FABRICATION AND EVALUATION OF ORAL CONTROLLED RELEASE OF MUCOADHESIVE ALGINATE MICROBEADS CONTAINING FLUVASTATIN SODIUM

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ABSTRACT

Objective: The aim of this study is to prepare oral controlled release (CR) of mucoadhesive alginate microbeads encapsulating fluvastatin by gastroretention technology.

Methods: The mucoadhesive microbeads containing fluvastatin were produced using emulsification internal gelation technique. The effect of different variables such as sodium alginate concentration and its combination with other hydrophilic polymers, and the effect of various curing agents on particle size, entrapment efficiency, and *in vitro* studies were evaluated.

Results: There was no marked change in drug entrapment efficiency, and dissolution studies occur during the stability studies of fluvastatin. The *in vitro* results give data that improvement in the CR of the drug from microbeads compared with marketed tablet. Hence, in this regard, to minimize the frequency of drug administration to reduce side effects. The optimum condition for the preparation of stable alginate beads and produce CR manner was occurred at a higher concentration of combined polymer mixture in equal ratios, i.e., 3% w/v. Infrared spectroscopic study (Fourier transform infrared) confirmed the no incompatibility between drug and other excipients. X-ray diffraction study and differential scanning calorimetry were provided evidence that successful entrapment of drug into the alginates microbeads and drug converted into amorphous nature. The efficiency of mucoadhesion strength of microbeads was determined by wash-off study.

Conclusion: The kinetic modeling of the release data indicates drug release from the microbeads follow anomalous transport mechanism and super Case-II transport mechanism. Drug release is a function of pH dependent and controlled drug release depends on type and concentration of polymer blend and curing agents. The release kinetics of drug from the alginate beads followed zero order.

Keywords: Fluvastatin, Emulsification internal gelation, Sodium alginate, Hydroxypropyl methylcellulose K4M, *In vitro* wash off study and swelling index.

INTRODUCTION

Orally administrated solid dosage forms possess large individual differences in the rate of bioavailability of many drugs, this occurs mostly because of the large variable gastrointestinal (GI) transit time of a tablet/capsule. In contrast to this, mucoadhesive drug delivery system designed to reduce both intra- and inter-subject variability in absorption of drug, to prevent sudden release of drug and provides drug release in controlled manner.

The polymers and transporters utilized in the arrangement are nontoxic, promptly accessible, and usually noncostly. Among completely various different polymers, Na-alginate remains as a polymer of decision for bead production. It is a bioerodible polyacid that has gained a unique property of gel network inside the sight of multivalent cations, for example, aluminum ions (trivalent) and calcium ions (divalent) in fluid media that happen chiefly at intersections in the G-G sequence rich chain region named as the "egg box junction." Bead production is gotten by the inclusion of the medication into a gel dispersion system, and the hydrophilic colloids at that point connect with polyvalent metal ions to obtain insoluble colloidal complexes (calcium alginate), drops that may cause to accelerate in the formation of cross-linked beads.

For soluble drug, carbopol gel could extend the release time but release pattern has significant fluctuation. Hence, in this study, polymer blend used containing hydroxypropyl methylcellulose (HPMC) K4M and sodium alginate was used in equal ratios and which acts as a matrix former to improve the rate of release of fluvastatin sodium from matrices. For many medications that are ingested basically from the proximal small intestine, controlled discharge in stomach would bring about improved [1-3] the rate of bioavailability. Different methodologies available to increase the gastrointestinal residence time, few of which include floatation system or swellable system, gas rafting system, and mucoadhesive system.

Fluvastatin sodium is an antilipidemic agent that competitively inhibits hepatic enzyme 3-hydroxy-3-methylglutaryl-coenzyme a reductase. It belongs to a class of medications called statins and is used to reduce plasma cholesterol levels and prevents cardiovascular diseases [4]. Its short biological half-life (3 h) and but it possesses low therapeutic concentration at the target site due to low rate of bioavailability (24%–29%) because of extensive presystemic metabolism, less GI residence time. The nature of drug is lipophilic. Nonetheless; this compound has a variable elimination half-life time around 1–6 h in people, requiring a dosing recurrence 20–40 mg twice a day. In the perspective on these attributes, numerous endeavors have been made to develop sustained-release (SR)/CR preparation with broadened clinical impacts and diminish dosing recurrence [5] and also prolong the duration of action of drug.

The emulsification internal gelation commonly formed by cross-linking of sodium alginate, yet the subsequent aluminum alginate microbead is absolutely compact, creating it very difficult to control the release of medication for a long period of time compared to other curing agents. These issues are generally confined by blending sodium alginate with completely other polymers, for instance, HPMC K4M, sodium carboxymethyl cellulose, and Carbopol 934P.

Sodium alginate polymer is a linear polysaccharide and unbranched, biocompatible, versatile, and non-toxic matrix of gel that protects the active components of factors such as humidity and heat and thereby improving stability and bioavailability [6]. Because of non-regular block carboxyl groups with the sodium alginate chains, these chains are highly prone to cross-link with divalence/trivalence cations which enables to form reticular hydrogel structure; hence, sodium alginate is broadly used mainly in application of the controlled drug delivery system nowadays. HPMC is a non-ionic cellulose-based compound because of its surface activity and high swelling capacity it makes to enhance drug loading and prolongs drug release.

It was discovered that the biocompatible microspheres had additional high selectivity for discharge of drug substance, in which they restrict drug release at the simulated gastric fluid (pH=1.2), however, permitted full maximum rate of the drug at the premises of higher pH atmosphere, i.e., intestines, implying that the materials are harmless and safe as a drug carrier for controlled release (CR) pattern of drug. This kind of science is reasonable for financially savvy make, prompting a desire that the methodology might be useful for scale-up to industrial production.

MATERIALS AND METHODS

Materials
The pure drug fluvastatin was obtained from AurobindoPharma Limited, Hyderabad, Telangana state, India. HPMC, sodium alginate was purchased from HiMedia, Mumbai, Carbopol 934, barium chloride ($BaCl_2.2H_2O$), calcium chloride ($CaCl_2.2H_2O$), aluminum chloride ($AlCl_3.6H_2O$), ethanol, and chloroform solution were obtained from QualigensFine Chemicals, Mumbai. All chemical agents used belonging to analytical high-grade quality.

Equipments
Digital electronic weighing balance (0.1 mg sensitive), Sonicator (Remi model, Mumbai, India), Sigma Blade Homogenizer (Remi model, India), Dessiccator (Borosilicate, Mumbai), Tablet disintegrating test apparatus (USP model, India), Dissolution test apparatus USP (model TDT-08L, Electro-lab, Mumbai, India), and Sonicator, Remi model, India. Double beam ultraviolet (UV)-visible spectrophotometer, Shimadzu, scanning electron microscope (SEM; S4100, Hitachi, Japan), X-ray diffractometer, Bruker spectrophotometer (Model-220, Germany) and Syringe needle 23 G, etc.

Preparation of fluvastatin microbeads
The fluvastatin based alginate microbeads were prepared by choosing emulsification internal gelation method. The mucoadhesive microbeads were prepared by taking fluvastatin 40 mg dissolved in purified mineral water and this solution was added to white viscous solution which is already prepared and this contained sodium alginate polymer solution. This polymer solution is prepared by taking sodium alginate polymer and dissolved in water by subjecting to homogenization process until sodium alginate is completely dissolves then keeps this homogenous polymer viscous solution inside sonicator to remove air bubbles completely. To the above physical mixture solution which is placed in a beaker, add selected cross-linking agent at 5% w/v concentration slowly into above glass beaker which is stirred with high frequency Remi modeled sigma blade homogenizer and keep on rotation for long time until it forms smooth, fine viscous solution completely and again keep the above solution for sonication up to 1–2 h to get free bubble solution finally. The above physical mixture solution containing drug with selected polymer of known concentration and cross-linking agent take into 10 ml syringe which is fixed with needle 22 gauge and pressed/extruded dropwise into 250 ml glass beaker which containing mixture of 100 ml light liquid paraffin oil (acts as a emulsifying agent) with 1.5% w/v concentration of span 80 and glacial acetic acid at 0.2% w/v. During the addition of drug-based polymer viscous solution dropwise, and it was sure to keep the minimum 10 cm distances between glass beaker and tip of needle throughout the process. The process of homogenization under 600 rpm by rotating with blade homogenizer was maintained continuously. After specified time, the above solution was filtered through filter paper placed over funnel, on filtration, it will form residue of insoluble high viscous smooth solid product and it was washed carefully 3–4 times with n-Hexane or chloroform solution to remove sticky fatty material on surface of small microbeads and these formed microbeads placed inside dessiccator until microbeads becomes completely dried.

In this current article, other hydrophilic polymers were also incorporated along with sodium alginate in equal ratios than alone sodium alginate polymer and different curing agents were added at 5% w/v concentration on optimized batch to evaluate the change in drug release pattern and other parameters, which were clearly mentioned in following Table 1.

