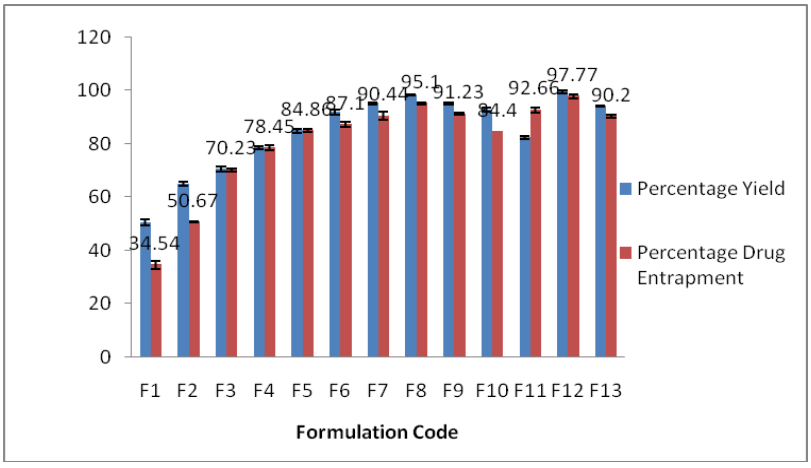

Figure 3: *Percentage Yield and Percentage Drug entrapment of different Atrovastatin microsphere*

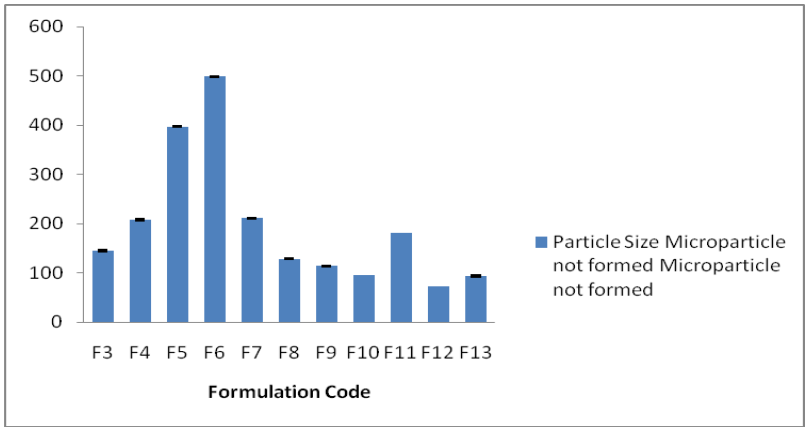

Figure 4: *Particle size of different Atrovastatin microsphere*

Micromeritic properties

Microspheres were characterized for their micromeritics properties such as angle of repose, compressibility index and Haussner's ratio. The results obtained for the said micromeritic properties are shown in the following Table 3.The good flow property of microspheres indicates that the floating microspheres produced are nonaggregated. Flotation might have been influenced by the low bulk and tapped densities.

Table 3: Micromeritic Properties of different Atrovastatin microsphere

S. No.	Formulation code	Angle of repose (Degrees)	Carr's index	Inferences	Hausner ratio	Inferences
1	F4	38.67±0.97	16.98±1.23	Good	1.13±1.83	Good
2	F5	31.85±0.68	10.06±1.45	Excellent	1.04±0.84	Excellent
3	F6	34.67±0.72	19.10±0.67	Good	1.20±0.98	Fair
4	F7	28.56±0.45	18.05±0.11	Fair	1.31±0.26	Passable
5	F8	27.43±0.38	09.54±0.98	Excellent	1.02±0.44	Excellent
6	F9	26.98±0.71	13.45±1.67	Good	1.12±0.62	Good
7	F10	25.11±0.55	15.02±2.01	Good	1.11±0.28	Excellent
8	F11	36.90±0.49	27.34±0.55	Poor	1.19±0.19	Fair
9	F12	28.23±0.23	6.03±0.99	Excellent	1.01±1.01	Excellent

