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Ionotropically Gelled Chitosan-alginate Complex Hydrogel Beads: Preparation, Characterization and *In-vitro* Evaluation

J.S Patil*, M.V. Kamalapur, S.C. Marapur, S.S. Shiralshetti and D.V. Kadam

Dept. of Pharmaceutics, BLDEA's College of Pharmacy, BLDE University campus, Bijapur - 586 103, Karnataka, India.

ABSTRACT

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Prolonged release drug delivery system of stavudine was made by ionotropic gelation and polyelectrolyte complexation technique. Cross-linking reinforced chitosan-Alginate complex beads were prepared by gelation of anionic sodium alginate, the primary polymer, with oppositely charged counter ion to form beads which were further complexed with chitosan as a polyelectrolyte. The effect of this polymer on release profile of drug was studied. Beads without chitosan complexation were also made. The reaction of chitosan-alginate complex dominates the formation of skin layer on the surface of beads. Stavudine an anti-retroviral drug was selected as novel drug for the experiment. The final formulations were subjected to in-vitro evaluation and several characterization studies. Batches with alginate alone showed Higuchi model and while chitosan-alginate showed zero order release. All the batches with copolymer showed sustained the drug release more than 12 hrs whereas with alginate alone showed up to 10 hrs. Batches with chitosan coating showed maximum drug encapsulation efficiency.

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

INTRODUCTION

Chitosan is a biopolymer, which could be used for the preparation of various polyelectrolyte complex products with polyanions such as corboxymethylcellulose, xanthan, alginate and gellan gum[1-3]. Chitosan-polyanion complexes have been widely investigated for applications like drug and protein delivery, cell transplantation, enzyme immobilization[4-6]. Among the chitosan-polyanion complexes, chitosan-alginate complex may be the important drug delivery system. The strong electrostatic interaction of amine groups of chitosan with the carboxyl groups of Alginate lead to the formation of chitosan- alginate complex.

Stavudine, 2', 3'-didehydro-3'-deoxythymidine (D4T) is a thymidine analog approved for the treatment of HIV infection[7] like other member of this class of antiretrovirals, its purported active metabolite, D4T-5'-triphosphate, is an inhibitor of the HIV reverse transcriptase and as a chain terminator during DNA synthesis. Stavudine is currently approved by US-FDA for the treatment of patients who have become intolerant or failed to response to zidovudine, didanosine or zalcitabine therapy. The mean serum elimination half life of stavudine was reported in the ranges between 1 to 1.67 hrs in adults. Stavudine is given 40 mg twice daily. The dose related adverse effect is peripheral neuropathy. Converting twice daily regimen of stavudine into once daily improve adherence and, therefore, enhances the effectiveness of antiretroviral therapy[8]. For many drugs, the optimal therapeutic response is observed only when adequate blood levels are achieved and maintained with minimal variation. Sustained release products have become important for the oral administration of many drugs because they give more consistent blood levels[9].

This work focused on the preparation of novel stavudine chitosan-alginate beads with inner cross-linked alginate core with calcium chloride and outer chitosan-alginate complex membrane. The one-stage procedure for the preparation of cross-linking reinforced chitosan- alginate beads was examined by dropping alginate solution into chitosan solution containing calcium chloride cross-linking agent. It has been found that the macromolecular chitosan rapidly bind onto the surface of alginate droplet, but were limited to diffuse into the inner core. In order to increase the stability of chitosan-alginate complex, chitosan solution, consisting of $CaCl_2$, was used for the gelation of alginate. The presence of calcium ions in the chitosan solution during the incubation had a great effect on the ability of a gel bead to bind chitosan. Chitosan was bound faster and to a higher extent with increasing concentrations of calcium chloride. The presence of calcium salt leading to the competition of gelling reaction and polyelectrolyte complex results in the formation of a more porous gel, allowing the diffusion of chitosan[10,11].

MATERIALS AND METHODS

Stavudine was a gift sample from Aurobindo Pharmaceutical Pvt. Ltd. Hyderabad, India. Sodium alginate was generous

***Address for Correspondence:**

J.S. Patil , Department of Pharmaceutics, B.L.D.E.A's, College of Pharmacy,BLDE University campus, Bijapur-586 103, Karnataka, India.

E-mail pharmajspatil@gmail.com

gift sample from Micro Lab Pvt. Ltd. Bangalore, India, and Chitosan was a gift sample from Central Institute of Fisheries and Technology, Cochin, India. All other chemicals, reagents and solvents used were of pharmaceutical or analytical grade.