CHARACTERIZATION STUDIES

Size distribution and size analysis
For determination of size distribution analysis, 200 mg of the cross-linked fluvastatin alginate microbeads of various sizes in a formulation were subjected to sieving method using a range of standard sieves grades and arranged one by one according to their mesh size and start

Table 1: Formulation composition and physicochemical properties of prepared fluvastatin microbeads

Polymers used	Batch code	Core: coat ratio	Yield (%)	Average particle size	Drug content	Percentage entrapment efficiency
Sodium alginate	FSF1	1:01	91.68	132.55±0.07	44.96±0.02	89.92±0.09
	FSF2	1:1.5	93.89	146.36±0.05	37.94±0.04	94.85±0.06
	FSF3	1:02	95.13	154.44±0.03	31.82±0.05	96.42±0.04
	FSF4	1:03	96.47	168.47±0.06	24.46±0.03	97.84±0.07
Sodium alginate+HPMC K4M	FSF5	1:01	92.12	135.13±0.05	44.13±0.09	88.26±0.05
	FSF6	1:1.5	92.35	157.24±0.09	37.34±0.08	93.95±0.09
	FSF7	1:02	94.27	168.49±0.08	32.23±0.09	97.66±0.08
	FSF8	1:03	96.45	180.29±0.07	23.41±0.05	93.64±0.04
Sodium alginate+carbopol 934P	FSF9	1:01	92.18	139.44±0.06	46.56±0.05	93.12±0.06
	FSF10	1:1.5	94.56	156.47±0.09	37.66±0.09	92.90±0.05
	FSF11	1:02	96.88	168.39±0.05	31.25±0.06	94.39±0.04
	FSF12	1:03	98.32	179.33±0.06	23.47±0.04	93.38±0.06
Sodium alginate+NaCMC 1100–1900Cps	FSF13	1:01	92.47	140.53±0.02	44.38±0.07	89.76±0.02
	FSF14	1:1.5	96.28	156.33±0.04	37.66±0.04	94.15±0.04
	FSF15	1:02	96.79	164.46±0.07	31.46±0.08	95.33±0.05
	FSF16	1:03	97.16	178.46±0.02	24.13±0.04	96.52±0.03
Sodium alginate+HPMC K4M	FSF17	1:03	96.36	174.32±0.05	23.27±0.04	93.08±0.07
Sodium alginate+HPMC K4M	FSF18	1:03	96.23	167.25±0.05	23.43±0.03	93.72±0.06

*Batches FSF1 to FSF16 cured with 5% $AlCl^{3+}$ with different polymersonly and FSF17 and FSF18 batches containing sodium alginate with HPMC K4M. and that cured with 5% w/v of $BaCl^{2+}$ and 5% $CaCl^{2+}$w/v. *All readings were taken in triplicate manner, i.e., n=3. Mean±SD. NaCMC: Sodium carboxymethyl cellulose, HPMC: Hydroxypropyl methylcellulose

the machine continuously according to the standard procedure. The amount of uniformed microbeads which were retained on different individual sieves was taken out and weighed accurately. Finally, the mean particle size of the fluvastain microbeads was determined by given the following equation.

$$\text{Mean particle size} = \frac{\sum \left(\text{Mean particle size of fraction} \times \text{weight Fraction} \right)}{\sum \left(\text{Weight fraction} \right)}$$

Percentage of yield
Percent yield defined as the percent ratio of practical yield to the theoretical yield. Mathematically, it is computed from the following equation.

$$\text{Percentage of yield} = \frac{\text{Practical yield}}{\text{Theroteical yield}} \times 100$$

Estimation of drug content of microbeads
Accurately 100 mg of microbeads were exactly weighed and shifted to a cleaned dry mortar and powdered by continuous grinding and dissolved in 100 ml of freshly prepared pH 6.8 phosphate buffer solution and filtered, suitably diluted the absorbance of the above solution, absorbance was measured at 304 nm by UV-visible spectrophotometer for fluvastatin.

Entrapment efficiency of microbeads
Entrapment efficiency was resolved using the formula. For calculation of drug content, 40 mg of beads were placed in 100 ml of 6.8 phosphate buffer for complete swelling at 37°C. The fluvastatin based alginate beads were crushed in a glass mortar with pestle and the above solution was then kept for 2 h to extract the drug completely and centrifuged to separate polymeric debris using centrifuge apparatus for 30 min. The solution was filtered, the clear supernatant solution was measured fluvastatin content using a UV-visible spectrophotometer at 304 nm.

$$\text{Entrapment efficeincy} = \frac{\text{Estimated percentage drug content}}{\text{Theoritical percentage drug content}} \times 100$$

$$\text{Drug loading} = \frac{\text{Total amount of drug in microbeads}}{\text{Weight of microbeads}} \times 100$$

Estimated percent drug content was determined from the analysis of 100 mg microbeads, and the theoretical percent drug content was calculated from the utilized core:coat ratio in the formulation of microbeads.

Morphological characterization of microbeads (SEM studies)
Tests were sputter-covered with gold under a vacuum before perception with a SEM at an increasing speed voltage of 10 kV.

Fourier transform infrared (FTIR) spectra analysis studies
Infrared spectra were recorded by utilizing a TENSOR 27 FTIR spectrometer (Bruker, Horiba, Oberursel, Germany). Scans were recorded more than 400–4000 cm^{-1} wavenumbers with a resolution of 2 cm^{-1}.

X-ray diffraction (XRD) studies
Pure fluvastatin sodium, microbeads loaded drugs were analyzed using an X-ray diffractometer (D8, Bruker, Germany) in the 2θ range 5–60° with a scanning velocity of 2° min^{-1}.

Differential scanning calorimetry (DSC) studies
For the determination of DSC studies, the microbeads of known quantity (2 mg) were heated from 40°C to 275°C using a temperature rate of 10°C/min. A nitrogen purge of 100 ml/min was fixed.

Determination of swelling index
It was determined by measuring the difference between the initial diameter of the microbeads and final diameter of the beads at time t. It can be calculated by the following equation.

$$\text{Sw} = \frac{\left(W_t - W_o \right)}{W_o} \times 100$$

Where,
S_w = percentage of swelling of microbeads, W_t = weight of the microbeads at time t, and W_0 = initial weight of the microbeads.

In-vitro wash-off test for microbeads
The mucoadhesive strength property of the cross-linked alginate microbeads was determined by conducting *in vitro* wash-off test. For doing of this test, few modifications were done to the disintegration test apparatus test. This test should be mimic same body atmospheric physiology conditions and for this reason, two freshly prepared buffer medium were used. During procedure acidic solution of 0.1 N HCl was used for 8 h and this acid solution replaced fast with 6.8 phosphate buffer solution and same continued for another 8 h. The important step involved in this test is use of fresh goat mucous membrane which surround the intestine of 3 cm×3 cm was rounded paddle completely at down place and keep and spread 100 fluvastatin microbeads exactly over mucous specimen and this paddle was connected to horizontal iron rod, which is clung to shaft of disintegration test apparatus (USP model) in the right position. During continuous up and down movement of shaft which is connected to the paddle that holds microbeads on mucous membrane and glass beakers contain acidic medium of 900 ml capacity for the first 8 h and the apparatus run under the temperature of 37±0.5°C and movement of paddle up and down were maintained 60 rpm without fail. The microbeads which are having maximum adhesive strength will stick long time to mucous membrane and not having mucoadhesive strength means, the beads will get detached in between study planned for 8 h. The results of *in vitro* wash-off test of three batches are shown in Table 2.

Dissolution studies
To simulate the release of drug in both the human stomach and intestine atmosphere, *in vitro* studies were carried out under both acidic medium (pH 1.2) for the first 2 h and alkaline (pH 6.8 phosphate buffer) conditions as well as separately for next 10 h using dissolution test apparatus USP model. Dried fluvastatin microbeads were precisely weighed equivalent to 40 mg and transferred into the dissolution basket

Table 2: *In vitro* wash-off test values for the fluvastatin sodium alginate microbeads prepared with combination of sodium alginate and HPMC K4M in 1:3 ratios by employing various cross-linking agents

Formulation	Percent of alginate beads adhering to tissue at 5 times (h)									
	0.1 N HCl, pH 1.2					Phosphate buffer, pH 6.8				
	1	2	4	6	8	1	2	4	6	8
FSF8	68 (1.5)	57 (1.8)	47 (1.2)	38 (1.8)	26 (2.0)	94 (0.5)	87 (1.0)	79 (1.0)	66 (1.0)	57 (1.0)
FSF17	66 (1.0)	54 (1.8)	40 (1.1)	32 (1.5)	22 (1.0)	92 (0.5)	85 (1.0)	76 (1.2)	64 (1.8)	53 (1.2)
FSF18	62 (1.8)	50 (1.2)	38 (1.8)	24 (2.0)	16 (1.8)	91 (0.5)	80 (1.0)	71 (1.5)	59 (1.5)	47 (2.0)
Microbeads prepared with EVA	48 (1.2)	22 (1.4)	4 (1.0)	-	-	42 (0.5)	18 (1.6)	2 (0.2)	-	-

*Each reading was taken in triplicate, i.e., n=3, Mean±SD. EVA: Ethyl vinyl acetate, HPMC: Hydroxypropyl methylcellulose

which composed of 900 ml freshly prepared dissolution mediums and paddle was rotated at 100 rpm under a temperature of 37±0.5°C. The dissolution medium containing drug released was sampled at various intervals of time up to 12 h by withdrawing sample hourly wise and then removed medium taking for sampling purposes and it was replaced by a fresh buffered medium with a similar quantity. The collected fluid was subjected to filtration by utilizing 0.22 μm filter paper, aliquots of filtrate were diluted suitably [7-9] and the absorbance reading of fluid was taken out using an UV-visible spectrophotometer by fixing the wavelength working at 304 nm. At last, the fluvastatin concentration was determined from the fluid using standardized calibration curve.

