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PREPARATION OF GASTRO RETENTIVE MUCOADHESIVE BEADS FOR ATORVASTATIN BY USING IONIC GELATION METHOD WITH DIVALENCE AND TRIVALENCE CURING AGENTS AND THEIR CHARACTERIZATION STUDIES

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Keywords:

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ABSTRACT: Atorvastatin alginate beads were prepared by ionic gelation method by using different curing agents and studied the different process variable like the effect of alginate concentration, the effect of rpm and curing agents on drug entrapment and to improve the bioavailability of the drug. The beads examined and qualified the all compatible studies. The amount of drug released in acidic medium was found to be less than alkaline medium and proved drug release is function of surrounding pH effect and beads prepared with aluminium chloride with physical mixture of sodium alginate and carbopol 934P at proportions of 3.3% produces more compacted, stabled and shows prolonged sustained release in comparison to barium chloride at same concentration of polymers. The size of beads, drug entrapment, and drug release depend upon variables like alginate concentration, type of curing agent, rpm, the valence of curing agent and addition of copolymer. The drug entrapment and physical stability will be improved by adding carbopol 934P along alginate polymer. DSC studies clarify Atorvastatin was dispersed in molecular level uniformly and XRD proves and indicating that crystalline conversion into the amorphous form of the drug after entrapment drug into the cross-linked alginate beads. By obtained data, it proved Atorvastatin beads forms suitable oral dosage forms and improved the rate of bioavailability and the data was fitted to Peppas equation and value of diffusional exponent n was found to be 1.216 indicating that the release of the drug from beads follows zero order super case II transport or non-fickian diffusion.

INTRODUCTION: Atorvastatin is an anti-cholesterol drug compound that acts selectively by inhibiting the intestinal absorption of cholesterol synthesis and minimizes cholesterol absorption. After consumption through oral administration, Atorvastatin undergoes a rapid rate of absorption and reach maximum plasma concentrations within the period of 1-2 h.

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Atorvastatin belongs to bio-pharmaceutics classification system class II category, having a low percentage of oral [1] bioavailability (12%) due to low aqueous solubility (0.1 mg/mL) [2] crystalline nature and hepatic first-pass metabolism. It has very good intestinal permeability.

Atorvastatin has low aqueous solubility resulting in low oral bioavailability and thus presents a challenge in formulating a suitable dosage form. Poor aqueous solubility hinders the i*n-vivo* performance of the drugs, causing a low rate of bioavailability, abnormal pharmacokinetic profile, inter-species variation and inter-subject leading to expensive and prolonged development [3, 4]. Given its long biological half-life and less bioavailability of

Atorvastatin and the incidence of associated side effects, we have chosen Atorvastatin as a model of the drug for developing the controlled release beads with sodium alginate as a coating polymer. Literature survey revealed that carbopol 934P is the polymer which shows good mucoadhesive properties, a high amount of drug entrapment efficiency and releases the drug in sustained release manner.

To improve bioavailability, physical, chemical properties of beads and swelling characteristics, the hydrophilic polymer in the form of carbopol 934P were added as a drug retardant enhancer at low concentrations only along with sodium alginate. This polymer blend swells and forms a mucilaginous gel by absorbing water and forming bonds with the mucus lining to get attached on its surface. It, therefore, provides a controlled release profile of drug delivery to improve the bioavailability of the drug.

Microencapsulation technology offers an alternative way of delivering the number of drugs. Careful and close consideration of the components of the encapsulation medium not only improves drug solubility but may also facilitate targeted and controlled drug delivery system. Sodium alginate is a mainly belongs to polysaccharide category and is said to be a natural polymer obtained from brown algae source and is belongs an anionic nature, biodegradable and biocompatible. Much of interest found in its significance as a vehicle for designing for sustained drug delivery system for a variety of reasons [5]. The alginates tend to form hydrogels in the presence of divalent cations naming Ca^{+2} and Ba^{+2} through ionically cross-linked [6] mechanisms. Upon discharging sodium alginate solution into di / trivalent curing ions at known concentrations, the rapid ion binding took place instantly and formation of a cross polymeric network that produces an inwardly moving gelling zone. Alginate moves from the gel core towards this gelling zone, leading to the deletion of alginate within the core [7, 8, 9].

The trivalent cation, *i.e.* $AlCl^{3+}$ orient into electronegative cavities like eggs arranged in an egg box and this binds alginate polymer close together by forming tight/compact junction zones and thereby causing gelation of the solution.

An insoluble calcium alginate matrix is formed by the cation exchange between $Na^{+,}$ and Ca^{+2} physical or chemical cross-linking of hydrophilic polymers are found typical approaches to form hydrogels. Alginate beads can provide sustained release properties and a more uniform distribution of drugs in throughout the gastrointestinal tract and have capable of undergoing swelling property with adhesion properties. These microbeads are discrete spherical microcapsules in appearance that serve as the solid substrate on which the drug is encapsulated or coated form [10, 11].

This work was primarily aimed at the development of Atorvastatin loaded alginate beads as a controlled release oral delivery system. Atorvastatin-loaded alginate beads were prepared, and the effects of the various process parameters were studied. Mainly process variables studied mostly a) the effect of conc. of sodium alginate, b) the effect of divalent curing agents and c) the effect of rpm and effect of hydrophilic polymer on DEE and *in-vitro* release studies. All the above said variables were evaluated, examined and determined their influence on the loading efficiency and the drug release were investigated.

MATERIALS AND METHODS: Atorvastatin pure sample was obtained from Aizant Company, Hyderabad, Telangana State, India, sodium alginate 45 cps (1% w/v solution in water at 25 °C), SD fine Chemicals. Mumbai, carbopol 934P, barium chloride, calcium chloride, aluminium chloride, potassium dihydrogen phosphate, plastic syringe and needle (0.55 × 25 mm), dispovan, potassium hydroxide pellets (E. Merck, India), ethanol and sodium lauryl sulfate were used. All used reagents were of best analytical grade.

The instruments used in these studies were Digital analytical weighing electronic balance, Dissolution test apparatus type II basket (Electro lab,TDT-08L), UV-visible spectrophotometer (Shimadzu U-1800 Japan), DSC Analyzer (TA-60WS, Shimadzu), FT-IR spectrophotometer (Shimadzu FTIR-8400, Japan), Magnetic stirrer (Remi equipments, Mumbai), Mechanical stirrer (RQ 122, Remi motors, Mumbai), Stage microscope (Olympus), Scanning electron microscope (SEM; S-4100, Hitachi, Japan). X-ray diffractometer (Pan Analytical X'pert PRO, India).

Preparation of Cross-Linked Alginate Atorvastatin Hydrogel Beads: Atorvastatin beads were prepared by using orifice gelation technique, which is said to be simple, economical and not requires much of organic chemicals as other methods and method of most appropriate process of different microencapsulation techniques. For the production of beads formation, 100 ml of different concentration ranging from 1 to 3% w/v aqueous solution of sodium alginate were prepared and to which add Atorvastatin 100 mg as a drug. After continuous homogenization done by using sigma blade mixer, it was discharged dropwise by using syringe needle (size-22) into the beaker containing 100 ml of an aqueous solution of 5% w/v barium chloride/calcium chloride solution and make stirring continuously by using the magnetic mixer at different RPMs from 100 to 300. While at discharging alginate solution into a beaker, keep the minimum distance between needle tip to curing solution at least 15 cms gap to get good spherical beads. Since sodium alginate gel can easily be formed by the ionic interaction in an aqueous medium, gel beads are commonly obtained by dropping solutions of sodium alginate into a solution which contains different divalent polyelectrolytes at 5% v/v. Formed soft beads cured for a period of 60 min in curing solution to promote harden the surface of soft beads and formed the barium/calcium alginate beads were separated by filtration or decantation process, obtained beads washed with distilled water continuously under tap water sensitively three times continuously to remove traces of curing medium, and make beads to dry by storing at room temperature.

TABLE 1: FORMULATION OF ATORVASTATIN MICROBEADS

Batch Code	D:P ratio	Curing agent	RPM	Curing time (h)	The volume of curing medium (ml)	Conc. of the curing agent (%)
AB1	1: 1000	$CaCl^{2+}$	100	1	100	5
AB2	1: 2000	$CaCl^{2+}$	100	1	100	5
AB3	1: 3000	$CaCl^{2+}$	100	1	100	5
AB4	1: 1000	$BaCl^{2+}$	100	1	100	5
AB5	1: 2000	$BaCl^{2+}$	100	1	100	5
AB6	1: 3000	$BaCl^{2+}$	100	1	100	5
AB7	1: 1000	$CaCl^{2+}$	200	1	100	5
AB8	1: 2000	$CaCl^{2+}$	200	1	100	5
AB9	1: 3000	$CaCl^{2+}$	200	1	100	5
AB10	1: 1000	$BaCl^{2+}$	200	1	100	5
AB11	1: 2000	$BaCl^{2+}$	200	1	100	5
AB12	1: 3000	$BaCl^{2+}$	200	1	100	5
AB13	1: 3000	$BaCl^{2+}$	300	1	100	5
AB14*	1:3300	$BaCl^{2+}$	200	1	100	5
AB15*	1:3300	$AlCl^{3+}$	200	1	100	5

* Contains one part of drug and 3g of sodium alginate with 0.3 g of carbopol 934P.

Calibration Curve of Atorvastatin: The calibration curve is obtained by dissolving pure Atorvastatin in both 0.1N hydrochloric acid 6.8 phosphate buffer medium and add 0.5% sodium lauryl sulphate. Absorbance measured spectrophotometrically at 245 nm against reagent blank. Calibration was to determine at what concentration it obeys Beer's Lambert's law, and for this different study concentration studied and which ranging from 5-30 µg/ml have been monitored.

Evaluations of the Powder Blend (Micromeritic Characterization):

The angle of Repose: It gives an indication by which it was easy to determine whether powder mixture flow is fair or good. An angle of repose was determined by using the funnel method.

The powder blend was poured through a funnel which is supported on a tripod stand that can be raised vertically until a specified cone height (h) was obtained. The radius of the heap (r) was measured the angle of repose (θ) was calculated by using the formula given below.

$$\text{Tan } \theta = h / r \text{ and } \theta = \text{tan-1 } h / r$$

Where, θ = Angle of repose, h = Height of the powder cone in cm and r is the radius of the powder cone in cm.

Bulk Density (Db): Bulk Density,

$$Db = M / V_0$$

Where, M = Mass of the powder sample and V_0 = Unsettled volume.

Tapped Density (Dt): Tapped Density,

$$Dt = M / V_f$$

V_f = Final tapped volume.

Carr's Compressibility Index (CI): It is an indication of the ease with which a material can be induced to flow, which is calculated as follows:

$$= (Dt- Db) / Dt \times 100$$

Where Dt= Tapped density and Db= Bulk density.

Hausner's Ratio: Hausner's ratio is an index of ease of powder flow and it is calculated using the following formula:

$$\text{Hausner's Ratio} = Dt / Db$$

Fourier Transform Infrared Red (FTIR) Studies: The FTIR analysis of pure drug, polymer, and drug-loaded beads prepared by both the ionic gelation method were analyzed with FTIR spectrophotometer (Shimadzu FTIR-8400, Japan). All the samples were crushed with potassium bromide to get pellets and analyzed the testing to determine compatibility/ integrity of drug in formulation among the used excipients [12].

Drug Entrapment Efficiency (DEE): The prepared drug-loaded microbeads were checked for their drug content. Accurately weighed 100 mg of the dried microbeads 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 spectro-photometrically at 243 nm by using UV-Visible spectrophotometer (UV-800 Shimadzu, Japan).

The entrapment efficiency was determined using the following equation.

$$\text{Drug entrapment (\%)} = \frac{\text{Actual drug concentration}}{\text{Theoretical drug concentration}} \times 100$$

$$\text{Drug loading (\%)} = \frac{\text{The weight of Drug in the sample}}{\text{Weight of sample}} \times 100$$

Scanning Electron Microscopy (SEM): The surface morphology of microbeads were investigated by using scanning electron microscope (SEM; S-4100, Hitachi, Japan). A known amount of sample was placed on a brass stub using double-sided adhesive tape and made electrically conductive by coating with a thin layer of gold and SEM images were observed at different accelerating voltage [13, 14].

Differential Scanning Calorimetry (DSC): [15, 16] Differential scanning calorimetry is an analytical technique used majorly to measure the amount of specific heat and enthalpies of the transition state. When a sample experiences a thermal transition, the power to the heater is adjusted to maintain the temperature. A signed proportional to the power difference is plotted on the recorder device. The area producing under the resulting curve is a direct measure of the heat of transition. DSC studies were carried out by using thermal analyzer (TA-60WS), Shimadzu which was calibrated against indium. A nitrogen purge (20 ml/min) was used throughout the runs A few milligrams (3-5 mg.) of the sample, were hermetically sealed into aluminium pans and heated under a nitrogen atmosphere with the heating rate of 10 °C/min. over a temperature of 300 ºC.

X-Ray Diffraction Study (XRD): [17] Change in the crystalline structure of the drug when loaded into alginate beads was measured by using X-Ray diffractometer [18] (Bruker Model-220, Germany USA). Pure Atorvastatin, pure sodium alginate and powdered microbeads were scanned at a different angle ranging from 5° to 55° diffraction angle (2θ) range.