Preparation of alginate beads[12]:

The beads were prepared by the unique ionotropic gelation technique. Sodium alginate solution was prepared by dissolving in deionized water and heated at 60 °C. Different concentrations of the drug was dissolved/ dispersed uniformly in 50 ml of alginate solution below 40 °C under continuous stirring. The stirring was continued after complete addition until a uniform dispersion was obtained. The resultant homogeneous bubble free slurry dispersion was dropped through a 21G syringe needle into 100 ml of calcium chloride solution containing chitosan in a definite proportion. The solution was kept under stirring to improve the mechanical strength of the beads and also to prevent aggregation of the formed beads. Immediate formation of small alginate beads took place after 5 min of curing time. The formed beads were collected by filtration and dried at 40°C. The alginate beads without chitosan were also prepared in the similar way.

Particle size Measurement[13]:

The prepared beads were subjected for particle size analysis using a digimatic micrometer (MDC-25S Mitutoyo, Tokyo, Japan) having an accuracy of 0.001 mm. The average diameter of the 100 particles per batch was calculated.

Drug entrapment efficiency[14]:

Known amount of beads (20 mg) were added to 20 ml USP phosphate buffer of pH 7.4 solution for complete swelling at 37 °C. The beads were crushed in a glass mortar with pestle the solution was than kept for 2 h to extract the drug completely and centrifuged to remove polymeric debris. The clear supernatant solution was analyzed for drug content using UV-visible spectrophotometer at 266 nm (Pharmaspec-1700, Shimadzu, Japan).

Dynamic swelling study[15]:

The dynamic swelling behavior of the beads was studied by mass measurement. The 50 mg of beads were incubated with 25 ml phosphate buffer solution pH 1.2 and pH 7.4 separately at 37 °C. The beads were taken out at different time intervals up to 12 hrs and blotted carefully without pressing hard to remove the excess surface liquid. The swollen beads were weighed using the electronic microbalance. The percent water uptake (Q) at different time intervals was calculated using the following equation.

$$Q = \frac{W_2 - W_1}{W_1} \times 100$$

Where W_1 is mass of the dry beads and W_2 is the mass of swollen beads.

Differential scanning calorimetric analysis (DSC):

Thermal behavior of the beads was examined by using a thermal analyzer (DSC Q20 V24.4 BUILD116, USA). The samples were heated from 0-300° at a heating rate of 10 °C /min under argon atmosphere in a micro calorimeter and then thermograms were obtained.

X-ray diffraction analysis (XRD):

The spectra were recorded using a Philips, PW-171, x-ray diffractometer with Cu-NF filtered CuK radiation. Quartz was used as an internal standard for calibration. The powder x-ray diffractometer was attached to a digital graphical assembly and computer with Cu-NF 25 KV/20 mA tube as a CuK radiation source in the 2 range 0-50.

Scanning electron microscopy (SEM):

The surface morphology of the beads was investigated using scanning electron microscope (JEOL, JSM-35CF, Japan). The beads were mounted onto stubs using double sided adhesive tape and sputter coated with platinum using a sputter coater. The coated beads were observed under SEM instrument at the required magnification at room temperature. The acceleration voltage used was 10 kV with the secondary electron image as a detector.

In-vitro drug release study[16]:

In vitro drug release study was carried out using a USP-XXIII dissolution apparatus (Electro lab-TDT 06P, Mumbai). The dissolution was measured at 37.0 ± 0.5 °C and 50 rpm paddle speed. Drug release from the beads was studied in 500 ml acidic medium (pH 1.2) for 2 hours and in alkaline medium (pH 7.4 phosphate buffer) till end of the study. At predetermined time intervals, 5 ml aliquots were withdrawn and replaced with the same volume of fresh solution. The amount of drug released was analyzed using UV-visible spectrophotometer at 266 nm.The release data were fitted to various mathematical models to know which model is best fitting the obtained release profile.