Kinetic studies

To evaluate the mechanism of drug discharge from the matrix tablets, the information got from the drug discharge studies was examined by the accompanying conditions. In all mathematical equations, Q is the measure of medication discharged at time t, M_t is the drug discharged at time t, M is the total measure of medication in the dosage form, F is the portion of the medication discharged at time t, K_0 is the zero-order release rate constant, and "n" is the diffusion exponent type which demonstrating the mechanism of drug release. The value of n=0.45 demonstrates Fickian type dispersion, the estimation of n somewhere in the range of 0.45–0.89 indicates anomalous transport and the value n=0.89 demonstrates Case-II transport model.

RESULTS AND DISCUSSION

On observation of micromeritic studies of alginate microbeads appeared as white, nearly spherical shape like and free-flowing of fluvastatin sodium microbeads were produced using the emulsification internal gelation method. The percentage yield varied from 91.68% to 98.32%, with the highest yield obtained for FSF12 batch. The results show that increase in concentration of polymer blend ratio improves product yield, i.e., concentration of polymer blend was forms direct proportional. However, mean particle size has been shown to be increased with increasing polymer blend concentration, which could be due to enhancement in relative viscosities of polymer. The flow property parameter values are mentioned in Table 1, the angles of repose were found in the range of 20.39±0.05–28.26±0.06 (IP limits 25–30) showing that the blend of powder was freely flowing. The values for the Carr's index were between 12.32±0.04% and 15.87±0.06% (IP limits 11–15%) indicating that all fluvastatin batches of powder blends were having good compressibility values. The Hausner's ratio was within the limits of 1.16±0.012–1.22±0.05 (IP limits 1.12–1.10). The results showed that all the batches showed good flow (rheological properties). The values of both tapped and bulk densities have shown good packing ability. The statistical data which is relevant to micromeritics of total formulations were shown in Table 3.

Effect of core:coat ratio on drug entrapment

In current article, a total of 18 batches have formulated and in all batches same quantity of drug was maintained and first four batches were designed only with combinations of drug and sodium alginate polymer (FSF1 to FSF4) was added. With different concentrations of sodium alginate ranging from 1%, 1.5%, 2%, to 3% w/v along with drug, DEE will be enhanced the reason for progressive increment in the first four batches due to the availability of abundant number of cross-linking sites along polymer chains and it will drag more drug inside and maximum cross-linking taking place.

Entrapment efficiency

The percentage entrapment efficiency values for the first four batches ranged from 89.92±0.09% to 97.84±0.07%. Hence, based on above evidence, it was proved that with increase in the concentration of polymers, i.e., polymer blend an increase in the drug entrapment efficiency of the microbeads was observed. It also depends on type of crosslinker used. In current studies, the three different cross-linking agents naming $AlCl_3$, $BaCl_2$, and $CaCl_2$ at 5% w/v level were used along with polymer blend as per described procedure. In above three curing agents, the different batches and $AlCl_3$ which is used as curing agent from FSF1 to FSF16 batches only will show maximum entrapment efficiency 97.84±0.07% because of it will form tight compact microbeads and not allow any leakage of drug during process of homogenization, microbeads obtained with $BaCl_2$ (FSF17 batch) show less DEE than FSF8 batch The entrapment efficiencies were generally higher for the formulations that cross-linked with Al^{3+} and Ca^{2+} as compared to the microbeads cross-linked with $BaCl_2$ 93.08±0.07% at the same concentrations of curing agents.

FTIR studies

The FTIR spectrum of pure fluvastatin sodium showed that characteristic peaks at wave numbers were 1013.16 cm⁻¹, 3688.12 cm⁻¹, 1214.96 cm⁻¹, and 1481.32 cm⁻¹ denoting stretching vibration of C-F stretching, O-H stretching, C-O stretching, and CH_3 deformations, the peak at 949.56 cm⁻¹ occurs due to aryl-F functional group, 1013.76 cm⁻¹ due to stretching vibration of C-F type, 949.56 cm⁻¹ corresponds to aryl-F, 1053.89 cm⁻¹ due to C-F stretching type, 1154.47 cm⁻¹ due to ether stretching, 1215 cm⁻¹ represents amine (C-N) group, 1481.32 cm⁻¹ due to CH_3 deformation, 1579.87 cm⁻¹ due to C=O stretching, 1620.28 cm⁻¹ due to the presence of carboxyl stretch C=O, 3375.41 cm⁻¹ due to aryl-H, 3580.60 cm⁻¹ due to C=O stretching, 3688.12 cm⁻¹ due to O-H stretching type, and 3894.24 cm⁻¹ due to O-H bending, respectively. The FTIR spectrum (Figs. 1-5) of optimized formulation showed that characteristic peaks at wave numbers were 1009.53 cm⁻¹, 3028.37 cm⁻¹ indicates C-H fetching, 3644.11 cm⁻¹, 1214.67 cm⁻¹, and 1480.31 cm⁻¹ denoting stretching vibration of C-N stretching, O-H stretching, C-O

Table 3: Flow properties of fluvastatin-based sodium alginate microbeads

Formulation	Angle of repose (θ)	Bulk density (g/cm³)	Tapped density (g/cm³)	Carr's index (%)	Hausner's ratio
FSF1	27.64±0.04	0.867±0.08	1.057±0.06	15.06±0.04	1.20±0.03
FSF2	25.73±0.06	0.886±0.04	1.063±0.04	14.18±0.02	1.20±0.06
FSF3	23.44±0.07	0.897±0.03	1.058±0.07	13.13±0.06	1.18±0.04
FSF4	20.39±0.05	0.913±0.07	1.059±0.02	12.32±0.04	1.16±0.07
FSF5	26.52±0.05	0.514±0.06	0.611±0.09	15.17±0.06	1.188±0.05
FSF6	26.14±0.07	0.522±0.05	0.619±0.08	15.087±0.05	1.185±0.05
FSF7	25.73±0.03	0.524±0.09	0.620±0.07	15.48±0.04	1.183±0.08
FSF8	26.71±0.04	0.527±0.07	0.622±0.05	15.07±0.03	1.180±0.09
FSF9	27.64±0.03	0.514±0.02	0.611±0.085	14.87±0.07	1.188±0.06
FSF10	26.93±0.05	0.519±0.05	0.614±0.05	15.10±0.07	1.183±0.08
FSF11	26.10±0.07	0.521±0.06	0.616±0.07	15.42±0.03	1.182±0.07
FSF12	26.75±0.04	0.531±0.08	0.627±0.09	15.31±0.05	1.181±0.09
FSF13	28.26±0.06	0.783±0.04	0.955±0.05	15.72±0.07	1.22±0.05
FSF14	26.45±0.07	0.799±0.05	0.958±0.04	14.36±0.05	1.20±0.02
FSF15	24.87±0.04	0.914±0.02	1.078±0.07	13.74±0.06	1.18±0.04
FSF16	22.66±0.03	0.928±0.08	1.076±0.06	12.71±0.04	1.16±0.03
FSF17	24.02±0.21	0.439±0.012	0.512±0.022	14.24±0.02	1.16±0.012
FSF18	25.22±0.13	0.445±0.009	0.522±0.012	13.94±0.03	1.17±0.09

*All readings were taken in triplicate (n=3). SD+Mean

stretching, and CH_3 deformations, 1561.69 cm^{-1} indicating C=C, and 1692.98 cm^{-1} corresponding to C=O, respectively. FTIR spectra of the physical mixture, i.e., optimized batch show almost nearest frequency range and near similar to the corresponding concurrent absorption of both the fluvastatin and added polymer molecules in same region of the FTIR spectra. Based on above evidence, the FTIR spectra of optimized batch depicted that there were no chances of any significant changes in the peak of fluvastatin sodium, as in making comparison of individual peaks as shown in pure fluvastatin (Fig. 1). The band at 3456 cm^{-1} is attributed to hydroxyl stretching in HPMC which was shifted to 3644.11 in optimized batch and also a peak at 1692.98 cm^{-1} which indicates the presence of C=O stretching in optimized batch, this clearly gives an idea about occurrence of acidic esterification between sodium alginate and HPMC. It is clearly suggesting that there were no physical or chemical interactions and there was no change in functional group of optimized batch, as shown in Fig. 5. From the Fig. 5, it was observed that similar peaks were also reported in optimized formulation, few of them listed here include. There was no marked change or large shifting of characteristic peaks in drug-loaded microbeads and which suggested that there were no significant drug-polymer interactions, which indicates the stable nature of the fluvastatin drug in optimized formulation. Frequency range of spectra of various polymers, drug and optimized are shown in Figs. 1-5.

