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Research Article

COLON SPECIFIC CHRONOTHERAPEUTIC DRUG DELIVERY FOR NOCTURNAL ASTHMA: EUDRAGIT S-100 COATED CALCIUM ALGINATE GEL BEADS-ENTRAPPED SALBUTAMOL SULPHATE

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ABSTRACT

Eudragit S-100 coated calcium alginate gel beads entrapped salbutamol sulphate as a model drug has been investigated in the present study. The microspheres were evaluated for the drug content, drug entrapment efficiency, particle size, surface morphology and in vitro drug release. Drug-polymer compatibility was investigated by FTIR spectroscopy and differential scanning calorimeter (DSC). Encapsulation efficiency was found to be high when cross-linking time is less. Scanning electron microscopy revealed that the microspheres were spherical and had a smooth surface. The drug dissolution study performed simulating gastrointestinal pH variance showed a burst release calcium alginate gel beads in initial hour, whereas eudragit S-100 coating allowed producing systems of controlled release system. The drug release was found inversely proportional to sodium alginate concentration, CaCl2 concentration and eudragit S-100 concentration.

Keywords: Drug delivery, Drug targeting, Polymer, Calcium alginate bead, Chronotherapeutic, Water uptake study,

INTRODUCTION

Among modified release oral dosage form, increasing interest has currently turned to systems designed to achieve delayed and pulsatile delivery of drugs. The Pulsatile release is described as fast release of certain amount of drug within a short time period after a lag time [1]. For chronotherapeutic time controlled system, when a lag time is needed, enteric coated formulations are utilized [2]. The possibility of exploiting pulsatile release for chronotherapy is quite appealing for those diseases, the symptoms of which recurs mainly at night time or early morning such as nocturnal asthma, rheumatoid arthritis and angina pectoris. Nocturnal asthma is defined as sleep related worsening in reversible airway disease. Symptoms generally include shortness of breath or wheezing at night. Asthma found to be approximately 70 fold more frequent between 4 and 5 am, during intended night time sleep than between 2 and 3 am, middle of the day time activity span [3]. It is reported that 94%, 74%, 64% & 39%, of the asthmatic patient had disturbed night time sleep at least once per month, once per week, thrice per week and every night respectively. The nocturnal worsening of asthma is a common phenomenon, which requires more attention. A commonly used approach for treatment of nocturnal asthma has been the evening use of sustained release theophylline [4]. Theophylline has been shown to adversely affect sleep quality. Salbutamol sulphate, a short acting β-2 agonist used to treat asthma, was selected as a model drug. Due to shorter duration of action of salbutamol sulphate most patients has left without bronchodilation at early morning during which intensity of asthma is maximum. Bogin et al. investigate the use of Proventil Repetabs (Pulsatile released Albuterol/Salbutamol) in patient with nocturnal asthma. They found that Proventil Repetabs was more effective in improving nocturnal asthma without sleep disturbance. The pulsatile release has so far mainly been achieved through hydrophilic or hydrophobic layer coating of a drug-loaded core, osmotic mechanism and swellable or erodible plugs sealing of a drug-containing insoluble capsule body [5]. Sod.Alginate is a natural polysaccharide, bio erodible polymer that has been widely used in modified release applications because it forms strong gels in aqueous media and is biodegradable. Sod. Alginate is naturally occurring substance from brown sea weed and algae. This has a unique property of ordered gel structure formation in the presence of multivalent ion such as aluminum and calcium ion, which take place mainly at "egg box junction" (G-G sequence rich chain).The gel with spherical shape is termed as alginate bead ,which is non toxic, highly biocompatible and having the ability of re-swell.. It is reported that alginate gel beads is one of the possible delivery carrier for a pulsatile release system of water-soluble macromolecular drugs such as protein and hormone. The multiparticulate dosage forms are gaining much interest over single-unit dosage forms for pulsatile delivery. The potential advantage of multiparticulate system include no risk of dose dumping, reduced risk of local irritation, less inter and intra subject variability and increased bioavailability [6] .The aim of the present study was to develop a pulsatile drug delivery system containing calcium alginate bead coated with pH-dependent polymers (Eudragit S-100).

