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Development and *In-Vitro* Characterization of Ca-Alginate Beads of Oxytetracycline Hydrochloride for Oral Use: Effect of Process Variables

Lalit Kumar Tyagi[1,*], Ravi Shekhar[2], Kalpesh Gaur[3], Mohan Lal Kori[4]

[1]Guru Nanak Institute of Pharmacy, Dalewal, Hoshiarpur, Punjab, India
[2]Department of Pharmacy, Dr B R Ambedker University, Khandari Campus, Agra, Uttar Pradesh, India
[3]Geetanjali Institute of Pharmacy, Geetanjali University, Manwa Khera, Udaipur, Rajasthan, India
[4]Vedica College of B. Pharmacy, A Constituent Institute of RKDF University, Bhopal, Madhya Pradesh, India
*Corresponding author: lktyagi13@yahoo.co.in

Abstract This work investigates the preparation of Oxytetracycline Hydrochloride loaded alginate beads to take advantage of the swelling properties of alginate beads for improving the oral delivery. Variations in polymer concentration, concentration of cross-linking agent and cross-linking time were examined systemically for their effects on the particle size, entrapment efficiency, percent yield, flow properties and *In vitro* drug release behavior. Calcium alginate (Ca-alginate) beads of Oxytetracycline hydrochloride were prepared by ionic-gelation method. Shape and surface characteristics were determined by scanning electron microscopy (SEM). Average particle size of drug-loaded beads was determined by sieving method. *In vitro* drug release behavior from Ca-alginate beads were carried out in simulated gastric fluid (SGF) for first 2 h and simulated intestinal fluid (SIF) for the next 6 h. SEM confirmed spherical shape of beads with rough and porous morphology. The average particle size of the beads was in the range of 470.96 ± 15.22 to 709.33 ± 16.28 µm. Results indicated that the average particle size and flow property of the beads increased with an increase in the concentration of polymer and the cross-linking agent as well as the cross-linking time. The entrapment efficiency and percentage yield was found to be in the range of 52.87 ± 1.56 to 61.76 ± 0.96 % and 69.98 ± 0.33 to 78.94 ± 0.43 % respectively. Concentration of the sodium alginate up to 1.75 % w/v, cross-linker concentration up to 2 % w/v and cross-linking time (30 min) shows highest percent entrapment efficiency (61.76 ± 0.96 %) and % yield (78.94 ± 0.43 %). A decrease in the rate and extent of drug release was observed with relative increase in the polymer concentration, cross- linker concentration and cross-linking time. No significant drug-polymer interaction was observed in DSC study. From this study it can be concluded that the natural polymer sodium alginate can prolong the release of Oxytetracycline Hydrochloride.

Keywords: *calcium alginate, beads, Oxytetracycline Hydrochloride, in vitro drug release*

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1. Introduction

There has been a growing interest in the use of natural polymers as drug carriers due to their biocompatibility and biodegradability. Sodium alginate (Na-alginate), is a sodium salt of alginic acid, a naturally occurring polysaccharides obtained from marine brown algae are known to be haemocompatible, nontoxic and do not accumulate in any organ of the body when taken orally [1]. Alginate can be considered as block polymers which mainly consist of mannuronic acid (M), guluronic acid (G) and are arranged in homopolymeric blocks (GG and MM) and in heteropolymeric blocks (MG) [2]. Sodium alginate is soluble in water and can be cross-linked in the presence of divalent or polyvalent cations such as Ca^{2+} and Zn^{2+}. The gelation phenomena can be explained by the egg-box model in which divalent cation bind to two carboxyl groups on the adjacent alginate molecules [3,4]. The hydrogel properties of calcium alginate beads have been proposed for controlling the release of small molecules and macromolecules [5].

Oxytetracycline hydrochloride (OTC HCl) is a broad spectrum bacteriostatic antibiotic commonly used for systemic therapy as well as locally for gastric or intestinal infections. It is presently considered as therapy of choice in papulopustulous acne, rosacea and perioral dermatitis as well as primary and secondary skin infections [6]. Conventional oral formulations of OTC HCl lead to gastrointestinal irritative effects such as stomach upset, epigastric burning, nausea and vomiting due to high solubility of this drug in the gastric fluid [7]. These adverse effects create a potential need to modify its release characteristics. Thus, this study was undertaken to develop prolonged-release formulation of OTC HCl using alginate beads. The development of formulation and characterization of the prepared calcium alginate (Ca-alginate) beads were investigated. Process variables such as polymer concentration, cross-linking

agent concentration and cross-linking time were varied. Drug-polymer interaction in the solid state were studied by differential scanning calorimetric analysis (DSC) and the surface characteristics were evaluated by scanning electron microscopy (SEM).

2. Materials and Methods

2.1. Materials

Oxytetracycline hydrochloride (Purity: 95.3 % and Lot No.: Y1-0803192-1) was obtained from Siemens Laboratory, Gurgaon, India. Sodium alginate (M.W.~147,000 and Lot No. E06Z/0406/2303/13) was purchased from S.D. Fine Chemical Ltd., Mumbai, India. Calcium Chloride was obtained from Qualigens Fine Chemical, Mumbai, India. All other chemicals of analytical grade were purchased from commercial sources.

