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Original research paper

Development of coated beads for oral controlled delivery of cefaclor: *In vitro* evaluation

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The aim of the present study was to develop and characterize coated chitosan-alginate beads containing cefaclor as a controlled release delivery system. Coated cefaclor beads were prepared by solvent evaporation techniques. Beads were found to be intact and spherical in shape. Their size range was 1.05 to 2.06. The loading efficiency showed maximum value when the concentration of cefaclor, chitosan and PEG 400 was 10 % (*m/V*), 0.5 % (*m/V*) and 2 % (*V/V*), respectively. Best retardation of cefaclor release from chitosan-alginate beads was achieved by coating with 15 % of shellac in formula F19. A significant antimicrobial activity ($p < 0.05$) against *Staphylococcus aureus* and *Klebsiella pneumoniae* was observed for formula F19 compared to the standard antibiotic disc. Furthermore, the simulated plasma profile showed the superiority of F19 in sustaining drug release for more than 12 h. Therefore, shellac coated chitosan-alginate beads could be considered a successful controlled release oral cefaclor dosage form.

Keywords: cefaclor, chitosan-alginate, shellac coated beads, modified release, oral delivery

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Development of sustained release dosage forms has attracted the attention of pharmaceutical manufacturers in recent years. Sustained release dosage forms can be formulated into a single unit dosage form such as tablet or capsule, or a multiparticulate dosage form such as pellet or bead. Recently, beads have attracted a considerable attention as potential drug carriers to obtain a controlled release, site-specific delivery and to increase drug bioavailability (1). Alginate and chitosan are the most widely used hydrogels in the formulation of such systems due to their high biodegradability and biocompatibility. Alginate is a natural polyacid and has a unique property of gel formation in the presence of multivalent cations such as calcium ions in aqueous media (2), while chitosan is a naturally occurring polysaccharide comprising glucosamine and *N*-acetyl-glucosamine with unique polycation characteristics (3). Alginate beads have been reported in some studies for the problems during beads preparation. Drug loss and shrinkage of

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the alginate polymer take place when subjected to an acidic medium, while it dissolves at higher pH values (2). On the contary, chitosan beads dissolve at low pH and are insoluble in alkaline media. Some studies reported that chitosan alone cannot control drug release due to its hydrophilicity (3). Therefore, the alginate-chitosan polyelectrolyte complex was considered to be a promising medium to reduce the porosity of alginate beads and decrease the leakage of the encapsulated drug (4). Furthermore, one approach to obtaining more controlled release of drugs is the formulation of coated drug beads. Various coating materials were used for this purpose, such as ethyl cellulose and Eudragit® (5).

In the present study, cefaclor was used as a model drug. Cefaclor is a broad spectrum antibiotic belonging to the family of second generation cephalosporins. Cefaclor is well absorbed in the body, with a peak serum concentration occurring within 30–60 min, which is significantly reduced in the presence of food, with no change in the total amount of drug absorbed. Cefaclor is rapidly excreted in urine, with an approximate half-life of two hours (6). Because of its short half-life and large dose, cefaclor is considered a good candidate for sustained release dosage forms.

The aim of the present study was to develop a controlled release delivery system of cefaclor using coated alginate-chitosan beads. The effects of stirring time and the addition of a solubilizing agent on the encapsulation efficiency, bead size and the drug release profile were investigated. In addition, the antimicrobial activity of the coated beads of cefaclor was also evaluated.

EXPERIMENTAL

Materials

Cefaclor was obtained as a gift from Julphar Pharmaceutical Company (UAE). Chitosan (CTS) medium molecular mass, dihydrogen sodium orthophosphate, disodium hydrogen orthophosphate and phosphate buffer saline (PBS) tablets (pH 7.4) were all purchased from Sigma-Aldrich (UK). Cellulose acetate phthalate (CAP), calcium chloride, ethyl cellulose (EC), and shellac (benzyl ethyl 2-methyl-4-(2-phenylethynyl)-1,4-dihydropyridine-3,5-dicarboxylate) were from BDH Chemicals (UK), β-cyclodextrin, sodium alginate (SA) and oleic acid (OA) sodium were from Hopkin and Williams (UK). All other chemicals were of analytical grade. *Staphylococcus aureus* (ATCC 25923) and *Klebsiella pneumoniae* (ATCC 15380) were obtained from the Ibn-Sina Center for drug quality control and analysis (Iraq).

