WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 12, Issue 19, 1159-1181. Research Article ISSN 2277– 7105

STUDIES ON ENTERIC COATED ATORVASTATIN CALCIUM LOADED MUCOADHESIVE SUSTAIN RELEASE MICROSPHERES

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Article Received on
20 Sept. 2023,

Revised on 10 Oct. 2023,
Accepted on 31 Oct. 2023

DOI: 10. 20959/wjpr202319-29614

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ABSTRACT

Atorvastatin calcium (ATC) is a water insoluble drug and is known to have antihyperlipidemic activity, which has low bioavailability and poor solubility. The present study aimed at developing and evaluating sustain release ATC-loaded mucoadhesive microsphere coated with poly methacrylate polymer. Atorvastatin calcium undergoes conversion to its lactate form under gastric acidic environment. To avoid release at gastric pH polymethacrylate (Eudragit- L100) coated mucoadhesive microsphere were developed. And also to enhance absorption at the duodenum and jejunum part of the small intestine. Ionotropic gelation technique was utilized used to formulate microspheres. Formulated microsphere were evaluated for percentage yield, drug content, drug entrapment efficiency, swelling index, in-vitro drug release. Beside this FTIR and DSC carried out for compatibility. Optimized formulation had percentage yield 98.14%, drug content > 94% w/w, entrapment efficiency > 94 %. Drug release at pH 1.2 which was observed to be successfully controlled in polymethacrylate coated microspheres. In vitro release data was fitted in mathematical model to confirm the release pattern of formulation. Ex-vivo permeation study was carried out by non- everted gut technique. Coated formulation F9B showed 49.67 ± 0.06 %. Microsphere of different size and drug content could be obtained by varying the formulation variables like polymer concentration. The multi-unit mucoadhesive microsphere of Atorvastatin calcium delivery system is accepted to provide clinician with new choice of an economical, safe and more bio- available formulation in the management of moderate to severe lipidemia. Therefore it may be concluded that enteric coated mucoadhesive microspheres are suitable delivery system for Atorvastatin calcium (ATC).

KEYWORDS: (Atorvastatin Calcium, Anti-hypertensive, Poly-methacrylate, Non-everted).

INTRODUCTION

Lipids are important biomolecules like cholesterol serves as both a precursor for bile acids and steroid hormones and is a necessary part of the human cell membrane. Any biomolecule in excess is harmful to human health similarly increase of various lipids (hyperlipidaemia) in the bloodstream results in a persistent health issue. Hyperlipidaemia is the main risk factor for coronary heart disease and a constant threat to coronary arteries.[1]

According to the World Health Organization (WHO) cardiovascular disease (CVD) continues to be the leading cause of death about 18 million deaths worldwide in 2016 were attributed to CVD or about one-third of all fatalities.[2],[3]

Atorvastatin Calcium (ATC) is a member of the drug used for lowering the cholesterol levels in body. It is competitive inhibitor of hydroxyl methyl glutaryl-coenzyme A (HMG-CoA) reductase followed mevalonate pathway.[4],[5]

Atorvastatin is administered in the active acid form and *in vivo* is metabolized by cytochrome P450 (CYP) 3A4 to o-hydroxyatorvastatin and p-hydroxyatorvastatin, which demonstrate pharmacologic potency equivalent to parent. Plasma exposure of o-hydroxyatorvastatin is similar to that of atorvastatin, whereas p-hydroxyatorvastatin represents <10% exposure of the total active species. Following the administration of atorvastatin, inactive lactone metabolites are also present in plasma, as atorvastatin lactone and the corresponding CYP3A4-mediated metabolites, o-hydroxyatorvastatin lactone and p-hydroxyatorvastatin lactone; plasma exposures of atorvastatin lactone and o-hydroxyatorvastatin lactone are equal to or greater than that of the respective acid forms.[6],[7]

Microspheres form an important part of such novel drug delivery systems that they can control the delivery of drugs from days to months therefore reducing frequent administrations and improving patient compliance and comfort. Certain problems regarding the drugs like high first pass metabolism, bioavailability of the certain drugs varies due to instability in acidic environment of stomach. To resolve such problems drug should be incorporated in the microspheres for sustained release using a suitable polymer. Different release profiles with desired release rates can be achieved by selecting polymers with different degradation

mechanism.[8]-[11]

This study was aimed to develop ATC loaded enteric coated mucoadhesive microsphere for drug release at the mucous layer of small intestine upper site. ATC microsphere was developed by ionic gelation method using sodium alginate and calcium chloride. Carbopol 974p was used to incorporate muco-adhesion property in the microsphere. Eudragit L100 which dissolves at pH greater than pH 6 which is used to coat the microsphere.

MATERIAL AND METHOD

Atorvastatin Calcium obtained as gift sample from (aquatic pharma pvt ltd). Carbopol and sodium alginate were provided by Oxford chemicals laboratory, Mumbai. Light Liquid Paraffin, Calcium Chloride and tween 80, eudragit L100 were obtained from Loba chemical laboratory, Mumbai. All other chemicals as well as Buffer salts used in experiments were analytical grade reagent. Jejunum part of small intestine collected from butcher shop.

Standardization and analytical method development

Atorvastatin Calcium was validated using UV-Spectrophotometer (V -1900, Shimadzu, Japan). Linearity, precision, accuracy, LOD, LOQ were carried out followed by spectral analysis. Calibration curve was plotted for atorvastatin calcium in Methanol AR grade, Simulated Intestinal fluid (SIF pH 6.8) buffer, simulated gastric fluid (SGF pH 1.2) and Physiological buffer (pH 7.4). The study was carried out in triplicate. The standard curve equation was obtained and concentration range of linearity was determined. Intra-day and Inter-day precision studies were carried out by performing the assay of the drug solution on the same day and on two consecutive days respectively.

Compatibility study between drug and polymer

Infrared Spectrum was taken by scanning the sample of pure drug and the polymer individually over a wave number 4000 to 500 cm^{-1} using Fourier transform infrared spectrophotometer (FT- IR, Shimadzu, Japan). The change in spectra of the drug in the presence of polymer was investigated which indicates the physical interaction of drug molecule with the polymer.

Formulation and optimization of microspheres

Microsphere were prepared using ionotropic gelation gelation method. Polymer aqueous solution prepared by drug accurately weighed dispersed in distilled water by sonication.

Different concentration of sodium alginate (1% w/v, 1.5%w/v, 2%w/v) were dispersed in above dispersion. Then separately prepared carbopol 974p solution added to dispersion to obtain polymeric solution. Polymeric solution added to optimized $CaCl_2$ Aqueous solution at 100 rpm. Solution using syringe with 26 gauze needle. Allowed to stand for 20 min for crosslinking. Then filtered and washed with water remove excess $CaCl_2$. Dried in oven at optimized temperature then stored in desiccator for further use.[12],[13]

Table 1: Composition of various formulation Atorvastatin calcium microsphere.