2.2. Methods

2.2.1. Preparation of OTC HCl loaded Ca-alginate Beads

Formulation and schematic illustration of OTC HCl loaded Ca-alginate beads formulations are shown in Table 1. OTC HCl loaded Ca-alginate beads were prepared by using ionic-gelation method [8,9]. Briefly, OTC HCl (2 % w/v) was added to an aqueous solution of sodium alginate and dispersed homogenously. This sodium alginate-drug dispersion was dropped manually through a needle (Size No. 26G) with the help of a hypodermic syringe into a calcium chloride ($CaCl_2$) solution which is used as cross-linking agent. Alginate gel beads were allowed to stand in $CaCl_2$ solution until they were fully formed. The beads were then separated by filtration on filter paper, washed three times with double distilled water and then beads were allowed to dry at room temperature in a desiccator until constant weight was achieved.

Table 1. Formulation and processing variables of OTC HCl loaded Ca-alginate beads

Formulation Code	Drug (% w/v)	Sodium Alginate (% w/v)	Cross-linking agent	Cross-linking time (min)
F_1	2	1.00	2 % (w/v) $CaCl_2$	30
F_2	2	1.25	2 % (w/v) $CaCl_2$	30
F_3	2	1.50	2 % (w/v) $CaCl_2$	30
F_4	2	1.75	2 % (w/v) $CaCl_2$	30
F_5	2	2.00	2 % (w/v) $CaCl_2$	30
F_6	2	1.75	5 % (w/v) $CaCl_2$	30
F_7	2	1.75	10 % (w/v) $CaCl_2$	30
F_8	2	1.75	2 % (w/v) $CaCl_2$	20
F_9	2	1.75	2 % (w/v) $CaCl_2$	10

Effect of polymer concentration (F_1 – F_5); Effect of cross-linker concentration (F_6 – F_7);
Effect of cross-linking time (F_8 – F_9).

2.3. Characterization of OTC HCl Loaded Ca-alginate Beads

2.3.1. Surface Morphology and Particle Size Analysis

The surface morphology of beads was studied by scanning electron microscopy (SEM) (JEOL JSM-1600, Tokyo, Japan) using gold sputter technique. The beads were vacuum dried, coated with gold palladium and observed microscopically. Average particle size of beads was determined by sieving method [10].

2.3.2. Determination of the Flow Property

The flow property of the beads was determined from the changes in the volume due to rearrangement and packing which occurs during tapping in a graduated measuring cylinder. Carr's compressibility index (%) and Hausner ratio were determined by using following formula [11]:

$$\text{Carr's compressibility index } (\%) = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \text{x} 100$$

$$\text{Hausner ratio } (\text{HR}) = \frac{\text{Tapped density}}{\text{Bulk density}}.$$

2.3.3. Determination of Drug Content and Entrapment Efficiency

Beads were accurately weighed and broken down using mortar and pestle. 10 mg of the crushed beads were taken into 10 ml of volumetric flask and add methanol up-to the mark, vortexed for 5 min and filtered through whatmann filter paper No. 4. The filtered samples were suitably diluted and analyzed for drug content spectrophotometrically (Shimadzu 1700, Japan) at 353 nm [12]. The entrapment efficiency was calculated by using the following formula [13]:

$$\text{Percent entrapment efficiency} = \frac{\text{Actual drug content in beads}}{\text{Theoretical drug content}} \text{x} 100.$$

2.3.4. Swelling Studies

The swelling rate of the Ca-alginate beads were carried out in two aqueous media: 0.1 N HCl (pH 1.2) as simulated gastric fluid (SGF) and phosphate buffer (pH 7.4) as simulated intestinal fluid (SIF). The beads were incubated in solutions at 37°C temperature. The swollen beads were removed at preset time intervals and weighed. The extent of swelling (% swelling) was determined by suspending the beads in 0.1N HCl (pH 1.2) solution at 37°C until equilibrium was achieved. The extent of swelling was determined by calculating the % weight uptake using the following equation [14]:

$$\% \text{ Water uptake} = \frac{\text{Ws} - \text{Wi}}{\text{Wi}} \text{x} 100$$

Where, Ws is the weight of beads in the swollen state and Wi is the initial weight of the beads.

2.3.5. *In vitro* Drug Release Studies

The *In vitro* drug release studies were carried out using USP (Type-I) dissolution test apparatus. The capsules

filled with drug-loaded beads (equivalent to 250 mg of OTC HCl) were put into the basket. Basket was placed in 900 ml of the dissolution medium at 37±0.5°C and rotated at 75 rpm. The release of drug from beads were monitored in 0.1 N HCl (pH 1.2) as simulated gastric fluid (SGF) for the first 2 h, followed by phosphate buffer (pH 7.4) as simulated intestinal fluid (SIF) for the next 6 h. At specified time intervals, 5 ml samples were withdrawn and immediately replaced with an equal volume of fresh dissolution medium. Samples were suitably diluted and analyzed spectrophotometrically (Shimadzu 1700, Japan) at 353 nm. All the tests were carried out in triplicate.

