**THE ROLE OF NEEDLE IN FORMULATION OF pH SENSITIVE SWELLABLE MICROBEADS PREPARED WITH HYDROPHILIC POLYMERS FOR ATORVASTATIN AND THEIR CHARACTERIZATION STUDIES**

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**ABSTRACT**

**Objective:** The aim of the study was to develop and characterize mucoadhesive microbeads for oral sustained release of atorvastatin by using hydrophilic polymers and application of different process variables in designing of pH sensitive swellable microbeads.

**Methods:** Microbeads were prepared by ionic gelation method. The compatibility studies of atorvastatin with polymers were investigated by differential scanning calorimeter and Fourier transform infrared spectroscopy studies. In this work process variable like optimization of curing agents and their quantity, effect of rpm, and their influence in drug entrapment were studied. Prepared beads were characterized for particle size, swelling index, erosion studies and drug release studies.

**Results:** Mixture of alginate and carbopol 934 P at 3.3 % w/v shows sustained release and good mucoadhesive capacity. Furthermore, drug loading and swelling increased with the use of a combination of polymers. On basis of in vitro release studies and swelling studies, it was observed that sodium alginate coated with carbopol 934 P showed sustained release of 84.5 % at end of 10 h in 6.8 phosphate buffer. The optimised batch followed peppas and higuchi release mechanism and releasing the drug by non-fickian transport.

**Conclusion:** The alginate beads with a combination of carbopol 934P showed a sustain release pattern. The release rate and swelling of atorvastatin beads could be adjusted by adding other hydrophilic polymers or by optimising curing agents, curing time and their volume. The zero order of drug release was confirmed for all the batches. The in vitro data was better fit to Higuchi’s diffusion model and diffusion rate limited.

**Keywords:** Atorvastatin, Mucoadhesive microbeads, Needle effect and Sustained release

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**INTRODUCTION**

Among the multiple routes of drug delivery [1], oral route is the most suitable, used and most accepted one by the patients for the delivery of the therapeutically active drugs over the past few years, many controlled releases, Controlled release drug delivery systems have been developed using a variety of innovative techniques with wide variety of polymeric matrices.

By adopting controlled drug delivery many of the obstacles, for example, they maximize drug absorption, reduce peak plasma fluctuations and minimize potential side effects etc [2, 3] will be overcome and getting much patient compliance [4] and improve the rate of bioavailability. Ionic gelation technique involves ionic cross-linking between polyelectrolyte biopolymers such as alginates, chitosan, etc. and counterions to produce a cross-linked matrix which would provide the desired controlled release rate of the drug molecule. From a polymer chemistry viewpoint, the development of interpenetrating network polymers (IPNs) of sod. Alginate is attractive because, by definition, the IPNs contain two polymers, each in a network form, which can be cross-linked in the presence of each other to give a three-dimensional network structure producing free volume for the easy encapsulation of drugs easily. Bio/ mucoadhesive [5] microspheres have few advantages such as marked efficient absorption and improved the rate of bioavailability of drugs owing to high surface to volume ratio, a much more close contact with the mucus layer following oral consumption and specific targeting of drugs to the desired absorption site.

Atorvastatin is a new class of lipid-lowering compound that selectively inhibit the intestinal absorption of cholesterol and decrease cholesterol absorption. After oral administration alone, atorvastatin is rapidly absorbed, reach maximum plasma concentrations within 1-2 h. Atorvastatin belongs to biopharmaceutics classification system class II category, showing low oral bioavailability (12%) [6], due to low aqueous solubility (0.15 mg/ml), high presystemic clearance in the gastrointestinal mucosa (80%), and extensive hepatic first-pass effect, thus their bioavailability depends on dissolution features. Its having halflife is 11-19 h in humans [7-9]. It is insoluble in aqueous solutions at pH 4 and below. It is insoluble in an aqueous solution of pH 4 and below, it is very slightly soluble in water and 7.4 pH phosphate buffer. The intestinal permeability of atorvastatin is high at the physiologically relevant intestinal pH [10]. However, it is reported that the absolute bioavailability of atorvastatin is 1% after a 40 mg oral dose [11]. In view of its long biological half-life and less bioavailability of atorvastatin and the associated side effects, we have chosen atorvastatin for developing the controlled release beads with sodium alginate as a coating polymer.

Polymers belong to the class of polysaccharides are widely used in oral drug delivery systems because of their simplicity to obtain the desired drug delivery system and drug release profile. In our recent paper, we have shown the ability of hydroxypropyl methyl cellulose K15M, carbopol 934P and polyvinyl pyrrolidine (PVP) polymers to form a high-viscosity hydrogels using a very small amount of hydrophilic materials. In our present study, we extend the investigation by using polymers in combination with Na. alginate to form hydrogel complex beads and to check the sustainability of the therapeutic effect of drug obtained from the matrix. Among existence of various natural polymers, alginites have been used as matrices to achieve controlled release drug delivery due to their hydrogel-forming properties. The sodium alginate [12] has been used as a controlled release matrix material in medicine and it can absorb water 200-300 times to their own weight. It consists of linear polymer of β-(1-4) mannuronic acid (M) and (1-4) guluronic acid (G) residues in varying proportions and arrangements. It has been shown that the G and M units are joined together anonymous. These residues are arranged in homopolymeric and heteropolymeric blocks.