Sr.no	Batch Code	Sodium alginate (%w/v)	Carbopol-p974 (% w/v)	Drug (mg)	Calcium chloride (%)
1	**F1**	1	0.25	40	5
2	**F2**	1	0.5	40	5
3	**F3**	1	0.75	40	5
4	**F4**	1.5	0.25	40	5
5	**F5**	1.5	0.5	40	5
6	**F6**	1.5	0.75	40	5
7	**F7**	2	0.25	40	5
8	**F8**	2	0.5	40	5
9	**F9**	2	0.75	40	5

Selection of salt as a cross linker for preparation of batches

Various salt were selected as a cross linking agent like calcium chloride aluminium chloride and ferric chloride and sodium chloride. Out of which calcium chloride was selected as it formed comparatively spherical and rigid microspheres.[14]

Selection of concentration of calcium chloride salt for preparation of microsphere

Table no 2: Batches for selection of crosslinking agent concentration.

Sr.no	Batch code	Sodium alginate (% w/v)	Carbopolp974 (% w/v)	Calcium chloride (% w/v)	Product characteristics
1	C1	1	0.5	1	Microsphere formed with tail and loses their shape after drying
2	C2	1	0.5	2	
3	C3	1	0.5	3	Microsphere formed with little tail and no loss of shape after drying
4	C4	1	0.5	5	Microsphere were formed spherical and no loss after drying.
5	C5	1	0.5	7	

Different concentration of 1%, 2%, 3%, 5% and 7% (w/v) were used out of which concentration of 5%w/v was selected as it formed comparatively rigid spherical microsphere and no loss of shape was observed during drying.

Formulation of batches of microsphere for sustained release of drug

Microsphere for sustain release of drug were prepared by ionotropic gelation method. Fixed amount of sodium alginate and Carbopol-974p was used to prepare microsphere with different concentration of calcium chloride as a cross linker and further batches were prepared by using different ration of sodium alginate and carbopol-974p. The concentration of sodium alginate and carbopol-974p were selected as per the viscosness of polymeric solution.

Selection of ration sodium alginate and carbopol-974p for preparation of batches

Concentration of 1%, 1.5% and 2% of sodium alginate was selected and 0.25%, 0.5% and 0.75% of carbopol-974p was selected based on the viscosity of the prepared polymeric solution.

Enteric coating of microspheres

There are various method of coating of the microspheres. Emulsion solvent evaporation method was incorporated for the coating of microsphere. Optimized batch of uncoated microsphere was coated with two different concentration of eudragit-L100. Core microsphere were dispersed in the light liquid paraffin oil containing span 80 (2% v/v) help of mechanical overhead stirrer at 1000 rpm. Separately prepared Eudragit L-100 (5%, w/V) in acetone and ethanol (ratio 1:2) was added drop wise with 1ml/min. Finally, encapsulated microspheres were filtered and washed with n hexane to remove the traces of oil and dried in a vacuum desiccator for 24h.[15],[16]

Table 3: Composition of enteric coated microspheres.

Sr.no.	Formulation	Core Microsphere (mg)	Eudragit-L100 (mg)	Core :coat
1	F8A	1000	250	4:1
2	F8B	1000	500	2:1

Characterization of uncoated and coated microspheres

Drug –excipient compatibility studies (FTIR analysis)

The IR absorption spectra of physical mixture of drug and polymer (1:1) and drug loaded microspheres were taken in the range of 400-4000cm^{-1} using potassium bromide disc method.

Micromeritic characterization of microspheres

The microsphere were characterized by their micromeritic properties such as particle size,

angle of repose.

Drug content, encapsulation and loading efficiency

Accurately weighed microspheres equivalent to 10 mg of the drug was crushed in glass mortal-Pestle and the powdered microsphere were suspended in 100 ml of pH 6.8 phosphate buffer. After 24 h, the solution was filtered using Whatmann filter paper. Of this, 1ml of the filtrate was taken and diluted to 10 ml. The absorbance was measured at 241nm for sustain release.[14]

$$\%\ Encapsulation = \frac{Drug\ initial\ amount - Free\ drug\ amount}{Drug\ initial\ amount} \times 100$$

Percentage yield

The product yields of microparticles of various formulations were calculated using the weight of final product after drying with respect to the initial total weight of the drug and polymerused for preparation of microparticles and percent yields were calculated as per the formula mentioned below.[14]

$$Percentage\ yield = \frac{Mass\ of\ microsphere\ obtained}{Total\ weight\ of\ drug\ and\ polymer} \times 100$$

Swelling study

The swelling index of the microspheres is an indication of the capacity of the beads to imbibe water and swell. Accurately weighed microspheres (50mg) were placed in petri dish containing pH 1.2 HCL buffer 30ml for 2 hr and subsequently transferred into pH 6.8 Simulated Intestinal Fluid (SIF) 30ml. At the end of every 1hr, the beads were removed from the swelling medium, soaked with tissue paper to absorb excess water on the surface and weighed. Then for every1hr, weights of the beads were noted.[14],[15]

Percent weight gained by the beads was calculated by the following formula:

$$\%\ Swelling\ Index = \frac{Ws - Wd}{Wd} \times 100$$

Where, Ws =weight of swollen beads, Wd = weight of dried beads.

***In-vitro* drug release studies**

The in vitro drug release studies were performed using Beaker method. The study was carried out using 500 ml of pH 1.2 buffer and phosphate buffer pH 6.8 maintained at 37 ± 0.5 ºC at a rotation speed of 50 rpm. Withdrawing 10 ml of sample and replacing it with equal amount

of fresh medium for preselected interval up to 12 hr, monitored progress of the dissolution. The release rate from these microsphere were conducted in a medium of changing pH by starting with microsphere in pH 1.2 for 2h and phosphate buffer pH 6.8 for further hours. The sample solution were analysed for atorvastatin calcium by UV absorbance at 242nm using UV spectrophotometer (UV -1900). Cumulative percentage of drug released was used calculated and mean of three determination was used in data analysis. Graph was plotted between cumulative percent drug release vs. time.[14],[15],[17],[18]

Mucoadhesive strength of microsphere

The mucoadhesive properties of the microspheres were evaluated by the in vitro wash-off test. A 2x2 cm piece of Goat stomach mucosa was tied onto a glass slide (3×1 inch) using thread. Microspheres were spread (∽100) onto the wet, rinsed, tissue specimen, and the prepared slide. Slide placed in beaker 45 degree angle to horizontal surface then thoroughly washed with PBS then number of microsphere still adhered to mucus membrane is counted.[11]

$$\%\ Mucoadhesive\ strength = \frac{No.\ of\ particle\ still\ adhered}{Initial\ no.\ of\ particle} \times 100$$

Kinetic treatment

The release data obtained were treated according to Zero order equation (Q= Ko*t); first order equation (InQ = Kf t); Higuch's equation ($Q = K_H t^{½}$); Korsmeyer and peppas equation ($F=(M_t/M)=Km\ t^n$) to find the equation with the best Fit. Where, Qand F are the amount and fraction of drug release at time t respectively; M_t is the drug release at time t, M is the total amount of drug in dosage form; Ko, Kf and KH are the zero order, first order and Higuchi's square root of time order release rate respectively; Km is the constant depend on geometry of dosage form; n is the Diffusion exponent indicating the mechanism of drug release. The n value is used to characterize different release mechanism and is calculated from the slope of the plotof log of fraction pf drug released (Mt/M) vs. log of time (t). If, n =0.45 indicate Non-fickian or anomalous transport, n = 0.89 to 1.00 indicate case –II transport and n>1 indicates Super case-II transport and n>1 indicates Super case –II transport.