2.3.6. Differential Scanning Calorimetry (DSC) Study

The differential scanning calorimetry profile of pure drug and physical mixtures of drug and excipients used in the formulations were recorded by Pyris Diamond DSC-4 (Perkins Elmer, Wellesley, MA) in order to assess the compatibility of excipients used in the formulation with the drug. Thermal behavior was studied under normal conditions with perforated and sealed quartz pans and with a nitrogen gas flow of 200 ml/min. The samples were heated at 10°C/min over temperature range of 26-475°C. The reference sample used in all determination was alumina. The spectra obtained were analyzed for incompatibility.

3. Results and Discussion

3.1. Effect of Processing Conditions on the MICROMERITIC PROPerties of OTC HCl Loaded Ca-alginate Beads

The gelled beads were formed by ionic interaction between the negatively charged carboxyl groups of sodium alginate and the positively charged counter ion such as Ca^{++}. Scanning electron micrograph of a dried OTC HCl loaded Ca-alginate beads showed that the beads have spherical and the rough surface, as well as presence of little porous and dispersion of drug as fine crystalline particles on the surface of beads were also observed (Figure 1 a & Figure 1b). The drug crystals observed on the surface were probably formed as a result of their migration along with water to the surface during drying.

OTC HCl loaded Ca-alginate beads were prepared by varying the concentration of Na alginate in the formulations to investigate the effect of this parameter on the micromeritic properties such as; particle size, % yield and flowability of the beads (Table 2). Percentage yield of formulations F_1, F_2, F_3, F_4 and F_5 was found to be 69.98 ± 0.33, 71.40 ± 0.52, 77.16 ± 0.51, 78.94 ± 0.43 and 77.95 ± 0.40 % respectively. It is evident that increasing the polymer concentration has resulted in an increase in the percentage yield. This effect can be explained by the fact that as the concentration of Na alginate increases, the quantity of the polymer becomes sufficient to cover OTC HCl particles completely and form beads. The % yield was found to be in the range of 69.98 ± 0.33 to 78.94 ± 0.43 % (Table 2). The % yield decreased with increasing sodium alginate concentration due to higher concentration of Na alginate (more than 1.75 % w/v) resulted in much higher viscosity of solution which resulted in the formation of lumps and hence decreased yields. Analysis of the particle size indicated that the average particle size increased significantly ($p<0.05$) with increasing the polymer concentration due to subsequent increase in the frequency of collision, resulting in the fusion of semi formed particles and as a consequence an overall increase in the size of the beads. Plot of the average particle size of the beads against the Na-alginate concentration exhibited close to linearity with r^2 value = 0.9711 (Figure 2). Other researchers have reported a similar relationship between the polymer concentration and the mean size [15].

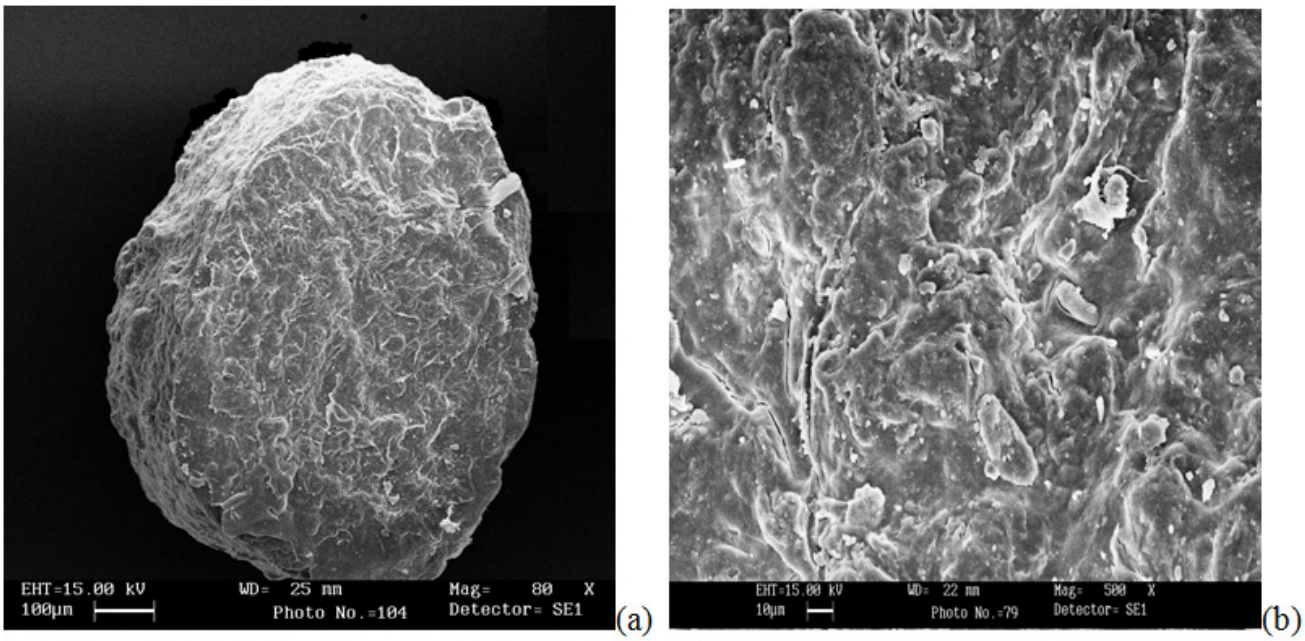

Figure 1. SEM images of OTC HCl loaded Ca-alginate beads. (a): at 80 X Magnification (b): at 500 X magnification

