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Formulation and Evaluation of sustained release Microspheres of Atorvastatin using Chitosan and Alginate

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ABSTRACT

Certain problems regarding the drugs like high first pass metabolism, also the bioavailability of the certain drugs varies due to instability in acidic environment of stomach. Hence, to resolve such problems the drug should be incorporated in the microspheres for sustained release using a suitable polymer. Natural polymer like chitosan gained great interest in pharmaceutical sector because of its advantages like biodegradability, biocompatibility, non-toxicity, non-immunogenicity and low cost. In the present study, formulation and evaluation of polymeric microspheres of Atorvastatin Calcium was carried out and the release profile of such drug using the alginate and chitosan was studied. Microspheres were prepared for sustained release of drug using chitosan and alginate polymers by ionotropic gelation method. Microspheres were spherical in shape, having good flow properties and further its encapsulation efficiency, swelling index, micromeritic study, in-vitro drug release study and stability studies were performed in order to characterize microspheres. Three different concentrations of sodium alginate (1%, 2% and 3%) 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 (88.36) was achieved with 2% w/v sodium alginate in combination with 3% chitosan (F6). Among the prepared formulations with respect to the entrapment efficiency, swelling studies and in vitro drug release, the alginate-chitosan microspheres prepared by ionotropic gelation using calcium chloride found to be better than ionically cross linked alginate spheres alone. Therefore, dual cross-linked, microspheres are promising carrier for sustained release of drug.

Keywords: Microspheres, chitosan, sodium alginate, ionotropic gelation.

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INTRODUCTION

The problems associated with various conventional delivery systems are poor patient compliance, frequent administration of drug, unavoidable fluctuations in plasma concentration time profile, incomplete absorption of drug in gastrointestinal tract, overmedication with drugs having small therapeutic index. Hence, sustained release delivery in the form of microspheres is a better alternative for effective and safer use of drugs and to achieve maximum therapeutic efficacy. Recently, dosage forms that can precisely control the release rates and target drugs to a specific body site have made an enormous impact in the formulation and development of novel drug delivery systems. [1-2]

Microspheres form an important part of such novel drug delivery systems. They have varied applications and are prepared using different polymers. Microspheres 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, also the bioavailability of the certain drugs varies due to instability in acidic environment of stomach. Hence, to resolve such problems the 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 mechanisms. Natural polymer like chitosan gained great interest in pharmaceutical sector because of its advantages like biodegradability, biocompatibility, non-toxicity, non-immunogenicity and low cost. [3-5]

The current research will be oriented towards the formulation and evaluation of polymeric microspheres of Atorvastatin Calcium and investigate the release profile of such drug using the alginate and chitosan.

MATERIALS AND METHODS

Materials

Atorvastatin Calcium was obtained from Lupin Pharma, Pune, India; Sodium Alginate was purchased from Loba Chemicals, Mumbai, India; Chitosan was obtained as gift samples from Nitta Gelatin, Cochin, India. All other ingredients used throughout the study were of analytical grade and were used as received.

Study of physical interaction between drug and polymer

Infrared spectrum was taken by scanning the samples of pure drug and the polymers individually over a wave number range of 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.

Preparation of microspheres [6-10]

Microspheres were prepared using ionotropic gelation method. Different concentrations of sodium alginate i.e. (1% w/v, 2% w/v and 3% w/v) were prepared by dissolving in water using magnetic stirrer and these solutions were dropped using a syringe with flat tip needle into the another solutions containing different ratios of calcium chloride (previously dissolved in acetic acid 1% solution) and chitosan respectively. Microspheres were formed immediately and were left into the original solution for 24 hours to ensure internal gelification. Then they were filtered, washed with alcohol and dried at room temperature. Microspheres were formed and showed satisfactory characteristics.

Selection of salt as a cross linker for preparation of batches [11-13]

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

Selection of concentration of calcium chloride salt for preparation of microspheres

Different concentration of 1% w/v, 3% w/v, 5% w/v, 7% w/v and 9% w/v were used out of which concentration of 3% was selected as it formed comparatively rigid microspheres and no loss of shape was observed during drying.

Formulation of Batches of Microspheres for sustained release of drug

Microspheres for sustained release of drug were prepared by ionotropic gelation method. Initially, sodium alginate was used alone with calcium chloride as a cross linker and further batches were prepared by using different ratio of sodium alginate and chitosan. The concentration of sodium alginate and chitosan were selected as per the factorial design.

Selection of ratio of sodium alginate and chitosan for preparation of batches

Concentration of 1% w/v, 2% w/v and 3% w/v of both sodium alginate and chitosan were used. Different ratios were selected as per full factorial design. 3^2 factorial design was used.