Preparation of cefaclor loaded beads

Cefaclor loaded beads were prepared using two polymers, alginate and chitosan--alginate combination. Table I summarizes the composition of the prepared cefaclor bead formulations.

Preparation of alginate beads

Alginate beads (F1) were prepared according to the method described by Kikuchi (7). Two grams of cefaclor were dispersed in 7 % (*m/V*) sodium alginate aqueous solution under shaking in a water bath at 40 °C until a homogeneous solution was obtained.

Table I. Composition of cefaclor loaded beads formulations

Formula	Cefaclor (%, *m/V*)	SA (%, *m/V*)	Formulation ingredients							
			Coagulation fluid		Post coagulation fluid			Coating solution		
			CTS (%, *m/V*)	$CaCl_2$ (%, *m/V*)	CTS (%, *m/V*)	SA (%, *m/V*)	$CaCl_2$ (%, *m/V*)	EC (%, *m/V*)	CAP (%, *m/V*)	Shellac (%, *m/V*)
F1	10	7	0	3	–	–	–			
F2	10	7	0.2	3	–	–	–			
F3	10	7	0.5	3	–	–	–			
F4	10	7	0.8	3	–	–	–			
F5	10	7	0.5	3	0.05	–	–			
F6	10	7	0.5	3	0.05	0.05	–			
F7	10	7	0.5	3	0.05	0.05	0.05			
F8	10	7	0.5	3	0.5	–	–			
F9	10	7	0.5	3	0.5	0.5	–			
F10	10	7	0.5	3	0.5	0.5	0.5			
F11	10	7	0.5	3				5	–	–
F12	10	7	0.5	3				10	–	–
F13	10	7	0.5	3				15	–	–
F14	10	7	0.5	3				–	5	–
F15	10	7	0.5	3				–	10	–
F16	10	7	0.5	3				–	15	–
F17	10	7	0.5	3				–	–	5
F18	10	7	0.5	3				–	–	10
F19	10	7	0.5	3				–	–	15

SA – sodium alginate, CTS – chitosan, EC – ethyl cellulose, CAP – cellulose acetate phthalate

The pH was adjusted to 5.5 with a few drops of 0.1 mol L^{-1} NaOH. A volume of 20 mL of the solution was dropped through a 20 gauge syringe needle into 100 mL of a 3 % (*m/V*) calcium chloride aqueous solution. The droplets were slowly stirred for 1 h. Then, alginate beads were separated from the solution, washed several times with distilled water and dried at 60 °C for 12 h.

Preparation of chitosan-alginate beads

Alginate-chitosan beads (F2-F4) were prepared by the ionotropic gelation method. Cefaclor was dispersed in a sodium alginate 7 % (*m/V*) aqueous solution. The solution was dropped through a 20 gauge syringe needle into 100 mL of a 3 % (*m/V*) of calcium chloride agitated solution (pH adjusted to 5.5) containing different concentrations of chitosan (0.2, 0.5 and 0.8 %, *m/V*) already dissolved in 1 % (*V/V*) acetic acid solution. Beads were left for 30 min, and washed twice with distilled water and dried at 50 °C for 4 h, and then at room temperature for 2 days for complete drying.