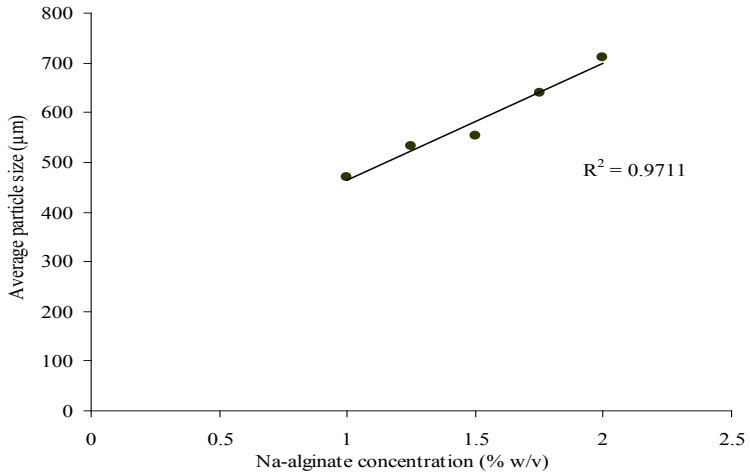

Figure 2. Relationship between Na-alginate concentration and average particle size of OTC HCl loaded Ca-alginate beads

Average particle size was found to vary from 639.86 ± 17.85µm (F_4), 646.91 ± 15.03 µm (F_6) and 649.26 ± 18.52 µm (F_7) on varying the cross-linker concentration from 2 %w/v, 5 %w/v, 10 %w/v respectively which indicated that increase in particle size was also observed with increase in the concentration of cross-linker. However, concentration of the cross-linker above 10 % w/v caused formation of irregular lumps due to extensive cross-linking of the guluronic acid unit of sodium alginate. When the cross-linking time was decrease, the average particle size reduced. Variation of the cross-linker concentration and cross-linking time was devoid of a significant change in the percentage yield.

Table 2 summarizes the flowability of OTC HCl loaded Ca-alginate beads exemplified by Carr's compressibility index [16] and Hausner ratio [17]. The flowability of OTC HCl drug was poor but the flowability of alginate beads was excellent. All beads formulation possessed Carr's compressibility index in the range of 5–15 and HR less than 1.25 suggesting excellent flow properties of all beads formulations unlike cohesive pure drug. However, a slight decrease in Carr's index and Hausner ratio of the beads was noticed by increasing the polymer concentration, cross-linker concentration and cross-linking time owing to formation of larger particles. As the particle size increases, the cohesivity of the particles decrease and beads formulation exhibited the low inter-particle friction resulting in good flow property.

3.2. Entrapment efficiency

The entrapment efficiency was found to be in the range of 52.87 ± 1.56 to 61.76 ± 0.96 % (Table 2). The entrapment efficiency increased progressively with increasing sodium alginate concentration. Increase in the Na-alginate concentration resulted in the formation of lager sized beads entrapping greater amount of the drug. This effect might have occurred due to the greater availability of active calcium-binding site in polymeric chains and consequently, the greater degree of cross-linking as the quantity of sodium alginate increased which generate more space for drug encapsulation [2]. Concentration of the sodium alginate up to 1.75 % w/v and cross-linker concentration up to 2 % w/v (F_4) shows highest percent entrapment efficiency i.e. 61.76 ± 0.96 %. However, further increase in the concentration of sodium alginate and $CaCl_2$ result in decrease in the entrapment efficiency. This could be due to the instant gelling of sodium alginate on addition of calcium chloride and squeezing out of the aqueous phase from the gel lattice which caused OTC HCl comes out from gel lattice along with squeezed aqueous phase. Variation in cross-linking time were also studied, entrapment efficiency decreases as the cross-link time decreases. Cross-linking time of 30 min was found to be optimum as it yielded highest entrapment efficiency; less cross-linking time resulted in incomplete gelling of sodium alginate [18].