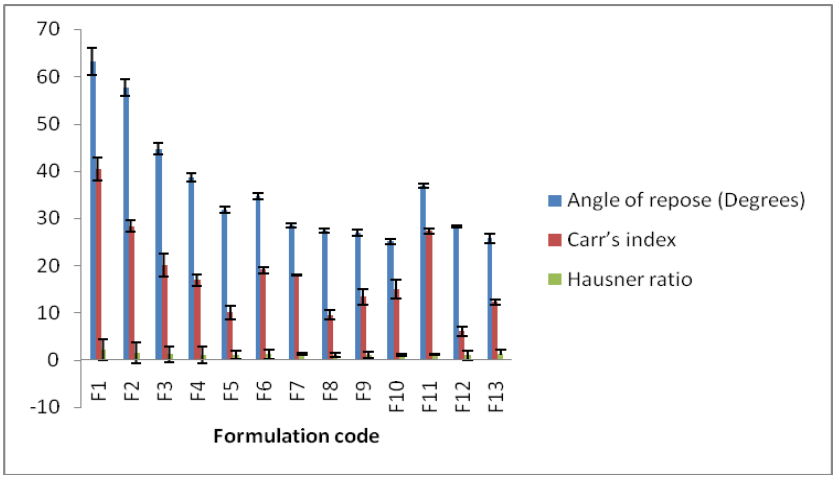

Figure 4: *Micromeritic Properties of different Atrovastatin microsphere*

The present research work revealed that Car's index was in the range of 6.03±0.99 to 40.34±2.451. The value of Hausner's ratio was between 1.02±0.44 to 2.14±2.18 and the values of angle of repose were found to be in the range of 25.11±0.55 to 63.12±2.89 for all the formulation which indicated the good flow potential. The better flow property indicated that the floating microspheres produced are non- aggregated.

Surface Morphology

Surface characteristics of floating microspheres were analysed using a scanning electron microscope. This technique is useful in the examination of internal and external morphology of floating microspheres. The SEM images of formulations were observed to be mostly spherical and exhibits smooth surface. Some microspheres in the images are broken, which might be due to handling and processing SEM revealed that floating microspheres were discrete, rough and spherical in shape. Some of the microspheres showed a dented surface structure, but they showed good

floating ability on the surface of the medium, indicating intact surface .the outer surface of the microspheres was smooth and dense while internal surface was porous.

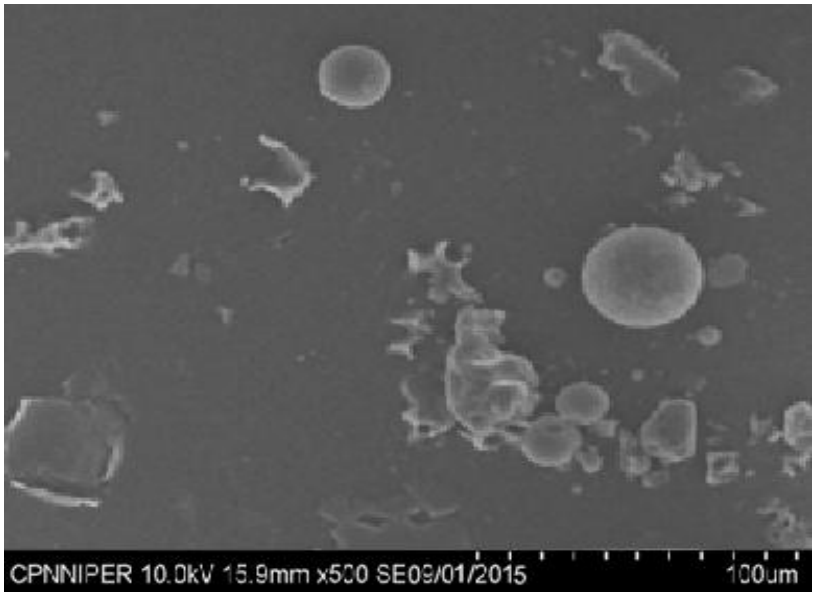

Figure 5: *Scanning electron photomicrograph of EC based floating microspheres*

***In-vitro* release studies**

Release of Atrovastatin from ethyl cellulose based microspheres was evaluated in 0.1 N HCl. Results showed that the optimized formulation showed an initial burst effect due to the presence of the drug particles on the surface of the microspheres. The microspheres sustained the drug release over 8h as shown in Figure no.6. Presence of pores in floating microspheres is responsible for faster dissolution rate. 50% of loaded drugs were released within 3hr and remaining 50% was released during next 8 hrs.