Morphology of microbeads

The formed microbeads are somewhat resembles with spherical in shape, discrete and surface covered with wrinkled layer which might be indication for occurrence of egg-box model structure that cross-linked by G-guluronic acid in sodium alginate molecules with polymer blend and $AlCl_3$.

The SEM photograph of fluvastatin sodium showed that microspheres were spherical in shape and also few microbeads posses surface are with smooth indicating uniform distribution of the drug in the polymeric matrix of the polymer. The SEM analysis also revealed that the prepared microbeads were found to devoid of aggregation microbeads and posses any kind of negligible surface charges this clearly indicating physical stability of microbeads. Significant effect was observed in shape of microbeads on increasing the equal concentration of polymer blend. The SEM of optimized microsphere formulation of fluvastatin sodium is shown in Fig. 6.

DSC studies

DSC studies were used to examine the crystallization and melting behaviors of pure drug and optimized batch. A sharp melting point at 120.89°C indicates its crystalline nature (Fig. 7). The temperature of the melting peak of fluvastatin in the microbeads, i.e., optimized batch
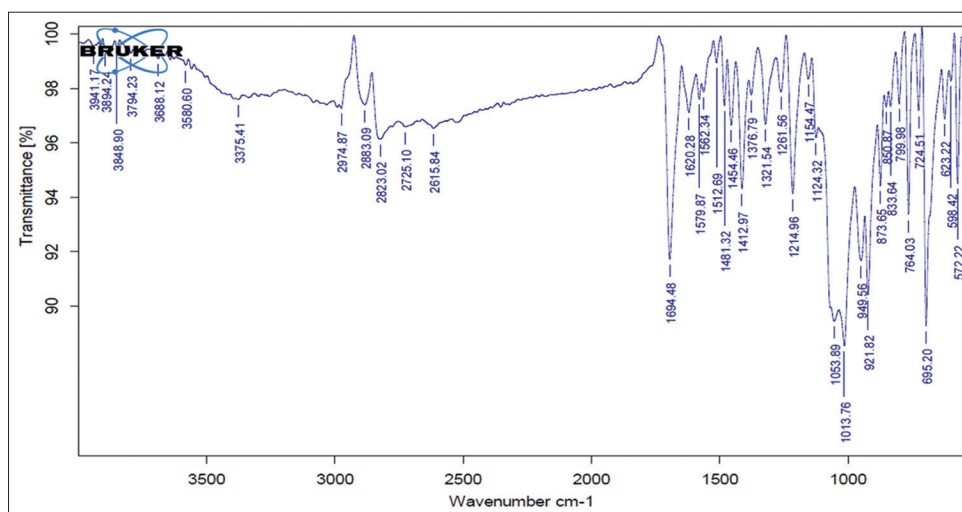

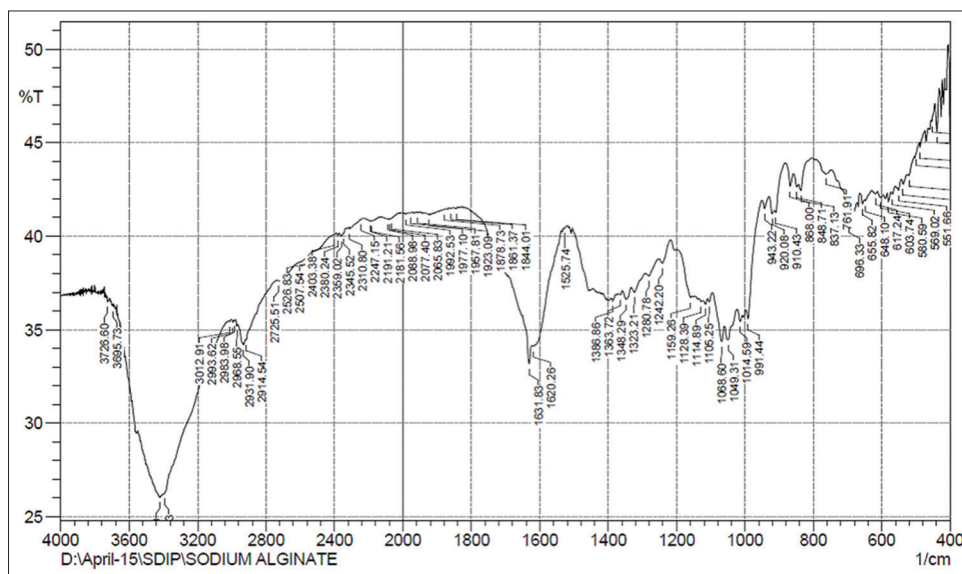**Fig. 1: Fourier transform infrared spectrum of pure fluvastatin sodium**

Fig. 2: Fourier transform infrared spectrum of pure sodium alginate
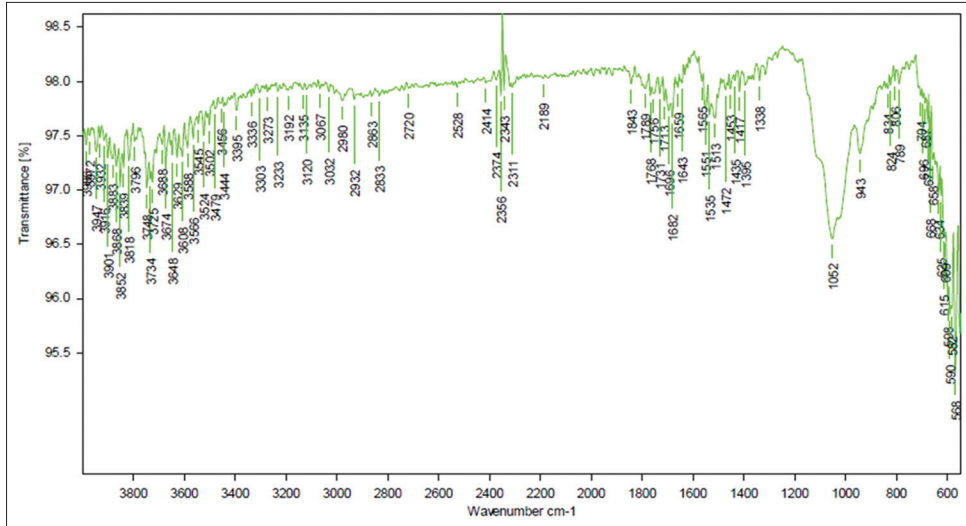
Fig. 3: Fourier transform infrared spectrum of pure hydroxypropyl methylcellulose K4M
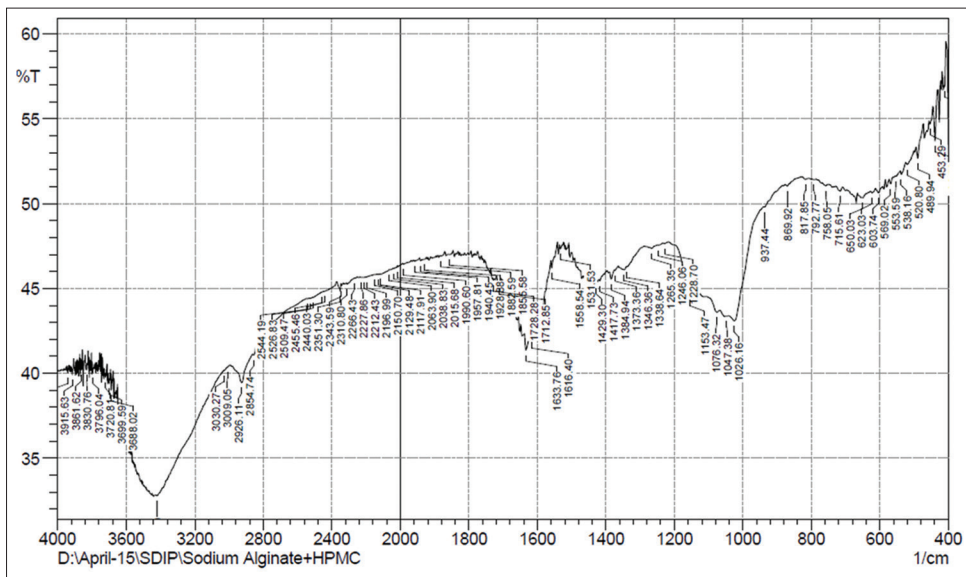