***Ex-vivo* permeation**

Non everted gut technique

Sac preparation collect the fresh sample from butcher shop, rapidly remove jejunum or duodenum of the intestine and divide into segment 5- 6 cm. Wash each segment with an ice-

cold physiological solution (eg. Oxygenated krebs solution pH 6.5 containing 7g/L sodium chloride, 0.34g/L disodium hydrogen phosphate, 0.207 g/L sodium dihydrogen phosphate and 46.8 mg/L magnesium chloride). In these method no need to evert the gut intestine clamp one end of the intestine and tie with silk braided suture and then fill with 10 ml suspension of 10 mg equivalent microsphere made in simulated intestinal fluid a 37ºC. Seal the filled intestine segment with a second tie using braided silk suture. Transfer the filled sac to the incubation flask containing 200 ml oxygenated krebs buffer solution at 37ºC. The sampling can be doneat different intervals.[19],[21]

Stability study

The stability of ATC microsphere were checked to assess the long-term usability of formulation. The stability study of formulation gives us idea about potential excipients reaction, long term drug stability and possible drug expulsion from formulation. It also assesses the stability of formulation at different environment and storage condition. The preparation was divided into three sets and was stored at 4℃, at room temperature (25℃) and at 40℃ (thermostatic oven). Formulation were tested at 0, 30, 60 and 90 days. The formulation was tested for entrapment efficiency and drug release by the method discussed earlier.

RESULTS AND DISCUSSION

Characterization of drug and Analytical study Organoleptic properties of atorvastatin calcium

Table 4: Organoleptic properties of atorvastatin calcium powder.

Sr.no.	Parameter	Result
1	Colour	White
2	Odour	Odourless
3	Appearance	Amorphous powder

Determination of melting point

The observed melting point of Atorvastatin calcium was between 175-178ºC by using themelting point checking machine.

Solubility study

Solubility study of Atorvastatin calcium was carried out and solubility found to be highly soluble in methanol and slightly soluble in ethanol and insoluble in water, n hexane, acetone and chloroform.

UV spectroscopy

The produced drug sample of Atorvastatin calcium with reference spectra as…

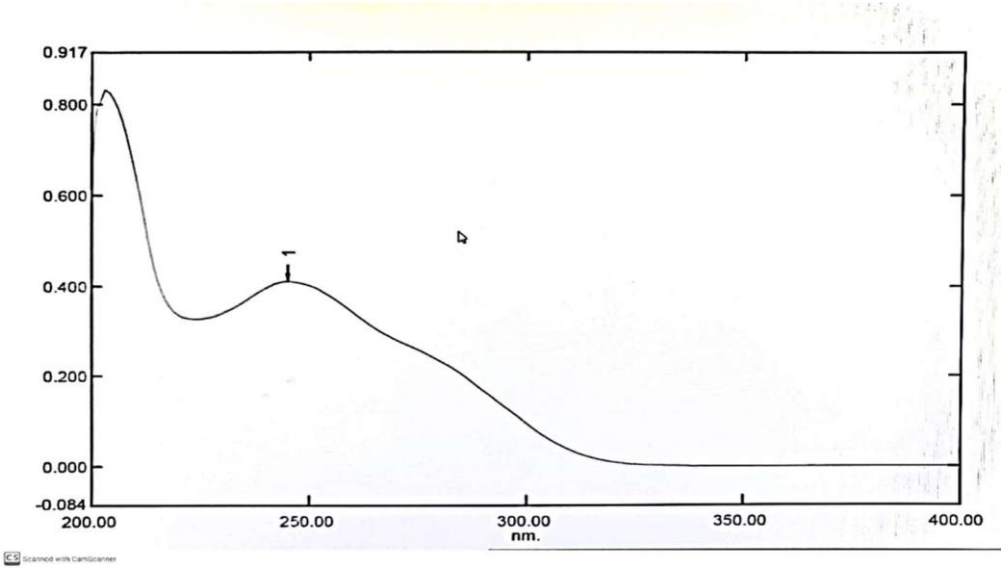


Fig. 1: Spectra of Atorvastatin calcium in methanol AR.

The solution of Atorvastatin calcium was found to exhibit maximum absorption at 256nm after scanning on the spectrophotometer which was reported as λmax in the literature as show in Figure 1.

Table 5: Standard Calibration curve of Atorvastatin calcium in methanol AR.

Calibration curve for	Atorvastatin calcium
Solvent	Methanol AR
Wavelength	245 nm
Equation of standard curve	Y=0.0459x-0.0021 R2=0.9998

Table 6: Calibration curve of Atorvastatin calcium in methanol AR.

Sr.no.	Concentration (µg/ml)	Absorbance
1	0	0
2	6	0.275
3	8	0.363
4	10	0.455
5	12	0.548
6	14	0.641

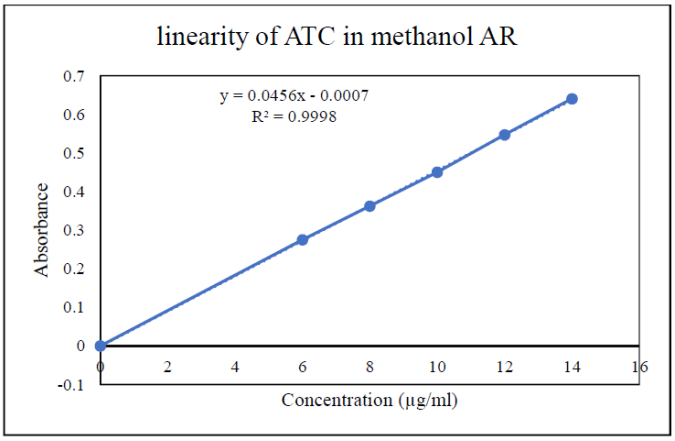
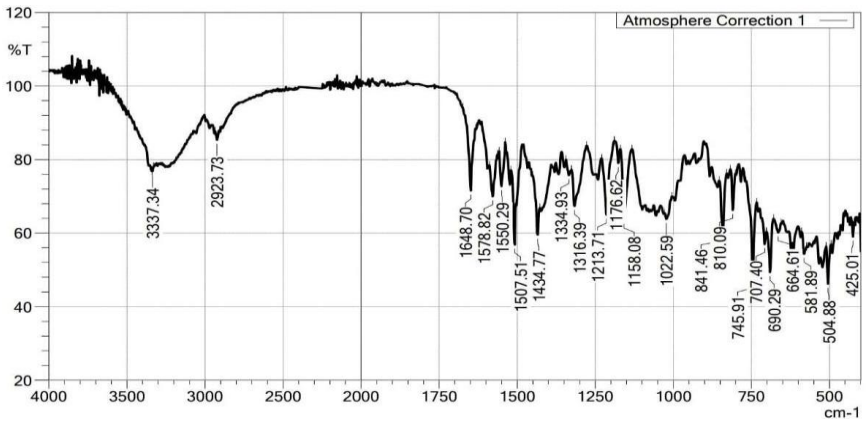


