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Original Article

DEVELOPMENT AND CHARACTERIZATION OF GASTRO RETENTIVE MUCOADHESIVE MICROBEADS CONTAINING SIMVASTATIN WITH DIFFERENT CROSS LINKING AGENTS

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ABSTRACT

Objective: The aim of the present work was to prepare and examine drug release of the oral controlled release microbeads using different curing agents by emulsification internal ionic gelation technique.

Methods: Cross-linked alginate microbeads were prepared with different cross linking agents by using mucoadhesive properties. The formation and compatibility of microbeads were confirmed by compatibility studies. Prepared microbeads evaluated for encapsulated efficiency, micromeritic properties, drug loading, *in vitro* wash off studies, *in vitro* dissolution studies, drug release kinetics and stability studies

Results: The *in vitro* drug release was influenced by both type of curing agents and type of polymers and no significant changes in characterization parameters was observed after 3 mo stability studies. The sustained release profile of optimized batch was found to be 99.66±0.18% in comparison to pure drug profile of 28.64±0.02% at 12 h release study. Results of both wash-off and *in vitro* studies suggests that batch (SF2) prepared with aluminium chloride has shown better mucoadhesive property. Drug release of optimized batch follows zero order with non fickian mechanism according to Korsmeyer-Peppas equation.

Conclusion: The data suggest the use of simvastatin mucoadhesive cross linked microbeads to offer the potential for oral controlled drug delivery with improved gastric retention and capable to provide sustained drug release by using cross linking agents.

Keywords: Simvastatin, Emulsification internal ionic gelation, Gatro-retentive drug delivery system, Mucoadhesive microbeads, *In vitro* study

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INTRODUCTION

Gastroretentive systems can remain in the gastric cavity for several hours and prolong the gastric residence time of drugs, maximum utilization of drug. The main aim of Gastro-retentive drug delivery systems (GRDDS) which prolongs the gastric residence time of the formulations by forming strong intimate contact, also improves solubility for drugs that are less soluble in a high pH environment and prolonged gastric retention improves bioavailability and hence therapeutic efficacy, reduced frequency of drug administration, reduction in dose size, improved patient compliance and/or to achieve a local effect in the stomach [1]. The controlled release systems have made significant progress in terms of clinical efficacy [2]. Of the several drug delivery systems used, multi-particulate drug delivery systems gained significant importance [3]. Simvastatin is used in treatment of hyperlipidemic patients and available in crystalline state with hygroscopic nature and belongs to BCS class II drug with a half-life of less than 3 h and bioavailability of less than 5% as its limited by absorption rate factor [4]. It often shows dissolution rate-limited oral absorption. Controlled release formulation (CR) describes sustained action along with its reproducibility and predictability of release of active drug ingredients from the drug delivery system [5]. Therefore, progressive results in both solubility and dissolution rate may lead to enhancement in bioavailability of drug. Oral controlled release particulate systems like microbeads, pellets, microspheres and micro particles etc. are becoming more effective than the single unit dosage forms, as these systems tend to distribute more uniformly over the entire region of gastro intestinal tract and high local drug concentration as well as risk of toxicity can be avoided. Multi-particulate systems also avoid the vagaries of gastric emptying and different transit rates; thereby release the drugs more uniformly [6].

Sodium alginate has been commonly used in the designing of GRDDS. Sodium alginate can be used as a major polymer because of its unique characters as biodegradable, bio-compatibility, non-toxic and in addition it has gained mucoadhesive properties also [7]. Hydroxypropyl methylcellulose (HPMC K4M) is a nonionizable water soluble polymer contains large number of hydroxyl groups which favors adhesion [8] and with its high molecular weight results in more viscous gel [9] and to provide drug release in slow manner. These polymers form a viscous gel layer once when they reach the contents of gastric fluids. Alginate easily forms cross-linking with polyvalent cations which result in the formation of stable gel-like matrices. In combination of above polymers it is possible to attain mucoadhesive drug delivery system [10]. These hydrophilic polymers were used in this work were to produce adhesion during hydration [11]. The Mucoadhesive dosage forms may facilitate to provide prolonged retention time at the application site, drug release occurs in a controlled rate for enhanced improvement of therapeutic activity [12]. Since this combination of hydrophilic polymers having characteristics in swelling/erosion concept [13], which can produce drug release in controlled release pattern.

MATERIALS AND METHODS

Materials

The pure drug simvastatin was obtained as a gift sample from Aurobindo pharma limited, Hyderabad, Telangana state, India. Hydroxypropylmethylcellulose K4M, Sodium alginate was purchased from Himedia, Mumbai, Sodium carboxymethyl cellulose 1100-1900cps, Carbopol 934, calcium chloride ($CaCl_2.2H_2O$), Barium chloride ($BaCl_2.2H_2O$), Aluminium chloride, N-Hexane, light liquid paraffin, Chloroformr, Hydrochloric acid, Potassium dihydrogen phosphate, Ethyl vinyl acetate (EVA) and Methanol solution were obtained from Qualigens fine chemicals, Mumbai. All chemical agents used were of analytical high grade quality.