Physical Evaluation Method:

Shear Stress Measurement:

Apparatus: The apparatus consists of two rectangular thick glass plates of uniform dimensions. One of the glass plates is mounted on a table permanently by using gum. The other glass plate is placed above the lower glass and is connected to a pan by a plastic wire and porcelain pulley. For any mucoadhesive material which is to be tested is placed between these two plates, it will require a certain force to detach the upper plate from fixed lower plate. The apparatus and arrangement are shown in **Fig. 22**.

***In-vitro* Release Studies:** [19] *In-vitro* release of prepared microbeads were assessed in the triplicate manner by using United States Pharmacopoeia (USP) Dissolution type II apparatus (Basket Type), model Electro lab at an accurate temperature of 37 ± 0.5 °C under 70 rpm. A freshly prepared 0.1N hydrochloric acid and phosphate buffer of pH 6.8 (900 ml) was used as dissolution mediums throughout the process alternatively to study drug release up to 10 h.

Atorvastatin microbeads containing 10 mg equivalent of the drug was placed basket and basket placed in 900 ml of dissolution mediums (0.1N HCl and 6.8 phosphate buffer solutions). During the studies, the revolution speed of the basket contains Atorvastatin cross-linked alginate beads were maintained at 70 rpm. At different time intervals, 5 ml of dissolution medium was collected, and the same dissolution media was always replenished with a fresh stock solution of 0.1N HCl solution and 6.8 phosphate buffer used only alternatively. Suitable dilutions were done with the fresh dissolution fluid only; the samples were analyzed for the drug concentration reading at different time intervals taken by using UV-Visible spectrophotometer (Shimadzu, Japan) at 243 nm [20].

Stability Studies: Stability studies were carried out for Atorvastatin beads at various temperatures as per guidelines of ICH guidelines. The samples of known weight were weighed in two sets and wrapped in a good quality butter paper and placed in sterilized petrie dishes. These containers were stored accurately at the ambient humid condition at room temperature (27 ± 2 °C) and elevated temperature (45 ± 2 °C) for the duration of 8 weeks exactly. Then Atorvastatin beads were analyzed for various physical changes such as size, shape, drug entrapment and drug release. The drug content was estimated for 2 weeks once as per the defined standard procedure. The drug samples were further scanned to observe any possible spectral changes even in a small level, and any changes mean they have been reported.

Release Kinetics: To study the release kinetics, data obtained from *in-vitro* dissolution study was fitted in various kinetic models: zero order as cumulative percent of drug released vs. time, first order as log cumulative percentage of drug remaining *vs.* time and Higuchi's model as cumulative percent drug released vs. square root of time, Hixon crewel describes the release from systems when there is a change in both surface area and diameter of particles.

To determine the mechanism of drug release [21] various equations mentioned above employed in this study and Korsmeyer-Peppas model due to their simplicity and easy applicability. The data was fitted into Korsmeyer and Peppas equation as cumulative log percentage of drug released *vs.* log time, and the exponent n value was calculated from the slope of the straight line. For slab matrix, if the exponent is 0.5, then the diffusion mechanism is fickian; if $0.5 < n < 1.0$, then it an indication of anomalous transport. If n is 1.0, it is case II transport and if $n > 1.0$, then it is confirmed as super case II transport or non-fickian diffusion.

RESULTS AND DISCUSSION:

Preparation of Calibration Curve in 0.1 N HCl: The aliquots of 0.2 to 4.0 ml of standard Atorvastatin stock-I solution consist of 0.2 mg/ml strength were transferred to series of 20 ml standard volumetric flask. The volume of each volumetric flask was made up to 20 ml with a freshly prepared solution of 0.1N hydrochloric acid, and 0.5% sodium lauryl sulphate only and absorbance reading were taken by using UV Visible spectrophotometer (Shimadzu 1700, Shimadzu Corp. Kyoto). The absorbance of above solution in each volumetric flask was measured at 243 nm against blank reagent. The following table shows the absorbance value of the different concentration of Atorvastatin in 0.1N hydrochloric acid at 24 nm. The calibration curve was plotted as shown in **Fig. 1** at a concentration range of 5-30 µg/ml.

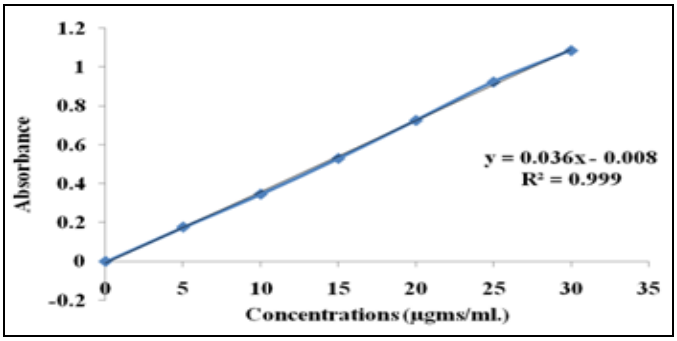

FIG. 1: STANDARD CURVE OF ATORVASTATIN CALCIUM WAS PREPARED IN 0.1 N HCl

After regression analysis of data as shown in **Table 2**, the value of R^2 was found to be 0.999 which indicate the exact accuracy of results.

TABLE 2: ABSORBANCE DATA OF PURE ATORVASTATIN IN 0.1N HCl SOLUTION AT 243 nm

S. no.	Conc. (µgm/ml)	Absorbance* (AM ± SD)
1	0	0
2	5	0.177 ± 0.004
3	10	0.347 ± 0.006
4	15	0.531 ± 0.007
5	20	0.727 ± 0.002
6	25	0.925 ± 0.001
7	30	1.087 ± 0.003

*Each value represents the Mean ± Standard deviation (n=3).

Procedure for Calibration Curve (6.8 Phosphate Buffer): Stock solution for pure Atorvastatin was prepared by dissolving accurately 100 mg of Atorvastatin 100 ml of methanol to get a final concentration of 1 mg/ml solution. Again, further 10 ml of above solution was pipetted into 100 ml of standard volumetric flask and made up to 100 ml with phosphate buffer pH 6.8 only to get 100 µg/ml solutions. Further 10 ml of this solution was pipetted into 100 ml volumetric flask and made up to 100 ml with phosphate buffer pH 6.8 only to get 10 µg/ml solutions of final concentration. From 10 µg/ml solution take 0.5, 1.0, 1.5.....3.0 ml solutions were pipetted into a series of 10 ml volumetric flask and were made up to 10 ml with phosphate buffer pH 6.8 to get 5, 10, 15, 20, 25 and 30 µg/ml solutions of Atorvastatin respectively. The absorbance of resulting solutions was measured at 243 nm against the blank. A graph was plotted by taking concentration on X-axis and absorbance on Y-axis.

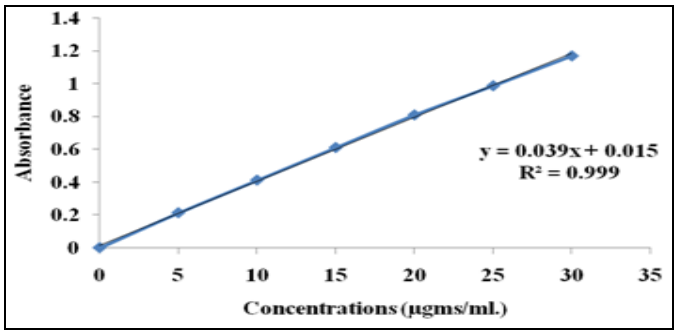

FIG. 2: STANDARD CURVE OF ATORVASTATIN WAS PREPARED IN 6.8 PHOSPHATE BUFFER

From the observed data, Atorvastatin calcium followed beer Lambert's law in the range of 5-30 µg/ml concentration. The equation of line was found to be Y=0.039x+0.015(R^2=0.999). Obtained correlation coefficient values indicated the linear correlation between concentration and absorbance from the observed data, Atorvastatin calcium followed beer Lambert's law in the range of 5-30 µg/ml and graph was showed in **Fig. 2**.

TABLE 3: ABSORBANCE DATA OF PURE ATORVASTATIN CALCIUM IN PHOSPHATE BUFFER pH 6.8 AT 243 nm

S. no.	Conc. (µg/ml)	Absorbance (AM ± SD) *
1	0	0
2	5	0.213 ± 0.004
3	10	0.412 ± 0.002
4	15	0.611 ± 0.005
5	20	0.81 ± 0.003
6	25	0.988 ± 0.007
7	30	1.17 ± 0.008

*Each value represents the Mean ± Standard deviation (n=3).

Drug Compatibility Studies (FTIR Studies): The FTIR analysis of pure drug, polymer, and drug-loaded beads prepared by both the methods were analyzed with FTIR spectrophotometer (Shimadzu FTIR-8400, Japan). FTIR spectra of pure drug, pure sodium alginate, pure carbopol 934P, and polymer blend, after completion of studies it was indicated that there no major interaction occurred between the functional groups of the drug and used polymer blend upon mixing.

FT-IR analysis was performed using a sample of Atorvastatin with various excipients at 1:1 mass/mass ratio. Of them, few are having intensities in broad range, small, weak and medium intensity. Atorvastatin showed the presence of band hydroxyl group present in the region of 3200 cm^{-1} to 3400 cm^{-1} range; secondary amine was present in 2400 region; amine and disubstituted alkane will appear in the range of 2200-2400 region and 2100 to 2400 cm^{-1} region at weaker intensities. The alkane groups will appear in ranges of 800 to 1200 cm^{-1} with medium intensities, and 1640 cm^{-1} region indicates for the presence of ketone functional group. The range of FTIR frequency for pure Atorvastatin, sodium alginate, carbopol 934p, and the physical mixture is shown in **Fig. 3**, **4** and **Fig. 5**. In spectrum of sodium alginate, peaks observed particularly at 3369.75 cm^{-1} ,1649 cm^{-1}, 1435 $cm^{-1,}$ and 1033 cm^{-1} are assigned to stretching vibrations of O-H and H-bonded –COO stretching asymmetric (very strong intensity), symmetric shape (medium intensity) and C-C, -C-O and –COC of very strong sharp intensities respectively. Besides, other peaks

appeared at 929.72 cm^{-1}, 883 cm^{-1}, 810 $cm^{-1,}$ and 748 cm^{-1} are attributed to –CCH deformation of assignment, C-C stretching with weak intensity and to C-H vibration of pyranose group.

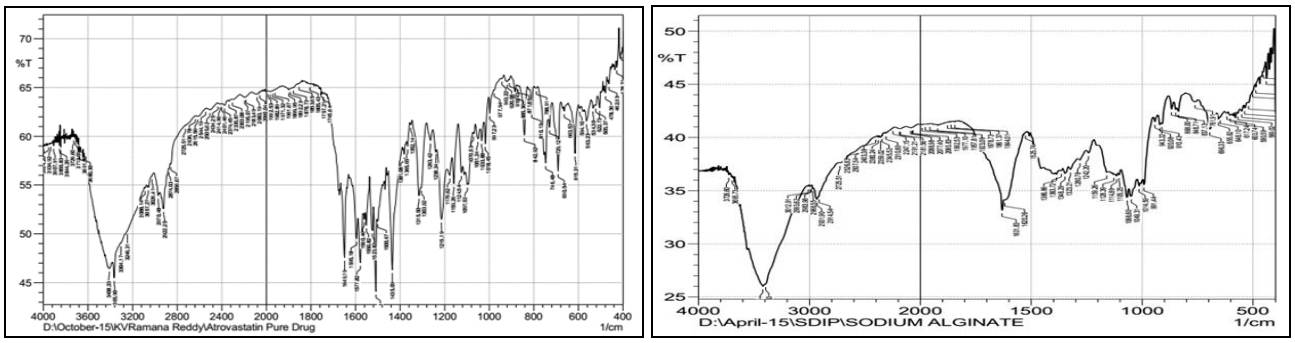

FIG. 3: FTIR SPECTRA OF PURE ATORVASTATIN (LEFT) AND PURE SODIUM ALGINATE (RIGHT)

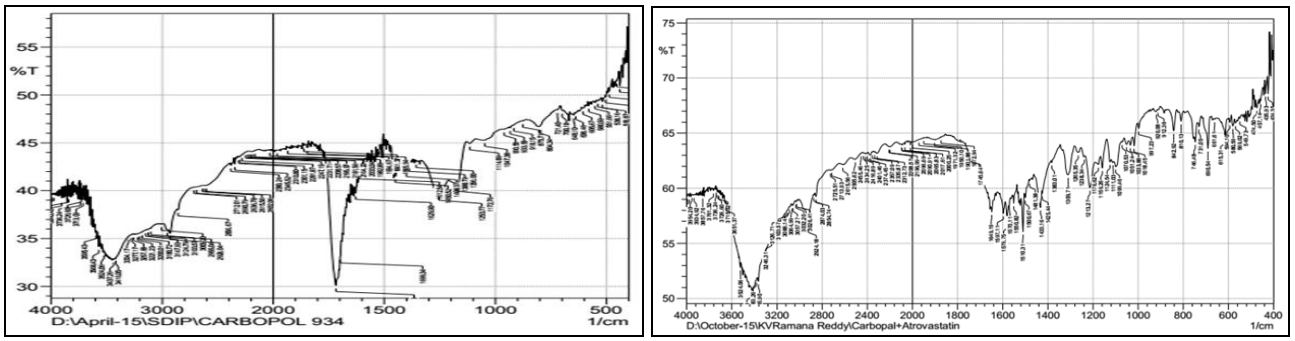

FIG. 4: FTIR SPECTRA OF PURE CARBOPOL 934P (LEFT) AND SPECTRA OF CARBOPOL 934P WITH ATORVASTATIN (RIGHT)