Preparation of multilayer chitosan-alginate beads (F5–F10)

Multilayer beads (F5) were prepared by placing the prepared cefaclor-chitosan alginate beads into 100 mL of the post coagulation solution, CTS solution 0.05 % (*m/V*), for 30 min with stirring. F6 was prepared by placing the beads into 100 mL of 0.05 % CTS and 100 mL of 0.05 % SA (*m/V*) for 30 min with stirring. F7 was prepared by incubation of chitosan-alginate beads in 100 mL of 0.05 % CTS and 100 mL of 0.05 % SA for 30 min, followed by 10 min stirring in 0.5 % (*m/V*) $CaCl_2$ aqueous solution. Indeed, F8, F9 and F10 were prepared using the same former steps but the concentration of coagulation solutions (CTS, SA and $CaCl_2$) was increased to 0.5 % (*m/V*) (Table I). Chitosan-alginate multilayer beads were washed with distilled water and dried in an oven at 30 °C for 24 h.

Preparation of coated beads

Cefaclor loaded chitosan-alginate beads were coated by the solvent evaporation technique. The beads were coated with ethyl cellulose (EC), cellulose acetate phthalate (CAP) or shellac. In brief, coating solutions were prepared by placing two grams of dried chitosan-alginate beads in 20 mL of each of the coating polymer solutions using different concentrations: 5, 10 and 15 % (*m/V*). Formulation compositions (F11-F19) are shown in Table I. The coating polymeric solutions were prepared as follows. Ethyl cellulose solution was prepared by dissolving EC in chloroform while CAP and shellac were prepared by dissolving the polymer in a 1:1 isopropanol/acetone (*V/V*) solution. The coating process was maintained at a stirring rate of 20 rpm and temperature of 50 °C until complete evaporation of the solvent took place. Coated beads were left overnight at room temperature to ensure solvent evaporation.

In vitro *evaluation of the prepared beads*

The prepared formulations were evaluated for their morphological features, particle size, yield percent, entrapment efficiency and *in vitro* release of cefaclor from the prepared beads.

Morphological and particle size analysis

An optical microscope (Eclipse TS 100, Nikon, Japan) was used to study the morphology and particle size of dried beads. The microscope eyepiece was fitted with a micrometer by means of which the particle size of the micro-beads could be determined. The particle size was measured as mean ± SD of 100 examined beads.

Entrapment efficiency

Ten mg of dried loaded beads were pulverized and incubated in 10 mL phosphate buffer (pH 7.4) at room temperature for 24 h. The suspension was then centrifuged at 6000 rpm for 30 min. The supernatant was assayed for cefaclor using a UV spectrophotometer (Cintra 5, GBC Scientific equipment, Australia) at λ_{max} of 280 nm. The supernatant from the empty beads was used as a blank.

In addition, the impact of some factors on the entrapment efficiency of cefaclor in chitosan-alginate beads (F5) was evaluated including the concentration of chitosan (0, 0.2, 0.5 and 0.8 %, *m/V*) and cefaclor (5 and 10 % *m/V*), stirring time (2, 30, 60 and 120 min) and additives' types and concentrations: PEG_{400} 1 and 2 % (*V/V*), oleic acid 1 and 2 % (*V/V*) and β-cyclodextrin 1 and 2 % (*m/V*). Different additives with different concentrations were added during bead preparation by adding the exact volume of the additive solution to the dispersion of cefaclor in the chitosan solution with stirring till a homogenous solution was obtained.

In vitro *release study*

The *in vitro* release study of the prepared formulae was carried out using a USP dissolution apparatus Type 1 (Copley, UK). The study was carried out by placing a weighed quantity of beads equivalent to 500 mg cefaclor into hard gelatin capsules, in 900 mL of different dissolution media ensuring sink conditions. The dissolution media used in the study were 0.1 mol L^{-1} HCl (pH 1.2) for 2 h, then acetate buffer (pH 4.6) for 1 h, followed by phosphate buffer (pH 6.8). The dissolution temperature was maintained at 37 °C with a rotation rate of 100 rpm. Filtered samples were withdrawn at certain time intervals and replaced with an equal volume of fresh medium. The samples were analyzed for cefaclor content using a UV spectrophotometer adjusted to 280 nm (7).