Accordingly, the objective of the current study was to optimise the composition of alginate beads with hydrophilic polymers to produce...
stable beads with optimum specifications. The major objective of the current experiment is made with different objectives i.e. the effect of conc. of sodium alginate, the effect of tri and divalent curing agents, the effect of rpm, effect of curing volume and effect of curing time.

In the present study, we have aimed to formulate the stable alginate beads by ion gelation technique with hydrophilic polymers naming carbopol 934P, polyvinyl pyrrolidone (PVP) and hydroxypropyl methyl cellulose K15 (HPMC K15). These polymers undergo ionic interactions with di and trivalent compounds, for this reason, we have used both metal ions as cad +2 and ald +3 as curing agents for the cross linking of alginate material and formed beads were evaluated the characterization of beads.

MATERIALS AND METHODS

Atorvastatin pure drug sample obtained from Aizant, Hyderabad, India, sodium alginate 45 cps (1% w/v solution in water at 25 ° C), carbopol 934P (SD fine chemicals, Mumbai), hydroxypropyl methyl cellulose K15 M, polyvinyl pyrrolidone, aluminium chloride dihydrate, calcium chloride, potassium dihydrogen phosphate, potassium hydroxide pellets (E. Merck, India), ethanol and sodium lauryl sulphate (SLS) were used. All other used reagents were of analytical grade.

Equipment

Weighing balance, sonicator (Remi model, Mumbai), homogenizer, (Remi, India),USP tablet disintegrating test apparatus, dissolution test apparatus USP, model TDT-08L, electro lab, uv-visible spectrophotometer, scanning electron microscope (SEM; S-4100, Hitachi, Japan), X-Ray diffractometer, Bruker spectrophotometer (Model-220, Germany) and syringe needles 22 G, 24 G etc.

Preparation of cross-linked swellable alginate hydrogel beads

Cross-linked alginate beads were prepared by adopting orifice gelation technique which is simple, easiest and simple method of entire microencapsulation techniques. For micro beads formation, 100 ml of 3% w/v aqueous solution of sodium alginate which contains atorvastatin 100 mg and 0.30 % w/v of different macroadsive hydrophilic polymers (which already dispersed in water and kept aside one night to develop interpenetrated network) naming carbopol 934P, polyvinyl pyrrolidone and hydroxypropyl methyl cellulose K15M. After uniform dispersion of above mucilage, it was discharged dropwise from syringe needle size-22 needle into 100 ml of an aqueous 5% w/v aluminium chloride/calcium chloride solution being stirred at different rpm. Keep distance between needle to curing medium at least 15 cms, gap to get stable spherical beads. Since sodium alginate gel can easily be formed by this ionic interaction in an aqueous medium, gel beads are commonly obtained by dropping solutions of sod. alginate into a solution which contains different divalent and trivalent electrolytes. Formed soft beads cured for a period of 30 to 60 min. into curing solution to harden the surface of soft beads. The concentration of Alcl+3 in the solution ranged from 5 % w/v. The stirring was continued for one hour and make sure that free air bubble should be made throughout polymer solution by subjecting to sonicator for more than 1 h and formed the aluminium alginate beads were separated by filtration and decantation, obtained beads washed with distilled water three times continuously to remove traces of curing medium, and make air dried overnight.

| Table 1: Formulation of atorvastatin microbeads |
|----------------|----------------|----------------|----------------|----------------|
| Batch code | Sodium alginate (% w/v) | Atorvastatin (mg) | Curing agent | Curing time (h) | RPM |
| A   | 2   | 100  | Cad2  | 2.5  | 0.25  | 100  |
| B   | 3   | 100  | Cad2  | 2.5  | 0.25  | 100  |
| C   | 4   | 100  | Cad2  | 2.5  | 0.25  | 100  |
| D   | 2   | 100  | AkB3  | 5    | 0.5   | 200  |
| E   | 3   | 100  | AkB3  | 5    | 0.5   | 200  |
| F   | 4   | 100  | AkB3  | 5    | 0.5   | 200  |
| G   | 3   | 100  | AkB3  | 5    | 1     | 200  |
| H   | 3   | 100  | AkB3  | 5    | 1     | 300  |

| Table 2: Formulation of optimised alginate beads with different hydrophilic polymers |
|----------------|----------------|----------------|----------------|----------------|
| Code | Polymers (3%)+0.3%w/v | Drug (mg) | Curing agent | Curing agent (%) | RPM | Curing time (h) | Curing volume (ml) |
| A1  | Sod. Alginate+carbopol 934P | 100 | Alcl+3 | 5 | 200 | 1 | 150 |
| B1  | Sod. Alginate+carbopol 934P | 100 | Alcl+3 | 5 | 200 | 1 | 200 |
| C1  | Sod. Alginate+carbopol 934P | 100 | Alcl+3 | 5 | 200 | 1 | 100 |
| D1  | Sod. Alginate+HPMC K15 | 100 | Alcl+3 | 5 | 200 | 1 | 100 |
| E1  | Sod. Alginate+poly vinyl pyrrolidine | 100 | Alcl+3 | 5 | 200 | 1 | 100 |

| Table 3: List of various optimised parameters |
|----------------|----------------|----------------|----------------|----------------|
| S. No. | Conc. of sodium alginate (%) | rpm | Curing medium (ml) | Curing time (min.) | Curing agent | Curing agent (%) | Syringe needle grade |
| 1   | 3   | 200 | 100  | 60  | Aluminium chloride | 5   | 22 G |