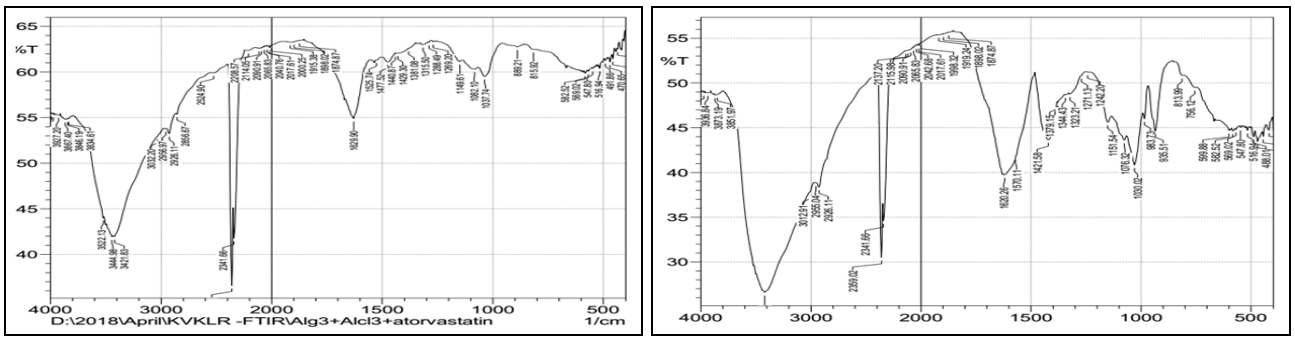

FIG. 5: FTIR SPECTRA OF MIXTURE CONTAINING ATORVASTATIN, SODIUM ALGINATE AND ALUMINIUM CHLORIDE (LEFT) AND MIXTURE CONTAINING ATORVASTATIN, SODIUM ALGINATE, CARBOPOL 934P (RIGHT)

The cross-linking process with aluminium ion caused an obvious. Shift to higher wave number and a decrease in intensity of COO^- and –C-C stretching peaks, and a decrease in intensity of 1033 cm^{-1} peak of alginate. The light changes in peaks due to the interference of aluminium ions in formation of cross-linked alginate beads. With the incorporation of Atorvastatin, the spectrum for the beads **Fig. 5** right side spectra) exhibited the peaks attributed to both aluminium alginate and Atorvastatin. The results revealed the compatibility of the drug with the excipients used in the formulation. The FTIR studies indicated the lack of drug-polymer interactions in all batches.

Micromeritic Characterization, Morphology and Particle Size of Atorvastatin Loaded Sodium Alginate Beads: Almost rheological parameters like bulk density, tapped density, compressibility index and angle of repose of prepared microbeads confirm desired flow and packaging properties. Of all, it is proved that increase in sodium alginate concentration from 1 to 3 % w/v, it leads to increase the particle size of microbeads and which result in a decrease in angle of repose. The values of both bulk density and tapped density of microbeads progresses as the concentration of sodium alginate increases from 1 to 3% w/v and this suggesting that the beads

formed at higher polymer concentration of sodium alginate, *i.e.* at 2 and 3% w/v are of compact in nature than 1% w/v of sodium alginate and with less intensity of porous surface than those prepared with less concentration of (1% w/v.) of sodium alginate only. The beads posses the requirement of good packability based upon good, acceptable ranges of both bulk density and tapped density values. The angle of repose was found to be 17.83 ± 2.06 to 25.85 ± 2.57 (º). The angle of repose is below that range of 30º which indicating good to excellent flow properties of the blend.

The characteristics like hausner's ratio and cars index ratio results indicate that prepared alginate beads formulated are having appreciate, desired compressibility and good flow properties. The powder blends of all the prepared formulations had hausner's ratio of less than 1.26 indicating the good flowability. The progressive results of small cross-linked alginate beads and their rheology suggest that microbeads can be easily handled throughout the process without any difficulty.

All the batches showed excellent flowability regarding angle of repose and compressibility index. All obtained alginate beads were subjected to SEM studies for determining exact shape and surface, and these studies reveal that formed beads are having nearly smooth with a spherical shape, nonporous and discrete in shape.

Normally, microbead obtained from natural polymers are not perfectly/exactly in spherical shape because of variations in the molecular weight and other properties of the polymer, but we obtained microbeads with a uniformly smooth surface, with no deformed surfaces particularly when alginate used in combination with carbopol 934P at low proportions. The surface of sodium alginate at higher concentration, *i.e*. at 3% w/v gives and forms rough surface small cracks along it. In comparison to barium alginate beads, calcium alginate beads develop cracks which are caused by collapsing of alginate polymer gel network during cross-linking time and during drying time, *i.e*. dehydration process. The degree of roughness on the surface of beads was high when formulated beads at low concentration than high concentration, *i.e.* 3% w/v. It proves that both natures of polymer and degree of cross-linking intensity had gained a significant effect on the morphological character of alginate beads. The size of alginate beads increased progressively upon increasing concentration of sodium alginate from 1% w/v to 3% w/v, and this could be due to an increase in the relative viscosity at higher concentrations of sodium alginate polymer, and we can observe this progress in batches of AB1, AB2 and AB3 for the beads obtained with calcium chloride as a curing agent at 5% w/v under 100 ml curing medium at 100 rpm gives particle size of 529.4 ± 1.32, 742.7 ± 1.06 and 1032.5 ± 2.04 µm respectively. The same phenomenon was observed for other batches which are prepared with barium chloride also. The size of beads in the $CaCl^{+2}$ solution was initially larger than those in the $BaCl^{+2}$ solution because the beads incubated in the $CaCl^{+2}$ solution did not form sufficiently compact and tight and had a low affinity than the beads incubated in the $BaCl^{2+}$ solution [22, 23].

Divalent curing agents naming barium and calcium ions are divalent; their bonding to sodium alginate is expected to occur in a planar two-dimensional network inside the lattice of the beads. Since barium ion has the largest radius, *i.e.* 1.74 Å compared to the other two cations (*i.e.*, 1.14 Å for Ca^{2+} and 0.68 Å for Al^{3+}, it is supposed to fill a larger space between the alginate molecules and producing a tight/compact arrangement with smaller void spaces.

Effect of Sodium Alginate Concentration: Sodium alginate concentration at 1% w/v, appeared to be too dilute to form stable, rigid, spherical shaped beads and forms loose crosslink network upon contact with curing agent at 5% w/v, but as the concentration raised to 2 to 3% w/v was desirable and forms stable cross-linked beads and progress viscosity of polymer solution also than comparison to earlier mentioned concentration, and makes easy extrusion through the 22 gauze needle without any difficulty.

At low polymer concentration (1 and 2% w/v), the orientation of polymeric network is loose with a greater hydrodynamic free volume which allows more of the liquid to be absorbed and produces a higher rate of swelling. This, in turn, facilitates diffusion of the drug molecule through the matrix and causes a higher amount of drug release.

On the other hand, at higher polymeric concentration, *i.e*. 3% w/v, an opposite phenomenon observed and resulting in the slower release of Atorvastatin.

The concentration of sodium alginate polymer influenced the control of drug release up to 10 h. The obtained statistical micro metric data of all batches are showed in **Table 4**.

TABLE 4: THE STATISTICAL DATA OF MICROMERITIC PROPERTIES OF BEADS

Batches	Mean particle size* (µm) (AM ± SM)	Angle of repose* (AM ± SM)	Bulk density* (AM ± SM)	Tapped density* (AM ± SM)	Carr's index* (AM ± SM)	Hausner's ratio* (AM ± SM)
AB1	529.4 ± 1.46	21.95 ± 1.47	0.194 ± 0.003	0.224 ± 0.01	13.39 ± 1.21	1.154 ± 0.004
AB2	742.7 ± 1.55	20.71 ± 2.06	0.19 ± 0.023	0.244 ± 0.001	18.44 ± 0.87	1.226 ± 0.005
AB3	1032.2 ± 1.09	17.83 ± 2.06	0.202 ± 0.01	0.256 ± 0.003	21.09 ± 1.21	1.267 ± 0.009
AB4	595.2 ± 0.63	22.12 ± 0.52	0.195 ± 0.003	0.215 ± 0.007	9.302 ± 0.21	1.102 ± 0.003
AB5	789.3 ± 1.46	23.24 ± 1.14	0.217 ± 0.004	0.261 ± 0.006	16.85 ± 0.14	1.202 ± 0.01
AB6	1060.4 ± 0.20	25.67 ± 1.55	0.208 ± 0.001	0.254 ± 0.003	18.11 ± 1.41	1.22 ± 0.04
AB7	480.4 ± 1.46	25.05 ± 1.54	0.198 ± 0.002	0.239 ± 0.001	17.15 ± 1.02	1.207 ± 0.003
AB8	670.7 ± 1.55	24.89 ± 0.24	0.201 ± 0.001	0.254 ± 0.003	20.86 ± 0.85	1.263 ± 0.002
AB9	930.2 ± 1.09	24.63 ± 2.53	0.228 ± 0.002	0.271 ± 0.003	15.86 ± 0.21	1.188 ± 0.004
AB10	550.2 ± 0.63	24.83 ± 1.44	0.194 ± 0.002	0.243 ± 0.001	20.16 ± 1.21	1.250 ± 0.031
AB11	685.4 ± 1.46	25.85 ± 2.57	0.202 ± 0.001	0.253 ± 0.001	20.15 ± 1.31	1.252 ± 0.001
AB12	950.9 ± 0.20	25.74 ± 0.97	0.215 ± 0.002	0.268 ± 0.002	19.77 ± 0.81	1.246 ± 0.002
AB13	850.9 ± 0.40	40.82 ± 2.07	0.698 ± 0.231	0.499 ± 0.103	28.51 ± 0.32	0.714 ± 0.31
AB14	1134.9 ± 0.20	25.13 ± 1.56	0.245 ± 0.02	0.320 ± 0.037	4.89 ± 0.027	1.306 ± 0.047
AB15	1095.2 ± 1.32	24.96 ± 2.39	0.279 ± 0.004	0.267 ± 0.005	20.76 ± 0.72	1.262 ± 0.007

*Each value represents the Mean ± Standard deviation, (n=3)

FIG. 6: ATORVASTATIN SODIUM ALGINATE WET BEADS PREPARED WITH BARIUM CHLORIDE AND CALCIUM CHLORIDE AS CURING AGENTS

Furthermore, polymer blend at the ratio of carbopol 934 P to sodium alginate of 0.3:3 ratio forms and gives well smooth/soft surface microbeads. The drug entrapment efficiency is more in beads designed with 3% w/v than 2% w/v concentration and the reason for this was explained elaborately in following words. Thus, the total polymer concentration of 3% w/w was used for subsequent batches to continue further studies.

An increase in concentration of sodium alginate resulted in production of larger and stable microbeads, which in turn entrapping higher amount of Atorvastatin drug during gelation process and this could be due to availability of greater quantity/number of active calcium/barium binding sites in the sodium alginate polymeric chains and consequently it results in the greater degree of cross-linking sites as the quantity of sodium alginate increased from 1 to 3% w/v. The concentration of polymer is directly proportional to the particle size, swelling index, and drug entrapment efficiency which might be due to the formation of the denser network upon addition of cross-linking agent and presence of higher molecular weight polymer.

The used divalent cations in this study, $CaCl^{+2,}$ and $BaCl^{+2}$ are used in high concentrations used as crosslinkers, *i.e*., at 5% w/v leading to form beads that associated with tight/compact gel matrix nearly sphere-like formation and forms low structural rearrangement to reduce the size of beads. The size of Atorvastatin loaded alginate beads depends not only on the concentration of sodium alginate as a polymer and also it will be affected by other

process variables, among those mainly by rpm is one of the parameters which determines the size of cross-linked alginate beads and concentration and type of curing agent *i.e.*, di/trivalence. The statistical data regarding particle size were summarized elaborately in the following table for each batch. It has been already reported that divalent curing agents mainly calcium cations bind preferentially to the poly guluronic acid units of alginate in a two-planar dimensional network, producing the so-called as egg-box pattern [24] structure like. At higher alginate concentration more, a number of both homo and heteropolymeric blocks of alginate are present that to be cross-linked, so that it obviously leads to maximum drug entrapment.

The Effect of Cations on Swelling: Because of the largest size of Ba^{2+} between the two crosslinking cations (*i.e.* 1.74 Å) its diffusion from the beads into the outer solution is relatively slow as compared to that of Ca^{+2}, this consequently leads to lower water uptake than of the beads crosslinked with calcium chloride. Hence, it can be indicating that the nature of crosslinking cations exerts a great influence on the rate of swelling and degradation behavior of cross-linked alginate beads. Moreover, the beads crosslinked with Ba^{+2} possess fair stability with a minimum percentage of water uptake. The beads formulated with aluminium chloride possess a low degree of swelling than barium and calcium alginate beads, and this could be due to a tight network of gelled beads and cross-linking done in the three-dimensional network. Reduced rate swelling of beads observed with low porosity on the surface of beads was observed with trivalent curing agent only, so that which leads to the low amount of buffer intake and less swelling during dissolution process happens when beads designed with aluminium chloride as a curing agent than calcium chloride as a curing agent.