Table 4: Percentage Cumulative drug release of Pure Drug and Formulation F12

S. No.	Time (hr)	Percentage Cumulative release (mean ± SD) of Pure Drug	Percentage Cumulative drug release (mean ± SD) of Formulation F12
1.	0	0	0
2.	0.5	7.31 ± 1.21	15.54 ± 0.99
3.	1	13.18 ± 1.09	26.32± 1.08
4.	2	19.59 ± 0.23	35.67 ± 1.17
5.	3	26.31 ± 0.87	44.89 ± 0.84
6.	4	32.52 ± 0.92	56.32 ± 0.39
7.	5	37.15 ± 0.67	65.90± 0.56
8.	6	42.43 ± 0.33	74.79 ± 0.38
9.	7	47.54±0.65	81.29 ±0.82
10.	8	53.88±0.83	88.42 ±0.61
11.	9	59.09±0.26	92.90 ±54

All values are represented as mean ± standard deviation (n=3)

It was observed that as the 2.5 % concentration of ethyl cellulose will have limited brust effect. The increased ethyl cellulose concentration leads to increased density of polymer matrix in to the microspheres which results in an

increased diffusional path length and consequent retardation in drug release. In case of controlled or sustained release formulations, diffusion, swelling and erosion are the three most important rate controlling mechanisms.
From the graph it was found that microsphere exhibit release in controlled manner and sustained manner as compare to pure drug.

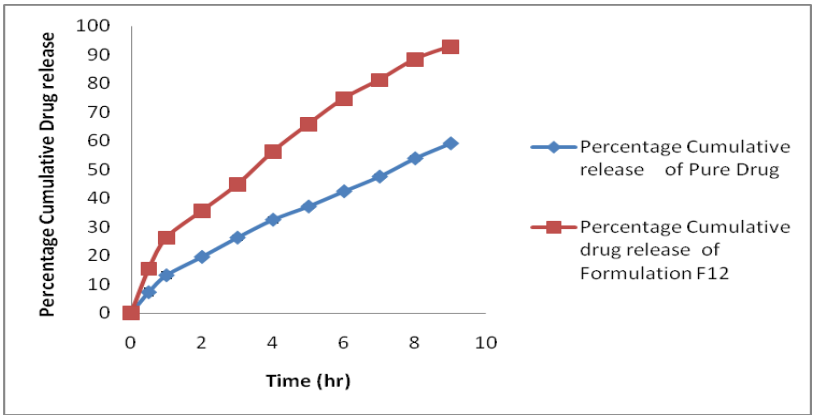

Figure 6: *Percentage Cumulative drug release of Pure Drug and Formulation F12*

Conclusion

By studying all the experimental results Controlled release floating microspheres of Atorvastatin with a matrix structure were prepared successfully by the emulsion solvent evaporation matrix erosion technique. The preparation method was simple and inexpensive. The obtained microspheres were fine and free flowing, the method followed was economical to get reproducible microspheres and the drug polymer ratio had an impact on the drug encapsulation efficiency and in vitro release.

A higher encapsulation efficiency of drug was obtained in formulations F8, F12 and due to higher Drug-Polymer ratio. The results indicated formation of microspheres with different and reproducible size ranges, uniform shape and smooth outer surfaces. The different size of the produced spheres affects significantly the drug loading efficiency, the release profiles and the dose of the released drug. The smaller size formulations F12 showed the burst release of the drug in comparison to the large size formulations.

The drug release from the microspheres prepared in formulation F12achieved its Target i.e. 92.90 ±54% and drug to polymer ratio was most constant and controlled.

This resulted in a homogeneous distribution of drug within the polymer matrix, reduction of the burst effect and the release of drug at a low and uniform rate.

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