Fig. 4: Fourier transform infrared spectrum of physical mixture containing sodium alginate with hydroxypropyl methylcellulose K4M
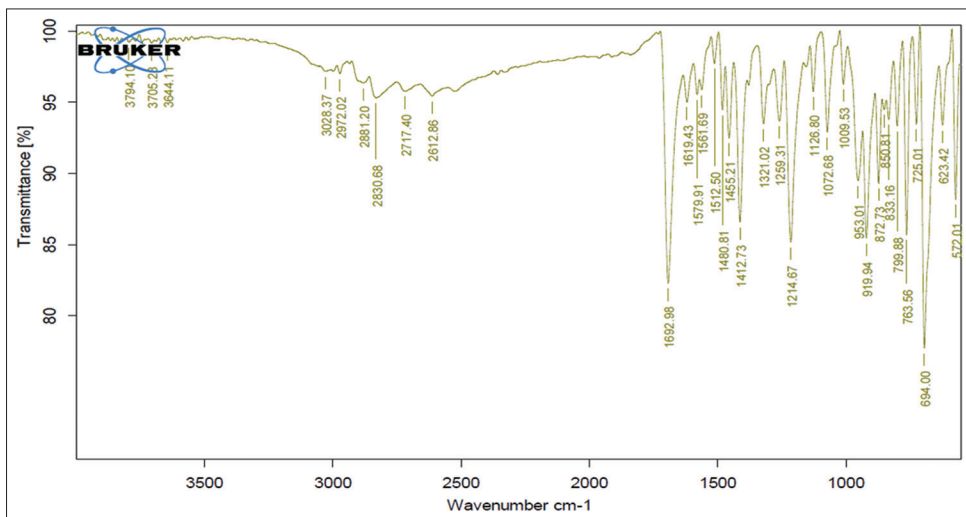

Fig. 5: Fourier transform infrared spectrum of optimized formulation

FLF8 exhibited same a sharp endothermic peak as in pure drug at 114.35°C reflecting that near almost same temperature and other peaks at 178.11°C and 283.17°C indicates components were interacted each other while and evaporation of solvent on heating during the process. On examination of both DSC spectra of pure drug and optimization batch, the disappearance of endothermic peaks (178.11°C) in optimized batch revealed and proves that fluvastatin exists in optimized batch (FLF8) in an uncrystallized form rather than exists in crystalline form as in pure drug and drug molecules have been distributed throughout polymer matrix bead and it also explained as the DSC thermogram of optimized fluvastatin batch does not show marked profound shifts in peaks in comparison to pure DSC spectra of fluvastatin indicating compatibility and thermographic results not showing any sign of reaction and existence of stability after the preparation.

XRD properties
The X-ray powder diffraction technique acts as powerful analytical tool in the identification of crystalline and non-crystalline phase. The characteristic diffraction peaks of fluvastatin sodium are at the

characteristics diffraction peaks of fluvastatin are at (2θ) 11.1311°, 11.9581°, 12.8641°, 13.9792°, 15.552°, 16.455°, 17.3989°, 18.6393°, 19.6992°, 20.6741°, 21.5800°, 22.3000°, 23.8534°, 24.6427°, 25.6400°, 26.0836°, 26.6400°, 28.1321°, 30.3000°, 31.9000°, 32.3200°, 33.8940°, 34.9600°, 35.5000°, 36.4200°, 37.0000°, 38.9920°, 41.0975°, 42.1366°, 42.1366°, 42.8481°, and 44.4573° indicates the crystalline nature of the drug. Moreover, peaks of individual fluvastatin drug were still detectable in their respective optimized formulation with dramatically maximum reduction in intensity of peaks, and in optimized batch the much diffused pattern manner was observed in FSF8 batch which gives clear indication of conversion of crystalline state to the amorphous nature of drug. Thus, there is no indication of any interaction were identified in optimized formulation under the standard conditions. The X-ray powder diffraction studies of fluvastatin sodium and optimized batch are shown in Fig. 8.

Wash-off studies
The microbeads consisting of sodium alginate alone and in combination with HPMC K4M, exhibited good mucoadhesive properties as observed in *in vitro* wash-off test when compared to microbeads prepared with non-mucoadhesive polymer ethyl vinyl acetate (EVA). The wash off was slow in the case of microbeads consisting of alginate-mucoadhesive polymers (hydrophilic nature) when compared to that of ethyl cellulose microspheres (Table 3). The wash off was faster at simulated intestinal pH (phosphate buffer 6.8) than comparison to that at simulated gastric pH (1.2). Swelling of the study depends on that the solubility, hydration, and mucoadhesivity of the polymers depend on the pH of the medium used. The rapid wash-off observed at simulated intestinal pH may be due to the ionization of carboxyl acid group in sodium alginate polymer and other functional groups present in the polymers, which increase
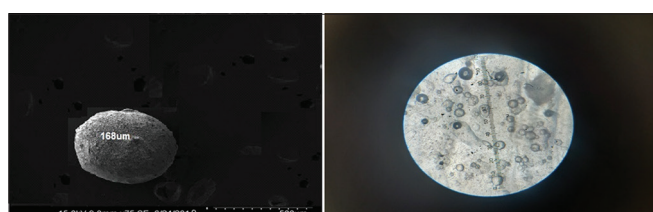

Fig. 6: Scanning electron microscope photograph of fluvastatin microbeads (left side) photographic image (right side)
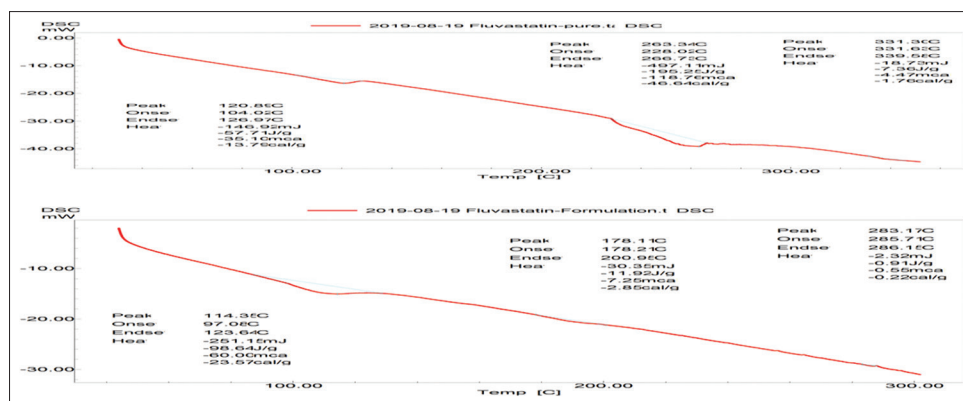

Fig. 7: Differential scanning calorimetry thermogram of pure fluvastatin (above spectra) and optimized batch (below spectra)
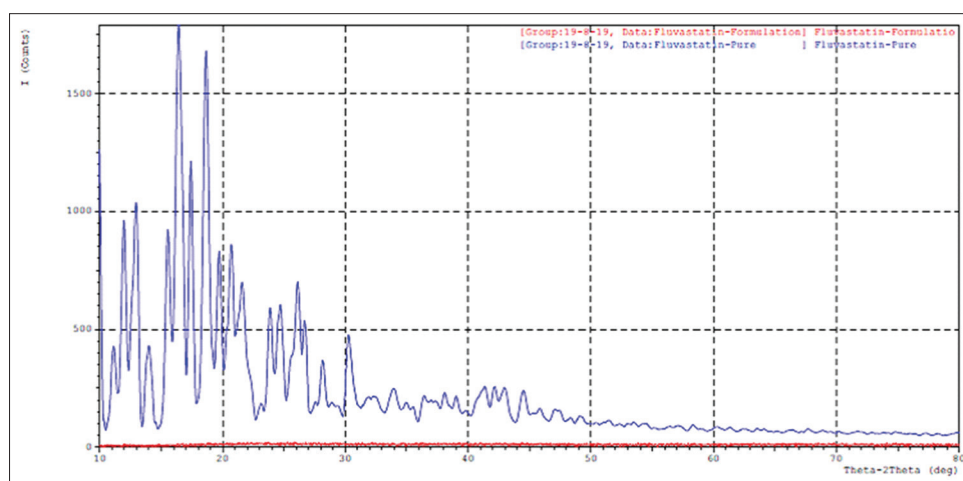

Fig. 8: X-ray diffraction spectra of pure fluvastatin drug (above spectra) and optimized batch (below spectra)

their solubility and reduce adhesive strength. The mucoadhesive behaviors of various microbeads formulations are shown in Fig. 9. The developed mucoadhesive microbeads would adhere strongly because of high viscosity of polymer blend, maximum swelling of microbeads in alkaline medium than acidic medium and thereby to enhance the surface area of microbeads to the gastrointestinal mucous walls and also cause high relaxation of polymers chains and resisting gastric emptying time. This would cause to ensure the prolong residence time at the mucous absorption site to promote further intimate contact with the absorption surface and thereby to enhance the rate of bioavailability of drug.
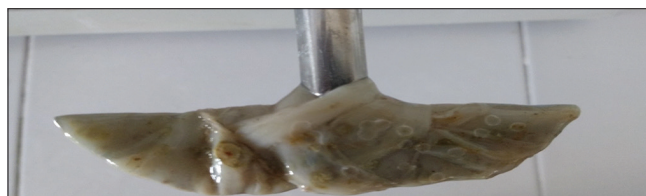
Determination of swelling index