Scanning Electron Microscope (SEM) Studies: After subjecting of SEM studies and close examination of all Atorvastatin loaded cross-linked beads with different curing agents at 3% w/v of sodium alginate and 3.3% w/v of sodium alginate and carbopol 934 as a physical polymer mixture, formed beads showed nearly spherical, discrete like shape, and accompanied with both rough surface as well as smooth surface **Fig. 7**, **8** and **9**.

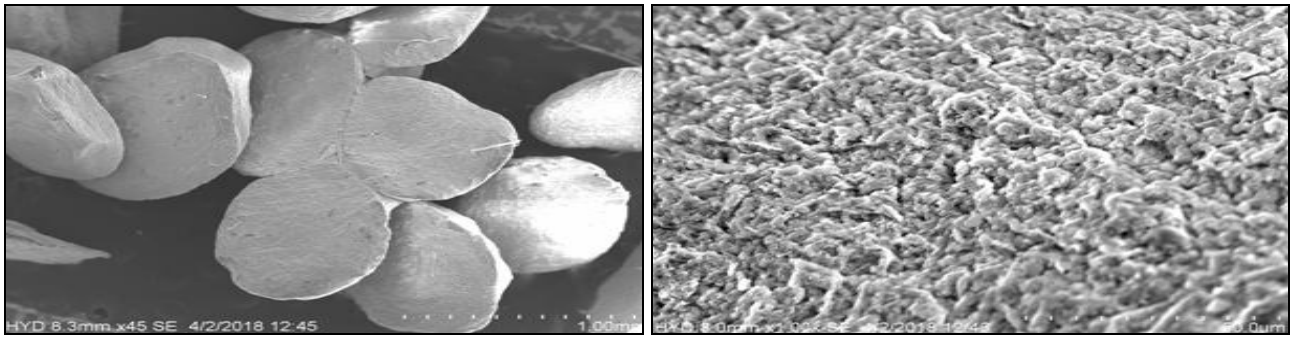

FIG. 7: SEM CONTAINING 3% SODIUM ALGINATE MICROBEAD WITH CARBOPOL 934P PREPARED WITH 5% w/v. BARIUM CHLORIDE AS A CURING AGENT BY USING IONIC GELATION METHOD. LEFT SIDE CONTAINING INDIVIDUAL BEADS AND RIGHT SIDE CONTAINS SURFACE OF BEAD WITH 1000 MAGNIFICATION

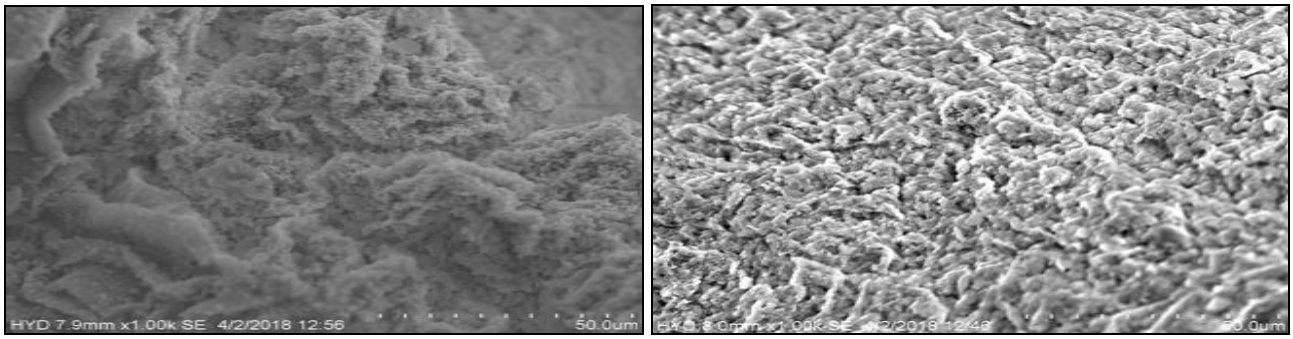

FIG. 8: SEM PICTURES CONTAINING 3% w/v SODIUM ALGINATE WITHOUT DRUG PREPARED WITH 5% BARIUM CHLORIDE (LEFT SIDE) AND SODIUM ALGINATE WITH CARBOPOL (0.3% w/v) CONTAINING DRUG PREPARED WITH 5% BARIUM CHLORIDE (RIGHT SIDE)

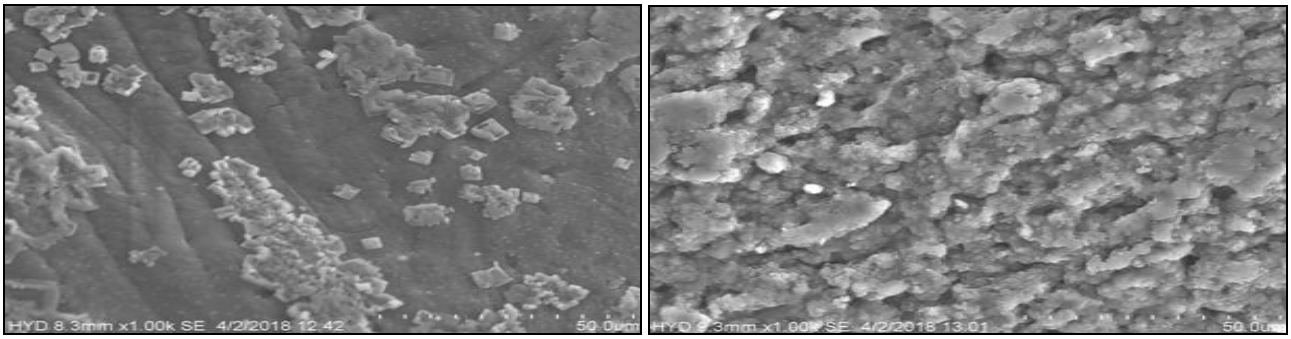

FIG. 9: SEM PICTURES CONTAINING 3% w/v SODIUM ALGINATE WITHOUT DRUG PREPARED WITH 5% CALCIUM CHLORIDE (LEFT SIDE) AND SODIUM ALGINATE WITH CARBOPOL (0.3% w/v) CONTAINING DRUG PREPARED WITH 5% CALCIUM CHLORIDE (RIGHT SIDE)

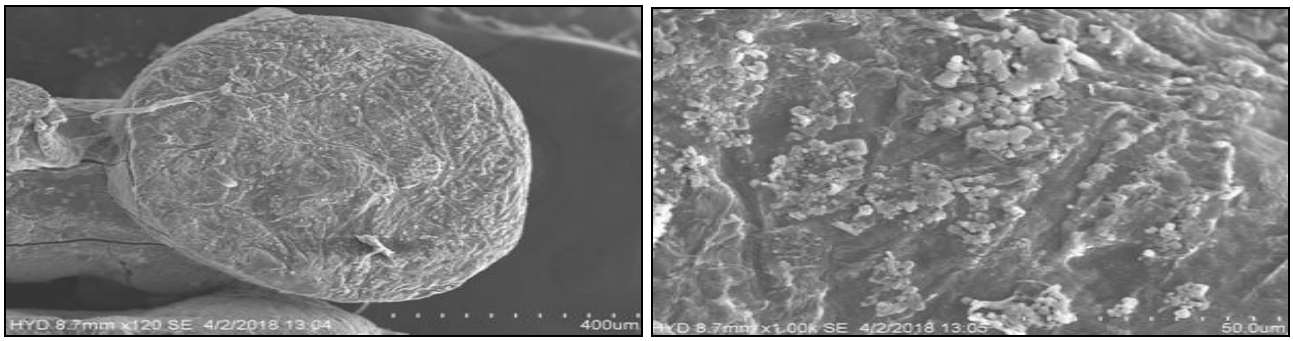

FIG. 10: SEM PICTURES CONTAINING SODIUM ALGINATE MICROBEADS WITH CARBOPOL 934P PREPARED WITH ALUMINIUM CHLORIDE (RIGHT SIDE) AND ALUMINIUM CHLORIDE AS A CURING AGENT AT 5% w/v. BY 1000 MAGNIFICATION (LEFT SIDE)

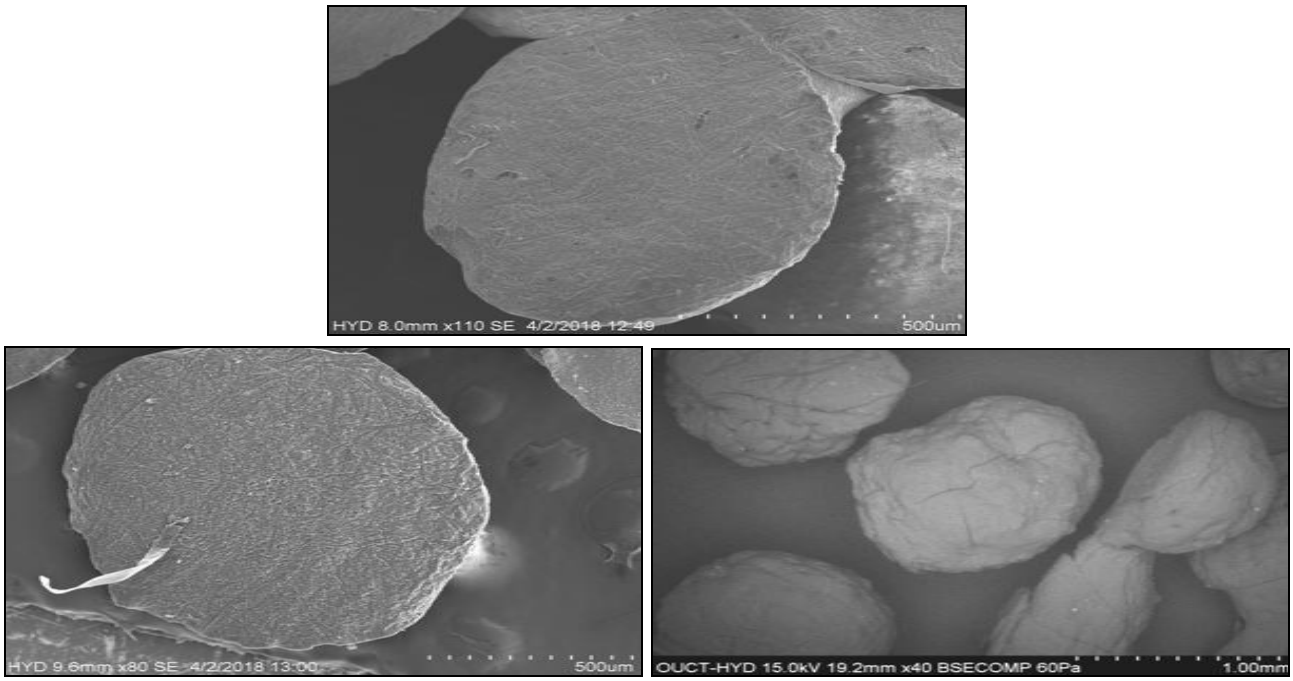

FIG. 11: SEM PICTURES CONTAINING SODIUM ALGINATE (3% w/v) MICROBEADS WITH CARBOPOL 934P (0.3% W/V) PREPARED WITH CALCIUM CHLORIDE (LEFT IMAGE) BARIUM CHLORIDE (MIDDLE IMAGE) AND ALUMINIUM CHLORIDE (RIGHT PICTURE) AS A CURING AGENT AT 5% w/v

It is amazing to see that the presence of little porous in appearance particularly beads prepared with calcium chloride as a curing agent, and deposition of drug crystals on the surface of beads non uniformly was also observed. The beads formulated with both sodium alginate and carbopol in 3:0.3 proportions in combination with drug shows beads with smooth and thick in appearance and forms large size beads than prepared without carbopol 934P and sodium alginate only.

The deposition of drug crystals on the surface of cross-linked alginate beads took place as a result of migration/diffusion of the drug along with curing medium to the surface during drying at room temperature. The development of few cracks also seen on the surface of alginate bead which caused by collapsing of alginate polymer network during cross-linking mechanism and drying period.

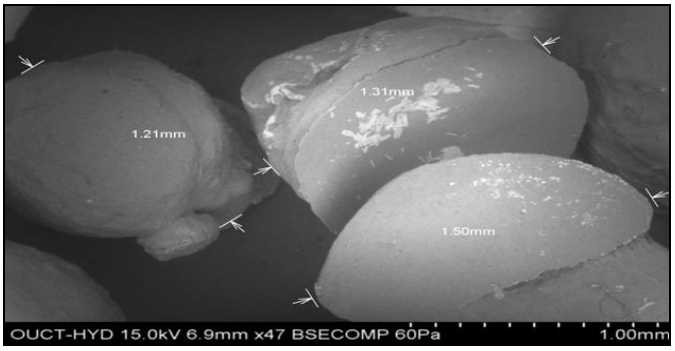

FIG. 12: SEM PICTURES FOR OPTIMIZED BATCH CONTAINING SODIUM ALGINATE MICROBEADS WITH CARBOPOL 934P PREPARED WITH ALUMINIUM CHLORIDE BY IONIC GELATION METHOD ONLY

This indicates that polymer nature, polymer concentration, a combination of polymer, type of crosslinking agent, the degree of cross-linking intensity had a major role and marked effect on the morphological character of developed cross-linked Atorvastatin alginate beads. The beads prepared with trivalence curing agent showed a high degree of cross-linking intensity than compared with divalent curing agent ex. calcium chloride and barium chloride and show less rigid gelled matrix than comparison to more rigid matrix surface with aluminium chloride at higher concentrations as a curing agent. The SEM picture for cross-linked aluminium alginate beads predicted clearly in **Fig. 10**.