MATERIALS AND METHODS

Materials

Salbutamol sulphate was a generous gift from Cipla (Rangpoo, Sikkim India). Sodium alginate was obtained from Indian research product (Chennai, India). Eudragit S-100 was procured from Loba Chemicals (Mumbai, India). Light liquid paraffin, Calcium chloride, acetone and *n*-hexane were procured from Ambica Scientific Pvt. Ltd. (Palampur, India). All other chemicals used were of analytical reagent grade.

Table 1: Composition of microparticle by ionic gelation method

Formulation	Drug : Polymer	Sod. alginate (% w/v)	$CaCl_2$ (M)	Cross linking time (min)
F 1	1:1	1.5	0.05 M	5
F 2	1:1	1.5	0.2 M	5
F 3	1:1	2.0	0.05 M	5
F 4	1:1	2.0	0.2 M	5
F 5	1:1	3.0	0.05 M	5
F 6	1:1	3.0	0.2 M	5
F 7	1:1	3.0	0.2 M	20

Methods

Preparation of Salbutamol loaded Calcium alginate beads

Sodium alginate was dissolved in double-distilled water at a concentration (1.5–3.0% w/v). Accurately weighed salbutamol sulphate (drug: polymer, 1:1) was then added under stirring. The dispersions were dropped through a syringe (no.23) at a dropping rate of 1ml/min in to a 250 ml of CaCl2 solution (at a concentration 0.05 M and 0.20 M) under gentle agitation at room temperature. The calcium alginate beads formed were allowed to stand in gelling solution for a time period (5 to 20 min), then collected by filtration and washed twice with 100 ml double-distilled water, consecutively. Then the beads were dried at 50^{0}C in an oven for 24 hrs [7, 8]

Particle size and surface morphology analysis

Particle size determination of calcium alginate beads was performed by optical microscopy using a compound microscope. The Ferret's diameter of at least 300 beads was measured using a calibrated ocular micrometer and the average particle size was determined using the Edmondson's equation

$$D_{\text{mean}} = \frac{\sum nd}{\sum n}$$

Where, n stands for number of beads counted and d for mean size range. Uniformity index was determined by the following equation:

$$UI = \frac{D_W}{D_n}$$

Where, Dw and Dn stands for weight average diameter and number average diameter respectively and calculated as follow

$$D_W = \frac{\sum N_i D_i^4}{\sum N_i D_i^3} \qquad D_n = \frac{\sum N_i D_i}{\sum N_i}$$

Where, Ni is the number of particle with Di diameter. Values of UI ranging from 1 to 1.1 and 1.1 to 1.2 indicate monodisperse and nearly monodisperse particles. The values higher than 1.2 have been regarded as indicative of particles with broad particle size distribution. [9,10] The shape and surface morphology of the core and coated beads were examined using SEM (JSM-5610; Tokyo Japan).The Beads were fixed with carbon tape, mounted on aluminum stubs and then coated with platinum, keeping the acceleration voltage at 20 kV.

Determination of encapsulation efficiency

The amount of salbutamol sulphate present in calcium alginate beads was determined by extraction in distilled water. 50 mg of the crushed and powdered alginate beads was taken and extracted in distilled water and stirred for 15 minute at 1500 rpm. The solution was filtered and diluted with 0.05M NaOH and absorbance was measured UV-Spectrophotometrically (UV-Vis 1800 Shimadzu) at 276nm by following formula [11]

$$EE(\%) = \frac{W_t}{W_o} X\ 100$$

Where Wt stands for weight of salbutamol sulphate loaded in beads and Wo for total weight of starting salbutamol sulphate.

Determination of alginate bead water content

Each Calcium alginate bead was weighed before and after drying (50^{0}C for 24 hr) and the mean water loss (WL) was calculated according to the following equations [12]

$$W_l\% = \{(W_o - W_d)/Wo\} * 100$$

Where, W_0 and W_d stands for initial weight and final weight after drying respectively.