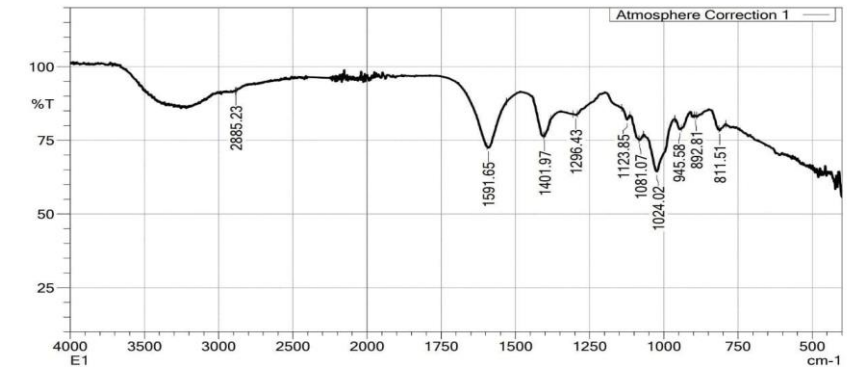
Fig. 2: Graph of linearity of atorvastatin calcium in methanol AR.

FTIR spectroscopy

It was observed from the FTIR graph as shown in Figure A (Atorvastatin calcium), Figure B (sodium alginate), Figure C (carbopol-974p) and Figure D (physical mixture) that there was no shifting of peaks of atorvastatin i.e., characteristic peak of the halide group (O-H stretching) at 3337 cm-1 in sustained release formulation. Hence, there was no interaction between drug and polymer.



A



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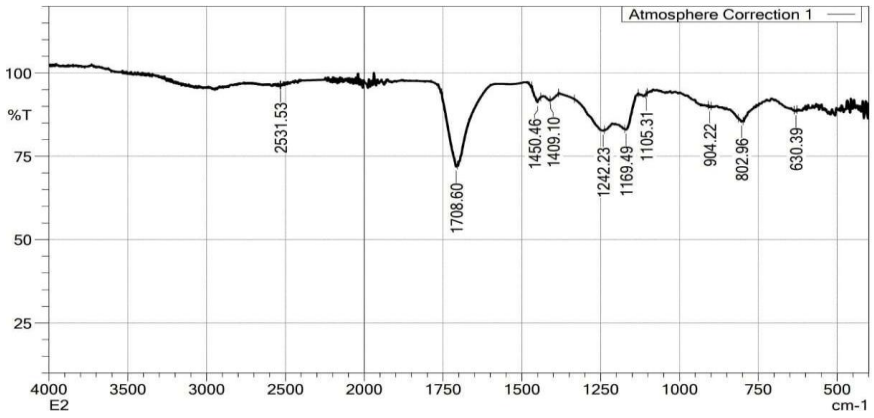

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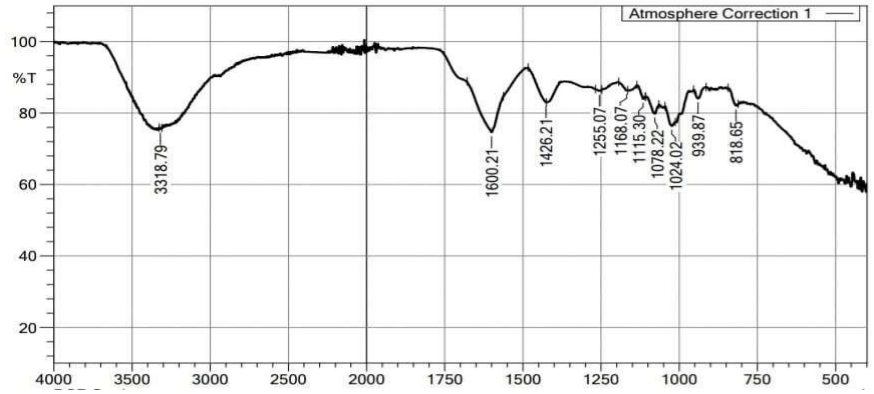


D

The microspheres of different formulations were evaluated for product characteristics and particle size, angle of repose. An angle of repose of less than 30 degrees indicates good flow properties.

Table 6: Average Particle Size and shape of Atorvastatin calcium microsphere.

Formulation Code	Average Particle Size	Shape of particle
F1	611.552 ±4.05	Loss of shape after drying
F2	647.976 ±3.09	Little tail
F3	681.61 ±4.09	spherical
F4	705.048 ±4.08	Little tail
F5	737.992 ±4.05	Spherical
F6	745.648 ±4.27	Spherical
F7	716.648 ±4.12	Spherical
F8	**774.648±4.27**	**Spherical**
F9	850.048 ±4.38	Spherical

Encapsulation and loading efficiency

Three different concentrations of sodium alginate (0.5%, 1% and 1.5%) (w/v) were used. The higher encapsulation efficiency was observed as the concentration of alginate increased. This is due to the greater availability of active calcium binding sites in the polymeric chains

and consequently the greater degree of cross linking.

The highest encapsulation efficiency (95.80%) was achieved with 1.5% (w/v) sodium alginate in combination with 0.5% (w/v) carbopol. Good drug loading efficiency was achieved for all formulation since Ca++ and OH- of carbopol 974p does not compete with each other and react with –COO- of Sodium alginate resulting in more compact structure.