Yield: The percentage yield value was found to be increased with a gradual increase in concentration of sodium alginate polymer from 1 to 2% w/v and this happens due to the increase in viscosity of physical mixture as concentration of sodium alginate increased from lower to higher concentration and which in turn leads to promote thickness of microbead surface and formation of maximum cross-linking density at higher concentration of sodium alginate. But above 2% of alginate, *i.e.* at 3% w/v of sodium alginate, the yield value was subjected to decrease little bit than 2% w/v alginate solution and this occurred in both curing agents and as well as at 100 and 200 rpm also, the reason could be due to wastage of alginate mixture during discharging from needle into curing solution. The least value was found to be 44.5% w/v for AB4 batch, and maximum yield value was found to be 80.43 for AB15 batch. The percentage of yield for all batches was summarized in following and **Table 5**.

Effect of RPM on Yield: At slow rpm, the beads not developed cross-link properly in its strength and at higher rpm surface of beads were disrupted because of generation of high shear force. At 100 rpm it gives less yield than compared at 200 rpm because at 100 rpm beads will develop loose crosslink network with the improper surface with weak-walled alginate beads and formed soft surface beads than compared to beads prepared at 200 and 300 rpm. In contrast to beads designed at 200 rpm, the beads formulated at 300 rpm the yield value was reduced to marked level and surface of cross-linked alginate microbeads will be almost damaged and improper in both their size and surface and this occurs because of generation of higher energy caused by mixing solution at higher rpm rate (300) which will throw the soft beads against walls of container during curing time and leads to formation of damaged beads, so by observing previous situations, only 200 rpm was considered as a best of choice and finally optimized for carrying further studies than prepare beads at 100 and 300 rpm. Accordingly, the stirring rate was kept and finalized at 200 rpm, and same 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 of the polymer mixture.

Drug Entrapment Efficiency: Drug entrapment efficiency ranges from 28.22 ± 1.43% to 58.90 ± 0.53%. Good entrapment efficiency was achieved by increasing the sodium alginate polymer concentration from 1 to 3% w/v [26]. The drug entrapment efficiency was also related to the particle size of microbeads, availability of cross-linking sites on alginate chains and type of curing agent used in this study. It is proved that; the entrapment efficiencies were determined to increase as their mean particle sizes of beads were increased. It is very clear that upon increasing concentration of polymer from 1 to 3% w/v, it

reduces loss of diffused drug in curing medium during cross-linking process due to formation of dense matrix structure and increase in cross-linking density of cross-linking network as increase in no. of apparent cross-linking sites at higher concentrations of sodium alginate than low alginate concentration.

Of both curing agents calcium chloride and barium chloride, the beads prepared with barium chloride as a curing agent shows maximum drug entrapment than beads prepared with calcium chloride and this could be due to the formation of pores occurs/as seen on surface of calcium alginate beads and reduced size of calcium alginate beads in diameter than barium chloride alginate beads and it also said to be due to the reason of tight cross-linking in the presence of barium chloride over calcium chloride as curing agents at same concentrations. The percentage of drug entrapment for all different batches was showed in **Table 5** and displayed in **Fig. 13**.

TABLE 5: DRUG ENTRAPMENT EFFICIENCY, YIELD AND MEAN PARTICLE SIZE OF CROSS-LINKED ATORVASTATIN ALGINATE BEADS

Code	Drug encapsulated efficiency* (%)	Yield (%)
AB1	28.22 ± 1.43	47.32
AB2	37.45 ± 1.76	72.30
AB3	50.28 ± 1.03	69.53
AB4	32.05 ± 1.84	44.35
AB5	40.65 ± 1.93	69.53
AB6	52.84 ± 1.21	66.05
AB7	35.21 ± 3.62	68.21
AB8	40.06 ± 1.82	77.20
AB9	53.43 ± 0.98	75.50
AB10	38.83 ± 1.87	67.46
AB11	46.85 ± 1.52	72.04
AB12	54.24 ± 1.05	70.25
AB13	40.03 ± 2.93	65.32
AB14	55.65 ± 2/41	78.60
AB15	58.90 ± 0.53	80.43

*Each value represents the Mean ± Standard deviation (n=3).

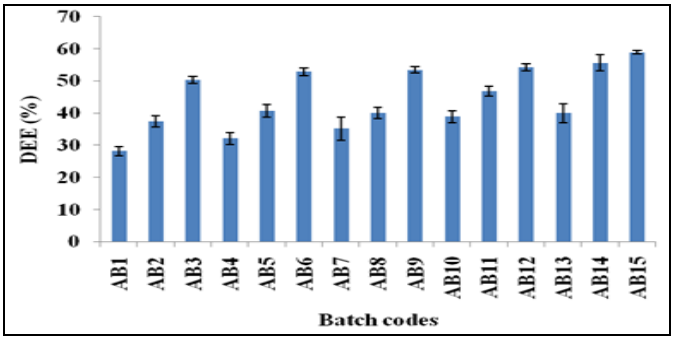

FIG. 13: COMPARISON OF PERCENTAGE OF DRUG ENTRAPMENT EFFICIENCY OF DIFFERENT BATCHES

Beads prepared with aluminium chloride as a curing agent forms low porosity on surface of alginate beads than comparison to both barium alginate beads and calcium alginate beads and it occurs due to high magnitude of tight cross-linking network occur with trivalence curing agent only, *i.e.* $AlCl^{3+}$ and existence of three dimensional network occurs with $AlCl^{3+}$ than two dimensional network formed with barium chloride/calcium chloride to alginate chains.

The batch AB15 and AB14 contains physical mixture of carbopol 934P and sodium alginate polymers at proportion of 0.3:3% w/v and provides more drug entrapment than AB12 and AB13 batches, because upon extra addition of carbopol 934P to sodium alginate polymer leads to extra entrapment of drug than those batches AB12 and AB13 done without carbopol 934P. This extra amount of carbopol forms very thick gel matrix beads, smooth surface beads and decrease the porosity of alginate beads further so that it prevents leakage of drug migration into curing medium during cross-linking done one hour to promote harden the surface of soft beads. At high concentration of aluminium chloride hydrogel matrix is seen as rigidly and fragile, so that drug loss polymer matrix is low and results in the high percentage of drug entrapment.

RPM Effect: Stirring speed is a second important parameter next to sodium alginate concentration and place importance role in the cross-linking step because it provides the sufficient energy to diffuse $CaCl^{+2}$ / $BaCl^{+2}$ in the form of the curing/curing agent into the sodium alginate bead. The mean particle size of sodium alginate microbeads decreased from 1025 ± 1.3 µm to 980.4 ± 1.56 µm with increasing stirring rate from 200 to 300 rpm during process and this difference suggests that particle size of beads was controlled by subjecting rpm from lower rpm to higher rpm during gelation process but at same time it can also be observed that it may result in formation of irregularly shaped microbeads when rpm set at 300 and same results observed/reported by Jain *et al*., 2004 in his studies. The microbeads formed at 100 rpm leads to form coalescence and formation of little aggregation of beads when alginates used at 3% w/v concentration medium stirring speed, *i.e.* at 200 rpm results in high shear and kinetic energy

than at 100 rpm and thus prevent microbeads agglomeration. The above data proves/suggest that up to a certain range of rpm, *i.e.* 100 to 200 rpm, it can predict the reduce in of size of sodium alginate beads but beyond certain range, *i.e.* from 200 to 300 rpm it gives different results with respected to bead size, *i.e.* size is reduced further and formed improper, damaged surface beads and this could be happened due to the reason of generation of high energy which throws all the soft formed beads to the surrounding walls of glass beaker and few are damaged by bombarding/by throwing against blades/wings of sigma blade mixer during gelation process.

At this higher stirring speed, *i.e.* 300 rpm, a vigorous, uniform, increased mechanical shear might have taken place. The low, stirring speed, *i.e.*, 100 rpm might have decreased the uniformity of mixing force throughout viscous solution of sodium alginate, and this force is not enough to promote the penetration/diffuse of curing agent, *i.e.* $Cacl^{+2}$ and $BaCl^{+2}$ into alginate beads, thereby it gives/forms small size alginate beads than that of beads obtained at 200 rpm, and it was observed decreasing in mean particle size of alginate beads under 100 rpm at 1% w/v of sodium alginate particularly in batches of AB1, AB4 because of low viscosity of alginate solution at 1%.

On decreasing the stirring speed from 300 to 200 rpm, it was observed an increase in mean particle size of beads and avoids the formation of cracked and damaged soft beads. Nevertheless, a stirring speed of 200 rpm was found to be optimal, yielding microbead of uniform size to the desired level and with a narrow size range and spherical shape among 100, 200 and 300 rpm. The results suggest that the mean particle size of cross-linked alginate microbeads was inversely proportional [27] to the stirring speed, as increasing the stirring speed resulted in small sized, damaged microbeads. This suggests that the size of the microbeads formed during ionic gelation might, therefore, be closely related to the size of the final microbeads. An inverse relationship takes place between mean size and stirring speed. The 200 rpm only was considered as an optimized parameter regarding rpm to continue the further studies. The beads formed at higher rpm produce improper and small damaged beads than prepared same at lower rpm [28].

Differential Scanning Calorimetry: The DSC data provides both qualitative and quantitative knowledge about physicochemical state inside microbeads of drug DSC thermogram of pure sodium alginate, pure carbopol, and pure Atorvastatin and cross-linked optimized batches were showed in **Fig. 14**, **15** and **16**.

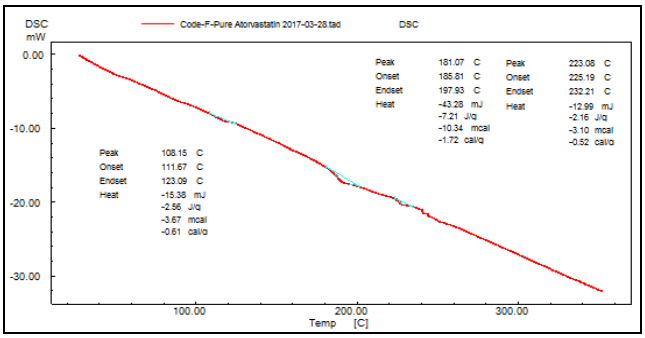

FIG. 14: DSC SPECTRA OF PURE ATORVASTATIN

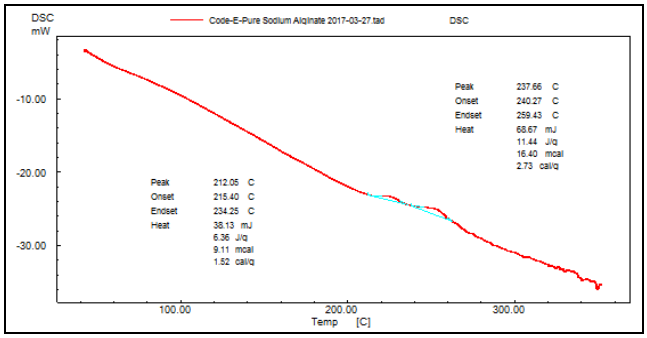

FIG. 15: DSC SPECTRA OF PURE SODIUM ALGINATE

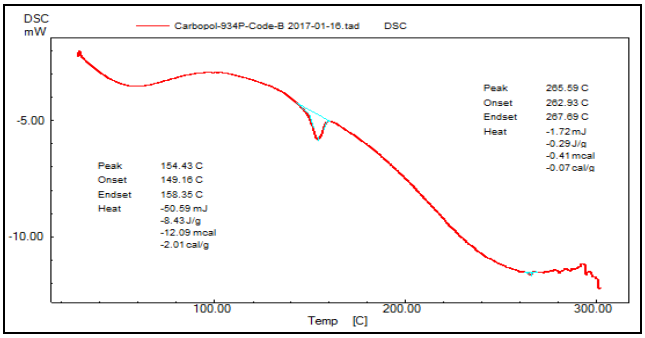

FIG. 16: DSC SPECTRA OF PURE CARBOPOL 934P

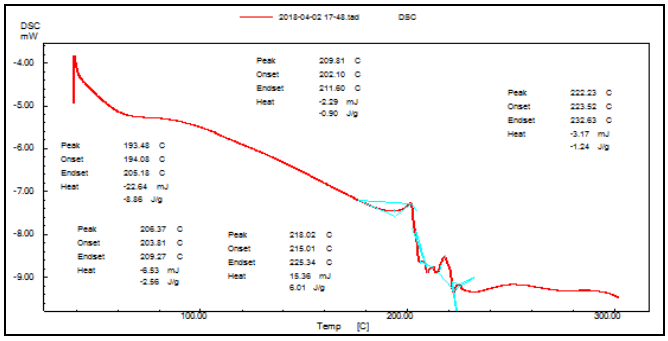

FIG. 17: DSC SPECTRA OF ATORVASTATIN MICROBEADS CONTAINING SODIUM ALGINATE, CARBOPOL 934P PREPARED WITH CALCIUM CHLORIDE AS A CURING AGENT

The DSC thermogram of pure sodium alginate showed both endotherm and exothermic at nearly 212.05 °C and 237.66 respectively. The DSC spectra of pure carbopol 934P show one endotherm at 154.43 °C and exothermic at 265.59 °C and this second exothermic peak can be due to degradation of carbopol 934P upon the further heating process. In pure Atorvastatin show endothermic at three different places and first is at 108.15 °C, 181.07 °C as on onset peak and exothermic peak at 223.08 °C. In a physical mixture of cross-linked Atorvastatin alginate beads prepared with aluminium chloride containing carbopol 934 P, sodium alginate and shows various endothermal and exotherm peaks with slight modification and pure Atorvastatin peak at 57.97 °C and 173.01 °C was appeared but slightly shifted from 181.07 °C to 173.01 °C that indicating absence of any interactions between the drug and polymers used in formulation.