Table 2. Effects of formulation and processing variables on the micromeritic properties and entrapment efficiency of OTC HCl loaded Ca-alginate beads

Form. code	Particle size* (µm)	% Yield* (w/w)	Carr's Index (%)	Hausner ratio (HR)	Entrapment efficiency* (%)
Pure Drug	-	-	47.62	1.90	-
F_1	470.96 ± 15.22	69.98 ± 0.33	14.25	1.24	56.36 ± 2.00
F_2	532.06 ± 13.77	71.40 ± 0.52	13.10	1.22	59.89 ± 0.70
F_3	553.66 ± 15.04	77.16 ± 0.51	12.65	1.18	60.39 ± 1.30
F_4	639.86 ± 17.85	78.94 ± 0.43	12.15	1.14	61.76 ± 0.96
F_5	709.33 ± 16.28	77.95 ± 0.40	10.65	1.12	59.93 ± 1.06
F_6	646.91 ± 15.03	77.36 ± 0.42	11.76	1.14	56.24 ± 2.30
F_7	649.26 ± 18.52	76.96 ± 0.38	11.44	1.13	52.87 ± 1.56
F_8	624.50 ± 18.25	77.65 ± 0.74	12.10	1.15	59.72 ± 1.26
F_9	618.78 ± 20.35	78.20 ± 0.36	12.60	1.17	54.46 ± 0.87
n = 3, values are expressed as a mean ± S D					

Effect of polymer concentration (F_1 – F_5); Effect of cross-linker concentration (F_6 – F_7);
Effect of cross-linking time (F_8 – F_9).

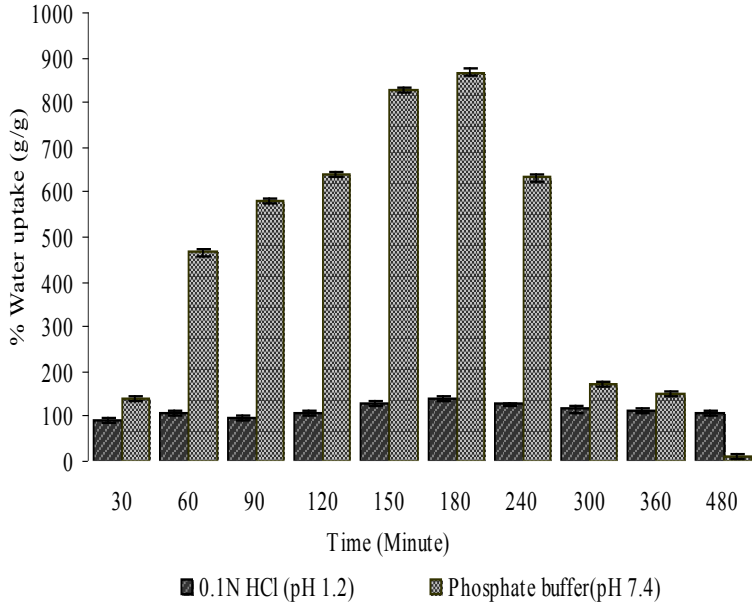

Figure 3. Swelling behavior of OTC HCl loaded Ca-alginate beads in 0.1 N HCl (pH 1.2) and phosphate buffer (pH 7.4)

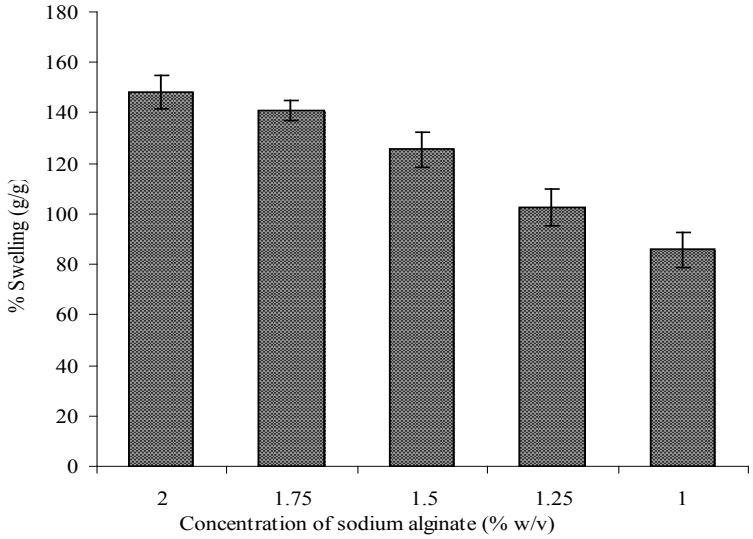

Figure 4. Effect of the polymer concentration on the extent of swelling of OTC HCl loaded Ca-alginate beads

3.3. Swelling Behavior

The swelling behavior of alginate polymer is the major factor for controlling the drug release from the beads system. The percentage water uptake of OTC HCl loaded Ca-alginate beads was determined in 0.1 N HCl (pH 1.2) and phosphate buffer (pH 7.4) [19]. As shown in Figure 3, the beads exhibited highest water uptake in phosphate buffer (pH 7.4), while lowest water uptake was noticed in 0.1 N HCl (pH 1.2). Maximum water uptake was obtained at 2-3 hours in phosphate buffer pH 7.4, subsequently erosion and breakdown occurred. These results suggested that the dried beads will swell slightly in stomach and they are subsequently transferred to upper intestine, where the beads begin to swell more and behave as matrices for controlled release of loaded drug.