Equipments

Digital electronic weighing balance (0.1 mg sensitive, Shimadzu, Mumbai), Sigma blade homogenizer (Remi model, India), Desiccator

(Borosilicate, Mumbai), Sonicator (Remi model, Mumbai,India), Dissolution test apparatus USP (Model TDT-08L, Electro-lab, Mumbai, India), Tablet disintegrating test apparatus (USP model, India), Double beam UV-Visible spectrophotometer, (Elico SL-210), Fourier-transform infrared spectrophotometer (FTIR, Bruker, X-ray diffractometer (Model PW/1710, Poland), Differential scanning calorimetry (Netzsch, Japan) Scanning electron microscope (S-3700 N SEM, Hitachi) and Syringe and Needle 23 G, etc.

Table 1: Formulation of simvastatin microbeads with various curing agents

Batch code	Polymers used	Percentage of polymers (% w/v)	Curing agent (5% w/v)
SF1	Sodium alginate	3	$Alcl^{+3}$
SF2	Sodium alginate+Hydroxy Propyl Methyl Cellulose K4M	1.5:1:5	$Alcl^{+3}$
SF3	Sodium alginate+Carbopol 934P	1.5:1:5	$Alcl^{+3}$
SF4	Sodium alginate+Sodium CMC 1100-1900cps	1.5:1:5	$Alcl^{+3}$
SF5	Sodium alginate+Hydroxy Propyl Methyl Cellulose K4M	1.5:1:5	$Bacl^{+2}$
SF6	Sodium alginate+Hydroxy Propyl Methyl Cellulose K4M	1.5:1:5	$Cacl^{+2}$

*All batches containing core and coat ratio is 1:3.

Methods

Preparation of simvastatin loaded microbeads

The simvastatin loaded cross-linked alginate microbeads were prepared by choosing emulsification internal gelation method. Simvastatin equivalent to 20 mg was dissolved in sufficient amount of methanol on a magnetic stirrer by stirring at 250 rpm at room temperature only. Simvastatin solution was incorporated in a beaker containing mixture of sodium alginate and different types of hydrophilic polymers with different concentrations (listed in table 1) with continuous stirring using homogenizer at a speed of 400 rpm to obtain homogenous viscous solution. This solution is kept inside sonicator for 2 h in order to remove air bubbles completely. Take the above solution in a beaker and add 30 ml of selected cross-linking agent at 5% w/v drop wise in a beaker which is stirred undisputedly with sigma blade homogenizer at 600 rpm and keep on mixing for 30 min. The above physical mixture solution take into 10 ml syringe which is fitted with 22 gauze needle and pressed gauze then discharge solution drop wise into 250 ml glass beaker containing mixture of 100 ml light liquid paraffin oil and concentration of span 80 (1.5 % w/v) and glacial acetic acid at 0.2 % w/v. During the addition of syringe solution, it was sure to keep the minimum 10 cm distance between tip of needle and beaker. The above viscous solution was stirred at a speed of 600 rpm by using sigma blade homogenizer continuously for a period of 30 min and this solution in later filtered through filter paper, it will form high viscous product with insoluble residue and in order to remove stickiness of viscous product, it was washed continuously four to six times with concentrated n-Hexane solution until sticky fatty material has entirely removed and place wet microbeads in desiccator overnight until it becomes completely dried and collect dried cross linked microbeads.

Characterization of simvastatin microbeads

Determination of maximum wavelength for pure simvastatin

Weigh pure simvastatin 10 mg exactly and transferred to 10 ml standard volumetric flask. Add few drops of ethanol until all simvastatin solubilized completely. Withdraw the 1 ml of above solution and made up to final volume 10 ml with distilled water to get final concentration of 10 µg/ml. The absorbance of the final solution was scanned in the UV wave length of 400-200 nm against distilled water as a blank solution

Micromeritic properties of the microbeads

The prepared cross linked microbeads were characterized for angle of repose, tapped density, bulk density, carrs index and Hausner's ratio [14].

Percentage of yield

Percent yield defined as the percent ratio of practical yield (obtained dried microbeads) to the theoretical yield (weight of used polymers and drug weight). Mathematically it is computed from following equation.

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

Size Distribution and size analysis

The size of microbeads will be determined by sieving method. The amount of uniformed microbeads which were retained on different individual sieves were taken out and weighed accurately. Finally the mean particle size of the microbeads was determined by using following equation.

$$\textit{Mean Particle Size} = \frac{\sum(\text{Mean Particle Size of Fraction} \times \text{Weight Fraction})}{\sum(\text{Weight Fraction})}$$

Entrapment efficiency of microbeads

Entrapment efficiency was resolved quantitatively by using the following formula. For calculation of drug content, 20 mg of beads were placed in a cleaned dry mortar and crushed to fine powder. Limited amount of methanol was added to the grinded powder to dissolve simvastatin completely and later sonicated for 30 min in order to get clear solution. The above solution was filtered through a membrane filter (0.45 µ). The clear filtered solution was diluted with little amount of methanol and subsequently with distilled water and drug concentration was measured using a UV-Visible spectrophotometer at 233 nm.

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

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

Drug content

Few microbeads samples were selected randomly and powdered to fine state by grinding in mortar. Weigh the above powder which is equivalent to 100 mg was transferred into 100 ml standard volumetric flask containing few drops of ethanol and shake it vigorously until completely solubilized and made to the final volume by distilled water only. The flask was placed in a sonicator till powder is completely solubilized. The above solution was filtered by using filter paper (0.45 µm pore size) and from this 1 ml was taken and transferred to 100 ml volumetric flask which was made up to the mark by distilled water. The absorbance of the solution was measured using double beam UV/Vis spectrophotometer against the distilled water as a blank at 233 nm for estimation of simvastatin.