Determination of swelling rate

The swelling ratios of beads were determined by immersion in phosphate buffer saline (PBS 7.4) at room temperature for 24 hrs, with gentle shaking. At specific time point (0.5, 1,2,4,6,8,12, and 24 hrs) sample were removed and rinsed with double distilled water (Fig.3). The alginate beads remained intact during the process and no microscopic pores were visible. The shape of alginate beads also remained same. Alginate beads were then blotted dry and the swollen weight was measured and the swelling ratio (Esw) was calculated according to equation as follows [13, 14]

$$E_{sw} = [(W_{sw} - W_0)/W_0]\ X\ 100$$

Where W_{sw} stands for weight after swelling and W_0 for initial weight

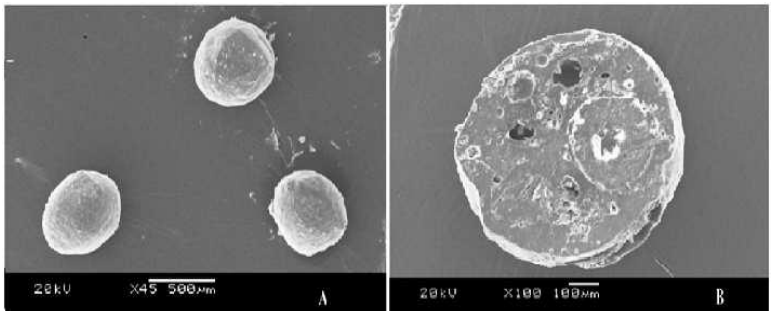

Fig. 1: SEM photograph of calcium alginate bead (A) and cross section of calcium alginate bead (B)

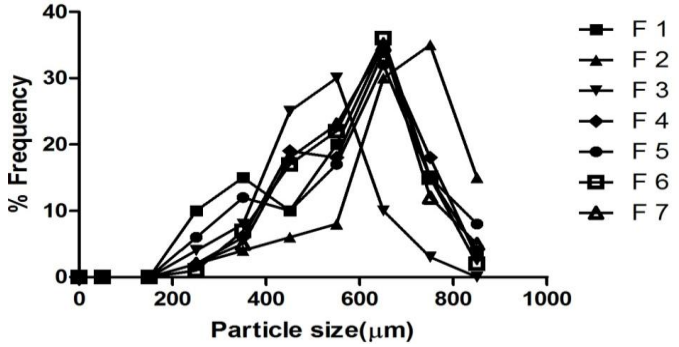

Fig. 2: Frequency Destribution of of different alginate core microparticle

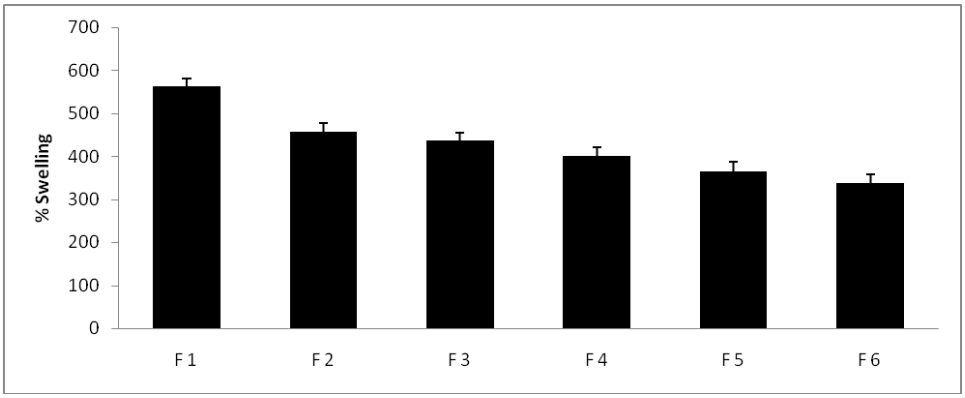

Fig. 3: Comparison between swelling properties of microparticle by ionic gelation.