Experimental design

Determination of absorption maxima

10 µg/ml solutions were taken to determine absorption maxima. Initially blank buffer solution was kept and scanned in the region of 200-400 nm by using UV-visible spectrophotometer. Then drug sample was kept for analysis and scanned in the above region.

Drug entrapment efficiency

The prepared drugloaded microspheres were checked for their drug content. Accurately weighed 100 mg of the dried microspheres were powdered and dissolved in 100 ml phosphate buffer (pH 6.8). The solution was kept overnight and then filtered using 0.45 µm cellulose acetate syringe filter, after series of suitable dilutions with phosphate buffer were done, solutions were filtered and analyzed spectrophotometrically at 243 nm by using uv-visible spectrophotometer (UV-800 Shimadzu, Japan). The entrapment efficiency was determined using the following equation:

$$\text{Drug entrapment} = \frac{\text{Actual drug concentration}}{\text{Theoretical drug concentration}} \times 100$$
Determination of swelling properties

The dynamic swelling property of microbeads was determined by placing beads in both, i.e., acidified 6.8 pH phosphate buffer medium under standard conditions. Prepared microbeads of known weight were placed in dissolution solution for specified time and were maintained 37±0.5 °C temperature with 70 rpm. The swollen microcapsules were collected by decantation at different intervals of time. The wet weight of the swollen alginate cross-linked microcapsules was determined by first blotting the particles with filter paper to remove absorbed water on the surface and then weighing immediately again on an electronic balance. The difference in given values gives the percentage of swelling of microcapsules in the dissolution media and it was calculated by using equation.

\[
S_{w} = \frac{(W_{t} - W_{0}) \times 100}{W_{0}}
\]

Where \( S_{w} \) = percentage of swelling of microcapsules/beads, \( W_{t} \) = weight of the microcapsules at time \( t \), and \( W_{0} \) = initial weight of the microcapsules.

Erosion studies

The dried alginate beads were weighed and placed in a dissolution basket, which contains phosphate buffer (pH 6.8) at 70 rpm. After every 1h intervals of time, the swollen beads were removed from the basket carefully and dried at room temperature. The beads were dried after water was been removed, and the dried beads were reweighed. The beads were then placed back in the basket. This entire process lasted 11 h. Erosion study was performed using the following formula.

\[
\% \text{ weight loss} = \frac{(W_{0} - W_{t}) \times 100}{W_{0}}
\]

Where \( W_{0} \) = Initial beads weight and \( W_{t} \) = weight of beads after drying.

Mucoadhesive testing by in vitro wash-off test

The functionality mucoadhesive property of the alginate base microbeads was evaluated by in vitro wash off test. Collect freshly excised pieces of the intestinal mucosa (3 × 3 cm²) from sheep were hermetically sealed onto the arm of a USP tablet disintegrating test apparatus. The tissue specimen was given a slow, regular up-and-down movement in the disintegration fluid at 37 °C contained in a one-liter basket vessel and maintain baskets move up and down continuously at 70 rpm. After the definite time intervals, the machine was stopped and the number of microcapsules still adhering to the tissue was counted. (Gastric pH) 0.1 HCl, pH 1.2 and (intestinal pH) phosphate buffer of pH 6.8 were used as test fluids. The difference in adherence of swelled alginate beads to glass slides before and after were given knowledge of the mucoadhesive strength of microbeads.

Scanning electron microscopy

The surface morphology of microbeads was investigated by using scanning electron microscope (USA model). A known amount of sample, the few milligrams of the sample, were hermetically sealed into aluminium pans and heated under a nitrogen atmosphere with the heating rate of 10 °C/min. [16-18]

Differential scanning calorimetry

DSC studies were carried out on the thermal analyzer (TA-60WS), shimadzu. A few milligrams of the sample, were placed on a brass stub using double-sided adhesive tape and made scanning electron microscope (USA model). A known amount of sample was determined by first blotting the particles with filter paper to remove absorbed water on the surface and then weighing immediately again on an electronic balance. The difference in given values gives the percentage of swelling of microcapsules in the dissolution media and it was calculated by using equation.

\[
S_{w} = \frac{(W_{t} - W_{0}) \times 100}{W_{0}}
\]

Where \( S_{w} \) = percentage of swelling of microcapsules/beads, \( W_{t} \) = weight of the microcapsules at time \( t \), and \( W_{0} \) = initial weight of the microcapsules.