Fourier-transform infrared (FTIR) spectroscopy analysis

The IR spectra of pure simvastatin and physical mixture containing simvastatin and excipients were obtained using FTIR spectrophotometer. The above samples weighed approximately 1 to 2 mg were blended with potassium bromide and compressed highly with maximum pressure to form disks and these disks were scanned from 4000 to 400 cm^{-1}.

Differential scanning calorimetry (DSC) studies

DSC evaluation was performed to find out the physical state of the simvastatin in microbeads using a DSC instrument and it helpful to study their thermal behavior of samples. For analysis, nearly 4 mg of sample was taken within a sealed aluminium crucible and it placed

into a temperature controlled DSC cell and the temperature range was measured from 30 to 400 ° C and samples were scanned at speed of 20 °/min.

X-Ray diffraction studies (XRD studies)

X-ray diffraction patterns were recorded using Cu Kα radiation, usually operated at 40 kV voltage and 40 mA current. The microbeads samples were recorded 0.025° step resolution, in the 2θ ranges from 10° to 60° for the simvastatin, blank and loaded microbeads.

Scanning electron microscopic (SEM) studies

The purpose of this study is to confirm the morphology characteristics i.e. shape, size and surface (texture) of dosage form. Tests were sputter-covered with gold under a vacuum before perception with a scanning electron microscope at an increasing speed voltage of 10 kV.

***In vitro* wash off test**

The mucoadhesive property of prepared microbeads will be determined by conducting *in vitro* wash off studies. The mucoadhesiveness of prepared microbeads will be compared with ethyl vinyl acetate microbeads (Non mucoadhesive material). A fresh piece of intestinal mucosa approximately 2x2 cm was obtained from goat and fix onto a glass slides (3x1 inch) with cyanoacrylate glue. These two glass slides were connected with a suitable support which is connected to a shaft which is facilitated with up and down movement with electric motor (Modification of disintegration test apparatus, USP). About 100 microbeads were spread uniformly onto each wet rinsed mucosa specimen and without delay the support was hung on to a arm of tablet disintegration test apparatus. When the machine is plugged on, slide was given series of up and downward movement continuously in a beaker which contains 900 ml test fluids. The whole assembly was maintained a temperature of 37 °C though out the procedure. The test was performed first in 0.1N HCl for 2 h and later in 6.8 phosphate buffer solution. At the end of 1,2,4,6, 8 and 10 h the machines were stopped for a while and count the number of microbeads still adhering/sticking on to mucosa was counted and run the machine until end of the last period.

Dissolution test

In vitro drug release of the samples was carried out in accordance to the USP dissolution method (Type II dissolution apparatus, basket type, Electrolab TDT-08L). The dissolution mediums (900 ml of 0.1N HCl for first 2 h and 900 ml of 6.8 phosphate buffer from 3rd h onwards to 12 h) was placed alternatively in the dissolution basket and maintain a temperature of 37±0.5 ° C and agitation was started at 100 rpm. To the above buffer medium add 1 % Tween 20. One milliliter of sample was withdrawn periodically at every 1 h intervals of time up to 12 h. After every withdrawal of sample, the dissolution medium was replaced with the same quantity of fresh buffer each time throughout the procedure to maintain sink conditions. Withdrawn samples were filtered through filter paper (0.45 µm pore size) and after series of appropriate dilutions the samples were analyzed for the concentration of simvastatin using double beam UV/Vis spectrometer at 233 nm [15]. The cumulative percentage drug release was calculated. Each absorbance reading was taken in triplicate manner (n=3).

Release kinetics

In vitro drug release data was subjected to different kinetic models like zero order, first order, Higuchi matrix, Korsmeyer-Peppas in order to investigate the release pattern of all formulated batches. For each kinetic model, correlation coefficient (R^2) was calculated. To characterize the drug release rate (kinetics) in different experimental conditions, both $T_{50\%}$ and $T_{80\%}$ were calculated from dissolution data according to the following equations.

$$T_{50\%} = (0.5/k)^{1/n}$$

$$T_{80\%} = (0.8/k)^{1/n}$$

Stability studies

Accelerated stabilities studies for the optimized batch were stored in stability chamber under different conditions of 25°±2°/60±5 % RH and 40±20 °C/75±5 % RH. The samples were examined for drug content after the time intervals of 90 d. The drug content, entrapment efficiency, in the samples was analyzed spectrophotometrically.

RESULTS AND DISCUSSION

Determination of λmax for pure simvastatin

After scanning of simvastatin solution containing concentration of 10 µg/ml using double beam spectrophotometer in the range of 200 to 400, the maximum absorbance was taken place at 233 nm. Each reading taken in triplicate manner i.e. n=3.

