International Journal of Pharmaceutical Development & Technology

e ISSN - 2248 - 910X

www.ijpdt.com

Print ISSN - 2248 - 9096

FORMULATION AND EVALUATION OF MICROSPHERES FOR IMMEDIATE AND SUSTAINED RELEASE OF DIFFERENT DRUGS USING SAME POLYMER

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ABSTRACT

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. Microspheres form an important part of such novel drug delivery systems. They have varied applications and are prepared using different polymers. Certain problems regarding the drugs like high first pass metabolism, also the bioavailability of the certain drugs varies due to the 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. The current research will be oriented towards the formulation and evaluation of polymeric microspheres of different drugs like atorvastatin calcium and amlodipine and investigate the release profile of such drug using the same polymer chitosan.

Keywords: Atorvastatin calcium, Amlodipine, Chitosan, Sodium alginate, Ionotropic gelation method.

INTRODUCTION

Microspheres can control the delivery of drugs from days to months therefore reducing frequent administrations and improving patient compliance and comfort. Different release profiles with desired release rates can be achieved by selecting polymers with different degradation mechanisms. Use of natural and thus biodegradable polymers to achieve desired release profile is widely accepted practice in pharmaceutical formulation. In this experimental work, cellulose derivatives like Chitosan and Sodium alginate were used to prepare microspheres [1].

Combination of drugs to mitigate different symptoms or aetiologies has been a trend. Its significance is well established and validated through several studies but there may be several consequences regarding the combination of drug in unit dosage form that are undesirable. Different absorption site of each drug is one of the most critical point that plays an important role in the success of the multi drug containing dosage form. In this study an attempt was made to release Atorvastatin and Amlodipine at two different sites via unit dosage form i.e. capsule. Single polymer i.e. Chitosan was used to avoid biocompatibility issue and Sodium alginate to sustain the release of drug [2].

MATERIALS AND METHODS

All the chemicals used were laboratory grade. All the procedures used were validated. Materials: Chitosan (Nitta Gelatin, Cochin, Kerala), Sodium Alginate (Loba Chemicals), Atorvastatin Calcium (Lupin Pharmaceuticals, Pune) and Amlodipin (Zim Laboratories, Nagpur), Calcium Chloride, Tripolyphosphate (TPP), Acetic Acid and Ethyl Alcohol.

Method of preparation of microspheres

For sustained release of drug:

Accurately weighed amount of sodium alginate (1%) was dissolved in water by using magnetic stirrer. In another beaker 1% chitosan previously dissolved in acetic acid solution (1%) was mixed with calcium chloride solution in water. Sodium alginate solution was added drop wise using a syringe with flat tip needle into a chitosan-calcium chloride solution. Microspheres formed immediately and were left into the original solution for 24 hr to ensure internal gelification. Then they were filtered, washed with alcohol and dried at room temperature [3-5].

For immediate release of drug

Different concentration of chitosan i.e.0.5% w/v,

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1% w/v and 1.5% w/v was prepared in 1% v/v acetic acid solution. Fixed concentration of drug (amlodipine) was dispersed in chitosan solution under continuous stirring until a uniform dispersion was obtained.

Swelling index

The swelling index of the microspheres is an indication of the capacity of the microspheres to absorbed water and swell. For estimating swelling index, the microspheres (50) were weighed initially then suspended in pH 1.2 and 7.4 phosphate buffer. After every 1 h microspheres were removed, surface water trapped with tissue paper and weighed. The increase in weight of microspheres used for calculation of swelling index [6].

$$\text{Swelling index} = \frac{\text{Weight of wet microspheres x 100}}{\text{Weight of dry microspheres}}$$

Determination of encapsulation efficiency

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, 1ml of the filtrate was taken and diluted to 10ml. The absorbance was measured at 246 nm for sustained release and at 360 nm for immediate release of drug [7].