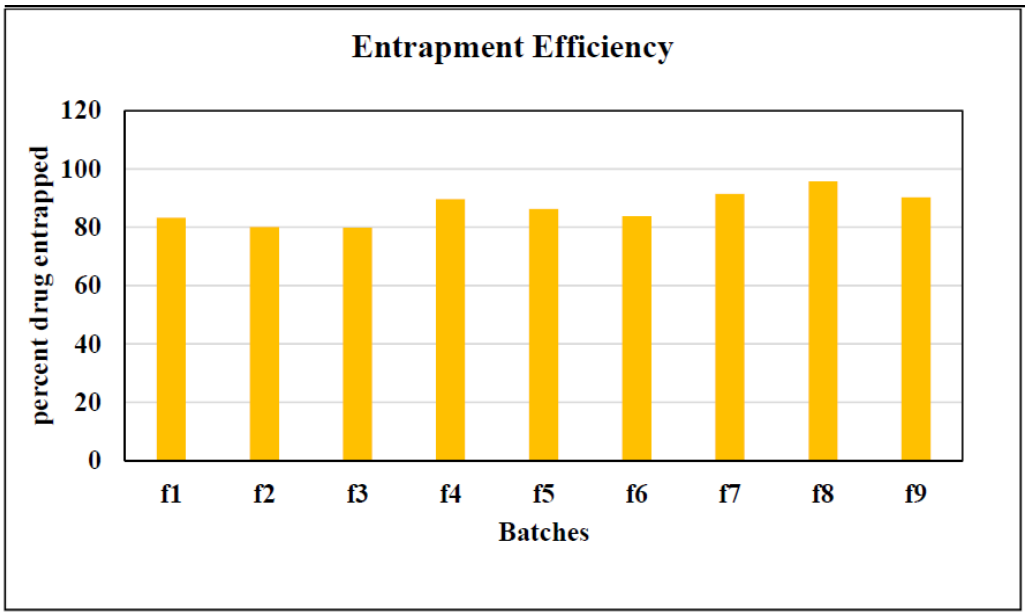


Fig 3: Graph of entrapment efficiency.

Table 7: Drug loading and drug entrapment of Atorvastatin calcium microspheres.

Formulation code	Actual Drug Content (mg)	Theoretical Yield (mg)	% Drug Loading	% Drug Entrapment
F1	8.32	10	9.005195	83.29
F2	8.01	10	7.368831	80.13
F3	7.97	10	6.485714	79.77
F4	8.96	10	7.083117	89.60
F5	8.63	10	6.823377	86.31
F6	8.37	10	5.602597	83.75
F7	9.14	10	5.966234	91.43
F8	9.58	10	5.602597	95.80
F9	9.01	10	4.927273	90.16

Table 8: Entrapment efficiency of coated microsphere.

Batch code	% Entrapment Efficiency
F8A	**94.35±2.56**
F8B	**95.10±1.64**

Swelling index

It was observed that all formulations showed comparatively lower swelling index in pH 1.2 simulated gastric fluid buffer than in pH 6.8 Simulated Intestinal Fluid buffer. It was found that the microspheres shrink in acidic pH, this could be well justified due to the fact that, Sodium alginate is compatible with most anionic substances and with few cationic substances, and it shows higher stability against external factors if it is conditioned in the form of a dry powder than in the form of a solution. With acids, sodium alginate gradually forms a gel of alginic acid at low pH values; at elevated pH values, alginic acid dissolves and restores its original viscosity. In alkaline environment, sodium alginate can withstand short periods of time, since pH values higher than 11 reduce its viscosity.at acidic pH strong interaction occurs between two carboxyl group of Alginate which is due to the formation of intermolecular and intramolecular hydrogen bond (polyelectrolyte complex) between the two polymers.

The increased swelling of microspheres in pH 7.4 phosphate buffer was due to, firstly, the breakage of H-bond, which reduces the interaction between the polyelectrolyte and ionization of carboxylic group of alginate results in swelling of microspheres network with subsequent imbibitions of fluid. Secondly, the ionization of cross linked calcium salt increase and the process of exchange of Ca2+ for sodium start. As Ca2+ ions are replaced by Na+ ions, the dense cross linked structure starts to get loosened and water starts getting absorbed into the microspheres.

Table 9: Swelling index of various formulation.

Sr. no.	Batch code	Swelling Index	
		pH 1.2	pH 6.8
1	F1	28.6	391.4
2	F2	25.6	382.4
3	F3	20.2	361.4
4	F4	18.6	298.2
5	F5	16.2	343.8
6	F6	16.6	374.2
7	F7	15.8	363.8
8	**F8**	**14.4**	**261**
9	F9	19.6	312.2

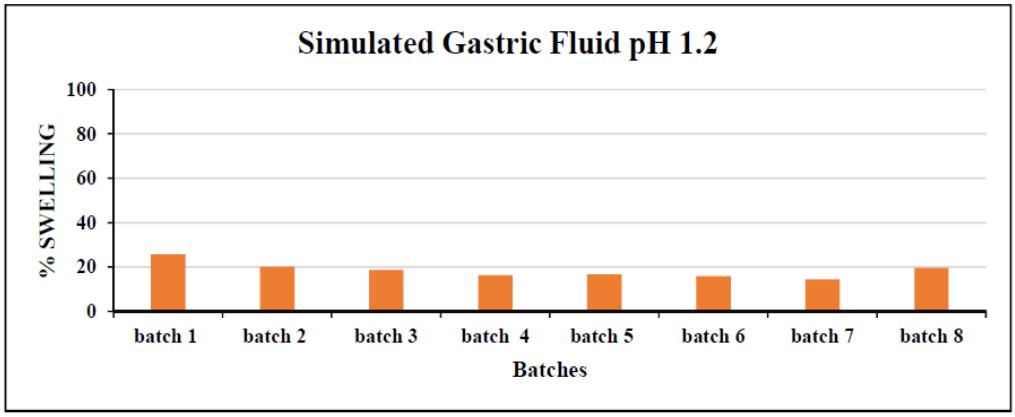


Fig. 4: Graph of swelling index of microsphere in gastric fluid pH 1.2.

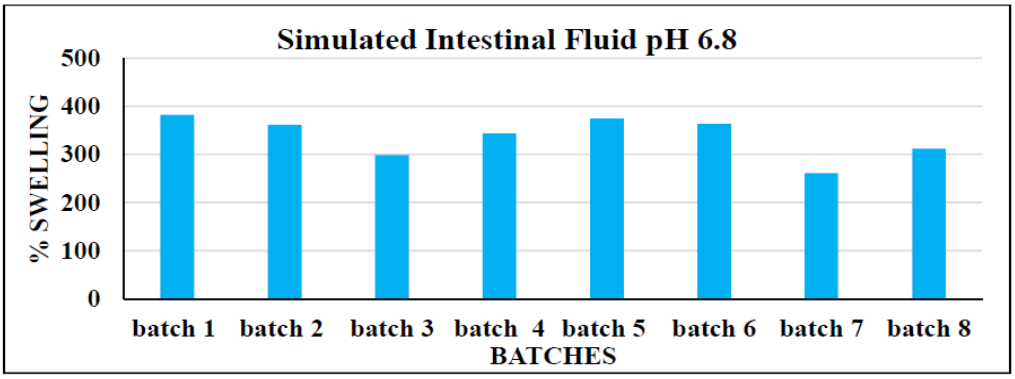


Fig. 5: Graph of swelling index of microsphere in simulated intestinal fluid pH 6.8.