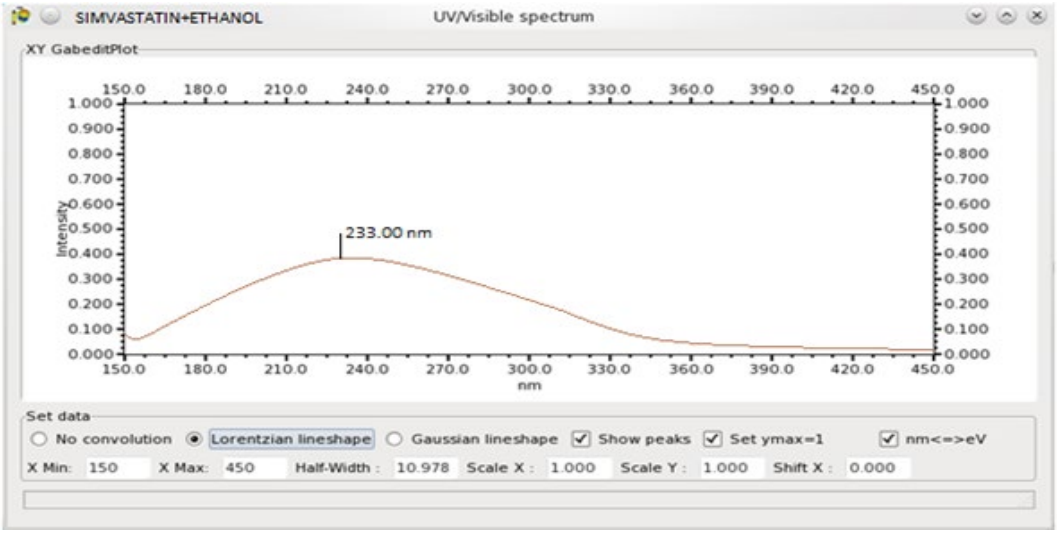

Fig. 1: UV Spectrum of pure simvastatin

The λ max of pure simvastatin was found to be 233 nm by scanning the diluted sample whose concentration ranging from 2-20 µg/ml by using double beam UV-Visible spectrophotometer. The obtained UV λmax spectrum was shown in the fig. 1.

Micromeritic properties of microbeads

The rheological parameters like angle of repose, tapped density, bulk density and packing properties (table 2) confirms that microbeads having fair flow properties. All the batches showed

angle of repose value within the accepted range of 21.16° to 23.66° which is the considerable range for microbeads to show good flow property while formulating in the desired dosage form. Each reading was taken in triplicate manner.

Table 2: Flow properties of various batches containing simvastatin microbeads

Batch code	Angle of repose (θ)	Bulk density (g/cm³)	Tapped density (g/cm³)	Carr's index (%)	Hausner's ratio
SF1	21.92±0.93	0.704±0.77	0.824±0.15	14.56±0.04	1.17±0.18
SF2	21.16±0.01	0.941±0.02	1.072±0.02	12.82±0.02	1.14±0.05
SF3	22.17±0.04	0.812±0.03	0.926±0.05	12.81±0.04	1.14±0.02
SF4	22.89±0.05	0.897±0.03	1.058±0.07	13.13±0.06	1.16±0.03
SF5	22.45±0.03	0.799±0.05	0.958±0.04	14.36±0.05	1.20±0.02
SF6	23.66±0.05	0.928±0.08	1.076±0.06	12.71±0.04	1.16±0.03

*All values are expressed as mean±SD (n=3).

The microbeads prepared with aluminium chloride as a curing agent will form small particle size in comparison to microbeads prepared with divalency curing agents naming calcium chloride and barium chloride. The main reason for above statement is change in development of cross linking network inside lattice of alginate beads during cross linking time. The order of magnitude of cross linking network is more in aluminium chloride (Trivalency ions) than remaining curing agents (Divalency ions). The presence of extra charge in Al^{+3} owns large cross linking network during curing time. The small shrinkage of microbeads occurs only when using $Alcl^{+3}$ as a curing agent.

Entrapment efficiency and drug loading

The entrap efficiency of simvastatin was found to be directly proportional to the concentration of the sodium alginate, low concentration of physical mixture will give lower viscosities which leads to lower encapsulation of drug. Of all three curing agents, $AlCl^{+3}$ which is used as curing agent from batch SF1 to SF4 will only show maximum entrapment efficiency o because tight compact of microbeads that has been formulated with Al^{+3} and not get leakage from surface of wet microbeads during curing time and process of homogenization, in contrast microbeads prepared with $BaCl^{+2}$ and $Cacl^{+2}$ (SF5 and SF6 batches) as curing agents soft walled cross linked microbeads will formed and it shows less entrapment efficiency. The reason for less entrapment efficiencies is due to easy leakage of drug from the soft surface of microbeads during curing time and homogenization. The entrapment efficiency of the various formulations was found in between 90.28±0.03 to 97.16±0.02 % and details were shown in table 3. Of all batches, SF1 batch shows less percentage of efficiency as it contains only single polymer, in contrast to this, more entrapment was observed when microbeads prepared with blend of polymers (SF2 to SF6 batches). The presence of more functional groups present in combination of polymers guarantees the more entrapment of drug during the cross processing linking time.

The less entrapment was observed in SF5 and SF6 batch relatively to microbeads prepared with trivalency curing agent (SF2 to SF4 batches). The difference in percent of entrapment efficiency occurs due to leakage of drug during curing time and homogenization period from the surface of wet microbeads and this is particularly observed when microbeads designed with divalency cross linking agent.

Table 3: Evaluation of simvastatin microbeads

Batch code	Yield* (%)	Average particle size (µm)	Drug content	Entrapment Efficiency* (%)
SF1	98.65	173.42±0.06	24.29±0.03	85.16±0.02
SF2	97.45	167.25±0.05	23.44±0.05	93.68±0.03
SF3	98.36	181.33±0.06	23.68±0.03	94.72±0.04
SF4	97.16	180.41±0.03	24.13±0.04	96.52±0.03
SF5	96.36	181.32±0.04	23.41±0.03	91.64±0.02
SF6	96.23	176.37±0.02	23.32±0.03	90.28±0.03

*All values are expressed as mean±SD (n=3).