RESULTS AND DISCUSSION

UV spectra (\( \lambda \) max) of atorvastatin calcium

10 µg/ml solutions of atorvastatin were taken to determine absorption maxima. Atorvastatin sample was observed scanned in the range of 200-400 nm. Absorption maxima were found to be 243 nm. Hence all further analysis was carried out at 243 nm.

Atorvastatin solubility in various buffers

Pure drug atorvastatin undergoing solubility less than 25.0 µg/ml. Atorvastatin has the highest solubility in pH 6.8 phosphate buffer with 1.5% (w/v) sodium lauryl sulphate (217.1 µg/ml). The addition of SLS increased the solubility of atorvastatin in pH 6.8 phosphate buffer. The solubility of atorvastatin was highest in pH 6.8 buffer compared with other buffers; hence, further dissolution testing of atorvastatin microspheres was performed by using pH 6.8 phosphate buffer with the addition of sodium lauryl sulphate in desired concentrations of 0.5%, 1%, and 1.5% (w/v). The solubility values of atorvastatin in different buffer medium were predicted in above table 4.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Medium</th>
<th>Solubility mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.0 Phosphate buffer</td>
<td>20.12±1.12</td>
</tr>
<tr>
<td>2</td>
<td>6.8 Phosphate buffer</td>
<td>70.12±2.34</td>
</tr>
<tr>
<td>3</td>
<td>7.4 Phosphate buffer</td>
<td>68.89±0.52</td>
</tr>
<tr>
<td>4</td>
<td>0.5 % SLS in 6.8 Phosphate buffer</td>
<td>72.5±2.64</td>
</tr>
<tr>
<td>5</td>
<td>1 % SLS in 6.8 Phosphate buffer</td>
<td>115.1±27.36</td>
</tr>
<tr>
<td>6</td>
<td>1.5 % SLS in 6.8 Phosphate buffer</td>
<td>217.2±0.89</td>
</tr>
<tr>
<td>7</td>
<td>0.5 % SLS in Phosphate buffer 7.4</td>
<td>90.34±0.56</td>
</tr>
<tr>
<td>8</td>
<td>1 % SLS in Phosphate buffer 7.4</td>
<td>97.12±2.45</td>
</tr>
<tr>
<td>9</td>
<td>1.5 % SLS in Phosphate buffer 7.4</td>
<td>126.23±2.24</td>
</tr>
</tbody>
</table>

\*n = 3 (average±SD)
FTIR studies [22, 23]

FT-IR spectra were recorded on a bruker spectrophotometer by using KBr discs in the range of 4000-450 cm\(^{-1}\). FT-IR analysis was performed using a sample of atorvastatin with various excipients at 1:1 mass/mass ratio. In the FTIR spectrum of sodium alginate powder, the various distinct peaks of alginate evident are those of the carbonyl at 1609 cm\(^{-1}\), hydroxyl group at 3429 cm\(^{-1}\), and carboxyl and carboxylate groups between 1000 and 1400 cm\(^{-1}\). The absorption band around 2880, 1656, 1421 and 1080 cm\(^{-1}\) corresponds to the stretching of -CH, COO\(^-\), C-O-C, -CH and -CH\(_2\), respectively.

FTIR spectra of atorvastatin showed presence of their characteristics band at 3362 cm\(^{-1}\) for (-OH), 3236 cm\(^{-1}\) for (-NH), 2971 cm\(^{-1}\) for (-CH\(_3\)), 2824 cm\(^{-1}\) for (-CH aromatic), 1649 cm\(^{-1}\) for (C=O), 1578 cm\(^{-1}\) for (C-N), and 1432 cm\(^{-1}\) for (C-C) functional groups. The cross-linking process with aluminium ion caused an obvious shift to higher wave number and a decrease in intensity of COO\(^-\)-stretching peaks, and a decrease in intensity of 1031 cm\(^{-1}\) peak of alginate. The results clearly revealed the compatibility of the drug with the excipients used in the formulation.

Effect of concentration of sodium alginate

Alginate derivatives are having mucoadhesive properties. In this study sodium alginate used as a coating material and that will be used in combination of different hydrophilic polymers in little concentrations were used. From the observed data, it was observed that increase in the molecular weight of the mucoadhesive polymers increases the drug entrapment efficacy. This can be due to increase in the viscosity of the polymeric solution which, the greater availability of aluminium binding sites in the network of polymeric chains. As a result formation of the more intact, denser matrix network, entrapping a higher amount of drug.

Overall results show that combination of alginate with other hydrophilic polymers, the release becomes more sustained. The decrease in conc. of sodium alginate, it gives less compact beads and provides lesser no. of binding sites along alginate chains for Ca\(^{2+}\)/Al\(^{3+}\) ions resulting in a compact gel with less viscosity, which in turn increases the influx of divalent ions leading to decrease in encapsulation efficiency.

The higher the amount of sodium alginate in the beads, the lower the swelling rate that more sustained release was observed with the combination. From the studies, it was confirmed that stable beads were formed in sodium alginate at 3% w/v only then 2% w/v. The beads prepared with 2 % w/v were not observed much stable after drying time and they become changes in their appearance. At 4% w/v. sodium alginate solution is making difficulty in passing through syringe needle grade no. 22 at the time of discharge into curing medium because of their higher viscosity. So it is confirmed that out of all concentrations best one found to be 3% w/v. and same used in further studies.