The extent of swelling (% swelling) of the bead was determined in 0.1 N HCl (pH 1.2) by using different concentration of sodium alginate polymer. The results showed that swelling was related to the polymer concentration, more swelling was found for beads containing high polymer concentration (Figure 4). This effect may occur due to increase in beads volume as the concentration of sodium alginate increased [20].

3.4. *In vitro* Drug Release

To study the effect of sodium alginate on OTC HCl release, sodium alginate was used at five different concentrations: 1.0 (F_1), 1.25 (F_2), 1.50 (F_3),1.75 (F_4) and 2.0 (F_5) %w/v. *In vitro* drug release study shows that the release of drug from F_1 formulation was found to be 67.96 ± 2.52 % at pH 1.2 within 2 h. After 2 h, the calcium alginate beads (F_1) disintegrated and lost remaining drug within 3 h in dissolution medium SIF (pH 7.4). Figure 5 indicate that with increase in concentration of sodium alginate (F_2, F_3, F_4, and F_5), the release of entrapped drug during first 2 h in SGF was significantly reduced and more sustained the drug release. The release of OTC HCl from F_2, F_3, F_4 and F_5 formulations was found to 68.36 ±

1.82, 67.24 ± 2.44, 48.22 ± 1.46 and 46.82 ± 1.38 % respectively at pH 1.2 within 2 h but after 2 h, only F_4 and F_5 followed the drug release pattern extending up to 8 h. A decrease in the rate and extent of release was observed with relative increase in the polymer concentration in beads, can be attributed by the increase in the extent of swelling and gel layer thickness that acted as a barrier for penetration of dissolution medium within polymer matrix thereby retarding the diffusion of OTC HCl from swollen alginate matrix [21]. The effect of cross-linker concentration on drug release was shown in Figure 6. The result shows that the release of OTC HCl from the beads decreased with increased cross-linking agent concentration. However, except for 2 % w/v $CaCl_2$ (F_4), the drug release from F_7 formulation (5 % w/v $CaCl_2$) and F_7 formulation (10 % w/v $CaCl_2$) did not vary significantly ($p>0.5$). OTC HCl release from beads was also decrease and more sustained with increasing the cross-linking time, F_8 and F_9 caused no significant change ($p >0.5$) in the amount of drug release (Figure 7). This could be observed due to the increasing cross-linking density that hindering drug release from beads.

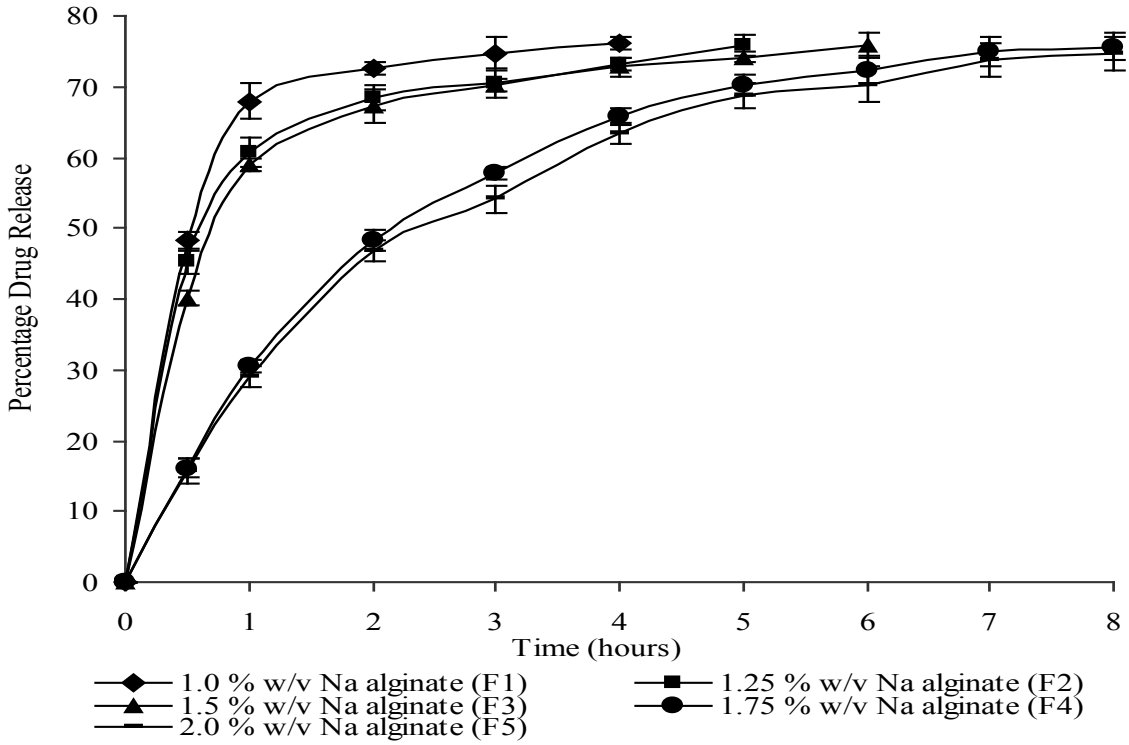

Figure 5. Effect of polymer (sodium alginate) concentration on release characteristics of OTC HCl from Ca-alginate beads