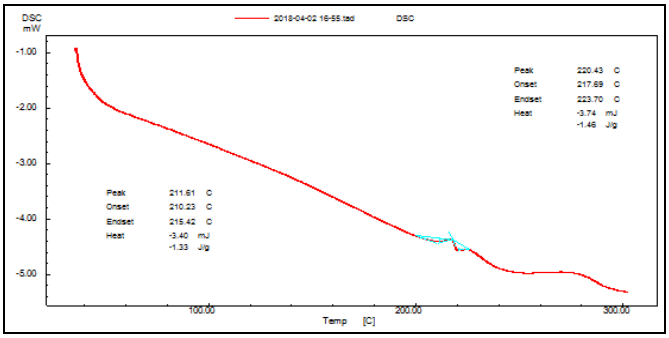

FIG. 18: DSC SPECTRA OF ATORVASTATIN MICROBEADS CONTAINING SODIUM ALGINATE, CARBOPOL 934P PREPARED WITH BARIUM CHLORIDE AS A CURING AGENT

And it is sure that enthalpy decrease when compared with pure drug and it is due to result of plasticization effect that occurs of reduction in attractive forces between alginate polymer chains and due to the partial reduction in crystallinity nature. The DSC study suggest that Atorvastatin did not dissolve polymer mixture completely and it seems even be involved slightly in cross-linking process. The changes in endotherm in the optimized batch may be due to Atorvastatin was dispersed in molecular level uniformly. There is a decrease in peak intensities for Atorvastatin loaded aluminium alginate beads; this could be because the drug may have existed inside the cross-linked beads in semi-crystalline form only. The sodium alginate exists as an amorphous form in a pure state, and it becomes trapped among the growing crystals of Atorvastatin so that as a result of high entangled nature of polymer chains the movement of amorphous sodium alginate polymer becomes restricted lightly and semi-crystalline phase formed.

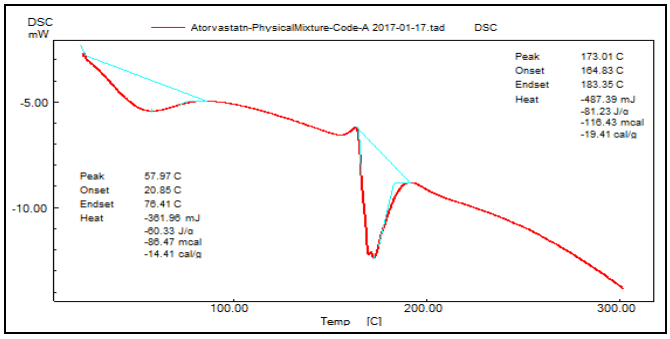

FIG. 19: DSC SPECTRA OF ATORVASTATIN MICROBEADS CONTAINING SODIUM ALGINATE, CARBOPOL 934P PREPARED WITH ALUMINIUM CHLORIDE AS A CURING AGENT

In DSC of a physical mixture of optimized batch, there is neither disappearance of existing peaks, nor new peaks appeared, and this indicates that there is no polymer drug interaction occurred in aluminium alginate beads congaing Atorvastatin as a drug. One interesting point is that one peak observed with decreased intensities that reflects to the occurrence of the dilution effect.

X-Ray Diffraction Studies (XRD): In order to confirm the physical state of Atorvastatin in beads, the X-ray diffraction studies of pure drug, added polymers were carried out, and X-ray diffraction pattern of the pure Atorvastatin has shows characteristic high-intensity diffraction peaks between 0° and 80 (2θ) due to its crystalline state of drug and few of major points discussed herein an elaborated manner. For pure Atorvastatin it shows peaks at different positions and most important diffractions 10.256°, 14.136°, 16.8427°, 19.2200°,

22.883°, 26.1533°, 28.0200°, 30.6125°, 32.9700°, 43.0700°, and 43.636°, *etc*. The diffractograms of both pure drug and optimized batch beads are almost identical in appearance. It is confirmed that the reflections of the pure drug match satisfactorily with the reflections of the drug in the optimized micro beads formulation. The disappearance of few intense peaks in a physical mixture of optimized batch indicates that drug has been dispersed uniformly in molecular level throughout the surface of beads.

Thus, it can be concluded that the polymorph of the pure drug was the little bit same as that of Atorvastatin and sodium alginate polymorph incorporated in microbeads, indicating the amorphous of dispersion of drug after entrapment into the cross-linked alginate beads and there is no major transformation took place majorly during the manufacturing process and storage. The changes in the crystalline state of Atorvastatin occurred during ionic gelation method.

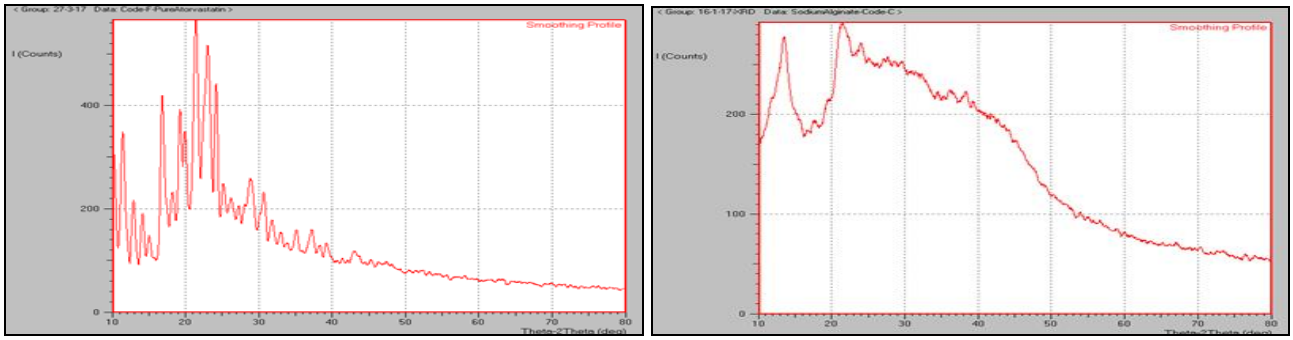

FIG. 20: XRD SPECTRA OF PURE ATORVASTATIN (LEFT IMAGE) AND PURE SODIUM ALGINATE (RIGHT IMAGE)

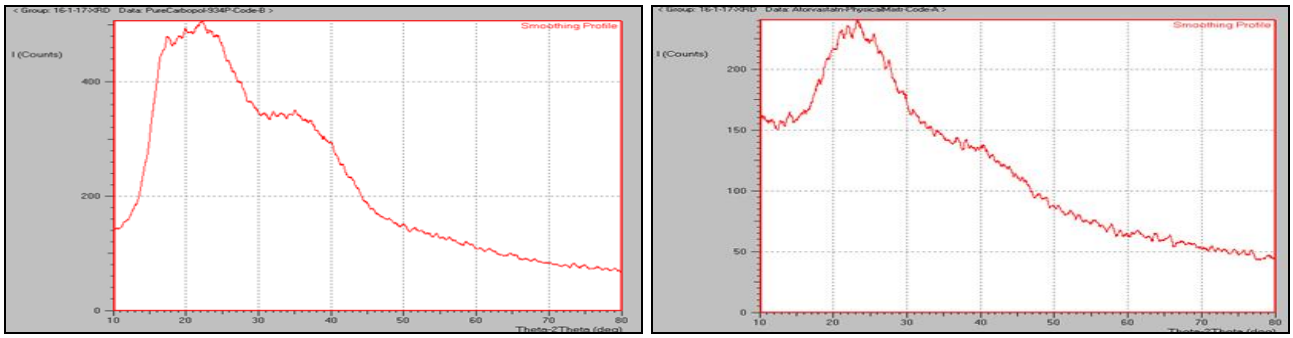

FIG. 21: XRD SPECTRA OF PURE CARBOPOL 934P (LEFT IMAGE) AND PHYSICAL MIXTURE (RIGHT IMAGE)

When aluminium chloride used in high concentrations there was no much significant changes in peak position takes place and few found except as a decrease in the magnitude of intensity of peaks and it occurs due to dilution effect of Atorvastatin drug with aluminium chloride all other peaks occurred with lower intensities than pure Atorvastatin, so it can clearly suggest that drug, sodium alginate, carbopol 934P, and aluminium chloride as a physical mixture (**Fig. 22** right side image) at high concentration and no significant interactions took place.

Physical Evaluation Method:

Shear Stress Measurement:

Procedure: Various concentrations of muco-adhesive material solutions such as a clear, viscous solution of sodium alginate ranging from 1%, 2% and 3% w/v were prepared freshly. A few drops of the viscous solution is placed in between two plates, and a one-hundred-gram weight was placed on the upper plate to spread the solution equally in all directions in between the two glass plates. After a span of 15 min, the weight kept on the upper plate is removed carefully. The weights which hold on the pan are gradually increased until the upper plate is detached slowly from the fixed lower plate.

The experiment is again performed by prolonging the contact time of the viscous solution between the two plates for different intervals of time. The weight in gm hold on glass surfaces is noted exactly. Similarly, the test is repeated three times by using the following solutions. The higher weight required to detach the plate represents the better mucoadhesive property of the materials.

The different weights for each material at their respective concentration are represented in **Table 6** and histogram at different time intervals was shown in **Fig. 23**. The comparative mucoadhesive properties of the tested materials of different batches AB1, AB2 and AB3 and consist of sodium alginate in range of 1%, 2% and 3% w/v only. The mucoadhesive property of the sodium alginate depending upon concentration, of all three different concentrations among 1, 2 and 3% w/v and 3% w/v possess the maximum mucoadhesive property and were on par in comparison to less percentage of sodium alginate regarding mucoadhesive strength.

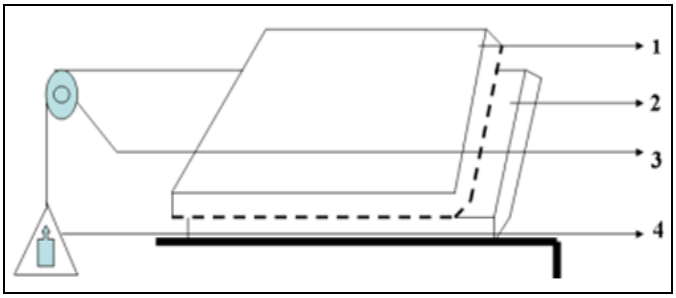

FIG. 22: APPARATUS FOR SHEAR STRESS MEASUREMENT. 1. An upper glass plate, 2. A lower glass plate, 3. Pulley and 4. Weight pan.

Upon increasing concentration of sodium alginate, the viscosity of solution leads to develop and it restrict free movement and develops the more sticky and strength, so that it takes more time to detach plate from the surface of the lower glass plate when compared to the low concentration of sodium alginate of 1 and 2% w/v contained in AB1, AB2 and AB3 batches, with regards to the above-discussed subject, high mucoadhesive strength occurs at high concentration of sodium alginate only and as per this high alginate concentration, *i.e.* 3% w/v was considered and optimized in further studies.

TABLE 6: STATISTICAL REPORT OF SHEAR STRESS MEASUREMENT

Batch code	Conc. of polymer (% w/v)	Weight in gm required to detach plate at different intervals*		
		15 Min	30 Min	60 Min
Sodium alginate	1	139.03 ± 1.45	159.75 ± 1.35	201.82 ± 1.03
	2	189.23 ± 1.32	206.36 ± 2.02	258.38 ± 1.86
	3	201.23 ± 2.01	237.39 ± 1.54	280.72 ± 2.75

*Each value represents the Mean ± Standard deviation (n=3).

On basis of above statistical data **Table 6**, it was proved that concentration of sodium alginate was directly proportional to shear stress and takes offers more weight in gm to detach plate as concentration of polymer increases in comparison to low concentration of sodium alginate (201.82 ± 1.03) against high concentration of sodium alginate (280.72 ± 2.75) by the end of 60 min.

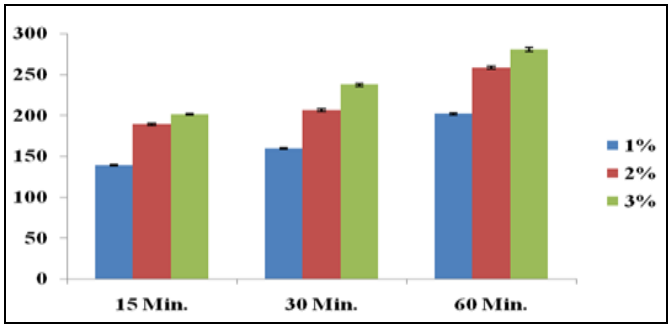

FIG. 23: HISTOGRAM CONTAINING SODIUM ALGINATE IN DIFFERENT BATCHES AB1 (1 %), AB2 (2%) AND AB3 (3%) AT DIFFERENT TIME INTER-VALS. X-axis represents time (min.) y-axis indicates in weight (gm).