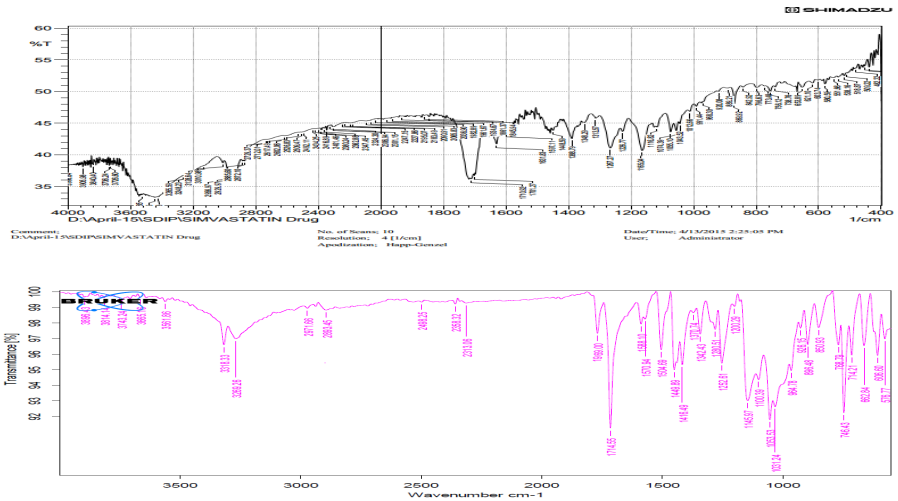

Fig. 2: FTIR image showing pure simvastatin (Above spectra) and simvastatin loaded microbeads (Below spectra)

Infrared spectroscopy

Fig. 2 shows IR spectrum of Pure simvastatin drug and drug with all excipients which used in formulation having characteristic absorption band in the following regions. 3392.78 cm^{-1}(alcoholic O-H stretching vibration), 2933.68 cm^{-1}(methyl and methylene C-H asymmetric and symmetric vibration, C=O stretching 1961.67 cm^{-1}, 1701.27 cm^{-1} (lactone C=O and ester C=O stretching), 1267.27 cm^{-1} (methyl and methylene C-H bending vibration) and 1065.10 cm^{-1}(ester C-O-C bending vibration) respectively.

FTIR spectrum of co-grinding mixture showed peak values at 3373.81, 2923.48, 1923.45, 1695.06, 1264.23 and 1012.00 cm^{-1} which showed that the intensities of these peaks were reduced in the co-grinding mixture and slight shift in the peak values indicates, the excipients and simvastatin were mixed without any chemical interactions. The reduced in intensities and number of peaks gives clear indication of reducing the crystalline nature and making the drug amorphous which helpful in better absorption. The obtained FTIR spectras was shown in fig. 2.

DSC studies

DSC thermogram of pure drug simvastatin showed sharp endotherm peak at 136.47 ° C corresponds to its melting point. There was no appreciable change in the melting endotherms 136.52 ° C for optimized formulation compared to that of pure simvastatin. This thermographic results show that the drugs retain its identity in the optimized formulation. This observation also confirmed the absence of chemical interaction of drug with additives during process, further it also supports the results of IR spectroscopy. The obtained DSC spectras was shown in fig. 3.