3% sodium alginate>2 % sodium alginate
Effect of stirring

The rate of stirring has much influence in the formation of stable beads. After getting discharging of sodium alginate solutions into curing medium, beads were formed because of gelation reaction between alginate and curing agents. At slow rpm, the beads not developed properly in its strength and at higher rpm surface of beads were disrupted because of generation of high shear force. The stirring rate during crosslinking is believed to be a determining typical factor that affects the bead size and the yield. With very high stirring rate i.e. at 300 rpm being responsible for the reduction in the yield. Accordingly, the stirring rate was kept at 200 rpm and was maintained with all formulation. This ensures that any variation in the bead size or morphology will be due to the variation in the composition.
Effect of curing time
The curing time has a major role in developing rigid layer during gelation reaction. Upon extending curing beyond 30 min to 60 min beads becomes many compacts in comparison to 10 min and 20 min. It was also confirmed that at 60 min the diffuses into curing medium and becomes less in drug encapsulating efficiency and makes delay in drug release. So curing time 1 h was selected for remaining studies.

The extent and rate of cross-linking depend on the valency of a cross-linking agent, molecular size of the drug and concentration of the agent as well as on the speed and curing time during processing. The water soluble and low molecular mass drugs have poor entrapment compared to insoluble and large molecular mass drugs.

The percentage entrapment efficiency decreased with an increase in the time of exposure to the cross linking agent may be due to the increased release of the drug from the matrix at the longer time of exposure. Since prolonged exposure in the curing medium caused greater loss of drug through cross-linked alginate microsphere. Accordingly, the cross linking time was kept constant (1 h) in all formulations.

Effect of curing volume
It was studied the effect of the volume of curing reagent on drug entrapment efficiency and drug release rate the microbeads were formulated by taking different volumes (50, 100 and 150 ml) of the curing agent’s solution. The entrapment efficiency was found to be increased with increase in the volume of curing agent up to 150 ml and then entrapment efficiency has decreased the reason was contributed to chances of leaching of the drug in bulk volumes of the curing solution. The atorvastatin microbeads formulated by taking 100 ml volume as curing reagent were showed good entrapment efficiency and desired drug release rate. Hence, 100 ml of curing reagent was selected for further studies. Dissolution profiles of the optimised batch with different curing medium i.e. differ in volume were showed in fig. 4.

Fig. 4: Dissolution profiles of the optimized batch with different curing medium 100 ml and 200 ml (left side) and with different curing agents (right side). Results are given in mean of triplicate.

Effect of curing agent
In present studies alginate beads prepared with different curing agents naming calcium chloride and aluminium chloride. The divalent compound CaCl$_2$ form two-dimensional network when compared to aluminium chloride as it forms a three-dimensional network. Curing agent aluminium chloride has gained three-dimensional networks in its orientation because of greater valence i.e. three in comparison to two of CaCl$_2$ and compact beads were formed with aluminium chloride at 5% w/v and make delay in drug release observed relatively. At lower concentrations of calcium chloride/aluminium chloride, the beads showed larger pores due to insufficient cross-linking which has resulting in lower drug entrapment, so for further studies was implemented in all formulations as it gives close compact network in the orientation of drug molecules in the polymer.

Although the aluminium has a small size as compared to calcium, the release of drug from the hydrogel beads cross-linked with aluminium chloride was found to be more sustained as compared to the beads crosslinked with calcium chloride. The reason is that aluminium carries an extra charge than calcium chloride. This three-dimensional bonding results in extended cross-linking sites throughout whole beads. Hence, it releases the drug at a slow manner as compared to divalent compound calcium chloride. Dissolution profiles of the optimised batch with different curing agents were showed in fig. 4.

Role of needle and its position
Information of stable spherical shape beads selection of needle size and its orientation plays a vital role. In the case of alginate which contains carbopol 934P [24] microspheres owing to its high viscosity in comparison to PVP and HPMC K15, the beads develops tail while in passing through the needle into the curing medium, so beads not forms spherical shape and gives an ovate/cylindrical shape like.

So to avoid abnormal shape of beads, the position of needle have to keep in divert position like, this modification has to make only in formulation contains sodium alginate in combination with carbopol 934P only. As the size of needle increases, the diameter of the needle decreases. The alginate beads prepared with different sizes of needles were observed.

Morphology and size distribution
Morphology of the various formulations of sodium alginate microspheres prepared was found to be in discrete and spherical appearance. The SEM photomicrographs of the dried alginate microspheres are shown in fig. 5. The surface of the alginate microspheres was rough due to a higher concentration of sodium alginate and uniformly dispersed of drug molecular level in the alginate matrices.

Effect of sodium alginate on drug entrapment efficiency
The higher viscosity of sodium alginate with different hydrophilic polymers at selected concentration will leads to decrease the rate of diffusion of drug into curing medium which would result in higher drug entrapment efficiency, this may be reason to greater availability of no. of active binding sites along polymeric chains and ultimately the degree of cross linking sites as the amount of alginate increased.