RESULT AND DISCUSSION

The formulation compositions of various batches are shown in Tables 1. The drug entrapment efficiency (DEE) of the prepared beads was carried out by swelling method and the results are summarized in Table 2. The drug entrapment efficiency was found to be in the range of 44.89±0.052 to 72.42±0.013 %. The results indicate that the DEE of the beads

Table 1: Composition of hydrogel beads of stavudine

Batch code	Drug (Parts)	Sodium alginate (Parts)	Chitosan (Parts)	Calcium chloride(%)
Fa1	1	1	1	3
Fa2	1	2	1	3
Fa3	2	1	1	3
Fa4	1	1	1	5
Fa5	1	2	1	5
Fa6	2	1	1	5
Fa7	1	1	-	3
Fa8	1	2	-	3
Fa9	2	1	-	3

Table 2: The data of bead size, DEE and swelling behavior after 12 hrs in pH 1.2 and pH 7.4 phosphate buffer

Batch code	Average size(µm)	DEE (%)	Swelling in pH 1.2	Swelling in pH 7.4
a1	734±2.33	68.77±0.094	114.8±0.11	112.7±0.14
Fa2	793±3.20	75.48±0.033	145.2±0.83	130.6±0.78
Fa3	838±2.31	64.30±0.020	122.2±0.42	115.1±0.21
Fa4	644±2.23	68.44±0.021	132.6±0.31	126.9±0.43
Fa5	653±3.32	73.21±0.032	154.8±0.26	150.0±0.62
Fa6	718±3.76	64.18±0.067	140.0±0.31	135.3±0.90
Fa7	623±2.09	50.34±0.087	106.0±0.64	102.4±0.23
Fa8	633±2.21	60.20±0.036	120.0±0.31	110.0±0.11
Fa9	731±3.54	48.09±0.021	110.6±0.42	105.8±0.94

prepared with lower concentration of polymer was lowest as compared to those prepared with higher concentration of polymer. The higher concentration of polymers in their ionized state resulted in intense cross-linking. Hence, as the polymer concentration increases, it increases the drug encapsulation. The bead diameter varied from 617±2.32 to 823±2.31 mm for different batches. The results indicate that as the amount of alginate increased, the size of beads also proportionally increased (Table 2). This could be attributed to the increase in micro-viscosity of the polymeric dispersion due to increasing alginate concentration, which eventually led to formation of bigger beads. The release of the entrapped drug from hydrogels depends on the swelling behavior, because swelling is directly proportional to drug release in case of hydrogels. As the hydrogel swells, the pores of network open and release of the entrapped solute occurs. Therefore the dynamic swelling study of the prepared beads was carried out in both phosphate buffer pH 1.2 and pH 7.4 and the results are shown in table 2. The swelling behavior of beads was expressed as the ratio of initial weight of beads to the final weight of swollen beads as a function of time. The swelling of beads depends upon the concentration of polymer. The swelling of the beads increased with an increasing amount of polymer in the beads. In intestinal pH, the protonated amino groups of chitosan get deprotonated and at the same time carboxyl groups of alginate ionize, which weakens the electrostatic interactions, thus making the bead structure loose resulting in increased swelling. In an effort to investigate the possible physical and chemical interactions between drug and polymer, we have analyzed pure stavudine, placebo beads and drug-loaded beads using DSC instrument (Fig.1).The DSC thermogram showed a sharp endothermic peak at 171.17 °C for pure drug which indicates the melting point of drug. In placebo beads, thermal transition at 178.32°C can be seen, which is attributed to the melting point of the sodium alginate polymer. In drug-loaded beads, the endothermic peak was not seen. This indicates that the drug was uniformly dispersed in an amorphous state in the polymer matrix. Hence, the evaluation of the thermograms clearly revealed no physical interaction between the polymer and the drug in the beads. The crystalinity of the drug in formulation was investigated by XRD study (Fig.3). The X-ray powder diffraction patterns of pure drug, drug loaded alginate beads revealed that the intensity of the peaks for the pure drug was sharp. But when it was incorporated into the polymer matrix, the drug peaks showed a loss of sharpness due probably decreased crystallinity of the drug. The surface morphology was examined by SEM studies (Fig.2). The SEM microphotographs revealed that the beads were spherical in shape having smooth and dense surface with inward dent and shrinkage due to the collapse of the wall of the beads during dehydration. The minute pores and cracks were also found on the surface of the beads. The permeability of drug through cross-linking reinforced chitosan-alginate beads was

Fig. 1: DSC thermograms of A) stavudine. B) Sodium alginate. C) Chitosan. D) Drug free alginate-chitosan beads. E) Drug loaded alginate- Chitosan beads