In-vitro drug dissolution studies

The cumulative percent drug release curve of the drug loaded sodium alginate microspheres showed the drug release from the microspheres decreased as the concentration of sodium alginate increased suggesting that drug release could be controlled by varying the polymers. It can be attributed to increase in the densities of the polymer matrix resulting in larger microspheres and this in turn increase the diffusional path length, which the drug molecules have to traverse during diffusion. Thus in order to control the release carbopol-974p was blended with alginate matrix. The sustained drug release about 58.08% was found to be in formulation F8 containing 2% w/v sodium alginate and 0.5% w/v carbopo-974p and hence was designated as optimized batch.

Cumulative percent drug release from optimized batch F8B enteric coated microsphere in SGF pH found to be negligible and release found in SIF pH 6.8 was sustained approximate 51±0.23 % within 8hrs. The negligible release in SGF pH1.2 because insolubility of the eudragit-L100 in the acidic pH.

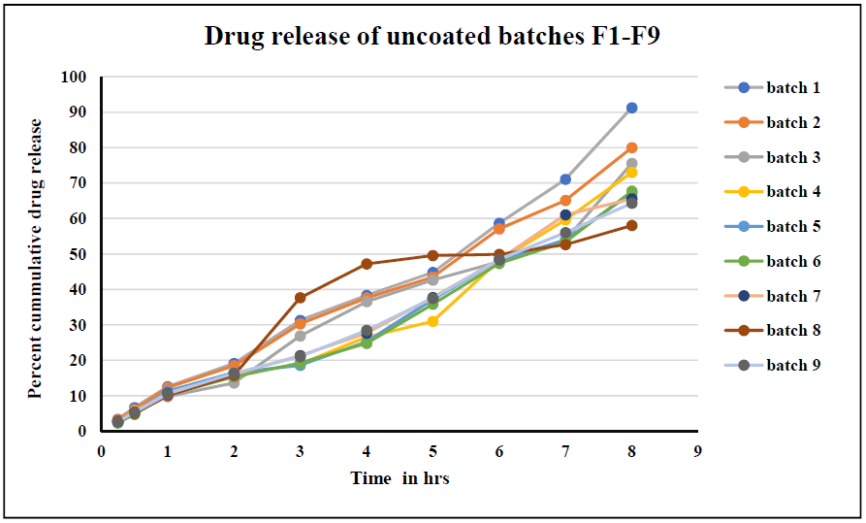


Fig. 6: Percentage cumulative drug release of mucoadhesive uncoated microsphere.

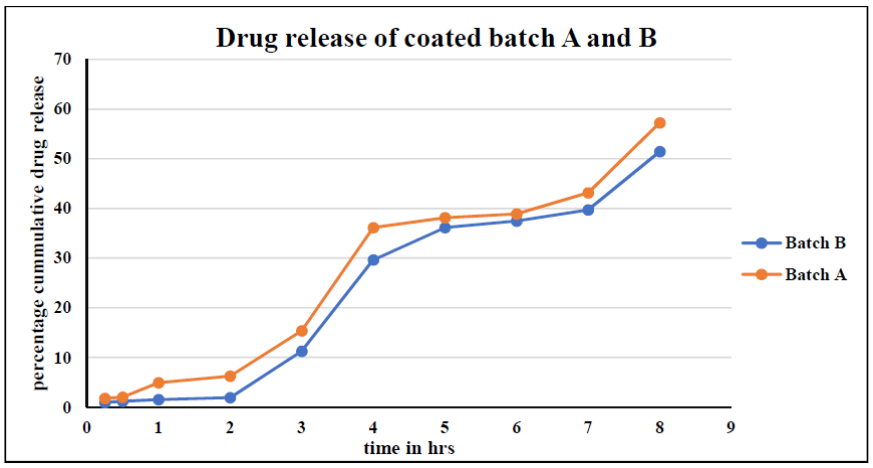


Fig. 7: Percentage cumulative drug release of enteric coated microsphere Mucoadhesive strength.

Mucoadhesive strength of formulations was found to be directly proportional to the concentration of the polymers. As the concentration of carbopol-974p increases the mucoadhesive strength found to be increased. Mucoadhesive strength of formulation was found between 72.67-99.33%.

Table 10: Mucoadhesive Strength.

Batch	Mucoadhesive strength
F1	72.67
F2	79.67
F3	85.00
F4	86.00
F5	92.00
F6	92.67

F7	92.33
F8	99.00
F9	99.33

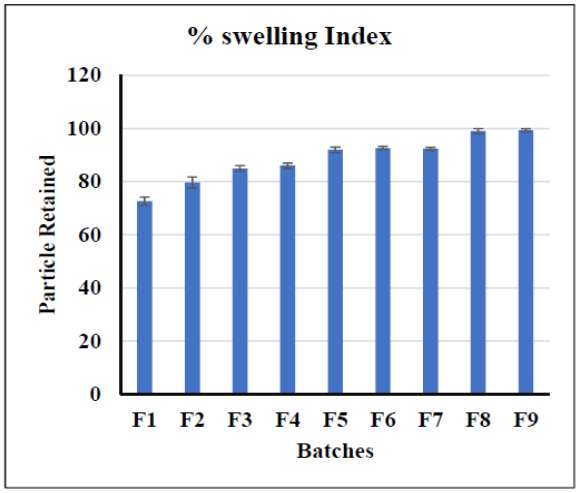


Figure no.8: Mucoadhesive Strength of Batches.

Ex vivo permeation

Non everted gut technique modified

Permeation study was performed on the plain drug and the Batch B and it was observed that the permeation of the drug from the batch B was less than the plain drug because the sustained release of the drug from the prepared microsphere.