Evaluation of microspheres [14-15]

Drug-excipient compatibility studies (FTIR analysis)

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

Micromeritic characterization of microspheres [16]

The microspheres were characterized by their micromeritic properties such as particle size, angle of repose, bulk density, tapped density, Carr's index and Hausner's ratio. Angle of repose was determined by funnel method. Bulk density and tapped density were determined by cylinder method, and Carr's index (CI) was calculated using the following equation.

Carr's index = (TD-BD) × 100/TD.

Hausner's ratio was calculated using following equation Hausner's ratio = TD/BD.

Table 1: Formulation of Batches containing sodium alginate alone

Formulation code	Drug concentration (%w/v)	Sodium alginate concentration (w/v)	Calcium chloride concentration (w/v)
A1	1	1	3
A2	1	2	3
A3	1	3	3

Table 2: Formula for various batches depending upon factorial

Formulation code	Drug (%w/v)	Sodium alginate (%w/v)	Chitosan (%w/v)	Calcium Chloride (%w/v)
F1	1	1	1	3
F2	1	1	2	3
F3	1	1	3	3
F4	1	2	1	3
F5	1	2	2	3
F6	1	2	3	3
F7	1	3	1	3
F8	1	3	2	3
F9	1	3	3	3

Drug content, encapsulation and loading efficiency [17]

Accurately weighed microspheres equivalent to 50 mg of the drug was crushed in glass mortar-pestle and the powdered microspheres were suspended in 100 ml of pH 7.4 phosphate buffer. After 24 h, the solution was filtered using Whatmann filter paper. Of this, 1 ml of the filtrate was taken and diluted to 10 ml. The absorbance was measured at 246 nm for sustained release.

$$\% \text{ Encapsulation efficiency} = \frac{\text{drug initial amount} - \text{free drug amount}}{\text{drug initial amount}} \times 100$$

$$\% \text{ Loading efficiency} = \frac{\text{drug initial amount} - \text{free drug amount}}{\text{Weight of drug microspheres}} \times 100$$

Table 3: Different ratio of chitosan and sodium alginate

S. No.	Sodium alginate/ chitosan ratio	Product characteristics
1	1:1 1:2 1:3 1:4	Threads were formed instead of microspheres
2	1:5 1:6	Irregular shapes of microspheres were formed with no rigidity
3	1:7 1:8 1:9	Microspheres were formed with no rigidity

Table 4: Effect of different salts on preparation of microspheres

Formulation code	Drug	Sodium alginate/ chitosan ratio	salts	Product characteristics
P1	1	1:1	Calcium Chloride	Microspheres were formed, spherical in shape and shows rigidity.
P2	1	1:1	Aluminium Chloride	Irregular shaped disc like structures were formed
P3	1	1:1	Sodium Chloride	No formation of microspheres
P4	1	1:1	Ferric Chloride	Microspheres were formed but shows discolouration due to reaction between chitosan and ferric ions.

Table 5: Different concentration of calcium chloride

Formulation code	Drug	Sodium alginate/ chitosan ratio	Concentration of calcium chloride	Product Characteristics
C1	1	1:1	1	Microspheres were formed but not strong enough and lost their shape while drying
C2	1	1:1	3	Microspheres were formed, strong enough and no loss of shape while drying
C3	1	1:1	5	
C4	1	1:1	7	Microspheres were formed, but show shrinkage while drying.
C5	1	1:1	9	

Table 6: Micromeritics Characterization of various batches

Batches	Mean Particle Size	Angle of Repose	Bulk Density	Tapped density	Carr's Index	Hausner's Ratio
A1	550.4±0.01	21.95°	0.348±0.01	0.426±0.05	13.188	1.222
A2	610.7±0.02	20.71 °	0.328±0.08	0.401±0.07	12.186	1.213
A3	700.5±0.03	17.83 °	0.333±0.07	0.409±0.08	14.528	1.227
F1	598.3±0.08	18.68 °	0.330±0.09	0.399±0.04	13.250	1.208
F2	625.2±0.07	19.21 °	0.428±0.08	0.485±0.05	11.700	1.132
F3	650.2±0.06	18.10 °	0.450±0.04	0.515±0.06	12.630	1.144
F4	680.4±0.08	17.92 °	0.373±0.06	0.430±0.02	13.559	1.156
F5	700.4±0.04	17.54 °	0.283±0.02	0.314±0.04	10.790	1.121
F6	726.3±0.07	15.42 °	0.333±0.03	0.383±0.07	13.158	1.150