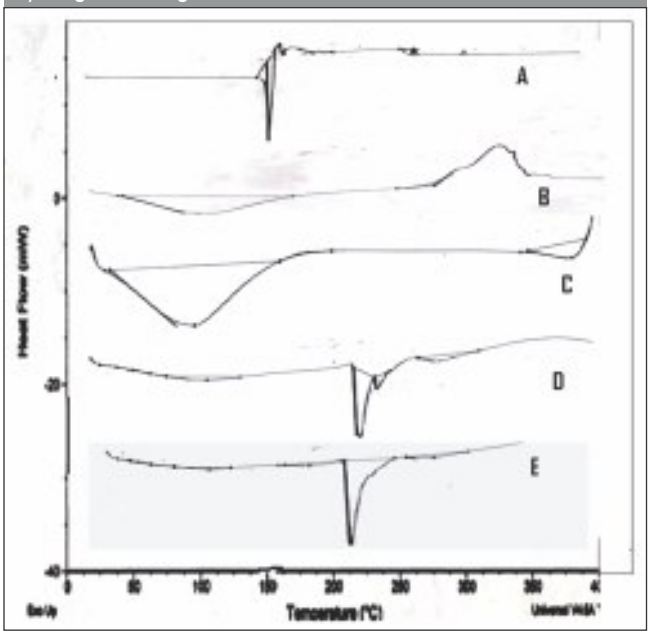


Fig. 2: SEM Photographs of A) Drug free alginate-chitosan beads. B) Drug loaded alginate- chitosan beads

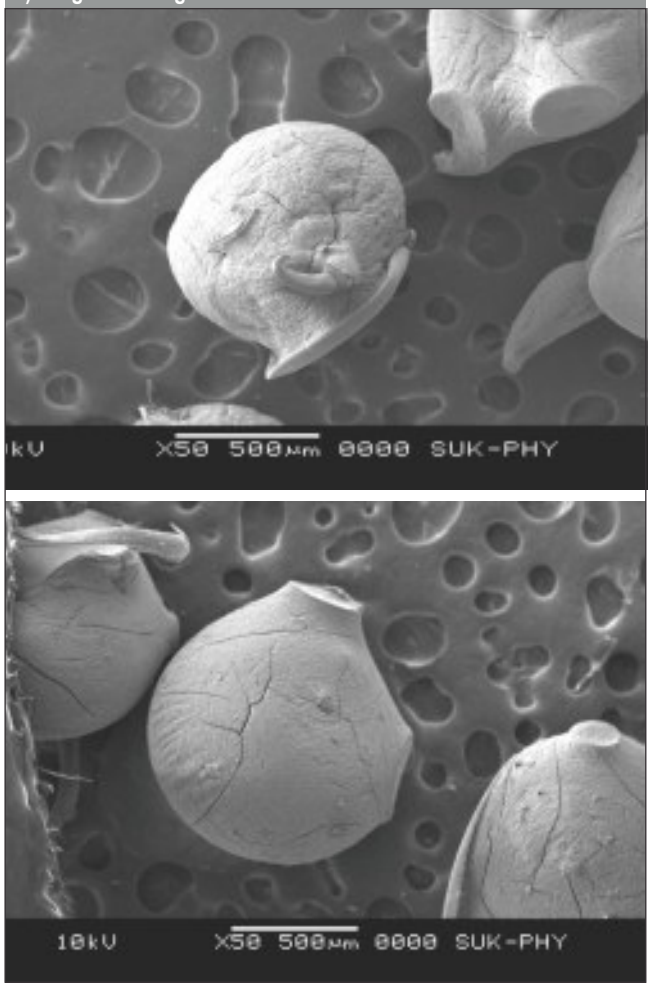


Fig. 3: XRD Spectrum of A) Pure stavudine B) Drug loaded alginate-Chitosan beads

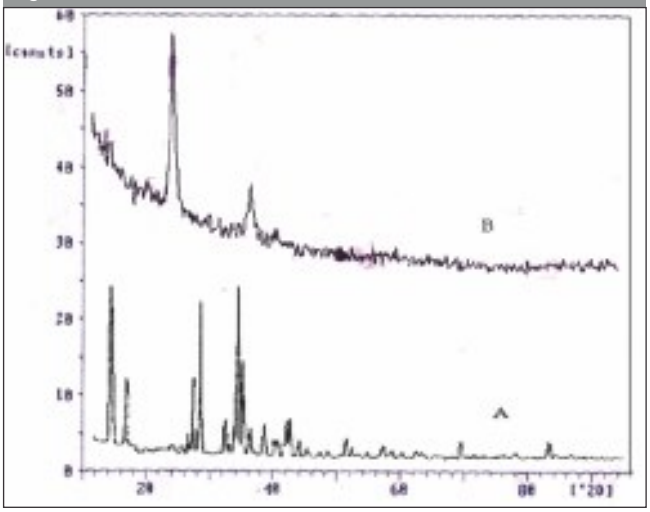
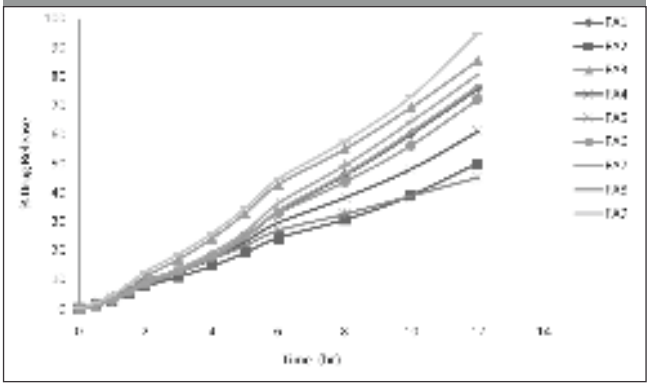