Incorporation efficiency of microspheres
The incorporation efficiency increased progressively with increasing sodium alginate concentration. An increase in the alginate concentration resulted in the formation of larger microspheres entrapping greater amounts of the drug. This may be attributed to the greater availability of active calcium-binding sites in the polymeric chains and, consequently, the greater degree of cross-linking as the quantity of sodium alginate increased.

The incorporation efficiencies were generally higher for the beads cross-linked with Al$_3^+$ as compared to the beads cross-linked with Ca$_2^+$. This may be attributed to the formation of nonpores alginate beads due to an increase in the apparent cross-linking density in presence of cations i.e. Al$_3^+$ which prevent the diffusion of the drug out of the beads at the time of curing.
Table 5: Entrapment efficiency, percentage yield observed from the atorvastatin mucoadhesive microbeads formulated by employing various formulations

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Theoretical drug content (mg)</th>
<th>Practical drug content (mg)</th>
<th>Encapsulation efficiency (%±SD)</th>
<th>Yield (%±SD)</th>
<th>Size (µm) (%±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>20</td>
<td>11.78</td>
<td>58.90±0.53</td>
<td>47.67±1.78</td>
<td>1095</td>
</tr>
<tr>
<td>B1</td>
<td>20</td>
<td>12.20</td>
<td>61.21±1.34</td>
<td>48.34±2.23</td>
<td>1074</td>
</tr>
<tr>
<td>C1</td>
<td>20</td>
<td>11.01</td>
<td>55.05±0.41</td>
<td>48.79±0.59</td>
<td>1016</td>
</tr>
<tr>
<td>D1</td>
<td>20</td>
<td>10.78</td>
<td>53.90±0.32</td>
<td>51.23±0.36</td>
<td>988</td>
</tr>
<tr>
<td>E1</td>
<td>20</td>
<td>11.56</td>
<td>57.80±2.12</td>
<td>69.24±0.34</td>
<td>985</td>
</tr>
</tbody>
</table>

*n = 3 (average±SD).

The low incorporation efficiency of alginate beads cross-linked with CaCl2 could be attributed to the formation of porous beads ensuring the diffusion of the drug out of the beads at the time of curing.

Swelling index

Polymer swelling and cross-linking are intimately connected to the release kinetics of the drugs from the swollen matrix. In order to investigate this effect, swelling results were obtained. Swelling of the beads was measured in terms of their percentage of water uptake at a selected time intervals. Swelling of cross-linking alginate beads was observed little in acidic medium, in contrast to this, the rate of swelling will be more than triple times in presence of alkaline medium as it undergoes relaxation of the polymer.

Table 6: Percentage of swelling index with 3 % w/v sodium alginate with different hydrophilic polymers (0.3 % w/v). Each reading taken in triplicate

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Alg-carbopol 934P</th>
<th>Alg-HPMC K15</th>
<th>Alg-PVP*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 (Acidic medium)</td>
<td>0.75±0.23</td>
<td>1.5±0.12</td>
<td>3.37±0.23</td>
</tr>
<tr>
<td>2 (Alkaline medium)</td>
<td>1.12±0.34</td>
<td>4.5±0.09</td>
<td>8.87±0.69</td>
</tr>
</tbody>
</table>

*n=3 (average±SD)

Erosion test

Fig. 6: Erosion studies of different formulations, *Results are given in mean of triplicate

Wash off test

It gives knowledge regarding the ability of the polymer to adhere to the GI tract mucosa when it is thoroughly and continuously washed by 6.8 phosphate buffer/GI fluid. Microspheres with a coat consisting of sodium alginate and a mucoadhesive polymer exhibited good mucoadhesive properties as indicated by the in vitro wash-off test. Higher the adhesion to mucosa as well as greater
localisation of beads forms at the site of absorption if beads have a higher value of mucoadhesion rate.

Beads prepared with carbopol 934P has higher mucoadhesion value, the reason due to the free carboxyl group which has high ability to form Covalent bonding with the mucin sites which present in mucous membrane whereas HPMC K15 polymer contains hydroxyl (-OH) groups. A hydroxy group present in HPMC K15 is less polar in comparison to the free carboxyl group of carbopol 934P. Hence a carbopol bead shows higher mucoadhesion as compared to HPMC.

The study details reveals in vitro wash off was faster at simulated intestinal fluid (pH 6.8) than that simulated GI fluid (pH 1.2). pH of in vitro wash off medium has pronounced effect on solubility, hydration and mucoadhesiveness of the polymer. The polymers exhibiting relaxation property depends upon pH.

The solubility of beads was faster at 6.8 buffer medium may be due to ionization of carboxy acid group i.e. sod. Alginate and other polymers. The decreased mucoadhesion of beads in phosphate buffer may result from erosion of aluminium ions which form a cross-linked structure.

At higher solubility beads shows less mucoadhesive strength. The results of in vitro wash off test indicated that atorvastatin beads had fairly good mucoadhesive properties. The prepared beads would adhere to walls of mucus, thus restricting gastric emptying and prolong the residence tome of atorvastatin beads at absorption site, thereby it is possible to improve and increase the rate of bioavailability.