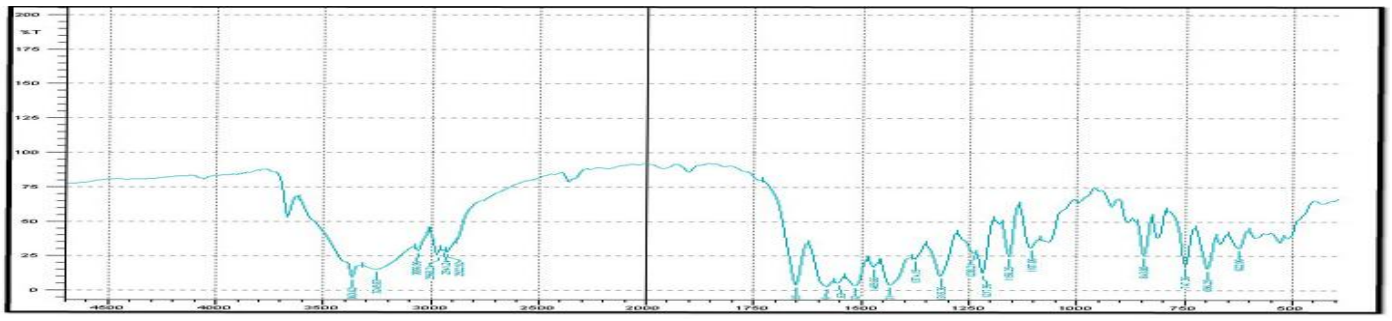

Fig. 1: Infrared Spectrum of Atorvastatin Calcium

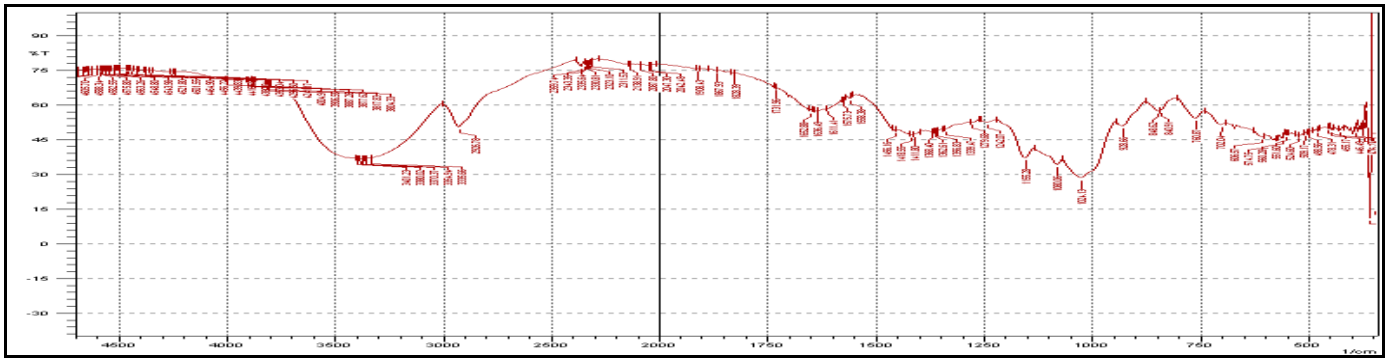

Fig. 2: Infrared Spectrum of Chitosan

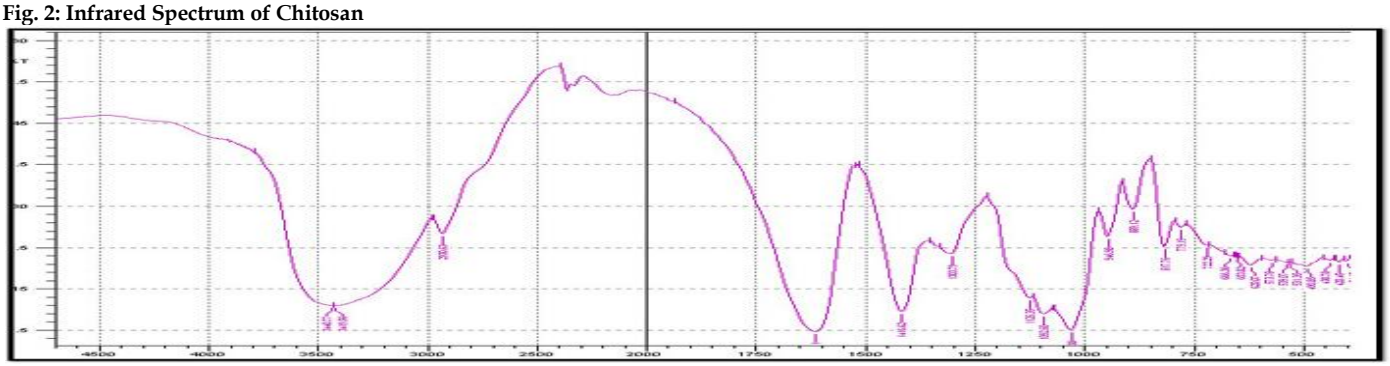

Fig. 3: Infrared Spectrum of Sodium Alginate

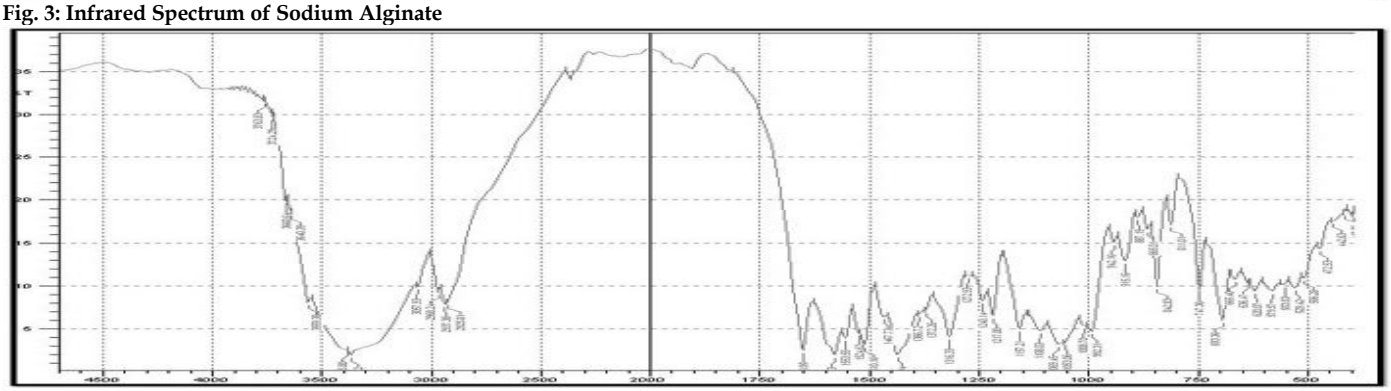

Fig. 4: Infrared Spectrum of sustained release formulation

Table 7: Swelling index of various batches

S. No.	Batch Code	Swelling Index	
		pH 1.2	pH 7.4
1	A1	350.14±0.012	759.25±0.017
2	A2	399.40±0.014	798.24±0.019
3	A3	450.25±0.017	851.54±0.016
4	F1	219.25±0.014	689.41±0.014
5	F2	178.54±0.012	640.88±0.012
6	F3	108.87±0.015	619.87±0.016
7	F4	250.96±0.019	789.79±0.012
8	F5	275.89±0.017	812.74±0.011
9	F6	299.34±0.016	830.77±0.013

Table 8: Kinetic Treatment of Drug Release Data of Various Batches

Batch code	Kinetic Equation				
	Zero Order Plot	First Order Plot	Higuchi Plot	Korsemeyer Peppas Plot	
	R^2	R^2	R^2	R^2	Slope
A1	0.9472	0.8451	0.9753	0.9931	0.865
A2	0.9489	0.8459	0.9854	0.9939	0.875
A3	0.9574	0.8574	0.9898	0.9941	0.869
F1	0.9874	0.8613	0.2569	0.9963	0.836
F2	0.9836	0.8717	0.2125	0.9970	0.854
F3	0.9812	0.8841	0.2014	0.9845	0.864
F4	0.9724	0.8782	0.1892	0.9942	0.865
F5	0.9805	0.8679	0.2094	0.9949	0.892
F6	0.9787	0.8694	0.2000	0.9941	0.863

Table 9: Stability studies of batches F4

S. No.	Parameter	Formulation Code	Sampling Intervals (Months)			
			0	1	2	3
1	Drug content	F4	39.25	39.04	38.84	38.40

Table 10: Cumulative % drug release from batches F4 during stability studies

S. No.	Storage Condition	Formulation Code	Sampling Intervals (Days)						
			0	15	30	45	60	75	90
1	4°± 0.5°C	F4	92.89	92.80	92.30	91.84	91.50	91.17	90.84