Kinetic analysis of in vitro *release*

The *in vitro* drug release profiles of all formulae were analyzed by fitting the first 60 % fraction of drug released to, the empirical equation proposed by Korsmeyer *et al*. (9):

$$M_t / M_\infty = k\, t^n$$

where M_t / M_∞ is the fraction of drug released at time t, k is the rate constant and n is the release exponent. The exponential release constant n was used to characterize the release mechanism. The rate constant k and the diffusion exponent were calculated from the intercept and the slope of a logarithmic plot of F *vs.* t.

Microbiological study

Antibiotic disc preparation. – Microbiological study of cefaclor released from the selected bead formulation was performed according to the standardized single disc method as described by Bauer *et al.* (10). Briefly, the standard antibiotic disc (15 μg per disc) was prepared by dissolving 1.5 mg of cefaclor in 100 mL of phosphate buffer (pH 7.4). One milliliter of this solution was placed on a previously prepared multi-filter paper disc and left to dry for 12 h at room temperature. Sample antibiotic discs were loaded with one milliliter of cefaclor solution released from F19 in phosphate buffer (pH 7.4) after 7 and 8 hours of dissolution. This concentration was measured against a previously prepared cefaclor calibration curve in phosphate buffer pH 7.4 and found to be 15 and 20 μg per disc, respectively.

Antibiotic sensitivity test

Antibiotic sensitivity test was carried out by transferring five colonies of each type of bacteria, *Staphylococcus aureus* and *Klebsiella pneumoniae* from the pure culture to 5 mL of a nutrient broth tube and incubated at 35 °C for 2–5 h to produce a moderately cloudy suspension, which was standardized by dilution in turbidity standard barium chloride solution (TSB), visually equivalent to the McFarland standard 0.5 (standard turbidity prepared by adding 0.5 mL of 1 % barium chloride solution to 99.5 mL of 1 % sulphuric acid). A volume of 0.1 mL of this suspension was added to non-cultured nutrient agar, spread using a sterilized swab over the entire agar surface and incubated for 24 h at 37 °C. The prepared standard and sample antibiotic discs were placed at suitable distances on the cultured nutrient agar medium and incubated for 24 h at 37 °C and the diameters of the produced inhibition zones were measured.

In vivo *convolution study*

Convolution was conducted to simulate the plasma concentration time curves for cefaclor from F13, F16, F19 and the uncoated bead formulations using spreadsheets. Although simulated data are not always close to real data, convolution is still a good tool to predict the time course of a drug in the body. Convolution was completed with the following assumptions: (*i*) drug elimination follow first-order elimination, (*ii*) no absorption phase is considered; drug is treated like a series of intravenous bolus injections with appropriate adjustment of bioavailability, (*iii*) absorption rate constant is greater than the dissolution rate constant, and (*iv*) the drug follows linear pharmacokinetics.

Simulated plasma concentration time profiles were compared to those of uncoated beads and the published data of plasma concentration time profiles for commercially available cefaclor formulations. The pharmacokinetic parameters (PK) of cefaclor were used as reported in literature to simulate cefaclor concentration. The following reported PK were used in the simulation: cefaclor bioavailability from immediate release formulations reported to be about 80 %, clearance 328.0 ± 83.1 mL min^{-1} and elimination half-life of 0.6 ± 0.1 h (11).

Statistical analysis

Results are expressed as mean ± SD for triplicate samples. The results were statistically analyzed to test any significant differences among the groups by the one-way analysis of variance (ANOVA) using SPSS statistical analysis program version 16.

RESULTS AND DISCUSSION

Morphological features of the prepared beads

Cefaclor loaded alginate beads (F1 to F4) using calcium chloride with or without chitosan were evaluated physically and found to be intact and spherical in shape. It was observed that the addition of chitosan to the coagulation solution produced beads with a smoother surface then that of alginate alone, as shown in Fig. 1.