The *In-vitro* Drug Release of Atorvastatin Alginate Beads: *In-vitro* release profiles of Atorvastatin loaded sodium alginate microbeads carried in both mediums namely in 0.1N hydrochloric acid and 6.8 pH phosphate buffer, and drug release pattern are shown in **Fig. 24**, **25**, **26**, **27** and **Fig. 28** respectively.

Sodium alginate is insoluble in acidic medium i.e.at 0.1N HCl solution, and at this low pH the total number of positively charged ion strength is high, and they reduce the intensity of electrical repulsion forces between negative charged sodium charged ions. Since, alginate is protonated into an insoluble form of alginic acid molecules, thus migration of dissolution medium through alginate polymer matrix is hindered to some extent and because of this event Atorvastatin drug released was minimal at acidic medium for first two hours under 70 rpm.

In later after replacement of acidic medium with 6.8 phosphate buffer the drug release is increased to more than 2 or 3 times level. At this alkaline pH deprotonation of alginic acid took place and it will highly favor dissolution medium easily/at faster rate into interior of cross-linked alginate beads and beads will get more percentage of swelling of alginate polymer by undergoing relaxation of sodium alginate polymer and drug release takes place rapidly in comparison to in acidic medium **Fig. 24** and **25**.

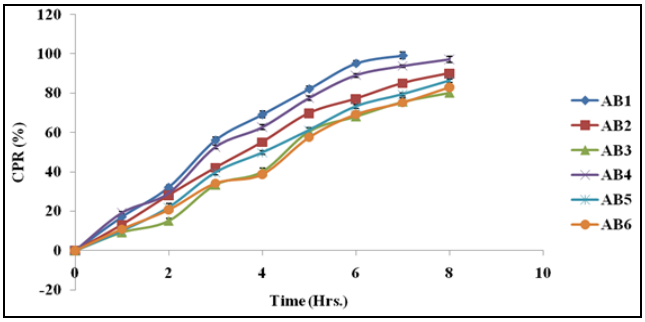

FIG. 24: DRUG RELEASE PROFILE OF ATORVASTATIN ALGINATE BEADS FROM BATCHES AB1-AB6. *Each value represents the Mean ± Standard deviation (n=3).

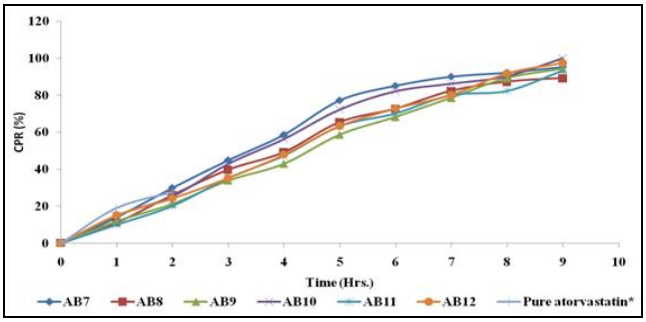

FIG. 25: DRUG RELEASE PROFILE OF ATORVASTATIN ALGINATE BEADS FOR BATCHES AB7 TO AB12 BATCH AND PURE ATORVASTATIN. *Each value represents the Mean ± Standard deviation (n=3).

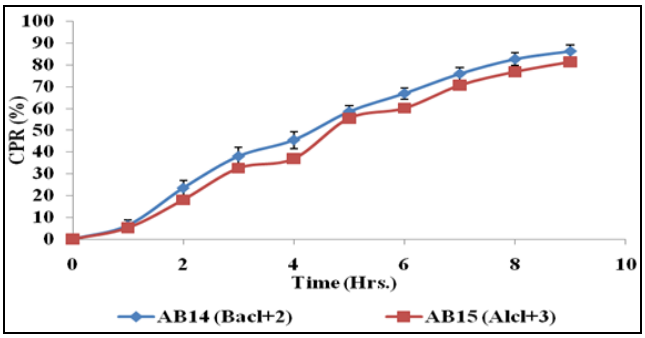

FIG. 26: DRUG RELEASE PROFILE OF ATORVASTATIN ALGINATE BEADS FORMULATED WITH DIVALENCE AND TRIVALENCE CURING AGENTS. *Each value represents the Mean ± Standard deviation (n=3).

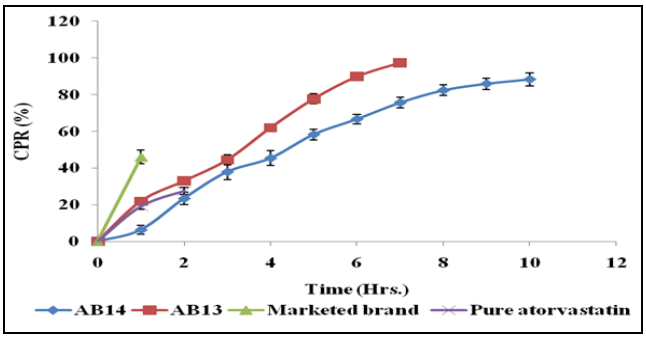

FIG. 27: DRUG RELEASE PROFILE OF BATCH 13, BATCH 14, PURE ATORVASTATIN AND MARKETED BRAND. *Each value represents the Mean ± Standard deviation (n=3).

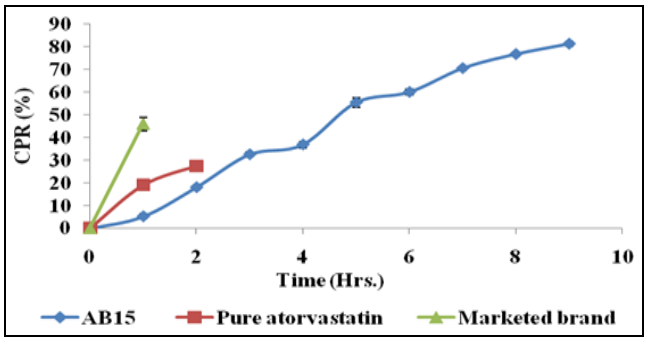

FIG. 28: DRUG RELEASE PROFILE OF OPTIMIZED BATCH (AB15), PURE ATORVASTATIN AND MARKETED BRAND. *Each value represents the Mean ± Standard deviation (n=3).

The drug release was totally depending upon, and it is affected by the concentration of polymer highly and at high concentration, *i.e*. 3% w/v. Diffusional resistance to Atorvastatin drug release also enhanced finally it leads to a decrease in the percentage of drug release, and it also depends upon majorly on the type of cross-linking used and, on their valence, also simultaneously. The extent of drug released based upon the presence of valence ions present on both divalent and trivalence curing agents aluminium chloride and barium chloride.

Of all batches, it was confirmed, and it indicates that release of drug was indirectly proportional to the amount of polymer concentration used, and at 3% w/v only sustained release and extends release observed than comparison to 1 and 2% w/v of sodium alginate concentration. As per this concept only 3% w/v recommended in subsequent batches naming both AB12 and AB13 batches.

The disintegration of alginate polymer was closely monitored by exchange of aluminium in aluminium chloride, calcium in calcium chloride and barium ion in barium chloride as a curing agent at 5% v/v concentration with potassium ions in present in dissolution medium and first 2 h drug release takes place less than 20% only and that occurs through small pores or cracks appeared on surface of alginate beads. The intensity of swelling is said to

be low in acidic medium for first two hours when beads placed in the acidic medium, in contrast, percentage swelling takes more in 6, 8 phosphate buffer mediums as alginate undergoing relaxation of polymer chains.

In **Fig. 26** the batches AB14 and AB15 composition contained sodium alginate with carbopol 934 P in 3.3% w/v but prepared with different curing agent barium chloride and aluminium chloride which was differ only in their valence of ions, it shows marked response towards drug release. The beads prepared with barium chloride forms only two-dimensional cross-linking network and forms large beads when comparison to beads done with aluminium chloride at the same concentration and the possibility of three-dimensional cross-linking network and surface of beads seems to be compact and smooth surface without much pores seen in SEM studies **Fig. 12** and mostly size of alginate beads produced were smaller because trivalence curing agent posses maximum cross-linking intensity than corresponds to divalent compound, *i.e*. barium chloride.

But here embarrassing moment is that than compared to size of beads and its influence to dissolution of beads, the intensity of cross-linking forms important step and it was higher in aluminium chloride beads than barium chloride, so aluminium chloride alginate beads will undergo less percentage of swelling rate because of tight cross-linking network and less number of pores that all leads to release the drug the slowly in a sustainable manner in comparison to barium alginate beads. Barium chloride has largest size 1.74 as compared to aluminium chloride ions 0.68 its is expected, and surely it forms strong, rigid microbeads with less number of smaller voids and absorbs only low dissolution medium during *in-vitro* studies. Overall to be explaining simply drug release was highly dependent upon contraction and relaxation of polymer chains that occurs in acidic and alkaline mediums. The aluminium crosslinked Atorvastatin microbeads form three-dimensional bonding structure with hydrophilic polymer carbopol 934P inside the microbeads. This orientation of three-dimensional bonding results in extended crosslinking through the whole microcapsule producing compact or hard and smooth surface microbeads which cause lower water uptake inside alginate beads and this leading to slow discharging of the drug in the 6.8 pH phosphate buffer medium up to 10 h. For batch 15 consist of aluminium alginate, Atorvastatin beads released drug at the end of 10 h will be 84.5 ± 2.74% only then compared to barium alginate Atorvastatin beads as a curing agent releases drug only 88.38 ± 3.58% respectively under same standard conditions.

Pure Atorvastatin drug release drug 27.45 ± 1.87 by the end of 2 h itself. The market brand (Zivast 10 mg) of Atorvastatin releases 46.02% of drug in the first hour only, so based upon above criteria, and upon comparison of different batches against marketed brand and pure sample only the batch AB15 is high suits to produce sustained release pattern and possible to minimize the intensity of producing side effects and to improve patient compliance.

The intensity of gelling cation on microbead swelling and concluded that calcium alginate microbeads were more prone [29] to swelling than the corresponding Ba-alginate beads. Swelling of microspheres is an important factor affecting the release of a drug incorporated in them. It has been found that drug release from highly hydrated carbopol 934P microbead is slower than that from alginate alone microbeads, *i.e*. without carbopol 934P. Slow cumulative drug release from microbeads may be attributed to the increased in the density of the cross-linking polymer matrix at 3.3% w/v and also to an increase in the diffusional path length that the drug molecules have to traverse. The swollen polymeric network might act as a barrier to penetration of the buffer medium, *i.e*. dissolution medium [30, 31], thereby rendering the suppressing the rate of diffusion of Atorvastatin drug molecule from the swollen polymeric matrix.

The physicochemical properties of drug and polymer, as well as the drug to polymer ratio, have been shown to govern the release of drug from formulations which could also change their release kinetics of drug. This proves that the amount of the sodium alginate and in combination with as a second polymer carbopol 934P could be used to improve drug entrapment, physical stability of Atorvastatin beads and to change/modulate the sustained release properties of the Atorvastatin

cross-linked alginate microbeads for designing of sustained drug delivery system that suitable for oral route of administration.

Kinetic Studies: The order of drug release from microbeads was described by using zero order kinetics, first-order kinetics, Korsmeyer Peppas and Higuchi equation. These results are given in following **Table 7**.

The release of drug from the cross-linked alginate microbeads containing both polymer blends fitted the and Hixson Crowell and Korsmeyer-Peppas models which state that drug release occurs from the surface of microbeads was totally controlled by a combination of both diffusion and erosion mechanisms from spherically shaped microbeads with changes took place with respect to diameter and surface area [32].

In all the batches from AB1 to AB15, the R^2 values of Higuchi matrix model were close to 1. The diffusion coefficient 'n' values ranged between 0.807 to 1.216. Since, the Regression coefficient values of Higuchi matrix were close to 1, this indicates the drug release follows matrix diffusion-controlled kinetics.

Also, the 'n' value indicates that the drug release mechanism from the optimized formulation was by super case II transport, in which a pronounced acceleration in drug release from the cross-linked alginate microbeads occurs toward the latter stages of drug release, which result in a more rapid relaxation-controlled transport [33]. The diffusion coefficient 'n' values of Peppas equation for all individual batches were showed and summarized elaborately at above **Table 7**.

TABLE 7: CORRELATION COEFFICIENT (R^2) VALUES IN THE ANALYSIS OF DISSOLUTION DATA OF ATORVASTATIN CROSS-LINKED ALGINATE BEADS AS PER ZERO-ORDER, FIRST-ORDER KINETIC, HIGUCHI ORDER, KORSMEYER- PEPPAS AND HIXSON CROWELL PLOT FOR ALL BATCHES

Batch code	Zero-order	First order	Higuchi plot	Korsmeyer Peppas		Hixson Crowell plot
	R^2	R^2	R^2	R^2	n	R^2
AB1	0.978	0.848	0.943	0.986	0.935	0.867
AB2	0.979	0.974	0.948	0.989	0.935	0.865
AB3	0.980	0.969	0.898	0.977	1.119	0.915
AB4	0.963	0.944	0.954	0.979	0.835	0.838
AB5	0.987	0.973	0.932	0.987	1.046	0.891
AB6	0.990	0.958	0.912	0.992	0.999	0.907
AB7	0.963	0.972	0.950	0.973	0.894	0.826
AB8	0.986	0.978	0.949	0.982	0.956	0.859
AB9	0.998	0.907	0.923	0.996	0.977	0.906
AB10	0.971	0.649	0.949	0.984	0.896	0.852
AB11	0.982	0.930	0.932	0.988	1.034	0.889
AB12	0.992	0.861	0.936	0.993	0.892	0.887
AB13	0.996	0.808	0.940	0.986	0.807	0.875
AB14	0.965	0.985	0.949	0.946	1.085	0.853
AB15	0.966	0.989	0.920	0.967	1.216	0.900

*Each value represents the Mean ± Standard deviation (n=3).