2	40° ± 0.5°C 75%RH	F4	92.89	92.75	92.24	91.78	91.41	91.07	90.67
3	60°± 0.5°C	F4	92.89	92.72	92.27	91.81	91.46	91.15	90.79

Swelling study [18-19]

The swelling index of the microspheres is an indication of the capacity of the beads to imbibe water and swell. Accurately weighed microspheres (50 mg) were placed in petri dish containing pH 1.2 HCl buffer (30 ml) for 2

h, and subsequently transferred into pH 7.4 phosphate buffer (30 ml). At the end of 1h, the beads were removed from the swelling medium, soaked with tissue paper to absorb excess water on the surface, and weighed. Then for every 1h, weights of the beads were noted. Percent weight gained by the beads was calculated by the following formula:

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

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 Dissolution Apparatus USP Type II (Rotating Paddle DISSO2000, Lab India). The USP rotating paddle method was selected to study the dissolution profiles from all formulations. The study was carried out using 500 ml of pH 1.2 buffer and phosphate buffer pH 7.4, maintained at 37°± 0.5° 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 h, monitored progress of the dissolution. The release rate from these microspheres were conducted in a medium of changing pH by starting with microspheres in pH 1.2 for 2 h and phosphate buffer pH 7.4 for further hours. The sample solutions were analyzed for atorvastatin calcium by UV absorbance at 246 nm using a UV spectrophotometer (UV-1700). Cumulative percentage of drug released was calculated and the mean of three determinations was used in data analysis. Graph was plotted between Cumulative percent drugs released vs. time.