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Experimental drug content x 100}}{\text{Theoretical drug content}}$$

Micromeritic Characterization of Microspheres

The microspheres are characterized by their micromeritic properties such as particle size, angle of repose, bulk density, tapped density, Carr's index and Hausner's ratio [8-10].

Particle size analysis

Particle size of microspheres was determined by using an optical microscope under regular polarized light, and the mean particle size was calculated by measuring 100-particles with the help of a calibrated ocular micrometer.

Angle of repose

Angle of repose of different formulations was measured according to fixed funnel standing method.

$$\theta = \tan^{-1} h/r$$

where, h is height of the heap, d is diameter of the microspheres heap that is formed after making the microspheres flow from the glass funnel.

Bulk and tapped densities

Bulk and tapped densities were measured by using 10 ml of graduated cylinder. The sample poured in cylinder was tapped for 100 times, then tapped volume was noted down and bulk density and tapped density were calculated.

$$\text{Bulk density} = \frac{\text{Mass of microspheres}}{\text{Bulk volume}}$$

$$\text{Tapped density} = \frac{\text{Mass of microspheres}}{\text{Tapped volume}}$$

Compressibility index or Carr's Index

Compressibility index or Carr's Index value of microparticles was computed according to the following equation:

$$\text{Carr (\%)} = \frac{\text{(Tapped density – Bulk density) x 100}}{\text{Tapped density}}$$

Hausner ratio

Hausner's ratio of microparticles was determined by comparing the tapped density to the bulk density using the equation [11]

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

***In vitro* drug release studies**

The *in vitro* drug release studies were performed using Dissolution Apparatus USP using simulated gastric fluid pH 1.2 for twelve hours. An accurately weighed amount of drug loaded mucoadhesive microspheres equivalent to 50 mg of cephalexin, was added to 900 ml of dissolution medium and the release of cephalexin, from mucoadhesive microspheres was investigated at about 100 rpm at temp 37 $^{\circ}$C ± 0.5 $^{\circ}$C. During dissolution 5 ml aliquot was withdrawn at different time intervals of 1 to 12 h and same was replaced with equal volume of fresh medium. The withdrawn samples were filtered through Whatmann filter paper no.42 and absorbance was measured at 263 nm using UV-Visible Spectrophotometer. Cumulative percent drug released was found out at each time interval and graph was plotted between cumulative % drug released and time in h.

Treatment of drug release data with different kinetic equations

Analysis of drug release from microspheres was performed with a flexible model that can identify the contribution to overall kinetics, mechanism of drug release and the dissolution data obtained for optimized formulation was treated with the different release kinetic equations [12].

RESULTS AND DISCUSSION

Microspheres were prepared using ionotropic gelation method, different concentrations of sodium alginate were prepared (1% w/v, 2% w/v and 3% w/v) and these solution was dropped into the another solution containing different ratios of calcium chloride and chitosan.

Microspheres were formed and showed rigidity. Hence, this approach was used for further preparation of batches.

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.

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. 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

Microspheres were prepared using ionotropic gelation technique. The concentration of the polymers and the concentration of the cross-linker agent were optimized by trial and error method. Three concentrations of the chitosan 0.5% w/v, 1% w/v and 1.5% w/v were tried which gave the formation of well formed microspheres. At concentrations above 1.5% w/v the viscosity was found to be very high as a result the drop wise extrusion of the polymer solution from the syringe became difficult to control. Thus, 0.5% w/v, 1% w/v and 1.5% w/v solution was chosen for the further studies. Similarly, a number of concentrations of Tripolyphosphate were tried as a cross-linker agent. Out of which 3% w/v and 6% w/v concentration of TPP were selected as it gave the formation of spherical and rigid microspheres. 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. In case of immediate release, the swelling of chitosan microspheres were increased with increasing chitosan concentration, in acidic pH, the swelling process involves the protonation of ammonium group of chitosan and mechanical relaxation of coiled polymeric chain.

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 incorporation efficiency (88.36) was achieved with 2 % w/v sodium alginate in combination with 3% chitosan (F6). In case of immediate release entrapment efficiency increases with increasing amount of chitosan.