It was determined by studying in two different dissolution media naming [10,11]. 0.1 N HCl for the first 2h and later the previous medium was replaced with 6.8 phosphate buffer medium under temperature at 37±0.5°C with 100 rpm and this test will be continued for 8 h with non-stop procedure using dissolution test apparatus USP model and basket (not paddle type) is used to hold microbeads inside which was ready to examine the swelling index. The measurement of swelling
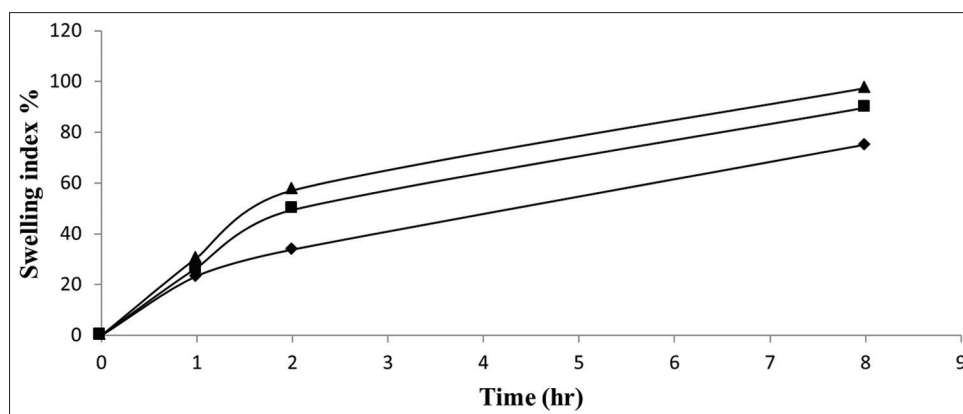
Table 4: Swelling index values of fluvastatin sodium alginate microbeads prepared with a combination of sodium alginate and HPMC K4M in 1.5:1.5 ratio by employing various cross-linking agents

Formulation code	Swelling index		
	Time (h)		
	After 1 h	After 2 h	After 8h
FSF8	23.45	33.82	75.20
FSF17	26.72	49.44	89.68
FSF18	30.67	57.13	97.47

*Each reading taken in triplicate manner, i.e., n=3. HPMC: Hydroxypropyl methylcellulose

Fig. 9: Adherence of fluvastatin-based sodium alginate microbeads around mucous tissue specimen during wash-off test

index of fluvastatin microbeads formulations using polymer blends of sodium alginate and HPMC K4M was carried out (FSF8 batch). This was conceptualized that high amount of polymer blend concentration, i.e., 3% w/v in equal proportions of sodium alginate and HPMC K4M absorbs huge quantity of solvent under alkaline conditions from the system in comparison to acidic medium and forms tight jelly like network around the surface of the microbeads with respect to the time t. This mechanical feature of surface hydrated polymer forms a viscous barricade that plays a major role in the whole amount of drug release rate. The swelling profiles for three different batches are shown in Fig. 9. However, interesting point in swelling rate after 8 h was observed in FSF8 batch than FSF17 and FSF18 that could be due to the reason of selection of different curing agents in above two different batches. In FSF8 batch been formulated using 5% w/v of $AlCl_3$, as it forms three-dimensional network in cross-linking and forms very rigid surface microbeads which does not allow easily diffusion of solvent into the interior surface of the cross-linked beads, so in connection to this point the microbeads prepared with FSF8 formulation they will not undergoes maximum rate of swelling, i.e., less swelling (75.20%/8 h) than other batches that designed with $BaCl_2$ (FSF17) and $CaCl_2$ (FSF18) at the same concentrations with same polymer blend (Table 4). The maximum rate of swelling 97.47%/8 h was observed in FSF18 batch, which formulated with $CaCl_2$ (divalency nature) and it allows easy diffusion of solvent into network of microbeads so that it leads to form maximum rate of swelling of all selected batches. The optimized batch containing HPMC K4M along with sodium alginate showed continuous increase in swelling. This may be again due to high proportion of polymeric system which retain water and form a thick swollen mass and minimize the erosion property of HPMC K4M (Fig. 10).

Model dependent kinetics

The drug release kinetics for all 18 batches was calculated using Microsoft Office Excel 2007 version. The release data were analyzed by fitting the profile of drug release of all the formulations into different kinetic orders which naming zero-order [12], first-order, Higuchi, and Korsmeyer–Peppas model, and regression coefficients (R^2) were calculated for all the formulations. The apparent dissolution rate constants K_o (mg/h), $T_{50(h.)}$ and $T_{90(h)}$. However, all batches posses maximum R^2 value (Regression coefficient) ranging from 0.9771 to 0.998 indicates zero-order drug release and maximum diffusion exponent value "n" 1.182 for optimized batch suggesting super Case-II transport mechanism.

The mechanism of fluvastatin based sodium alginate discharge from the hydrophilic matrix carrier system involves polymer swelling/erosion and non-Fickian diffusion of the drug. The parameters are shown in Table 5.

Fig. 10: Comparative swelling index plot of fluvastatin sodium alginate microbeads prepared with a combination of sodium alginate and hydroxypropyl methylcellulose K4M in 1:3 ratios by employing various types of cross-linking agents. FSF8 (◆◆◆) Fluvastatin sodium alginate microbeads prepared with a combination of sodium alginate and HPMC K4M in 1:3 ratio by employing aluminum chloride. FSF17(-■-) Fluvastatin sodium alginate microbeads prepared with a combination of sodium alginate and HPMC K4M in 1:3 ratio by employing barium chloride. FSF18(-▲-) Fluvastatin sodium alginate microbeads prepared with a combination of sodium alginate and HPMC K4M in 1:3 ratio by employing calcium chloride. *Each reading taken in triplicate manner, i.e., n=3.

Dissolution studies

The pattern could be controlled by the alginate gel disintegration in both 0.1 N HCl acid for the first 2 h and continued the study up to 12 h by replacing previous medium freshly prepared 6.8 phosphate buffer solution at 37±0.5°C under 100 rpm using paddle-type *in vitro* dissolution test apparatus. The breakdown or slow fragmentation of soft swellable alginate microbeads was monitored by the exchange of Al^{3+} from FSF1 to FSF8 batches with potassium ions present in the 6.8 potassium phosphate buffer dissolution medium. The drug release mainly relies on diffusion of drug through the small pores and cracks which are formed on surface of wet swellable microbeads during dissolution process under standard conditions.

The release behavior of alginate microbeads, produced by internal gelation with different gelling agents relies on the size and valence of the cations of the respective cross-linking agents at 5% w/v. To investigate this mechanism, the polymer blend consists of sodium alginate 1.5% w/v sodium alginate and 1.5% w/v of HPMC K4M were added with various cross-linking agents at 5% w/v concentrations naming $AlCl^{3+}$ (FSF8 batch), $BaCl^{2+}$ (FSF17), and $CaCl^{2+}$ (FSF18) and their impact on drug release was studied.

The slow release of fluvastatin through the insoluble cross-linked matrix of blend polymer occurs in 0.1 N HCl dissolution medium and very slow rate of swelling takes place in prepared microbeads and it results in only small quantity of drug release in acidic medium, in contrast to this, swelling and erosion of the microbeads prepared from polymer blend were highly observed in phosphate buffer of pH 6.8. Slow erosion of aluminum cross-linked fluvastatin based alginate microbeads could occur through slight degradation of sodium alginate backbone into smaller species.

The FLF8 batch in equal proportions of 1.5:1.5 containing polymer blend of sodium alginate and HPMC K4M retard or produces sustain the release of drug from the microbeads compared to other batches. The slow amount of drug release is due to swelling of HMPC K4M that makes in delays of drug release from the cross-linked microbeads. The drug release patterns of all eighteen batches are shown in Figs. 11-13. Due to the result of the rheology [13] of hydrated/swelled product, the swollen particles combine. This outcome in a non-stop viscoelastic matrix that fills the interstices, keeping the integrity of the microbeads, and hindering further entry of the dissolution medium into microbeads containing a higher amount (1.5% w/v) of HPMC K4M.

Table 5: *In-vitro* **dissolution kinetics parameters of fluvastatin microbeads prepared with sodium alginate alone and in combination with hydroxypropyl methylcellulose K4M/Carbopol 934 P/NaCMC in different ratios by employing aluminum chloride/barium chloride/calcium chloride as a cross-linking agent at 5% w/v**