Kinetic treatment [20]

The release data obtained were treated according to Zero order equation ($Q = K_0 t$); First order equation ($\ln Q = K_f t$); Higuchi's equation ($Q = K_H t^{1/2}$); Korsmeyer and Peppas equation ($F = (M_t / M) = K_m t^n$) to find the equation with the best Fit. Where, Q and 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; K_0, K_f and K_H are the zero order, first order and Higuchi square root of time order release rate constants respectively; K_m 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 mechanisms and is calculated from the slope of the plot of log of fraction of drug released (Mt / M) vs. log of time (t). If, n = 0.45 indicate Fickian Diffusion, n = 0.45 to 0.89 indicates Non-Fickian or anomalous transport, n = 0.89 to 1.00 indicate case –II transport and n>1 indicates Super case-II transport.

Stability study of optimized batch

The optimized batch of microspheres was kept at the chosen temperature and humidity for 3 month and was evaluated for different parameters such as drug content, *in vitro* drug release.

RESULTS AND DISCUSSION

FTIR spectroscopy

It was observed from the FTIR graph as shown in Figure 1, Figure 2, Figure 3 and Figure 4 that there was no shifting of peaks of atorvastatin i.e., characteristic peak of the halide group (C-F stretching) at 1107 cm^{-1} in sustained release formulation. Hence, there was no interaction between drug and polymer.

Evaluation of microspheres

The microspheres of different formulations were evaluated for product characteristics (as shown in Table 3, Table 4 and Table 5) and particle size, angle of repose, bulk density, tapped density, Carr's index and Hausner's ratio (Table 6). An angle of repose of less than 30 degrees indicates good flow properties. This was further supported by the lower Carr's index. Granules with Carr's index values around 21% and below are considered to have fair and excellent flow properties.

Encapsulation and loading efficiency

Three different concentrations of sodium alginate (1%, 2% and 3%) 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 (88.36) was achieved with 2 % w/v sodium alginate in combination with 3% chitosan (F6) (shown in Figure 5).

Good drug loading efficiency was achieved for all the formulation (A1-F6) since Ca^{++} and NH_3^+ of Chitosan compete with each other and react with $-COO^-$ of Sodium alginate resulting in more compact structure. Some drug was lost to the external phase during preparation and recovery. The optimized batch F4 showed the loading efficiency of 39.25.

Swelling Index

From the Table 7 and Figure 6 and 7 it was observed that all formulations showed comparatively lower swelling index in pH 1.2 buffer than in pH 7.4 phosphate buffer. It was found that the microspheres shrink in acidic pH, this could be well justified due to the fact that, at acidic pH strong interaction occurs between ammonium groups of Chitosan and carboxyl group of Alginate which is due to the formation of intermolecular and intramolecular hydrogen bond (polyelectrolyte complex) between the two polymers. Additionally, a repulsive force within the test microspheres is created due to protonation of primary ammonium group ($-NH_3^+$) of Chitosan. But because the force of H-bond is greater than the repulsive force, the microspheres are kept in a shrunken state in acidic medium.

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 Ca^{2+} for sodium start. As Ca^{2+} ions are replaced by Na^{+} ions, the dense cross linked structure starts to get loosened and water starts getting absorbed into the microspheres.