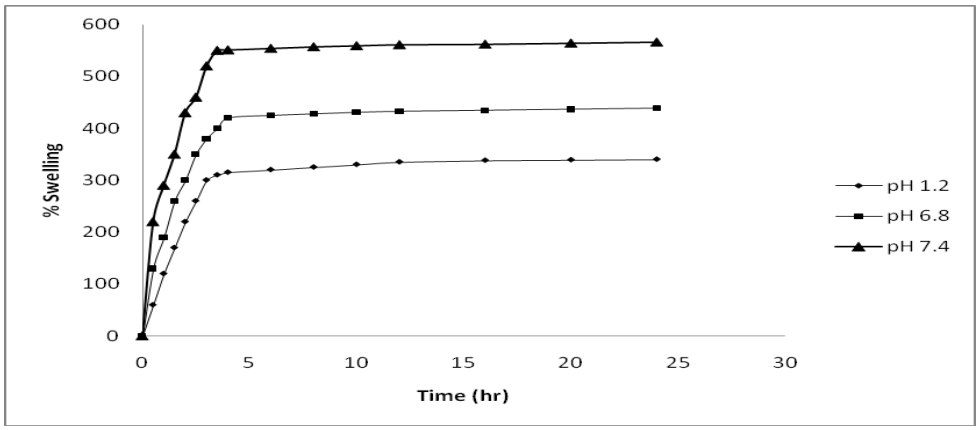

Fig. 4: Relationship b/w % swelling vs. different dissolution medium

Table 2: Effect of variables on physicochemical property of Calcium alginate beads

Formulation	Particle size (mm ± SD)	Uniformity Index	Practical yield (%)	Encapsulation efficiency (%)	Water uptake
F 1	0.670±0.011	1.2097	76.2±0.45	32.11±0.10	97.6±0.89
F 2	0.770±0.0109	1.005	77.9±0.36	48.11±0.81	95.6±1.2
F 3	0.530±0.016	0.99	78.98±0.92	54.23±0.17	95.4±1.5
F 4	0.680±0.013	0.9933	75.90±0.76	67.82±0.21	90.6±1.6
F 5	0.640±0.010	1.113	82.99±0.38	74.82±0.19	84.7±0.94
F 6	0.690±0.015	0.9978	88.46±0.35	86.05±0.72	79.3±1.6
F 7	0.670±0.0132	0.9936	84.05±0.63	68.09±0.67	82.6±0.98