Fig. 4: In- vitro Drug release profile of different formulation batches



examined by dissolution test. The *in- vitro* drug release profiles of alginate beads of stavudine with different polymer concentrations are shown in Fig.4. The rate and extent of drug release from prepared beads significantly decreased with an increase in alginate concentration. This could be attributed to the increase of alginate matrix density and in the diffusion path length which the drug molecules have to traverse (by formation of bigger sized beads). The drug release from these beads was characterized by an initial phase of high release (burst effect). However, as gelation proceeded (cross-linking of alginate with Ca2+ ions), the remaining drug was released at a slower rate followed by a phase of moderate release. This bi-phasic pattern of release is a characteristic feature of matrix diffusion kinetics[17]. The initial burst effect was considerably

Table 3: *In-vitro* drug release kinetics from different hydrogel beads

Batch Code	Zero order equation		First order equation		Higuchi Equation		Korsemeyer's Equation	
	n	r^2	n	r^2	n	r^2	n	r^2
Fa1	5.816	0.976	0.223	-3.96	15.02	0.742	1.610	0.858
Fa2	3.978	0.995	0.242	-3.79	10.48	0.808	1.373	0.848
Fa3	6.889	0.991	0.211	-3.92	18.03	0.789	1.610	0.813
Fa4	4.848	0.993	0.235	-3.89	12.72	0.795	1.448	0.860
Fa5	3.991	0.988	0.242	-3.76	10.76	0.884	1.251	0.810
Fa6	5.588	0.984	0.227	-3.95	14.54	0.765	1.538	0.863
Fa7	6.250	0.979	0.219	-3.96	16.19	0.752	1.573	0.855
Fa8	5.891	0.976	0.113	-3.96	15.22	0.743	1.606	0.850
Fa9	7.416	0.990	0.194	-3.52	19.41	0.786	1.537	0.832

reduced with the increase in alginate concentration. The initial burst effect from batches of chitosan-coated beads was considerably reduced when compared to the corresponding batches of non-coated beads. The fact is that chitosan coating over the beads resulted in better incorporation efficiency and formed a thick coating layer around the beads. This could be the reason for the observed decrease in the burst effect.

In order to investigate the mechanism of drug release, the data were fitted to models representing zero-order, Korsmeyer's and Higuchi's square root of time[18] (Table 3). The examination of the coefficient of determination (*R2*) indicated that drug release from the prepared beads followed a diffusion controlled mechanism, since the *R2* values for Higuchi's square root of time (from 0.729 to 0.813) was always higher compared to zero-order (from 0.969 to 0.995) and to the Korsmeyer's Equation ones (from 0.693 to 0.894). Since the release from the prepared beads followed a biphasic profile, it was decided to use a more stringent test in order to distinguish between the mechanisms of drug release. The release data were fitted to the Peppas exponential model[19] $Mt/M\infty = Ktn$, where $Mt/M\infty$ is the fraction of drug released after time *t, K* is the kinetic constant and *n* is the release exponent which characterizes the drug transport mechanism. The *n* values were in the range from 3.996 to 7.429 indicating that all the prepared formulations followed the non-Fickian diffusion controlled mechanism of drug release. Hence, it can be concluded from the study that, among the prepared formulations with respect to entrapment efficiency, swelling studies and in vitro drug release, the alginate-chitosan beads prepared by ionotropic geltion and polyelectrolyte complexation method found to be better than ionically cross linked alginate beads alone. Therefore, dual cross-linked beads are promising carrier for oral control release.

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