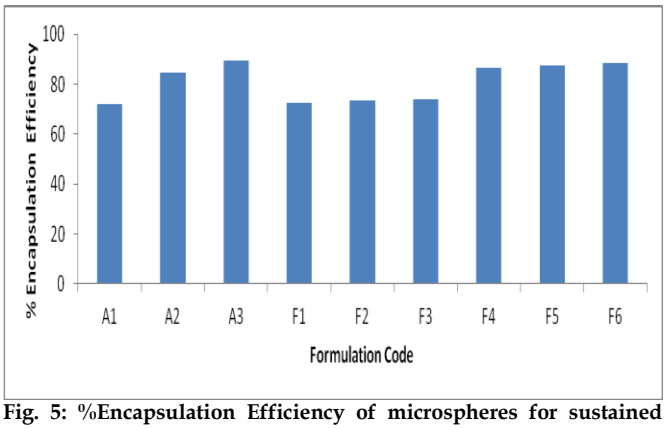

Fig. 5: %Encapsulation Efficiency of microspheres for sustained release of drug

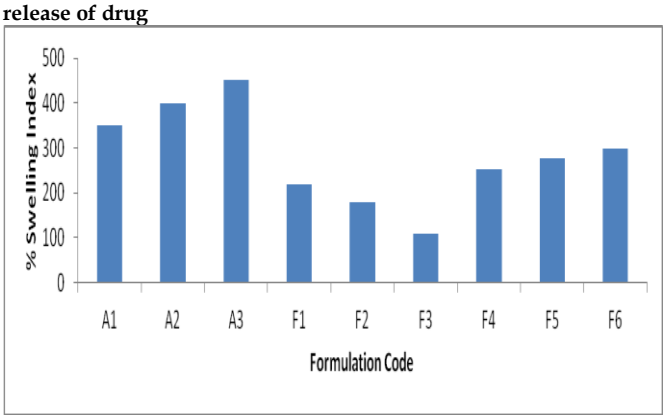

Fig. 6: % Swelling Index of Microspheres for sustained release of drug at pH 1.2

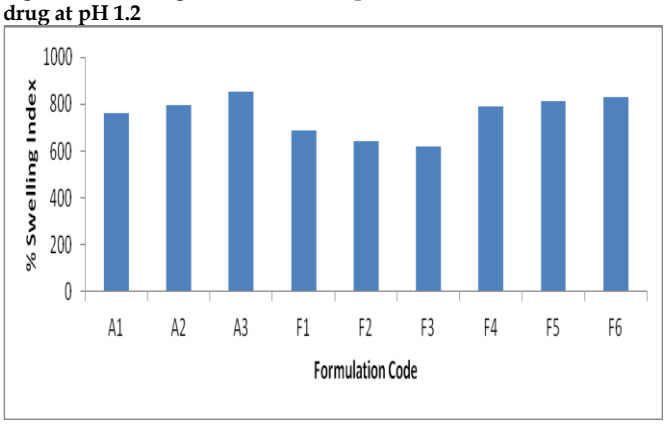

Fig. 7: % Swelling Index of microspheres for sustained release of drug at pH 7.4

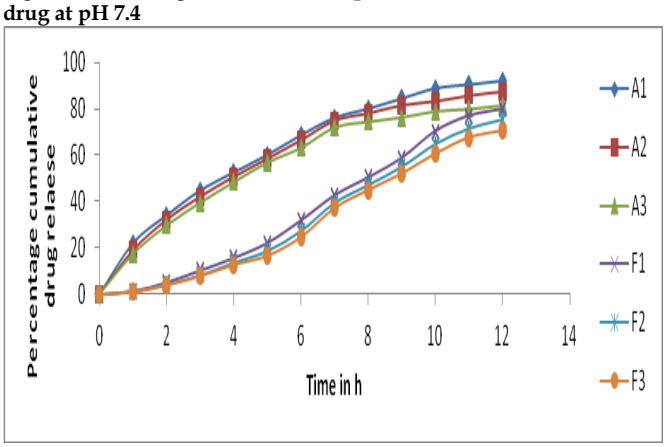

Fig. 8: Percentage Cumulative drug release (A1-A3, F1-F3)

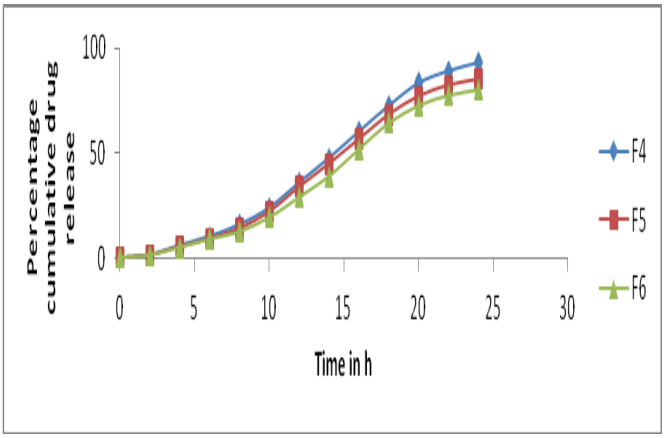

Fig. 9: Percentage Cumulative drug release (F4-F6)