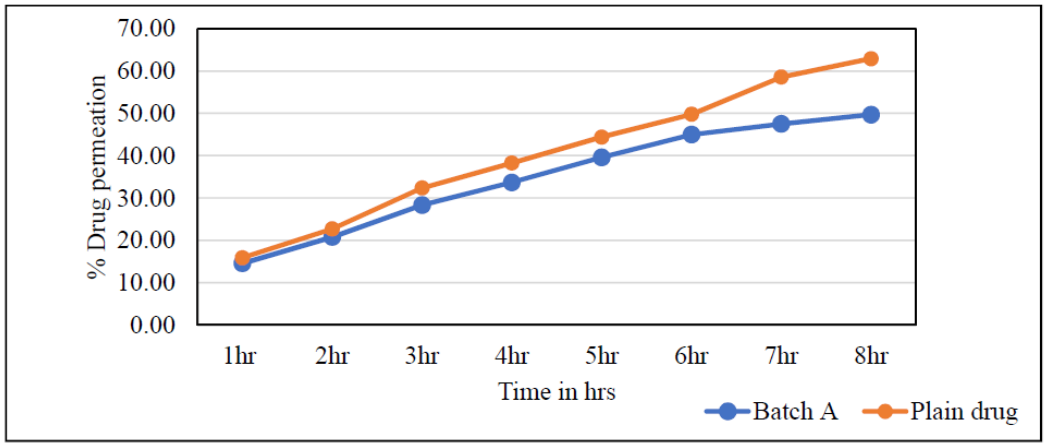
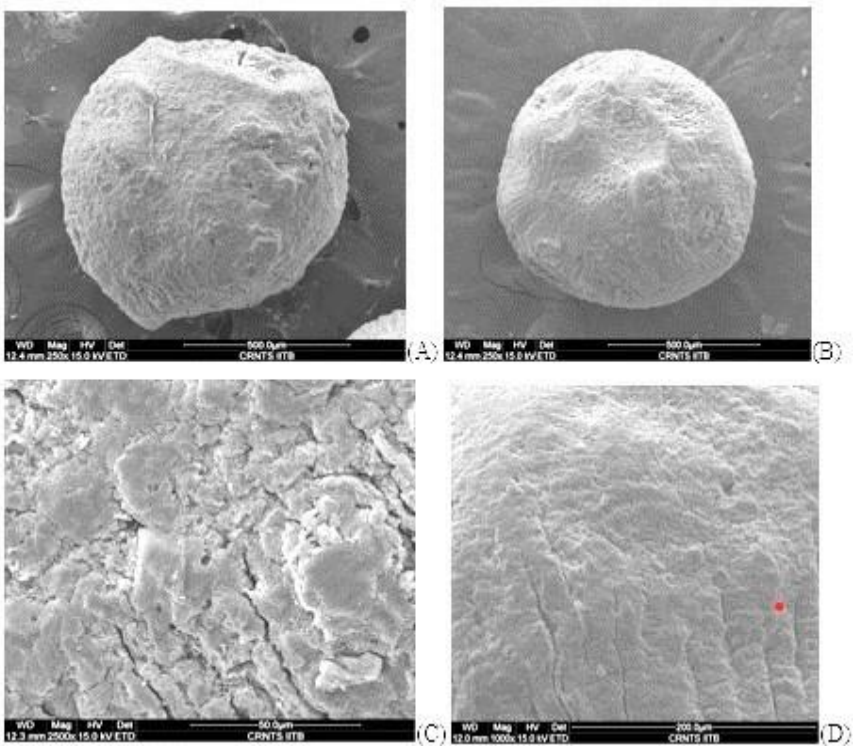


Fig. 9: Cummulative percent drug permeation of enteric coated and plain drug.

Scanning electron microscopy (SEM)

The SEM photomicrograph of microsphere are shown in figure.



A. DSEM image of uncoated mucoadhesive microsphere
B. SEM image of enteric microphere
C. Surface morphology of uncoated mucoadhesive microsphere
D. Surface morphology of coated microsphere

Effect of pH on drug release

It is clear that the release rate of atorvastatin was significantly larger in simulated intestinal fluid (pH 6.8) than in simulated stomach fluid (pH 1.2). A strong connection between the carbapol 974p ammonium groups and the carboxyl groups of alginate, which results from the creation of both intermolecular and intramolecular hydrogen bonds between the two polymers, was the cause of the low release in acidic media. Additionally, the protonation of carbapol p974's principal ammonium groups (-NH3 +) has resulted in the creation of a repulsive force inside the microspheres. The microspheres are maintained in a shrunken form in an acidic solution, and the medicine is released. However, in an alkaline environment, the H-bond was broken, which reduced the interaction between the polyelectrolyte and the polyelectrolyte. Ionisation of the carboxylic group of alginate caused the microsphere network to swell, which then allowed the drug to dissolve and be released by diffusion.

All batches except F2, F5 and F8 exhibit a greater rate of drug release, whereas batches F2, F5 and F8 exhibit a high initial release followed by a slower and finally a moderate rate of

drug release as gelation progressed.

Coated microsphere of Batch B shows almost negligible release at acidic pH with no significantchange in the drug content and drug entrapment efficiency.

Effect of polymer concentration on drug release

Slowest release was observed at batch F8 containing 2% w/v sodium alginate and 0.5 w/v carbopol-974p with 58.08% drug release in 8hrs thus these formulation were capable of controlling drug release and considered as optimized. Release rate was rapid with low concentration of polymer. Microsphere (F1, F2, F3, F4, F5) containing sodium alginate released more than 70% within 8 hrs these result suggested that higher polymer concentrationof carbopol 974p formed highly viscous microspheres network which sustained the drug release.

Kinetic treatment

The *in vitro* release profiles were applied on various kinetic models in order to find out the mechanism of drug release. The best fit with the highest correlation coefficient was shown in zero-order, Higuchi, and followed by first order equations. The rate constant were constants were calculated from the slope of the respective plots. The data obtained were also put in korsmeyer-peppas model in order to find out n value, which describes the drug release mechanism. All batches had found follow zero order release pattern.

Table 11: R^2 value for the release kinetics for formulation (F1-F9).

Batch	Zeroorder	Firstorder	Higuchi	Korsemayerpeppas	Hixon
F1	0.97	0.82	0.91	0.78	0.89
F2	0.99	0.91	0.94	0.81	0.95
F3	0.97	0.88	0.93	0.8	0.92
F4	0.94	0.86	0.86	0.71	0.89
F5	0.97	0.9	0.89	0.75	0.93
F6	0.97	0.91	0.9	0.76	0.94
F7	0.98	0.94	0.92	0.78	0.96
F8	0.93	0.87	0.91	0.78	0.92
F9	0.99	0.95	0.93	0.97	0.8