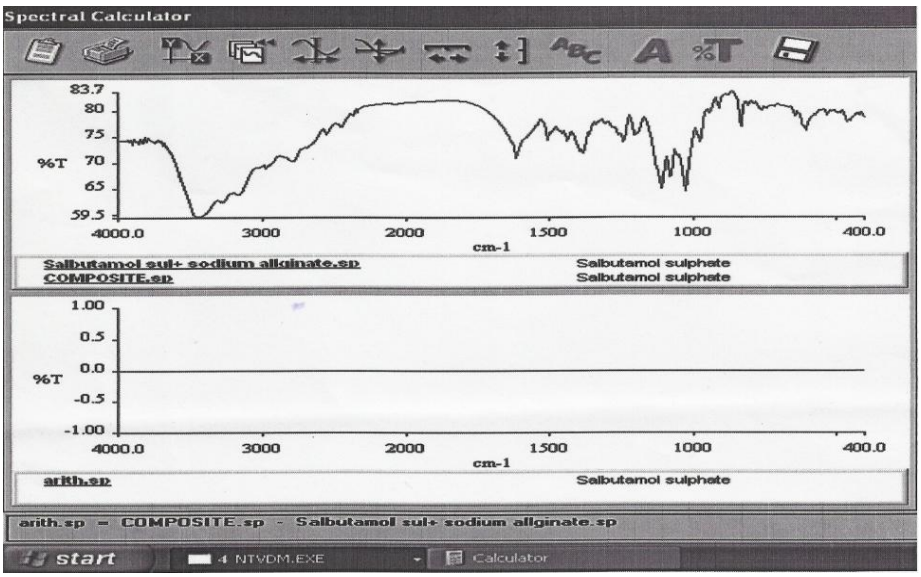

Fig. 5: FTIR spectra of Salbutamol and Alginate beads

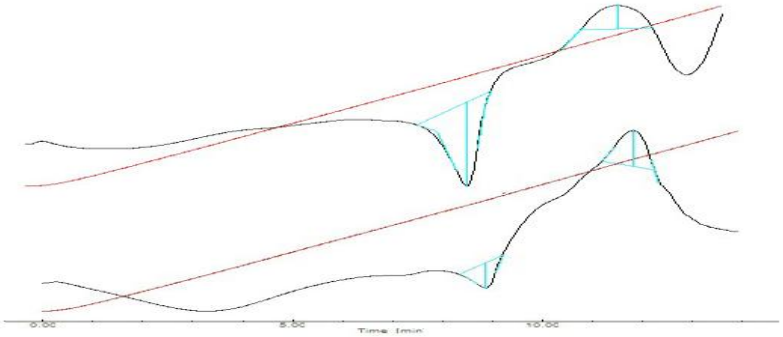

Fig. 6: DSC thermogram of salbutamol (A) and calcium alginate beads (B)

Coating of Calcium alginate bead

The enteric coated solution was prepared by dissolving eudragit in acetone (at different concentration 25, 50 and 75 % w/v). This solvent makes complete dissolution of eudragit S 100 while maintaining integrity of the calcium alginate bead. The optimized batch of calcium alginate bead was dispersed in the enteric coated solution. This dispersion was then poured in to 70 ml of liquid paraffin & stirred at 1500 rpm for 2 hr at room temperature to evaporate the solvent. Finally it was wash with n-hexane to remove paraffin then dry at 30 ^{o}C for 24 hr. [15]

Solid state analysis

Fourier Transform Infrared (FTIR) spectroscopy and Differential Scanning Calorimetry (DSC) analyses were carried out in order to investigate the possibility of any incompatibility between the drug and excipients. The FTIR spectra were recorded as KBr disc using FT-IR (Perkin Elmer, Model No. 883). The scanning range was 400-4000 cm-1 and the resolution was 2 cm-1. (Fig.5). DSC analyses were performed with a DSC-TA system (Perkin Elmer).All samples were sealed in a crimped Aluminum pan and heated at a rate 20^{0}C / min from 70 to 300 ^{0}C in an atmosphere of nitrogen gas by passing at a flow rate of 60 ml/min. An empty aluminum pan was used as reference. The straight line (Fig.5) shows that there is no incompatibility between drugs and alginate.

***In vitro* drug release study**

In vitro drug release studies of calcium alginate bead and coated beads were performed according to the method at 37^{0}C ± 0.5 ^{0}C and at a stirring rate of 50 rpm. Dissolution medium was varied according to the following sequence, in order to simulate pH conditions of the gastrointestinal tract: pH 1.1 0.1 M HCl solution (artificial gastric juice) for 2 hrs; pH 6.8 phosphate buffer (artificial small intestinal fluid) for 2 hrs; pH 7.4 phosphate buffer (artificial intestinal fluid) till the completion of the study. Samples were withdrawn at specified time interval replacing with blank. The samples were centrifuged, filtered and analyzed at 276 nm by UV-Spectrophotometrically [16]

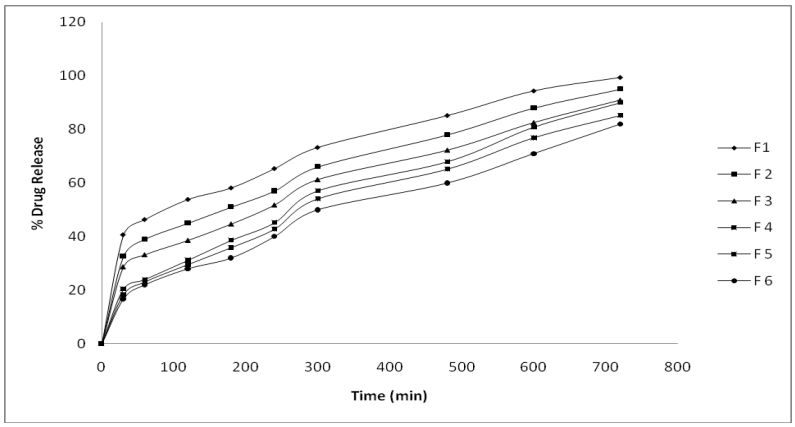

Fig. 7: Dissolution profile of different calcium alginate beads.