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 39.20.

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.

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. 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.

Table 1. Formulation of Various Batches for Sustained Release of Drug

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

Table 2. Formulation of Various Batches for Immediate Release of Drug

Formulation code	Drug level(%w/v)	Chitosan (%w/v)	Tripolyphosphate(%w/v)
I1	1	0.5	3
I2	1	1	3
I3	1	1.5	3
I4	1	0.5	6
I5	1	1	6
I6	1	1.5	6

Table 3. Swelling Index of Various Batches

Sr. 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
10	I1	450.65±0.013	--
11	I2	504.87±0.011	--
12	I3	550.21±0.010	--
13	I4	309.41±0.017	--
14	I5	375.39±0.014	--
15	I6	345.25±0.015	--

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

Table 4. Encapsulation Efficiency of Various Batches

Sr. No.	Batches	% Loading Efficiency	% Encapsulation Efficiency
1	A1	38.31±0.012	72.18±0.014
2	A2	39.42±0.010	84.74±0.015
3	A3	39.84±0.014	89.54±0.018
4	F1	38.40±0.016	72.45±0.012
5	F2	38.59±0.012	73.21±0.015
6	F3	38.73±0.017	73.91±0.014
7	F4	39.25±0.016	86.74±0.017
8	F5	39.42±0.012	87.61±0.012
9	F6	39.69±0.013	88.36±0.013
10	I1	39.21±0.014	88.03±0.015
11	I2	38.47±0.018	91.45±0.019
12	I3	39.14±0.014	94.21±0.014
13	I4	39.41±0.011	87.14±0.019
14	I5	39.25±0.013	92.45±0.017
15	I6	39.74±0.012	93.24±0.014

Table 5. Micromeritics Characterization of Various Batches

Batches	Mean Particle Size	Angle of Repose	Bulk Density	Tapped density	Cars Index	Hausner Ratio
A1	550.4±0.01	21.95º	0.348±0.01	0.426±0.05	13.188	1.222
A2	610.7±0.02	20.71 º	0.328±0.08	0.401±0.07	12.186	1.213
A3	700.5±0.03	17.83 º	0.333±0.07	0.409±0.08	14.528	1.227
F1	598.3±0.08	18.68 º	0.330±0.09	0.399±0.04	13.250	1.208
F2	625.2±0.07	19.21 º	0.428±0.08	0.485±0.05	11.700	1.132
F3	650.2±0.06	18.10 º	0.450±0.04	0.515±0.06	12.630	1.144
F4	680.4±0.08	17.92 º	0.373±0.06	0.430±0.02	13.559	1.156
F5	700.4±0.04	17.54 º	0.283±0.02	0.314±0.04	10.790	1.121
F6	726.3±0.07	15.42 º	0.333±0.03	0.383±0.07	13.158	1.150
I1	535.2±0.01	22.01º	0.347±0.01	0.398±0.09	13.45	1.154
I2	590.6±0.05	21.45º	0.359±0.07	0.405±0.06	11.280	1.127
I3	621.8±0.09	19.25º	0.318±0.01	0.361±0.04	11.846	1.135
I4	550.2±0.04	21.95º	0.289±0.05	0.339±0.08	14.840	1.174
I5	625.4±0.07	19.22º	0.348±0.09	0.426±0.09	13.188	1.222
I6	660.8±0.08	18.20º	0.328±0.07	0.401±0.01	12.186	1.213

Table 6. 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
I1	0.5894	0.5850	-1.254	0.8225	0.745
I2	0.5908	0.5872	-1.425	0.8232	0.785
I3	0.5998	0.5874	-1.652	0.8365	0.774
I4	0.9954	0.9989	0.7541	0.8547	0.863
I5	0.9862	0.9972	0.7341	0.9097	0.895
I6	0.9675	0.9622	0.7021	0.9196	0.874

Fig 1. Percentage Cumulative drug release (A1-A3, F1-F3)