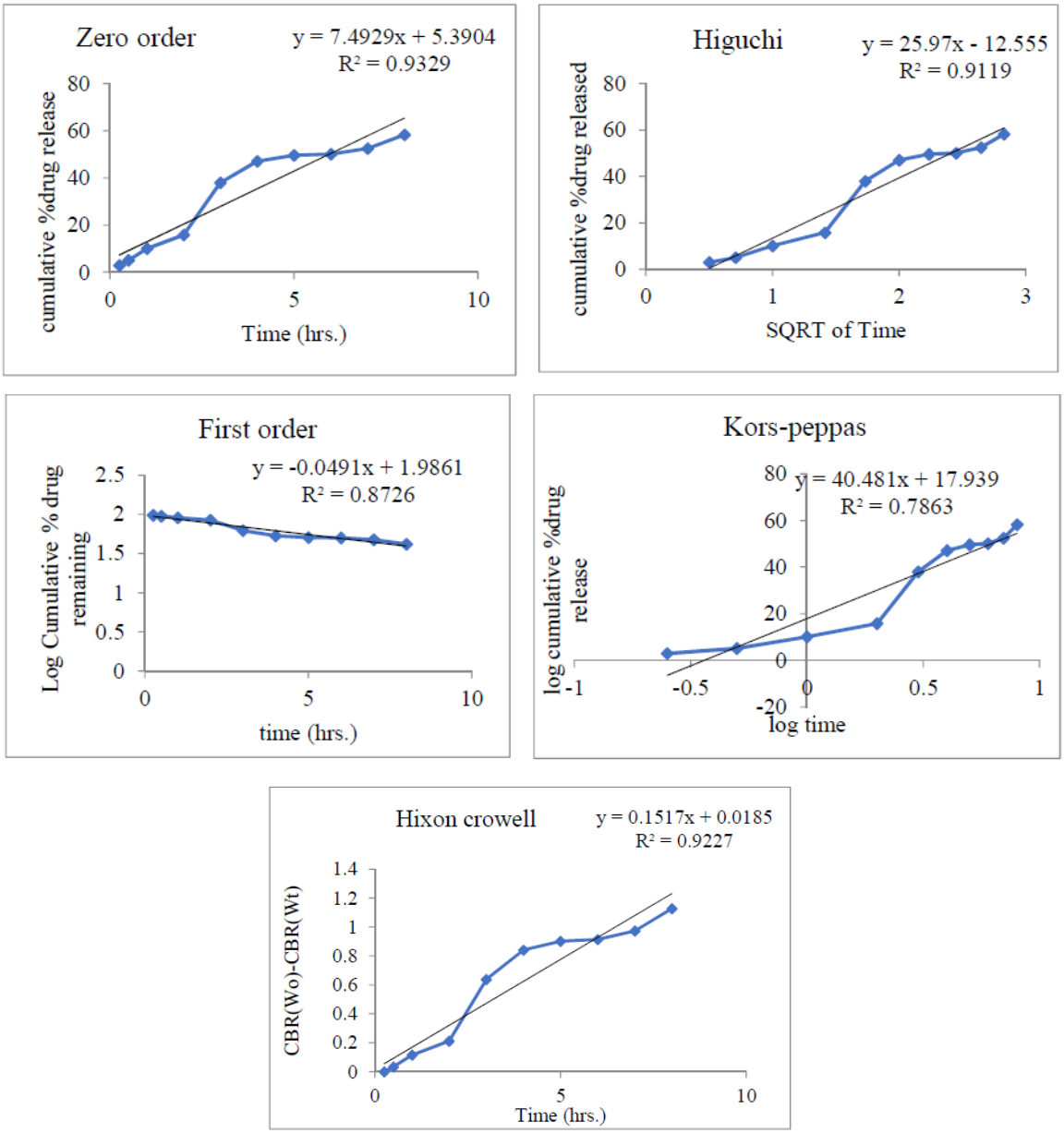


Fig no 10: Graphical Representation of the Kinetic Model for Drug Release of OptimizedBatch.

Stability study

The stability study was performed as per the ICH guidelines in which formulation was said tobe stable if its physical and chemical integrity remains intact over a period of time. After 3 months storage period, there were no change in physical property, colour and no liquefaction was observed. The drug entrapment efficiency was found to be maximum at room temperature (25±2^{0}C).

Thus the sample stored at 5±3^{0}C shows maximum buoyancy which might be due to aggregation of microsphere stored in refrigerated conditions. The size of the particle has an

inverse relationship with density. Hence a slight increase in buoyancy might due to an increase in aggregation of particle.

No considerable changes are found in the percent drug release from microsphere formulation at different storage conditions. Initially the floating mucoadhesive microspheres at 5±3^{0}C showed 51.42±1.37% drug release in 8 hr while at the end of 3 months the formulation showed 48.99±0.98%. While at the room temperature (25±2^{0}C) the release was initially found to be 50.42±1.37% and after 3 months the release was 50.75±0.98% thus the results of the stability studies of Atorvastatin calcium loaded enteric coated mucoadhesive microsphere as per the evaluation performed at 25±2^{0}C and 5±3^{0}C were shown that formulation was stable at both temperature conditions but it is most stable at room temperature as per results obtained from the stability study.

Table 12: stability study of Atorvastatin calcium (ATC) loaded enteric coated mucoadhesive microsphere at 5±3^{0}C.

Temperature	**5±3^{0}C**			
Days	1	30	60	90
Physical changes	NC	NC	NC	NC
Colour change	NC	NC	NC	NC
Liquefaction	NC	NC	NC	NC
In-vitro drugrelease (%)	51.42±1.37	51.22±0.30	50.37±0.87	48.99±0.98
Entrapment efficiency (%)	96.24±21	95.7±0.23	93.3±1.23	90.1±1.45

All result are expressed as Mean ± SD (n=3)

Table 13: stability of Atorvastatin calcium (ATC) loaded enteric coated mucoadhesive microsphere at 25±2^{0}C.

Temperature	**25±2^{0}C**			
Days	1	30	60	90
Physical changes	NC	NC	NC	NC
Colour change	NC	NC	NC	NC
Liquefaction	NC	NC	NC	NC
In-vitro drugrelease (%)	51.45±1.37	51.22±0.30	51.10 ±0.87	50.75±0.98
Entrapment efficiency (%)	96.24±2.1	96.19±1.5	95.65±1.3	95.2±2.4

All result are expressed as Mean ± SD (n=3)

CONCLUSION

The present study has been a satisfactory attempt to formulate a enteric mucoadhesive microsphere of Atorvastatin calcium with a view of improving its oral bioavailability and

giving a controlled released of drug. From the experimental result it can be concluded that, FT- IR study shows no significant of the peak therefore it confirm the short term stability of the drug in microsphere.

Biocompatibility polymer like sodium alginate, carbopol-974p, Eudragit-L100 can be used to formulate mucoadhesive released microsphere.

Identification of drug was carried out by physical characterization, UV Spectroscopy, IR Spectroscopy and by melting point determination.

Good percentage drug entrapment were obtained with both the polymer. The percentage drug entrapment were found to be 79.77 to 95.80 %. The size of preparation optimized batch 774.648±4.27. *In vitro* drug release of optimized batch was found to be in SGF was found to be approx. 15% and in SIF was found to be 58.08 ±0.4% and swelling index in SGF was 14.4 and in SIF was 261. Optimized formulation had good mucoadhesive property.

Enteric coated batch B showed negligible change in the drug content and drug entrapment efficiency. It successfully controlled the release at SGF pH1.2 without altering release at SIF pH 6.8 which ultimately result into prevention of conversion atorvastatin calcium acid into its lactate form and will help to reduce side effect like muscle stiffness due to lactate accumulation into skeletal muscle. The results of the stability studies of Atorvastatin calcium loaded enteric coated mucoadhesive microsphere as per the evaluation performed at 25±2^{0}C and 5±3^{0}C were shown that formulation was stable at both temperature conditions but it is most stable at room temperature as per results obtained from the stability study.

Microsphere of different size and drug content could be obtained by varying the formulation variables like polymer concentration. The multi-unit mucoadhesive microsphere of ATC delivery system is accepted to provide clinician with new choice of an economical, safe and more bio-available formulation in the management of moderate to severe lipidemia. Therefore it may be concluded that enteric coated mucoadhesive microspheres are suitable deliverysystem for atorvastatin calcium.

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