Formulation	Correlation coefficient (R^2)				Release kinetics			Diffusionexponent value(n)
	Zero order	First order	Higuchi	Peppas	K_o (mg/h)	$T5_0$ (h)	T_{90} (h)	
FSF1	0.9992	0.7245	0.8952	0.9902	5.17	3.86	6.96	0.8956
FSF2	0.9985	0.7326	0.9117	0.9948	4.82	4.14	7.46	0.8848
FSF3	0.9976	0.7683	0.9178	0.9946	4.41	4.53	8.16	0.8778
FSF4	0.9979	0.7709	0.9315	0.9968	3.56	5.61	10.11	0.8500
FSF5	0.9996	0.8056	0.9303	0.9990	4.72	4.23	7.62	0.9112
FSF6	0.9989	0.7971	0.9107	0.9998	4.4	4.5	8.18	1.082
FSF7	0.9986	0.8073	0.9214	0.9985	3.84	5.1	9.2	1.108
FSF8	0.9983	0.6380	0.9052	0.9991	3.28	6.09	10.97	1.182
FSF9	0.9976	0.7964	0.9404	0.9975	4.96	4.03	7.25	0.8418
FSF10	0.9998	0.8044	0.9268	0.9995	4.64	4.31	7.75	0.9427
FSF11	0.9994	0.8068	0.9228	0.9928	4.08	4.9	8.82	1.0301
FSF12	0.9998	0.7640	0.9261	0.9993	3.60	5.5	10.0	1.1035
FSF13	0.9778	0.7407	0.9089	0.9941	5.70	3.50	6.31	0.8883
FSF14	0.9771	0.7705	0.9167	0.9960	4.80	4.16	7.5	0.8802
FSF15	0.9910	0.7991	0.9278	0.9971	4.50	4.44	8.0	0.8748
FSF16	0.9978	0.8085	0.9352	0.9983	3.94	5.07	9.13	0.8649
FSF17	0.9990	0.7235	0.9126	0.9966	3.48	5.74	10.32	0.8801
FSF18	0.9976	0.7478	0.9168	0.9934	3.60	5.55	10.0	0.9023
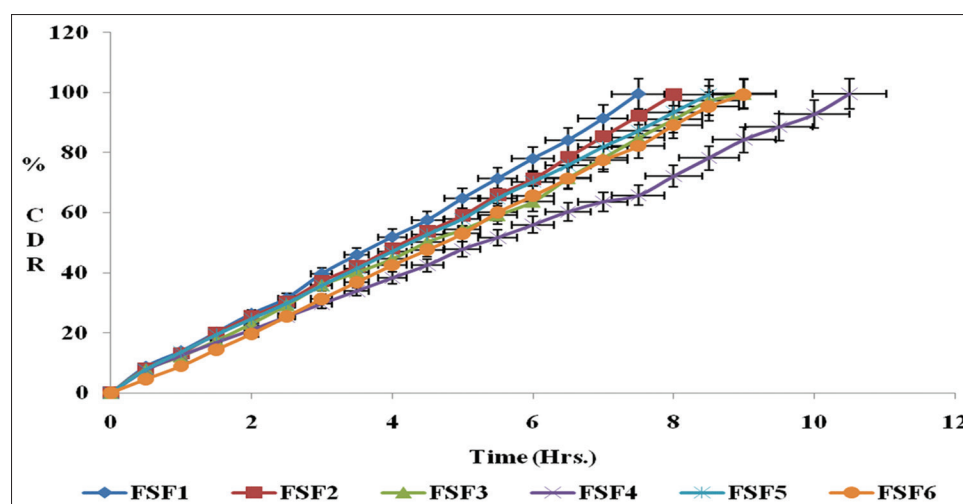
Fig. 11: Dissolution profile of batches from FSF1 to FSF6. Each value taken in triplicate, i.e., n=3. Mean±SD
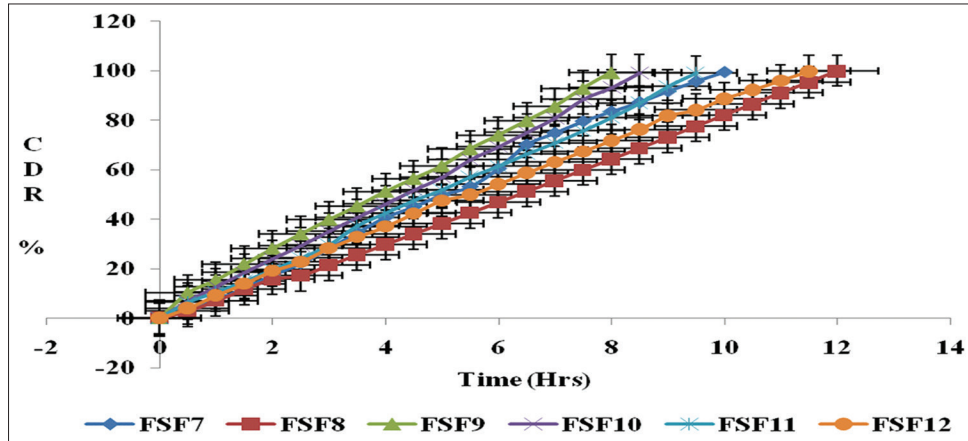
Fig. 12: Dissolution profile of batches from FSF7 to FSF12. Each value taken in triplicate, i.e., n=3. Mean±SD
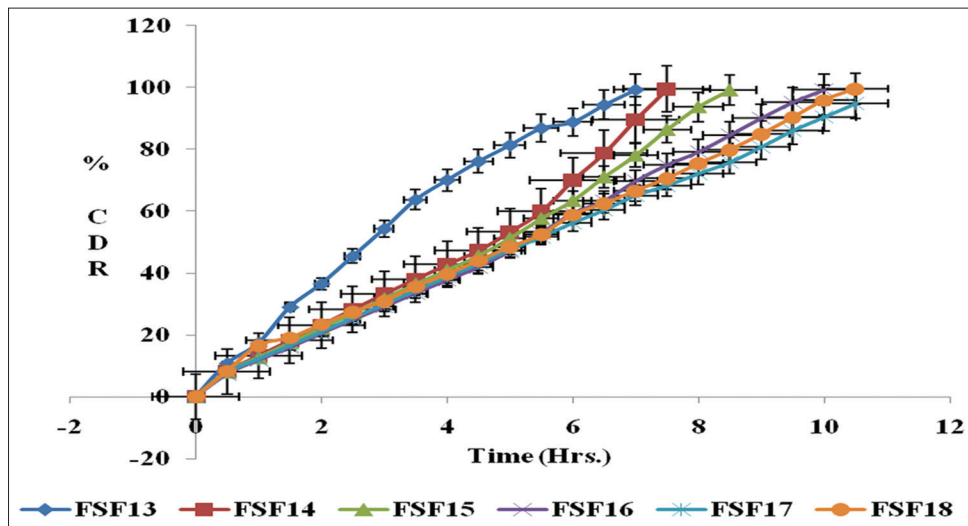

Fig. 13: Dissolution profile of batches from FSF13 to FSF18. Each value taken in triplicate, i.e., n=3. Mean±SD
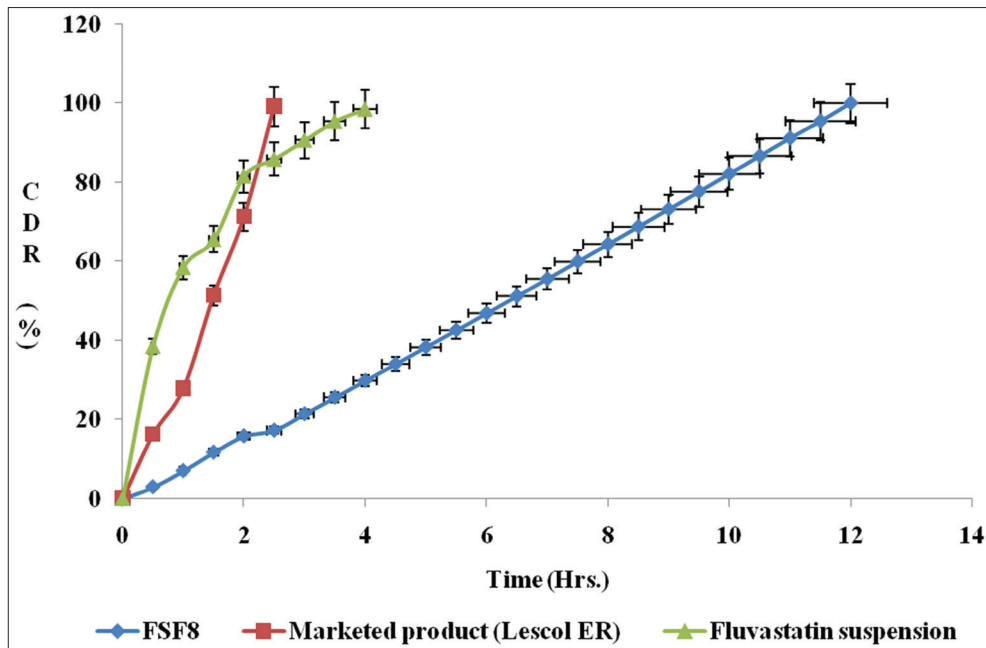

Fig. 14: Comparative dissolution profile of optimized batch FSF8, marketed product, and pure drug. Each value taken in triplicate, i.e., n=3. Mean±SD

The outcomes acquired can be clarified on the basis of the extent of cross-linking in the microspheres. In case of $CaCl_2$ and $BaCl_2$ used as hardening agents in batches (FSF18 and FSF17) and of their divalence nature, they form two-dimensional network bonding structure with sodium alginate chain inside the cross-linked alginate matrices. In case of Ba^{2+} it has the largest size (1.74 A°) when compared to the other two curing agents (0.68 A° for Al^{3+} and 1.14 A° for Ca^{2+}) it is expected to form strong alginate microspheres with little voids and minimum water uptake. Along these lines, the exchange of larger barium ions in the microbeads with potassium ions of dissolution medium (phosphate buffer, pH 6.8) and furthermore, their expulsion as insoluble barium phosphate was upset, and this resulting in delayed swelling rate of the microbeads and slow release of drug occurs. In case of calcium alginate microbeads, due to the smaller size of Ca^{2+} ions as compared to barium ions, it ensures fast removal of calcium as calcium phosphate from the microbeads due to process of ion exchange with potassium of 6.8 phosphate buffer medium and thus leading to greater amount of fluid uptake and this leads to cause maximum release of drug. In case of Al^{3+} alginate microbeads, this deferral was taken place and it was due to the ability of Al^{3+} to form three-dimensional bonding network bonding structure with the sodium alginate inside the microbeads. This three dimensional bonding results in an extended cross-linking through the whole microbeads, producing rigid and compact surface of cross-linked alginate microbeads with low amount of water uptake and it leading to slow removal of Al^{3+} due to ion exchange with potassium ions present in the 6.8 phosphate buffer medium. As a result, the swelling of the soft cross-linked beads is delayed and it leading to slow disintegration of microbeads.