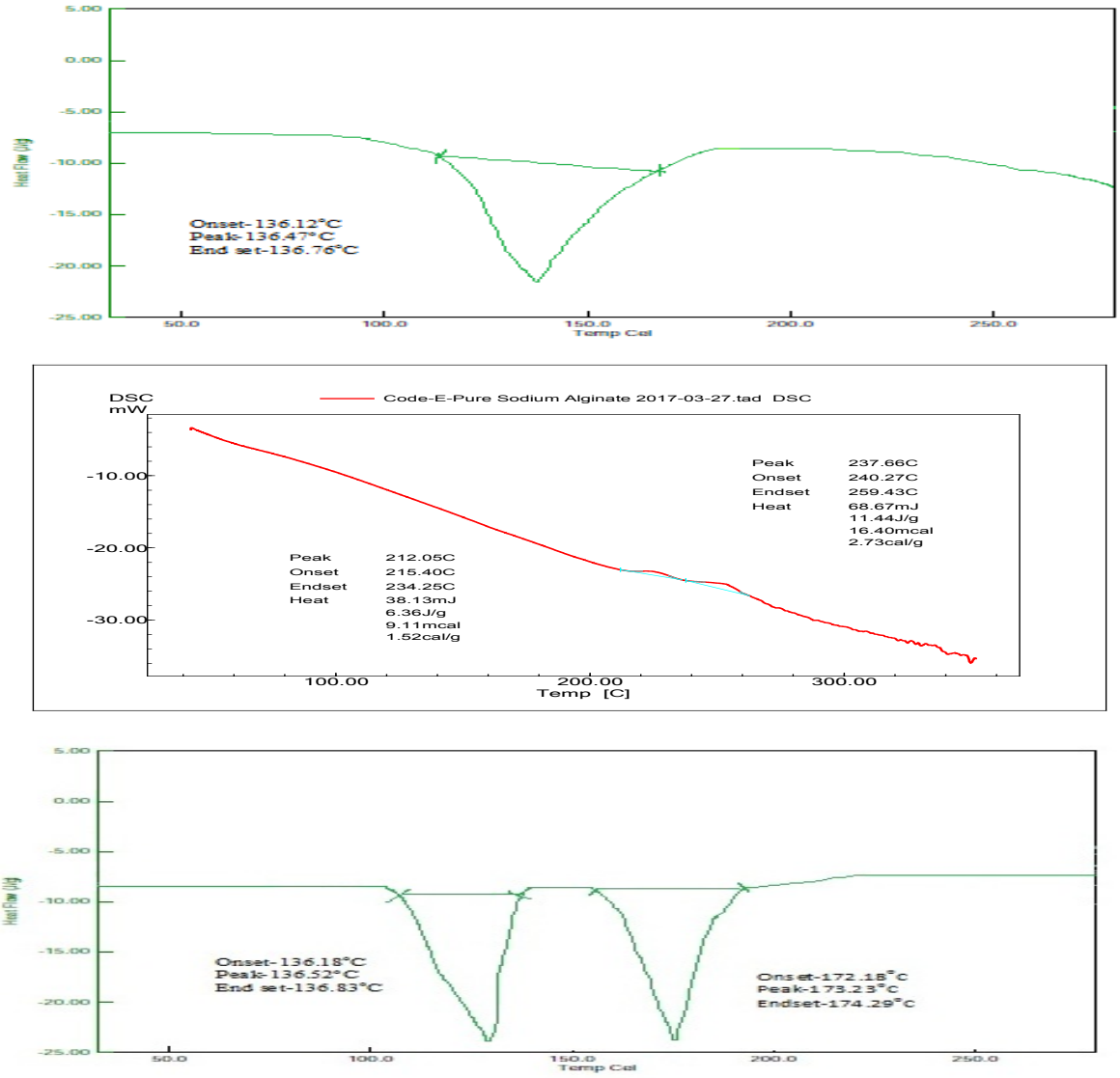

Fig. 3: DSC spectra of pure simvastatin (Above spectra), pure sodium alginate (Middle spectra) optimized batch (bottom spectra)

Powder X-ray diffraction (PXRD) studies

The diffraction pattern of the pure drug simvastatin shows a highly crystalline nature, indicated by numerous distinctive peaks at a diffraction angle of 2θ (28.4°, 24.6° and 15.2° throughout the scanning range. The results of the PXRD pattern of pure drug and optimized formulation are shown in fig 4. Pure drug and the optimized formulation showed the almost same peaks but with reduced intensity in height of peaks and in much diffused pattern were observed in optimized batch (SF2) which gives clear indication of conversion of crystalline state to the amorphous nature of drug. This decrease in crystallinity of drug present in surface of alginate beads which resulted in an increased dissolution rate of simvastatin and also it confirms the absence of chemical interaction of drug with the optimized formulation and well dispersion of drug in polymeric matrix beads.

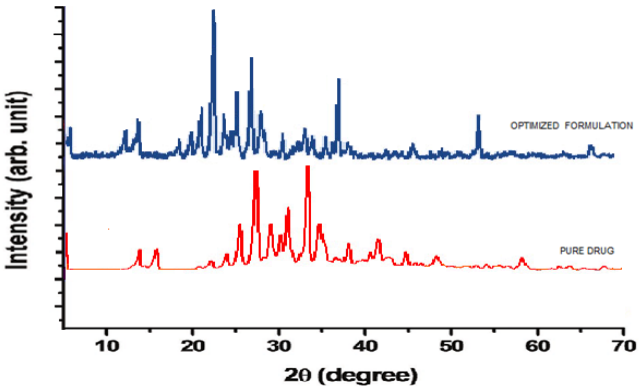

Fig. 4: XRD spectra of pure simvastatin and optimized formulation of simvastatin

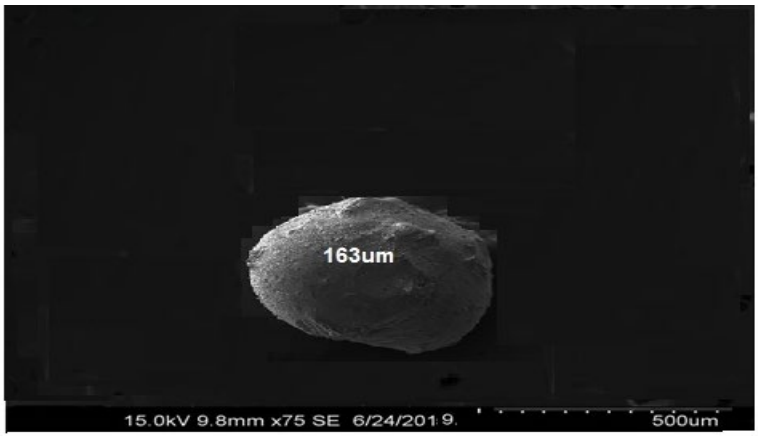

Fig. 5: SEM photograph of microbead

Table 4: *In vitro* wash-off test values for the simvastatin microbeads prepared with combination of sodium alginate and HPMC K4M in 1:3 ratios by employing various cross linking agents at 5 %w/v