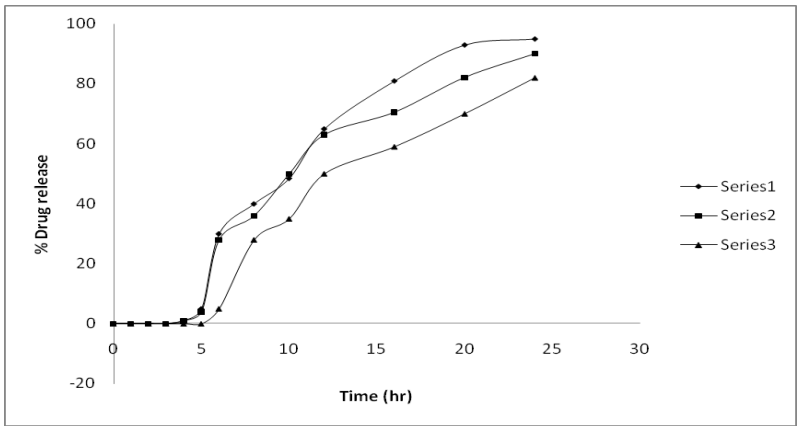

Fig. 8: Comparison b/w dissolution profile of coated calcium alginate beads

Kinetics of drug release from microparticle

The release from the different formulations was determined by curve fitting method for mathematical equation such as zero order (% Release vs. t), First order Kinetics (log (100 - % CR) vs. t), Higuchi diffusion equation (Q=Kt1/2) and Korsmeyer-Peppas equation (Mt/M∞ = Ktn), where Mt is the amount released at time t; Mt/M∞ is the friction released at time t, K is kinetic constant and n is the diffusion exponent [17]