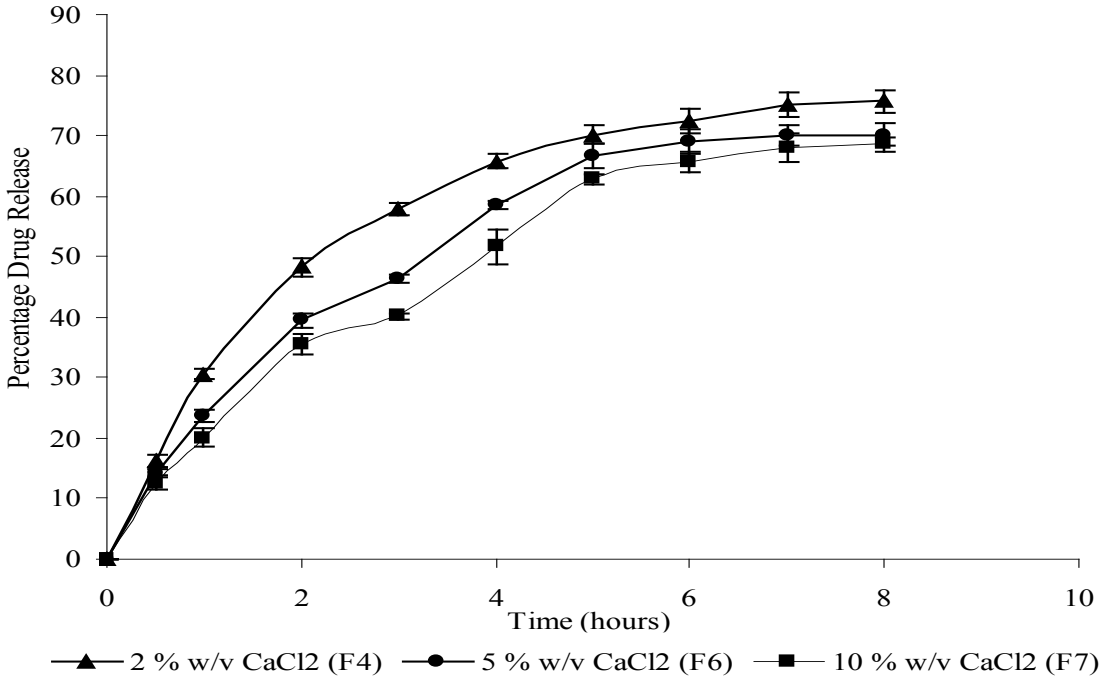

Figure 6. Effect of cross-linker (calcium chloride) concentration on release characteristics of OTC HCl from Ca-alginate beads

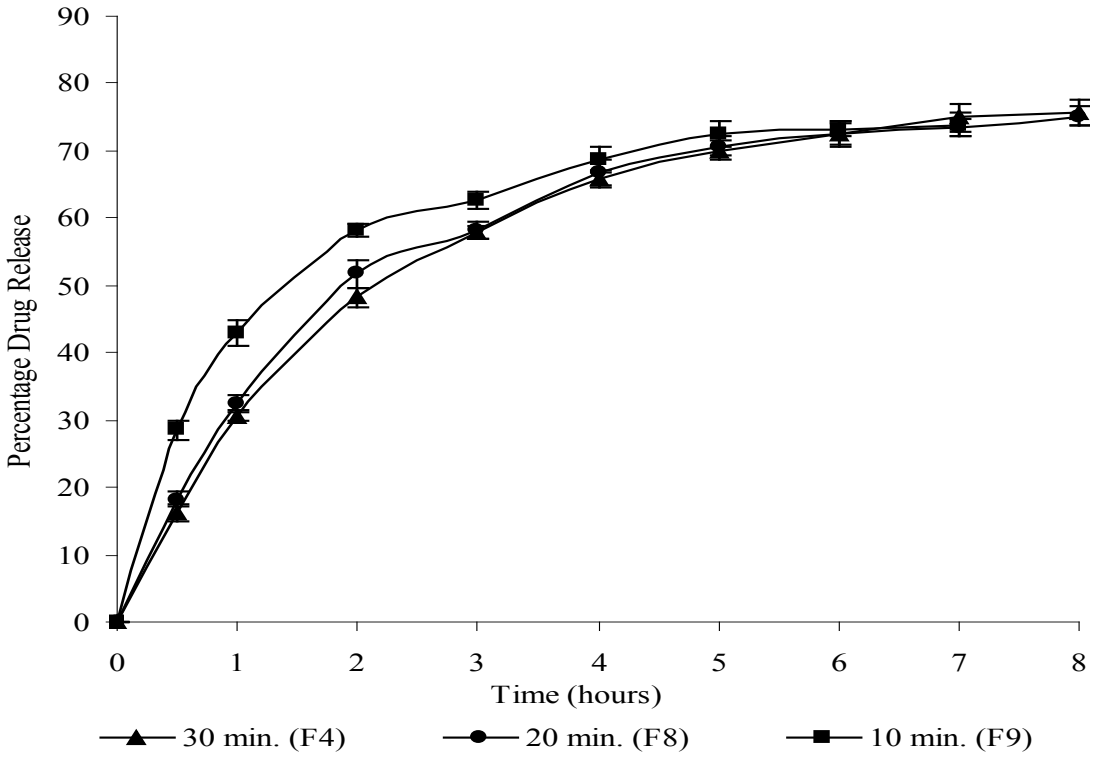

Figure 7. Effect of cross-linking time on release characteristics of OTC HCl from Ca-alginate beads