Batch	Percent of alginate beads adhering to tissue at 6 times (h)											
	0.1 N HCl, pH 1.2*						Phosphate Buffer, pH 6.8*					
	1	2	4	6	8	10	1	2	4	6	8	10
SF2	65±1.3	53±1.5	44±1.3	36±1.6	27±1.7	26±1.4	92±0.4	85±1.2	76±1.4	63±1.3	54±1.4	20±1.4
SF5	62±1.0	51±1.5	39±1.3	30±1.4	19±1.2	18±1.9	89±0.8	83±1.2	73±1.4	61±1.6	50±1.3	16±1.3
SF6	60±1.4	49±1.2	37±1.5	22±1.6	14±1.4	13±1.6	86±0.4	78±1.2	69±1.4	57±1.3	44±1.8	14±1.8
EVA microbeads	50±1.3	38±1.2	08±1.6	-	-	-	47±2.1	36±1.9	05±2.0	-	-	-

*All values are expressed as mean±SD (n=3).

Scanning electron microscopic study

SEM study revealed that the cross linked microbeads were nearly spherical in shape and arranged in discrete manner and shape of microbead was shown in fig. 5.

***In vitro* wash-off studies**

The wash off was faster at simulated intestinal pH (phosphate buffer 6.8) than comparison to that at simulated gastric pH (1.2) [16]. The rapid wash-off observed intestinal pH than at gastric pH, the reason may be contributed is due to the ionization of carboxyl acid groups [17] and other functional groups present along the polymers chain at alkaline atmosphere, which increases the solubility and reduce the mucoadhesive strength. The results of wash off test (table 4) suggest that mucoadhesive microbeads had fairly mucoadhesive properties.

***In vitro* dissolution studies**

Hydrophilic nature of HPMC K4M facilitates penetration of dissolution medium into the network structure of alginate polymer chain thereby leads to hydration of the polymer blend hence swelling of microbeads takes place during dissolution process. The drug release profile of different is shown in fig. 5. The formulated microbeads showed increase drug release profile in 6.8 phosphate buffer medium than 0.1N HCl medium comparatively. The main reason behind the increase in drug release is based on two principles, primary one is relaxation of polymers due to difference in osmotic pressure/increase stress of beads thereby it renders to increase in surface area of wet microbeads which leads to create micron size pores on surface of enlarged beads (swelled beads) and second principle is that disintegration of cross-linked alginates microbeads occurs through ion exchange between bound $Ca^{+2}/Ba^{+2}/Al^{+3}$ ions and sodium ions present in phosphate buffer medium. Formation of gelatinous layer during dissolution process acts as a boundary layer for drug that has to be released. At one stage after complete hydration of microbeads as the polymer chains becomes beyond levels of hydration, erosion takes place [18]. The increase or decrease of drug release depends upon nature of dissolution medium and the type of cross linking agent used. In this work two divalency and one trivalency curing agents were used for promoting cross-linking network (between carboxylate residues of

G and M-blocks of sodium alginate and curing agent) during curing time. Sodium alginate could form gel in the presence of different multivalent cations such as Al^{+3}, Ca^{+2} and Ba^{+2} by ionic cross-linking [19] to form microspheres/microbeads. Of entire study, decreased swelling rate was observed when microbeads prepared with aluminium chloride than calcium chloride and barium chloride as a curing/hardening agent. The intensity of cross-linking network was very compact with aluminium chloride in comparison to remaining two divalency curing agents. The extent of drug release depends upon swelling of microbeads in different dissolution mediums. Because of one extra valency electron present in aluminium chloride (Al^{+3}) as it favors extreme cross-linking network than with divalency curing agents (Ba^{+2} and Ca^{+2}) during gelation process. Therefore microbeads prepared with aluminium chloride as a curing agent showed better retarded effect when compared to other two curing agents (barium chloride and calcium chloride) over a period of 12 h

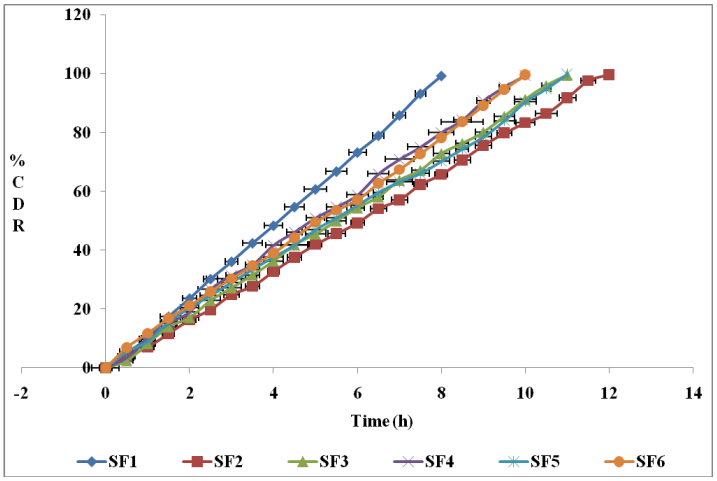

Fig. 5: Comparative dissolution profile of batches from SF1 to SF6. All values are expressed as mean±SD (n=3)

A previous study indicated that alginate beads gelled in Ba^{+2} solutions were larger than those gelled in Ca^{+2} solution [20, 21]. The microbeads cross linked with Ca^{+2} (SF6 batch) shrank fastly than remaining curing agents [22]. Based upon pattern of drug release it was clearly understood that drug release is pH dependent. The relaxation of polymer chains takes place in alkaline medium. The drug release mechanism from the microbeads was supercase 2ndtransport [23] as n value is more than unity. The optimized batch SF2 was shown its sustained release up to 12 h by releasing the 99.66% of drug in comparison to pure simvastatin sample which released 28.64% in same time under standard specific conditions.