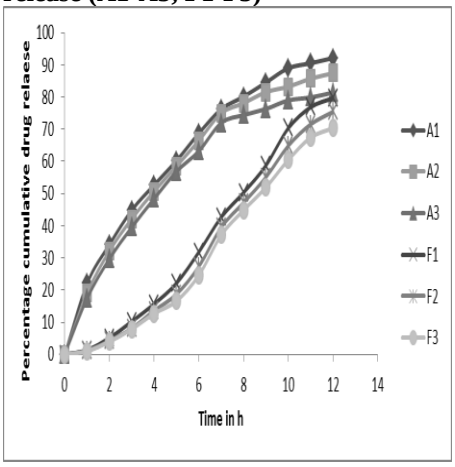


Fig 2. Percentage Cumulative drug release (F4-F6)

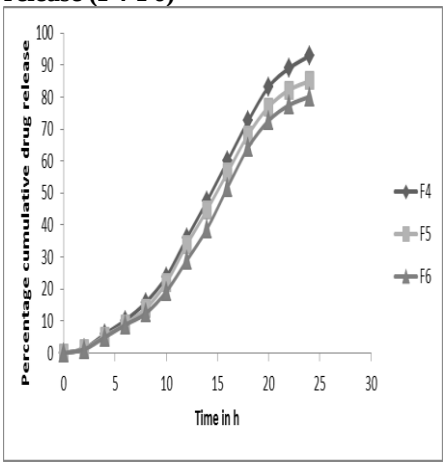
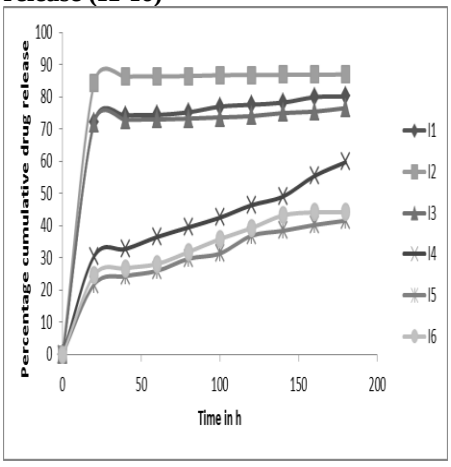


Fig 3. Percentage Cumulative drug release (I1-I6)



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.

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. The cumulative percentage drug release curve of the drug loaded chitosan microspheres showed increase rate of drug release at lower concentration of chitosan and TPP. The highest drug release about 86.94% was found to be in formulation I2 and hence was designated as optimized batch. Batch I3 showed high swelling index (550.21) as compared to the other batches. Swelling is directly proportional to the drug release. Swelling ability of ionic cross-linked microspheres is depending on the pH value of the swelling medium. Microspheres show high swelling degree at low pH. The higher swelling degree is attributed to the strong protonization of ammonium groups on chitosan, which bring strong electrostatic repulsion among intrachain and interchain of chitosan, resulting in the relaxation of the polymer network. Hence, increased the release rate of drug.

As the concentration of the TPP solution increased, the swelling capacity of the microspheres decreased as per table no. 15 batches I4, I5and I6 shows comparatively low swelling index 309.41, 375.39 and 345.25 as compared to the batches I1, I2 and I3 containing 6% TPP. These support that the more tightly cross linked chitosan matrix does not swell as compared to the loosely cross linked chitosan matrix. At low concentration of TPP, the chitosan network is loose and has high capacity to accommodate more solvent molecule therby inducing chitosan TPP matrix swelling.

CONCLUSION

Microspheres were prepared for sustained and immediate release of drug using a same polymer chitosan 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. In case of immediate release of drug, the Chitosan/TPP crosslinked microspheres of amlodipine were prepared by the ionotropic gelation method. The microspheres showed very high release of drug at pH 1.2. Release was found to be depend upon the various chitosan/TPP ratio.

ACKNOWLEDGEMENTS

Authors wish to thank ZIM Labs, Nagpur for providing amlodipine as a gift sample.

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