### Table 7: Result of in vitro wash-off test of micro beads in 0.1 N Hcl

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Alg. with carbopol 934P</th>
<th>Alg with HPMC</th>
<th>Alg with PVP*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>88.67±1.32</td>
<td>75.76±5.67</td>
<td>79.78±4.12</td>
</tr>
<tr>
<td>2</td>
<td>80.45±3.45</td>
<td>69.89±3.56</td>
<td>70.13±0.12</td>
</tr>
<tr>
<td>4</td>
<td>60.78±3.67</td>
<td>50.45±5.98</td>
<td>51.2±8.34</td>
</tr>
<tr>
<td>6</td>
<td>42.32±4.23</td>
<td>39.21±3.32</td>
<td>40.12±4.83</td>
</tr>
<tr>
<td>8</td>
<td>34.78±8.65</td>
<td>23.32±4.43</td>
<td>30.23±2.78</td>
</tr>
</tbody>
</table>

* n = 3 (average±SD)

### Table 8: Result of in vitro wash-off test of microcapsules in 6.8 phosphate buffer

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Alg. with carbopol 934P</th>
<th>Alg with HPMC</th>
<th>Alg with PVP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>75.12±1.23</td>
<td>60.34±1.56</td>
<td>70.23±5.45</td>
</tr>
<tr>
<td>2</td>
<td>50.23±2.45</td>
<td>41.23±3.45</td>
<td>47.56±2.45</td>
</tr>
<tr>
<td>4</td>
<td>29.56±0.23</td>
<td>20.12±4.56</td>
<td>25.67±6.03</td>
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<tr>
<td>6</td>
<td>15.23±8.95</td>
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<td>12.56±4.12</td>
</tr>
<tr>
<td>8</td>
<td>5.78±3.76</td>
<td>0</td>
<td>3.45±3.32</td>
</tr>
</tbody>
</table>

Every reading taking in triplicate i.e. n = 3 (average±SD)

### X-ray diffraction studies

The diffraction pattern of the pure drug showed characteristic high-intensity diffraction peaks at 8.94, 9.34, 10.01, 10.38, 11.65, 12.20, 16.84, 19.26, 21.36, 22.50, 23.11, and 23.51, which indicates that the drug is dispersed in the crystalline form that is also confirmed by DSC results. It is confirmed that the reflections of the pure drug match satisfactorily with the reflections of the drug in the microbeads formulation. Thus, it can be concluded that the polymorph of the pure drug was the same as that of atorvastatin in polymorph incorporated in microbeads, and there are no transformation took place during the manufacturing process and storage.
In vitro dissolution

Alginate is insoluble in acidic pH i.e. 0.1N HCl, and at low pH, the number of positively charged ions is high, and they minimise the electrical repulsion between the negatively charged alginate molecules. Alginate is protonated into the insoluble form of alginic acid molecules. Thus, penetration of dissolution fluid through the polymer matrix is hindered, and the percentage of drug released due to diffusion through the polymer network was minimal at pH 1.2 for all prepared beads.

The rate of drug release from alginate-hydrophilic polymers i.e. carbopol 934P, HPMC K15 M and PVP. Beads formulated with a higher degree of cross-linking was more sustained than the beads formulated with a lower degree of cross-linking. Again, the release rate of atorvastatin in acid was found to be slower and more sustained. This may be due to the stability of alginate at acidic pH and the conversion of calcium/aluminium alginate to the insoluble but swelling alginic acid.

The higher amount of drug release in 6.8 phosphate buffer than acidic buffer may be due to the higher swelling rate of the alginates in phosphate buffer medium. The presence of carbopol 934P and HPMC in the beads added to the network structure of the beads and in forced the degree of crosslinking due to its interaction with aluminium ions. This produces tight packing which disintegrates and dissociates slowly with subsequent slow release even in the intestinal phase.

When pH is increased i.e. at 6.8 pH phosphate buffer, drug release is increased up to the maximum level. Deprotonation of alginic acid occurs at higher pH values. It will draw dissolution fluid into the beads, which causes swelling of beads and burst apart of the drug.

As the polymer concentration increased, diffusion resistance to drug release also increased, which, in turn, decreased in drug release [24-26]. Positively charged groups of HPMC K15 strongly interact with alginate and atorvastatin ions, thus reducing both swelling and release probably due to protonation of carboxylated functional groups from Na-alginate molecules, and based on ficks law of diffusion, it may be inferred that as the viscosity of the formulation increases, molecular diffusivity (diffusion coefficient) and percentage release of drug also decreases.

The results indicated the more sustained effect with an increase in the viscosity of sodium alginate with different hydrophilic polymers. The first phase might be for the negligible dissociation of alginate beads in the acidic buffer and the drug release mainly based on drug diffusion through the formation of small pores and cracks upon swelling process. The second phase compared to initial phase exhibited a sustained release pattern, which was accompanied by rapid alginate disintegration of alginate in phosphate buffer than acidic medium. The sodium alginate combination with other hydrophilic polymers in the formulation greatly influenced the steady state release of atorvastatin from the alginate beads.