The higuch plot showed regression coefficient value of 0.920 in optimized batch, *i.e.* AB15 which suggesting the diffusion forms prominent role in controlled release formulation. The data were fitted to Korsmeyer Peppas equation and value of diffusional exponent n was found to be 1.216 indicating that the release of the drug from alginate beads shows super case II transport or non-fickian diffusion. Peppas equation with highest linearity (R^2=0.967) followed by Higuchi plot and first order (R^2=0.989) and this clearly suggest that drug diffusion occurs from polymer matrix and drug release found to be very close to Higuch kinetics and which explains that drug diffusion occurs at comparatively slower rate as the distance of diffusion is progresses or increased. The results indicate that the drug release slows down with increasing polymer concentration. The combination of polymers in the form of alginate and carbopol 934P leads to swelling much extent and thereby if forms strong a mucilaginous gel by absorbing water and forming bonds with the mucus lining to get attached on its surface. Hence, it was concluded that diffusion was the main key mechanism of drug release from the microbeads. Further, the obtained diffusion coefficient values are good indicators of

the fact that the Atorvastatin drug release from the formulation follows non-Fickian transport mechanism controlled by both swelling and relaxation of the polymer. It is therefore to provides and supports the basic concept of the controlled release profile of drug delivery and to improve the rate of bioavailability of drug [34, 35]. It can be explained that greater the amount of polymer, thicker the layer of polymer was formed around the drug particles and more effectively the polymer would hold the drug with itself. This had also been proved with the result of drug content. The same reason is also true for the batches prepared with the combination of another polymer.

Stability Studies: Stability studies on optimized batch AB15 has been carried out and shown no significant remark found in drug entrapment efficiency, particle size and *in-vitro* drug release when stored at accurately at both ambient humid condition at room temperature (27 ± 2 °C) and at elevated temperature (45 ± 2 °C) for duration of 8 weeks exactly. During a study at different intervals of time observation has been happened against any changes in appearance, DEE, and particle size parameters. The obtained data corresponding to stability studies before and after have summarized in following **Table 8**.

The comparison of cumulative percentage drug release of optimized Atorvastatin alginate beads (AB15) along with drug entrapment and mean particle size prepared on day 1 and after 8 weeks are given in **Table 8**.

TABLE 8: DATA OF STABILITY STUDY CONDUCTING ON DISSOLUTION DATA OF ATORVASTATIN ALGINATE BEADS BEFORE AND AFTER 8 WEEKS FOR OPTIMIZED BATCH (AB15) ONLY

Time (h)	Cumulative percentage drug release*		Mean particle size*(µm) (AM ± SM)		DEE* (%)	
	Before stability studies	After stability studies	Before stability studies	After stability studies	Before stability studies	After stability studies
0	0	0	1095.2 ± 1.32	1094.1 ± 2.03	58.90 ± 0.53	57.64 ± 3.10
1	5.21 ± 1.03	5.06 ± 3.03				
2	18.04 ± 0.87	17.02 ± 3.27				
3	32.54 ± 1.01	32.04 ± 4.03				
4	36.87 ± 1.30	36.78 ± 2.50				
5	55.43 ± 2.04	55.03 ± 1.95				
6	60.02 ± 1.13	59.20 ± 3.03				
7	70.52 ± 0.94	70.52 ± 0.94				
8	76.72 ± 0.79	75.05 ± 1.39				
9	81.26 ± 1.02	82.06 ± 1.78				
10	84.5 ± 2.74	85.3 ± 3.04				

*Each value represents the Mean ± Standard deviation (n=3).

The stability data which showed in **Table 8** proves that there was no significant change in the appearance of the alginate microbeads indicating that the formulations were good stable at different conditions during the storage period. The stability study was performed for the prepared formulation in accordance to ICH guidelines and results showed that the batch AB15 was stable in most aspects mainly with no physical change in swelling and there was no significant reduction in drug entrapment efficiency. Thus, we may conclude and report that the optimized Atorvastatin bead doesn't undergo any degradation in the stability period.

SUMMARY AND CONCLUSION: The present investigation describes a particulate drug delivery system for Atorvastatin cross-linked alginate beads prepared by the ionic gelation technique, which is simple, less economical and requires a minimum number of chemicals and equipment. The prepared alginate mucoadhesive microbeads were of a suitable size for oral administration and showed negligible both toxic and side effects. The formulation variables, polymer concentration, type of cross-linking agents and stirring speed influenced drug entrapment efficiency, the mean particle size, and *in-vitro* drug release characteristics of the microbeads. Release kinetic modeling of aluminium alginate atorvastatin microbeads indicate that drug release data fit well to Korsmeyer Peppas equation plot (R^2 =0.967) indicating the dissolution rate limited drug release from the formulation and drug release occurs from microbeads was occurs by a combination of both diffusion and swelling-erosion mechanisms from micro alginate beads with changes took place in

both diameter and surface area also, the n value (1.216) indicates that the drug release mechanism from the optimized formulation was found to be super case II transport.

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CONFLICT OF INTEREST: Nil

REFERENCES:

1. Taha EL, Al Saidan S, Samy AM and Khan MA: Preparation and *in-vitro* characterization of self nano emulsified drug delivery system of all-trans retinol acetate. International Journal of Pharmaceutics 2004; 28: 109-19.
2. Lau YY, Okochi H, Huang Y and Benet LZ: Pharmacokinetics of Atorvastatin and its hydroxy metabolites in rats and the effects of concomitant Rifampicin single doses: relevance of the first-pass effect from hepatic uptake transporters, and intestinal and hepatic metabolism. Drug Metabolism and Disposition 2006; 34: 1175-1181.
3. Verma S, Rawat A, Kaul M and Saini S: Solid dispersion: a strategy for solubility enhancement. International Journal of Pharmacy and Technology 2011; 3: 1062-1099.
4. Van de Waterbeemd H: The fundamental variables of the biopharmaceutics classification system (BCS): a commentary. Euro Jou of Pharma Sci 1998; 7: 1-3.
5. Das MK and Senapati PC: Evaluation of Furosemide loaded alginate microspheres prepared by ionotropic external gelation technique. Acta Poloniae Pharmaceutica 2007; 64: 253-62.
6. Yurdasiper A and Sevgi F: An overview of modified release chitosan, alginate, and eudragit RS micro particles. Jour of Chemical and Pharmaceutical Res 2010; 2: 704-21.
7. Thu B, Skjak-Braek, G, Micali F, Vittur F, and Rizzo R: The spatial distribution of calcium in alginate gel beads analyzed by synchrotron-radiation induced X-ray emission (SRIXE). Carbohydrate Research 1997; 101-105.
8. Sugawara S, Imai T and Otagiri M: The controlled release of prednisolone using alginate gel. Pharmaceutical Research 1994; 11: 272-277.
9. Ostberg T, Lund EM and Graffner C: Calcium alginate matrices for oral multiple unit administration: IV release characteristics in different media, International Journal of Pharmaceutics 1994; 112: 241-248.
10. Kumar R, Gupta RB and Betageri GV: Formulation, characterization, and *in-vitro* release of glyburide from proliposomal beads, Drug Delivery 2001; 8: 25-27.
11. Xing L, Dawei C, Liping X and Rongqing Z: Oral colon-specific drug delivery for bee venom peptide: development of a coated calcium alginate gel beads-entrapped liposome. Journal of Controlled Release 2003; 93: 293-300
12. Forizs E, Patrut A, Cozar IB and David L: Synthesis, structure elucidation of palladium complexes with theophylline. Academia Romana 2010; 55: 697-704.
13. Chang X, Weng T, Zhao X, Chen H, Gao Z and Yang Y: A study of micro emulsion systems for transdermal delivery of triptolide. Journal of Controlled Release 2004; 98: 427-36.
14. Yadav A and Jain DK: Formulation and evaluation of mucoadhesive microspheres of propranolol hydrochloride for sustained drug delivery. Asian Journal of Pharmacy and Medical Science 2011; 1: 1-8.
15. Cosijins A, Nizet D and Nikolokakis I: Porous pellets as a drug delivery system. Drug Development and Industrial Pharmacy 2009; 35: 655-62.
16. Deshpande RD, Gowda DV and Mohammed N: Design of *Pistacia lentiscus* (mastic gum) controlled release spheroids and investigating the influence of roll compaction. Industrial Crops Products 2013; 44: 603-610.
17. Kumar AN, Deecaraman M, Rani C, Mohanraj KP and Venkates K: Preparation and solid-state characterization of Atorvastatin nanosuspensions for enhanced solubility and dissolution. International Journal of Pharmtech Research 2009; 1: 1725-1730.
18. Swamy NG, Rupa V, Abbas Z and Dasankoppa FS: Formulation and evaluation of nano suspensions for enhancing the dissolution of poorly soluble mebendazole. Indian Drugs 2010; 47: 47-54.
19. Palem CR, Patel S and Pokharkar VB: Solubility and stability enhancement of Atorvastatin by cyclodextrin complexation. Journal of Pharmaceutical Science and Technology 2009; 63: 217-225.
20. Wei H, Qing D, De-Ying C, Bai X and Li-Fang F: *in-vitro* and *in-vivo* studies of pectin/ethyl cellulose film-coated pellets of 5-fluorouracil for colonic targeting. The Journal of Pharmacy and Pharmacology 2008; 60: 35-44.
21. Surjyanarayan M, Hemangini R, Bhavdip J, Mikesh P and Rajesh KS: Release kinetic modeling of Atorvastatin calcium loaded self-micro emulsifying drug delivery system. Elixir Pharmacy 2012; 53: 11725-11729.
22. Chuang JJ, and Huang YY, Lo H, Hsu TF, Huang WY, Huang SL and Lin YS: Effect of pH on the shape of alginate particles and its release behavior. International Journal of Polymer Science 2017; 1-9.
23. Loh QL, Wong YY and Choong C: Combinatorial effect of different alginate compositions, polycations, and gelling ions on microcapsule properties. Colloid and Polymer Science 2012; 7: 619-629.
24. Grant GT, Morris ER, Rees DA, Smith PJ and Thom D: Biological interactions between polysaccharides and divalent cations-the egg-box model. FEBS Letters 1973; 32: 195-198.
25. Ayak AK, Hasnain MS, Beg S and Alam MI: Muco-adhesive beads of Gliclazide: Design, development, and evaluation. Science Asia 2010; 36: 319-325.
26. Kaygusuz HF, Erim B and Eving AG: Cation effect on slow release from alginate beads: A fluorescence study. Journal of Fluorescence 2014; 24: 1: 161-167.
27. Shanmuganathan S, Shanumugasundaram N, Adhirajan N, Lakshmi RTS and Babu M: Preparation and characterization of chitosan microspheres for Doxycycline delivery. Carbohydrate Polymers 2008; 73: 201-211.
28. Kawashima Y, Handa T, Takeuchi H, Iwamoto T and Itoh K: Preparation of controlled-release microspheres of ibuprofen with acrylic polymers by a novel quasi-emulsion solvent diffusion method, Journal of Pharmaceutical Sciences 1989; 78: 68-72.

29. Darrabie MD, Kendall WF and Opara EC: Effect of alginate composition and gelling cation on microbead swelling. Journal of Microencapsulation 2006; 23: 613-621.
30. Filipovic-Grcic J, Becirevic-Lacan M and Skalko N: Chitosan microspheres of Nifedipine and nifedipine-cyclodextrin inclusion complexes. International Journal of Pharmaceutics 1996; 135: 183-190.
31. Hafner A and Filipovic-Grcic J: Development and *in-vitro* characterization of chitosan-based microspheres for nasal delivery of promethazine. Drug Development and Industrial Pharmacy 2007; 33: 427-436.
32. Odeku OA, Lamprecht A and Okunlola A: Characterization and evaluation of four natural gums as polymers in formulations of Diclofenac sodium micro beads. International Journal of Biological Macromolecules 2013; 58: 113-120.
33. Jacques CHM, Hopfenberg HB and Stannett V: Permeability of plastic films and coatings. Super case II transport of organic vapors in glassy polymers. Hopfenberg, Harold 1974; 73-86.
34. Prajapati SK, Tripathi P, Ubaidulla U and Anand V: Design and development of gliclazide mucoadhesive microcapsules: an *in-vitro* and *in-vivo* evaluation. American Association of Pharmaceutical Scientists 2008; 9: 224-230.
35. Sharma VK and Bhattacharya: Release of Metformin hydrochloride from ispaghula-sodium alginate beads adhered on cock intestinal mucosa. Indian Journal of Pharmaceutical Education and Res 2008: 42: 365-372.

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