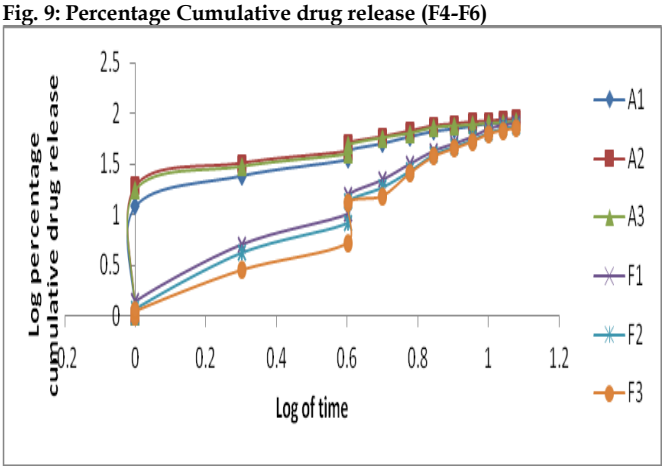

Fig. 10: Korsemeyer peppas plot of various batches (A1-A3, F1-F3)

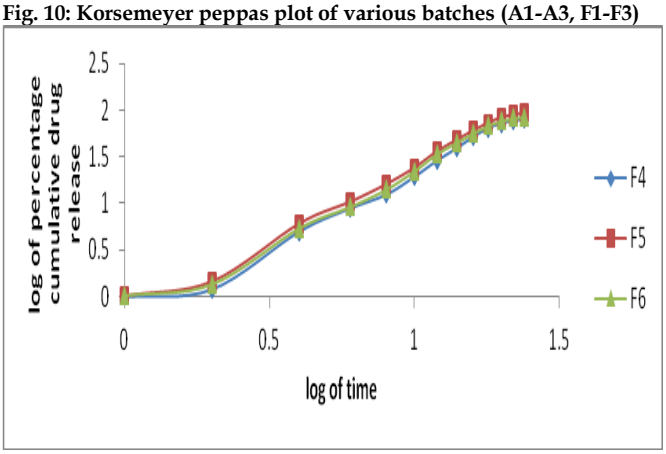

Fig. 11: Korsemeyer peppas plot of various batches (F4-F6)

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 chitosan was blended with alginate matrix. The highest drug release about 92.89% was found to be in formulation F4 containing 2% w/v sodium alginate and 1% w/v chitosan and hence was designated as optimized batch (shown in Figure 8 and 9).

Effect of pH on drug release

It can be seen that release rate of Atorvastatin in simulated intestinal fluid (pH 7.4) was relatively higher than in simulated gastric fluid (pH 1.2). Low release in acidic medium was due to strong interaction between ammonium groups of Chitosan and carboxyl group of Alginate which is due to the formation of intermolecular and intra molecular hydrogen bond between the two polymers. Additionally, a repulsive force within the microspheres has created due to the protonation of primary ammonium groups ($-NH_3^+$) of Chitosan. But because the force of H-bond is greater than the repulsive force, the microspheres are kept in a shrunken state in acidic medium and the drug is released slowly.

However, under alkaline condition there was breakage of H-bond which reduces the interaction between the polyelectrolyte and ionization of carboxylic group of alginate results in swelling of microsphere network with subsequent imbibitions of fluid and dissolution of drug followed by drug release by diffusion. Batches from F1- F6 had shown this type of release. However, the releases from batches A1-A3 were characterized by an initial phase of high release (burst effect). As gelation proceeded, the remaining drug was released at a slower rate followed by a phase of moderate release.

Effect of polymer concentration on drug release

Slowest release was observed in formulations (F4) containing 2% w/v sodium alginate and 1% w/v chitosan with 92.89% drug release in 24 hours Thus, these formulations were capable of controlling drug release and considered as optimized. Release rate was rapid with low percent polymer concentration. Microspheres (A1-A3) containing only sodium alginate released up to 90% of drug within 12 hours. These results suggested that, higher polymer concentration of chitosan formed a highly viscous microspheres network which sustained the drug release.

Effect of Polymer ratio on Drug Release

It was concluded that microspheres containing Chitosan (F1-F6) gave lower drug release than the microspheres containing only sodium Alginate (A1-A3). Since, the presence of chitosan increases the control of the release of drug from the microspheres, as at increasing concentration,(batch F4, F5 and F6 containing 1% w/v, 2% w/v and 3% w/v chitosan respectively) it can form a network of bonding between the two polymer chains. Hence, at increasing amount of chitosan concentration into the formulation, interaction between the two polymers might have been increased, forming a closer network, which should decreased the diffusion of drug outside the microspheres.

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 (Table 8). The rate 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. The n value of microspheres of different batches was ranged between 0.836 and 0.862, indicating that the mechanism of drug release was Non-Fickian or anomalous transport (release due to swelling, polymer chain relaxation and disentanglement) as shown in Figure 10 and 11.

Stability study

The results of stability study (Table 9-10) indicated that there was no significant variation in the drug release profile of the optimize batch F4 during the three month study therefore, it was concluded that the batch F4 was stable over the chosen temperature and humidity for 3 month.