Swelling of the polymer also leads to the formation of the matrix, thereby retarding the release of drug from the formulation [27, 28]. The higher mucoadhesion of carbopol 934P microspheres may also be attributed to the higher molecular weight of carbopol than HPMC [29]. These polymers are often used as a rate-controlling membrane to modulate the drug release from dosage forms with organic or aqueous coating techniques [30-33]. The principle of gelation or crosslinking of sodium alginate with aluminium chloride is based on the formation of a tight junction between the residues of guluronic acid. The number of the apparent cross-linking points within the formed aluminium alginate gel beads increased with increasing sod.
alginate concentration in the formulation. This increase in the intensity of crosslinking density delayed the alginate gel disintegration in 6.8 pH phosphate buffer due to the retardation of aluminium+ exchange with Na+. Increased alginate gel density per unit volume also affected the decreased pore size within the gels, and thus atorvastatin release becomes slow.

The release behaviour of alginate microspheres, produced by ionotropic external gelation causes precipitation with different gelling agent's ex. CaCl$_2$ and AlCl$_3$ depend upon the valency of ions and size of the cations of the respective cross-linking agent. To investigate this aspect, the sodium alginate (3.0% w/v) beads were prepared via cross linking in 5% w/v solution of CaCl$_2$ and AlCl$_3$ respectively.

Drug release from the microspheres depends on the viscosities of the various polymers. Their release profile in 6.8 phosphate buffer was depicted in fig. 10. The sustained release pattern was observed in all formulations. The results obtained can be explained on the basis of the extent of cross-linking in the microspheres. Calcium chloride being a divalent forms two-dimensional network structure with sodium alginate inside the alginate matrices. In the case of Al$_3$, alginate microspheres, the delay was due to the ability of Al$_3$ to form three-dimensional network structure with the sodium alginate inside the lattice of microspheres. This three-dimensional bonding results in an extended crosslinking through the whole microsphere, producing hard and compact alginate microspheres with less water uptake and thus leads to slow removal of Al$_3$ due to the exchange of ions with Na$^+$ in the 6.8 phosphate buffer. As a result, the swelling of the beads are delayed leading to slow disintegration/rupture of bead surface.

**Kinetic modelling in vitro dissolution data was analyzed by different kinetic models in order to find out the n value, which describes the drug release mechanism. The values of coefficient of correlation ($R^2$) obtained for the respective model are listed in following table 9. Models with the highest correlation coefficient ($R^2$) were judged to be the most appropriate model for the in vitro release study.**

The values of n for the release of atorvastatin from the alginate microspheres range between 1.376 and 2.927, indicating that the drug release from the microspheres followed the anomalous transport and super case-II transport mechanism controlled by swelling and relaxation of the polymer chains. Prepared micro beads were best fitted Higuchi model which indicated the drug release by diffusion in the slow and sustained way.

CONCLUSION

This study reveals that the sustained release of atorvastatin microbeads can be successfully achieved by ionotropic gelation technique using sodium alginate as a coating polymer and different hydrophilic polymers as release rate modifiers. To meet this objective, the microbeads were formulated by varying the processing conditions. The *in vitro* studies demonstrated that the microbeads formulated with 3% w/v sodium alginate as coating polymer with 0.3% w/v of carbopol 934P under the presence of 100 ml at 5% w/v aqueous aluminium chloride solution as curing reagent with 200 rpm offered sustained release rates for 10 h. The specifications of alginate beads depend on the composition of the beads. The addition of sodium alginate to viscosity modifiers i.e. as in form of carbopol 934P, pvp and hpmc k15 increases the drug entrapment efficiency and swelling property into these beads than alginate alone. As this method developed is highly simple, fast and reproducible. A delay in the release of atorvastatin was observed for the combination of sod. alginate and carbopol934P beads.

The value of n is 1.567 for the formulation which contains a combination of sodium alginate with carbopol 934P. The n value confirms that drug release from beads was followed super case-II transport mechanism indicates that drug control by swelling and relaxation of polymer chains. The optimized mucoadhesive microcapsules exhibited good flow properties, mucoadhesive potential, better drug entrapment efficiency, and the desired release characteristics. Matrix swelling induced by 6.8 phosphate buffer and drug release is proved to be a function of pH based. Thus, this study concludes that the performance of the microbeads can be altered by changing the processing variables involved in the production of stabilized cross-linked atorvastatin microbeads.

ACKNOWLEDGEMENT

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CONFLICTS OF INTERESTS

Declare none

REFERENCES


Table 9: Release kinetics data of all formulations

<table>
<thead>
<tr>
<th>Batch code</th>
<th>Zero order</th>
<th>First order</th>
<th>Higuchi matrix*</th>
<th>Korsmeyer Peppas*</th>
<th>Hixon crowell equation*</th>
<th>n value</th>
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<tr>
<td>D1</td>
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<td>Marketed brand</td>
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<td>$R^2=0.999$</td>
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<td></td>
<td>2.927</td>
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</table>

$'n$ = 3 (average±SD)