RESULTS AND DISCUSSION

The aim of the present work was to design a pH sensitive multiparticulate pulsatile drug delivery system of salbutamol sulphate for the treatment of nocturnal asthma. The delivery system was obtained in two steps (1) Preparation of core Ca-alginate beads by dropping aqueous solution of sodium alginate (1.5-5% w/v) into the coagulation fluid containing CaCl2 (0.05- 0.2M) (Table 1) The Ca-alginate bead was coated by pH sensitive polymer eudragit S-100 (1:1) by solvent evaporation method. In the initial step, cross linking time was gradually varied from 5-20 min and its influence on the physiochemical property of microbeads such as particle size, encapsulation efficiency and % yield was evaluated by keeping the sod. alginate [3% w/v] and Ca++ [0.2M]. As the cross linking time was increased from 5-20 min the encapsulation efficiency (EE %) was decreased (Table 2) for same Ca ++ and Sod. alginate concentration. This may be attributed to the high solubility of the drug, which is responsible for outwards diffusion of salbutamol sulphate during the bead formation. To assess the effect of Ca ++ and sod. alginate concentration for a given cross linking time (5 min), we selected a series of calcium alginate batches (Table 1). Table 2 showed the encapsulation (EE %) and particle size analysis of selected batches. The calcium alginate beads were almost spherical in shape (Fig.1). The UI data of all batches have narrow size range (below 1.2).The encapsulation efficiency of various formulation was in the range 32.11-86.05 % .This showed that when sod. Alginate concentration increases (1.5-3 %), the encapsulation efficiency of calcium alginate also increases. As the concentration of sod alginate increases number of binding sites for Ca++ ion also increases, stronger gel was formed compared to low concentration of sod. alginate, which decreases the outward diffusion of salbutamol sulphate during the calcium alginate beads formation. The result also indicated that when Ca++ ion increased for same sod. Alginate concentration EE (%) increased. This is attributed to the ability of more Ca++ ion per unit volume capable to interact with free carboxyl group of alginate and thus positively affecting encapsulation efficiency and reducing outwards diffusion of salbutamol sulphate, a highly water soluble drug during microbeads formation. The practical yield (%) was found to be in the range 76.2 ± 0.45 - 88.46 ± 0.35, indicating directly proportional to increase in Ca++ ion and sod. alginate concentration causing stronger gel formation as compared to low Ca++ ion and sod. alginate concentration. The result shows that water uptake was inversely proportional to sod. Alginate concentration and also calcium ion concentration. As the number of intermolecular cross linking formed between carboxyl group of alginate and Ca++ ion increased, stable gel was formed and water permeability of calcium alginate bead decreased. Fig.3 showed the swelling behavior of different calcium alginate beads at pH 6.8. The observed swelling rate of calcium alginate bead followed the order F1>F2>F3>F4>F5>F6. As the concentration of sod. alginate and Ca++ increases, the swelling of microbeads decreases. This is attributed to low water permeability of calcium alginate beads hence as Ca++ and sod. Alginate concentration increases, water could not diffuse into the matrix. Swelling property of calcium alginate beads depends upon the ionic strength, pH and ionic composition of the medium. When we studied the swelling of calcium alginate bead at different pH (1.2, 6, 8 & 7.4), the lowest swelling was found in pH 1.2 whereas highest swelling ratio was found in pH 7.4 (Fig.4.). Fig.5 shows the FTIR spectra of salbutamol and calcium alginate bead. The straight line in spectra shows that there is no incompatibility between drugs and alginate. The DSC thermogram (Fig.6) of salbutamol sulphate, and salbutamol loaded calcium alginate bead. The thermogram shows a sharp melting point peak with onset at 200.17^{0}C similarly thermogram of calcium alginate bead showed a depressed endotherm at near 210^{0}C, which could be due to the dilution effect of amorphous polymers. Fig. 7. Shows the drug release profile of different calcium alginate beads. The result shows that rate and extant of drug release from calcium alginate beads is inversely proportional to sodium alginate concentration and Ca++ ion concentration. Thus a slight change in concentration leads to significant changes in dissolution profile, which may be attributed to change in density of matrix as well as diffusion of water in matrix. This is so because too low concentration of Ca++ ion is not sufficient to form an insoluble hydrogel and also to increase the dissolution of drug by decreasing the tortuosity of path of diffusion. The release profile showed a burst release in the initial hour. In the first 30 min drug release was 16.71, 18.34 20.41, 28.7, 32.6 and 40.6 for F6, F5, F4, F3, F2 and F1 respectively. *In vitro* release study was analyzed using various mathematical models. The best fit model for formulation F1 and F2 was Korseymear Peppas model and F3, F4, F5 and F6 was Higuchi kinetic model, indicating that the release is by diffusion from these calcium alginate beads. The n value of Korseymear-Peppas model for all calcium alginate beads was below 0.45 indicating the drug release mechanism is non-fickian diffusion.

Coating of calcium alginate beads

Formulation F4 F5 and F6 were selected for coating with Eudragit S-100 as they showed good encapsulation efficiency, practical yield and release profile. This tends to dissolve at pH > 6.8. The coated level was varied from 25-75 % w/w and its influence on drug release was investigated by pH progression method to simulate gastrointestinal condition. The *in vitro* drug release profile of coated calcium alginate beads are shown in fig. 8**.** Complete absence of drug release was observed from eudragit coated calcium alginate beads in pH 1.2 to 6.8. As the coating level increases the rate and extent of drug release decreases. The curve fit model was performed to investigate the mechanism of drug release by regression value (r^2) along with rate constant (K). Based on regression value best-fit model for all coated calcium alginate beads was by first order kinetic and n value for coated calcium alginate is > 0.89, indicating supercase II transport. This indicates that chain relaxation along with swelling is responsible for drug release from coated calcium alginate beads.