Microspheres were prepared for sustained release of drug using chitosan and alginate polymers by ionotropic gelation method. Microspheres were spherical in shape, having good flow properties and encapsulation efficiency, swelling index, micromeritic study, in- vitro drug release study and stability studies were performed in order to characterize microspheres.

In case of sustained release of drug, among the prepared formulations with respect to the entrapment efficiency, swelling studies and in vitro drug release, the alginate-chitosan microspheres prepared by ionotropic gelation using calcium chloride found to be better than ionically cross linked alginate spheres alone. Therefore, dual cross-linked, microspheres are promising carrier for sustained release of drug.

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REFERENCES

1. Lee Y, Robinson J, In: Remington, The Science and Practice of Pharmacy, 20th ed., Vol 2, Lippincott Williams and Wilkins; 2000.p. 903-904
2. Lachman L, Liberman H, Kanig J, The theory and Pracice of Industrial Pharmacy, 3rd ed., Philadelphia, Lea and Febiger, 1986.p. 430.
3. Chien Y, Novel Drug Delivery Systems, 2nd ed., New York, Marcel Dekker, Inc.,1992.p. 1.
4. Vyas SP, Khar RK, editors. Targeted and controlled drug delivery novel carrier systems. New Delhi: CBS publishers and distributors; 2002. p. 417.
5. Jain NK, Controlled and Novel Drug Delivery. New Delhi: CBS publishers and distributors; 2005.p.536.
6. Ramdas M, Dileep KJ, Anitha Y, Willi P, Chandra PS. Alginate encapsulated bioadhesive chitosan microspheres for intestinal drug delivery. J Biomater Appl 1999;13(4):290-296.
7. Kamel AH. Alginate- diltiazem hydrochloride beads: optimization of formulation factors, *in vitro* and *in vivo* bioavailability. J Microenacapsul 2003;20(2):211-255.
8. Barhate SD, Rupnar YS, Sonvane RM, Pawar KR, Rahane RD. Formulation and evaluation of floating microspheres of ketorolac trometamol. Ind J Pharm Res Dev 2009;1(9):1-8.

9. Rasala TM, Lohiya GK, Kale VV, Avari JG. Comparative study of ionotropic gelation technique to entrap diltiazem HCl in mucoadhesive microparticulate system. J Pharm Res 2010;3(7):1531-1534.
10. Kumbar SG, Kulkarni AR and Aminabhavi TM. Cross linked chitosan microspheres for encapsulation of diclofenac sodium: effect of crosslinking agent. J Microencapsul 2002; 19: 173- 180.
11. Orienti I, Cerchiara T. Influence of different chitosan salts on the release of sodium diclofenac in colon-specific delivery. Int J Pharm2002;238;51-59.
12. Jain GK *et.al,* Enhanced bioavailability of nano-sized chitosan–atorvastatin conjugate after oral administration to rats. European J Pharm Sci 2011; 44; 241–249.
13. Burges DJ, Hickey AJ. Microsphere technology and applications. In: Swarbick J, Boyan JC, editors. Encyclopedia of pharmaceutical technology. Vol.10. New York: Marcel Dekker, Inc; 1994: 3.
14. Wittaya-areekul S, Kruenate J, Prahsarn C. Preparation and in vitro evaluation of mucoadhesive properties of alginate/chitosan microparticles containing prednisolone. Int J Pharm 2006; 312:113-118.
15. Silva CM, Ribeiro AJ, Figueiredo M, Ferreira D, and Veiga F. Microencapsulation of haemoglobin in chitosan-coated alginate microspheres prepared by emulsification/internal gelation. AAPS Pharm Sci Tech 2006;7(4):E903-913.
16. Poncelet D, Babak V, Dulieu C, Picot A. A physico-chemical approach to production of alginate beads by emulsification-internal ionotropic gelation. Colloids and Surfaces A: Physicochem Eng Aspects 1999;155:171-176.
17. Sweetman SC, Martindale. The complete drug reference. 33rd ed. Pharmaceutical press; London: 2000; p. 1421, 1321, 1265, 130.
18. Rowe RC, Sheskey PJ, Weller PJ. Editors. In: Handbook of Pharmaceutical Excipients. 6th ed. Pharmaceutical Press, London. 2001. p. 624, 326, 110.
19. United States Pharmacopoeia 30, NF 25, Asian Ed;, United States Pharmacopoeial Convention, 2007. pp. 1137, 1081.
20. Berthold A, Cremer K and Kreuter J. Preparation and characterization of chitosan microspheres as drug carrier for prednisolone sodium phosphate as model for anti-inflammatory drugs. J Control Release 1996; 39: 17-25.

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