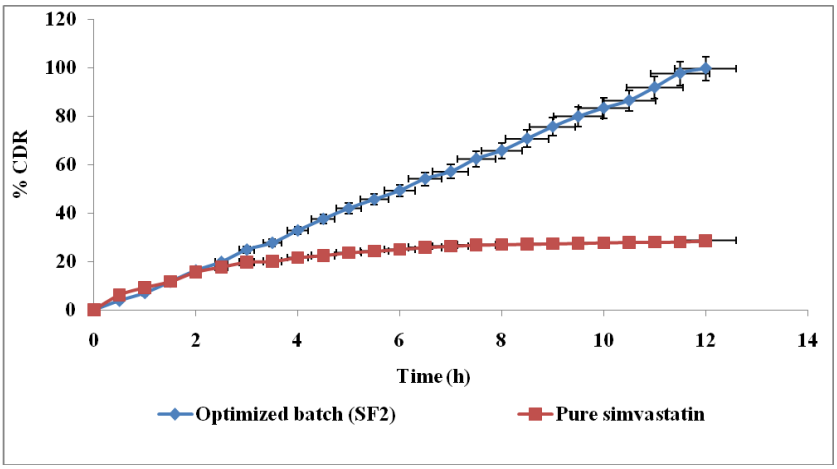

Fig. 6: Comparative dissolution profile of optimized batch and pure simvastatin. All values are expressed as mean±SD (n=3)

The release kinetics both $T_{50\%}$ and $T_{80\%}$ were calculated from dissolution data and is shown in table 5. The delaying of drug release was facilitated by using blend of polymers (SF2 to SF6 batches) and type of cross linking agents. The hindrance in drug release is possible when microbeads undergoing maximum cross linking network, thereby microbeads will develop densed walls and it was proved by using trivalency curing agent only. The controlled release phase may be attributed to the swelling or erosion of the polymer chains, which leads to the creation of small pores within the polymeric matrix [24].

Stability studies

The developed Simvastatin microbeads were observed for drug content, Percent encapsulation efficiency, Drug loading and dissolution kinetics after keeping the product in specified standard conditions of stability studies (table 6). Furthermore all characterization parameters of optimized batch remained unchanged at different storage conditions during the entire period of stability studies. It was shown that no change was observed with test results at the end of three months indicating that simvastatin remained chemically stable in cross-linked microbeads.

Table 5: *In vitro* dissolution kinetics parameters Simvastatin microbeads prepared with sodium alginate and polymer blend in equal ratios (1.5:1.5) by employing with various cross linking agents

Batch code	Correlation coefficient* (R^2)				Release kinetics*			Diffusion exponent value (n)*
	Zero order	First order	Higuchi	Peppas	K_o (mg/h)	T_{50} (h)	T_{90} (h)	
SF1	0.9997	0.8446	0.9185	0.9994	4.9	4.08	7.34	1.0673
SF2	0.9997	0.7791	0.9176	0.9986	3.34	5.99	10.77	1.0797
SF3	0.9997	0.7916	0.9214	0.9942	3.6	5.55	10.00	1.1035
SF4	0.9996	0.8006	0.9260	0.9983	3.97	5.03	9.06	1.0269
SF5	0.9986	0.7482	0.9329	0.9990	3.57	5.6	10.08	1.0412
SF6	0.9992	0.7572	0.9261	0.9985	3.92	5.1	9.18	1.0219

*All values are expressed as mean±SD (n=3).

Table 6: Drug content, encapsulation efficiency and dissolution kinetics of optimized simvastatin microbeads (SF2) stored at 25±2 °C/60±5% RH and 40±2 °C/75±5% RH

Storage conditions	Time interval	Drug content*	Encapsulation efficiency*	Release rate constant (mg/h) K_o	$T_{50\%}$	$T_{90\%}$
25±2 °C/	1st month	23.44±0.05	93.68±0.03	3.34	5.99	10.77
60±5% RH	2nd month	23.44±0.05	93.68±0.03	3.34	5.99	10.77
	3rd month	23.44±0.05	93.68±0.03	3.34	5.99	10.77
40±2 °C/	1st month	23.44±0.05	93.68±0.03	3.34	5.99	10.77
75±5% RH	2nd month	23.44±0.05	93.68±0.03	3.34	5.99	10.77
	3rd month	23.44±0.05	93.68±0.03	3.34	5.99	10.77

*All values are expressed as mean±SD (n=3).

CONCLUSION

The drug release of optimized batch (SF2) containing sodium alginate and hydroxyl propyl methyl cellulose K4M that cross-linked with 5% w/v aluminium chloride has sustained drug release up to 12 h. The extent of drug release rate, and mechanisms were found to be governed by the two parameters naming blend of polymers and type of curing agents used. Micro beads obtained were having spherical shape. The drug release mechanism was found to be super case II transport; dependent on both drug diffusion and relaxation of polymer chains. The results of *In vitro* wash-off test has proved optimized batch has better mucoadhesive property. Thus simvastatin microbeads retained for longer time in gastro intestinal region which leads to improve therapeutic effect of drug with better patient compliance.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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