CONCLUSION

Eudragit S-100 coated salbutamol sulphate loaded calcium alginate beads were realized to chronotherapeutic drug delivery for nocturnal asthma. The physicochemical properties and releases profile depends upon the calcium chloride concentration, sodium alginate concentration and cross-linking time. In addition the drug release from eudragit S-100 coated calcium alginate gel beads depends on the concentration of eudragit S-100 solution concentration. Eudragit S-100 Eudragit S-100 coated calcium alginate beads leads to prevent release in stomach pH and upper intestinal pH and rapid release of certain amount of drug on lower intestinal pH proved that it is a potential system for chronotherapeutic drug delivery.

REFERENCES

1. Suresh Sarasija, Stutie P. Chronotherapeutics: emerging role of biorhythms in optimizing drug delivery. Indian journal pharmaceutical science. 2005; 2, 135-40.
2. Patel N, Gayatri C. Comparative in vitro evaluation of enteropolymers for pulsatile drug delivery system. Acta Pharmaceutica Sciencia. 2009; 51, 243-50
3. Smolensky Michael, Bjorn Lemmer. Chronobiology and chronotherapy of allergic rhinitis and bronchial asthma. Advance drug delivery reviews; 2007; 10, 852-882
4. R M Bogin, Ballard RD. Treatment of Nocturnal Asthma with Pulsed-Release Albuterol.Chest; 1992; 102, 362-366.
5. Arora Shweta, Ahuja Alka. Pulsatile drug delivery systems: An approach for controlled drug delivery. Indian journal pharmaceutical sciences. 2006; 3, 295-300.
6. Roy Pallb, Sahiwala. Statistical optimization of ranitidine HCl floating pulsatile delivery system for chronotherapy of nocturnal acid breakthrough. Eur. J. Pharm Sci. 2009; 28; 4, 363-369

7. Y.Phalguna, B.S.Venkateshwarlu. HPMC microspheres of zidovudine for sustained release. International Journal of Pharmacy and Pharmaceutical sciences 2010; vol2, suppl4: 41-43.
8. Maja Simonoska, Crcarevska KG. Chitosan coated Ca–alginate microparticles loaded with budesonide for delivery to the inflamed colonic mucosa. European Journal of Pharmaceutics and Biopharmaceutics. 2008; 68, 565-78.
9. S.Jain, I. Biswal, A.Dinda. Modified sustained release chitosan-coated zidovudine microspheres. . International Journal of Pharmacy and Pharmaceutical sciences 2011; vol 3, supp4: 41-43
10. Nokhodchi A, Tailor A. In situ cross-linking of sodium alginate with calcium and aluminum ions to sustain the release of theophylline from polymeric matrices. IL Farmaco. 2004; 12, 999-1004.
11. Akihiko K, Minako K. Effect of Ca -alginate gel dissolution on release of dextran with different molecular weights. Journal of Controlled Release; 1999; 4, 21-28.
12. Gheorghe Fundueanu, Claudio Nastruzzi. Physico-chemical characterization of Ca-alginate microparticles produced with different method, Biomaterials 1999; 20, 1427-1435
13. Sevgi T, Omer HO. Formulation and investigation of nicardipine HCl–alginate gel beads with factorial design-based studies. European Journal of Pharmaceutical Sciences. 1998; 6, 241-246.
14. Mladenovska K, R. S. Raick. Colon-specific delivery of 5-aminosalicylic acid from chitosan-Ca-alginate microparticles. International Journal of Pharmaceutics. 2007; 342, 124-36.
15. Khan MS, Sridhar BK. Development and Evaluation of pH-Dependent Micro Beads for Colon Targeting; Indian J Pharm Sci. 2010; 1, 18–23.
16. Anandrao R, Kulkarni KS. In-vitro release kinetics of cefadroxil-loaded sodium alginate interpenetrating network beads. European Journal of Pharmaceutics and Biopharmaceutics 2001; 51, 127-33.
17. Olav Ghersd, Ian G. The enhancement of the bioadhesive properties of calcium alginate gel beads by coating with chitosan. Int. J .Pharm 1998; 175, 237-246.