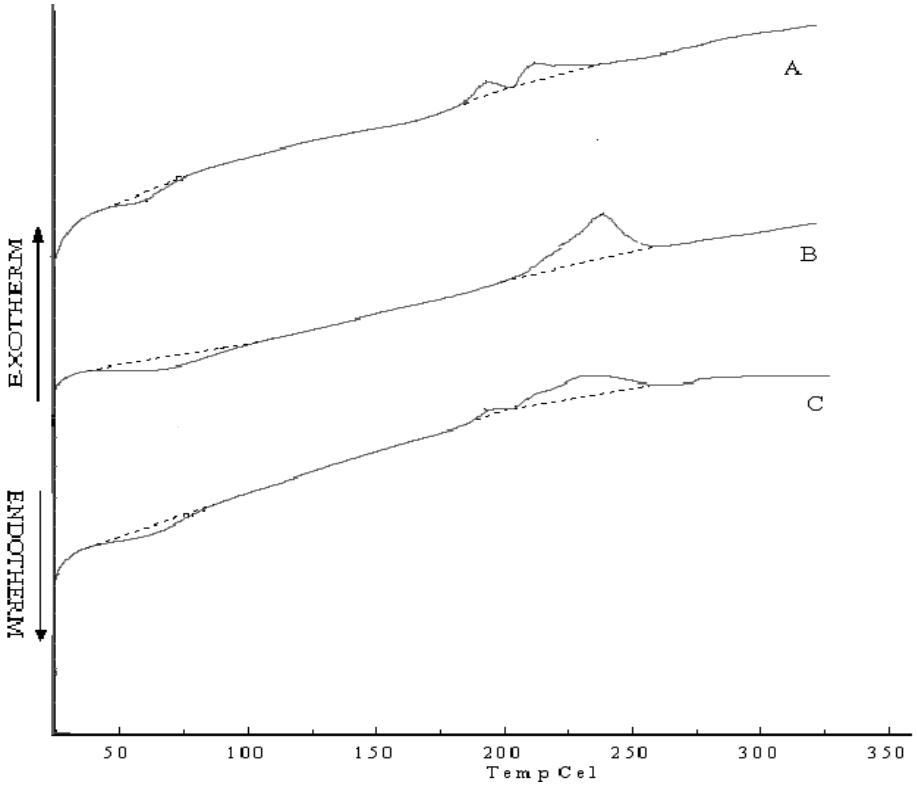

Figure 8. DSC thermo grams of pure drug, polymer and physical mixture of drug and polymer. Oxytetracycline hydrochloride (A), Sodium alginate (B), Oxytetracycline hydrochloride + Sodium alginate (C)

3.5. Differential Scanning Calorimetry (DSC) Study

Thermal behavior of Oxytetracycline hydrochloride and physical mixture with polymer (Na-alginate) was investigated by DSC. Figure 8 Shows the DSC thermo grams obtained during the heating stage for Oxytetracycline hydrochloride (A), sodium alginate (B), Oxytetracycline hydrochloride: sodium alginate mixture (C). The DSC thermogram of Oxytetracycline hydrochloride (A) showed one endothermic

peak at 61°C and two exothermic peaks at 195°C and 214°C. The DSC curve for physical mixtures of Oxytetracycline hydrochloride and sodium alginate (D) exhibited one endothermic peak at 62°C and two exothermic peaks at 194°C and 231°C. The exothermic peak at 231°C which show the peak of sodium alginate (Figure 8, B). This clearly showed that there were no changes in the DSC thermogram of pure drug in presence of Na-alginate, indicating absence of interactions between the drug and polymer.

4. Conclusions

In this study, Ca-alginate beads of Oxytetracycline Hydrochloride were prepared by using ionic-gelation method. The ability of calcium alginate beads to incorporate the OTC HCl and prolonged release of Oxytetracycline Hydrochloride has been investigated through variation in processing conditions. Variables such as polymer concentration, cross-linking agent concentration and cross-linking time were considered. Drug release and entrapment efficiency were affected by polymer concentration, cross-linking agent concentration and cross-linking time. From this study it can be concluded that a natural polymer sodium alginate can be used to prolong the release of Oxytetracycline Hydrochloride. DSC study did not reveal any significant drug interactions.

Acknowledgements

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Conflicts of Interests

The authors declare no conflict of interest

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