The percent of drug release for FSF8 batch to reach T_{90} is 10.97 h, towards FSF17 batch 10.32 h and for FSF18 batch it was took 10 h. Hence, on the basis of above long discussion regarding drug release data, only FSF8 batch will provide SR up to 12 h and it leads to reduce the frequency of drug administration, so to minimize the incidence of side effects, thereby to improve patience compliance.

There is change observed on surface of the microbeads after dissolution experiment showed presence of microns size of pores which gives the indication that the fluvastatin drug was released occurs through these pores slowly by swelling and the mechanism of drug release was completely swelling and diffusion-controlled based. The diffusion

Table 6: Drug content, encapsulation efficiency, and dissolution kinetics of fluvastatin sodium alginate microbeads stored at 25±2°C/60±5% RH and 40±2°C/75±5% RH

Storage conditions	Time interval	Drugcontent	Encapsulationefficiency	Release rate constant(mg/h) K_o	$t_{50\%}$	$t_{90\%}$
25±2°C/60±5% RH	1st month	24.40	97.60	3.28	6.09	10.97
	2nd month	24.38	97.52	3.28	6.09	10.97
	3rd month	24.35	97.40	3.28	6.09	10.97
40±2°C/75±5% RH	1st month	24.37	97.48	3.28	6.09	10.97
	2nd month	24.34	97.36	3.28	6.09	10.97
	3rd month	24.32	97.28	3.28	6.09	10.97

*Each value taken in triplicate. n=3

Table 7: I*n-vitro* dissolution data of fluvastatin cross-linked sodium microbeads stored at 25±2°C/60±5% RH and 40±2°C/75±5% RH after 3 months stability studies

S.No.	Time (h)	Initial	Percentage of fluvastatin sodium released (X±SD)					
			25±2°C/60±5% RH			40±2°C/75±5% RH		
			1st month	2nd month	3rd month	1st month	2nd month	3rd month
1.	0	0	0	0	0	0	0	0
2.	0.5	2.94±0.15	2.84±0.09	2.76±0.13	2.67±0.06	2.72±0.13	2.64±0.08	2.55±0.05
3.	1	7.02±0.08	6.93±0.14	6.86±0.06	6.75±0.13	6.81±0.11	6.73±0.09	6.66±0.08
4.	1.5	11.68±0.15	11.57±0.10	11.48±0.08	11.40±0.09	11.45±0.14	11.39±0.11	11.13±0.05
5.	2	15.81±0.11	15.72±0.11	15.64±0.05	15.64±0.05	15.57±0.06	15.43±0.10	15.36±0.11
6.	2.5	17.25±0.06	17.16±0.15	17.07±0.09	16.98±0.09	17.06±0.05	16.97±0.11	16.85±0.10
7.	3	21.42±0.11	21.33±0.13	21.26±0.11	21.14±0.15	21.19±0.13	21.12±0.06	21.04±0.13
8.	3.5	25.61±0.09	25.52±0.09	25.43±0.13	25.33±0.09	25.39±0.09	25.32±0.08	25.25±0.11
9.	4	29.82±0.11	29.73±0.06	29.64±0.15	29.54±0.06	29.59±0.15	29.53±0.13	29.46±0.09
10.	4.5	34.05±0.08	33.96±0.08	33.87±0.06	33.77±0.08	33.82±0.13	33.74±0.09	33.65±0.08
11.	5	38.31±0.06	38.22±0.10	38.13±0.09	38.02±0.13	38.09±0.10	38.02±0.15	37.94±0.06
12.	5.5	42.58±0.11	42.49±0.08	42.40±0.05	42.30±0.06	42.37±0.05	42.31±0.10	42.27±0.09
13.	6	46.88±0.06	46.80±0.11	40.71±0.06	46.60±0.11	46.66±0.11	46.59±0.06	46.43±0.11
14.	6.5	51.21±0.15	51.12±0.14	51.03±0.05	50.92±0.10	50.98±0.13	50.91±0.08	50.83±0.10
15.	7	55.55±0.09	55.47±0.09	55.39±0.11	55.26±0.15	55.32±0.05	55.26±0.06	54.97±0.13
16.	7.5	59.92±0.15	59.84±0.13	59.76±0.08	59.63±0.09	59.69±0.15	59.61±0.13	59.54±0.15
17.	8	64.31±0.08	64.22±0.11	64.13±0.10	64.02±0.06	64.09±0.09	64.03±0.11	63.94±0.10
18.	8.5	68.73±0.11	68.64±0.14	68.54±0.13	68.45±0.11	68.51±0.06	68.44±0.08	68.37±0.11
19.	9	73.16±0.06	73.08±0.12	72.99±0.09	72.90±0.09	72.99±0.08	72.92±0.09	72.57±0.13
20.	9.5	77.62±0.15	77.54±0.15	77.43±0.06	77.32±0.06	77.37±0.09	77.31±0.11	77.43±0.15
21.	10	82.1±0.09	82.07±0.13	81.91±0.08	81.80±0.08	81.86±0.05	81.80±0.09	81.73±0.09
22.	10.5	86.61±0.11	86.52±0.10	86.44±0.15	86.31±0.13	86.38±0.06	86.32±0.10	86.26±0.10
23.	11	91.13±0.08	91.07±0.11	90.95±0.10	90.83±0.10	90.89±0.10	90.83±0.13	90.76±0.13
24.	11.5	95.41±0.09	95.33±0.13	95.25±0.09	95.16±0.14	95.21±0.15	95.16±0.09	95.08±0.15
25.	12	99.98±0.12	99.90±0.10	99.82±0.11	99.72±0.13	99.79±0.09	99.73±0.06	99.66±0.08

*Each value taken in triplicaten=3. Mean±SD

exponent "n" for optimized batch is 1.182 that indicates drug release follows super Case II transport mechanism controlled by both swelling and relaxation of the polymer chains. The drug release of optimized batch FSF8 were compared to pure drug and marketed tablet of same strength of drug to elucidate the difference and progress in achievement of controlled drug release of optimized batch. The drug release profile of pure drug, optimized batch, and marketed product (Lescol ER) is shown in Fig. 14. All readings were taken in triplicate, i.e., n=3.

Stability studies

The optimized formulation FSF8 was subjected to stability studies at both room temperature i.e., 28°C and 45°C/75% RH and evaluated for their drug content, DEE, release rate constant and *in vitro* drug release. Only small negligible changes were observed in the different physicochemical parameters at different temperatures, i.e., 28°C and 45°C/75% RH which studied for 3 months under standard conditions and as per International Council for Harmonisation (ICH) guidelines (Tables 6 and 7). The details of *in vitro* data after stability studies done for 3 months at different temperature and humidity conditions are shown in Table 7, and details of drug content, entrapment efficiency, release rate constant, $t_{50\%}$ and $t_{90\%}$ with respect to monthly wise up to 3 months are shown in Table 6.

CONCLUSION

Among all the formulations, fluvastatin loaded mucoadhesive microbeads prepared with sodium alginate and HPMC K4M (FSF8 batch) in equal proportion of 1.5:1.5 shows CR up to 12 h. Effect of various factors such as concentration of polymer, combination of sodium alginate with other polymers, type of cross-linking agent effect, and concentration of polymer blend was studied. Using emulsification-internal gelation technique, it is possible to obtain a maximum drug entrapment efficiency of 97.84±0.07%. It was clear that as the quantity of the polymer is increases, the rate of drug release is reduced and microbeads prepared with $AlCl_3$ as a curing agent only provides CR of drug. The formed microbeads were spherical in shape and fluvastatin remained distributed in the polymer matrix at amorphous state which was proved by X-ray diffraction studies, it proves clearly that the rate of bioavailability of drug was enhanced. The prepared microspheres exhibited good mucoadhesive properties as observed in *in vitro* wash-off test when compared to a non-mucoadhesive polymer, EVA microbeads. The drug release mechanism was Case II transport kinetic model type which indicates drug release occurs by both swelling and relaxation of microbeads. There was no significant change in drug entrapment and drug release profile when stored at different storage atmospheric conditions as it is proved by conducting stability studies as per the ICH guidelines.

AUTHORS' CONTRIBUTIONS

The complete guidance of work, design of experiment, knowledge support, and help for the research work, experimental work, optimization and interpretation of analytical results was done by Mr. VenkataRamana Reddy K. Nagabhushanam MV and Sai Kishore V.

CONFLICTS OF INTEREST

Declared none.

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