Multilayer chitosan-alginate beads (F5–F10) were found to be intact and compact. Use of chitosan in the preparation of alginate beads resulted in spherical beads with a smooth surface. This may be attributed to the fact that chitosan bounds electrostatically to the alginate polymer molecules present on the surface of the beads. The beads that were additionally treated with sodium alginate (F6 and F9) were found to be clumpy after washing. An extra treatment with $CaCl_2$ (0.5 %, *m/V*) also resulted in smooth and spherical beads (F7 and F10) where Ca^{2+} ions were cross-linked with the outer most alginate layer of the beads to yield a complex structure (12).

Bead yield and bed size

The yield of prepared beads was almost 80 to 90 % for all the formulations. It was noticed that the bead yield was slightly lower (70–75 %) in the formulations where chitosan concentration was low (0.05 %, *m/V*).

Mean sizes of the prepared beads of different formulations are shown in Table II. The mean particle size of the prepared beads was in the following order: chitosan-alginate beads (1.05–1.37 mm) < chitosan-alginate multilayer beads (1.34–1.65 mm) < chitosan-alginate coated beads (1.68–2.08 mm). There was no significant difference in particle size between multilayer beads (F5, F6 and F7) and chitosan-alginate beads (F3 and F4). However, there was a significant difference ($p < 0.05$) in particle size between the multilayer bead formulae (F8, F9 and F10) and the chitosan-alginate bead formulae (F1, F2, F3 and F4). On the other hand, coated beads were significantly larger than chitosan-alginate single and multilayer beads.

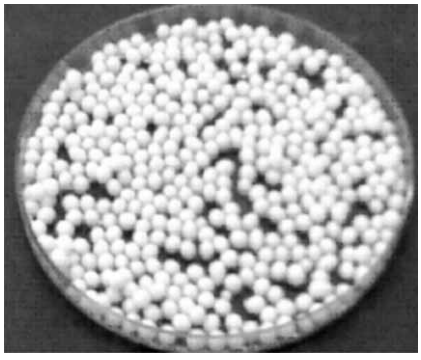

Fig. 1. Microscope picture of cefaclor loaded chitosan-alginate beads.

Table II. Factors affecting cefaclor entrapment efficiency in chitosan-alginate beads

Factor	Entrapment efficiency (%)[a]					
Chitosan (%, *m/V*)	0 (F1)	0.2 (F2)	0.5 (F3)		0.8 (F4)	
	18.9 ± 2.5	29.1 ± 4.1	49.0 ± 6.8		49.0 ± 9.6	
Stirring time (min)	2	30	60		120	
	63.2 ± 7.9	48.9 ± 6.86	34.1 ± 3.9		26.5 ± 5.4	
Additive	PEG 400 (% *V/V*)		Oleic acid (%, *V/V*)		β-Cyclodextrin (%, *m/V*)	
	1	2	1	2	1	2
	67.6 ± 10.6	72.7 ± 12.7	63.7 ± 8.3	64.5 ± 9.3	63.9 ± 9.6	64.2 ± 10.5
Cefaclor (%, *m/m*)	5	10				
	57.8 ± 7.6	72.7 ± 11.4				

[a] Mean ± SD, $n = 3$.

Entrapment efficiency

The effect of certain factors on the entrapment efficiency was studied on a selected cefaclor formulation (F5) (Table III). Increasing chitosan concentration in the coagulation solution was found to lead to significant increase in the drug entrapment efficiency with $p < 0.05$. Almost the same entrapment efficiency was obtained when chitosan concentration increased from 0.5 to 0.8 % in formulae F3 and F4, respectively. Formula F1, with no chitosan, showed the lowest drug entrapment efficiency (18.9 %). This may be attributed to gelation of the polymer at pH 4.5 where interaction between the carboxylic groups of the sodium alginate molecules and the protonated amine groups in chitosan results in the formation of a firm netlike complex of chitosan-alginate that tightly holds the drug inside the beads (13). However, the absence of chitosan in formula F1 resulted in inadequate cross-linking of polymer molecules and led to the formation of large pore size which permitted drug diffusion and consequently resulted in very low entrapment efficacy of cefaclor.

Increasing the stirring time during bead preparation resulted in a decrease in cefaclor entrapment efficiency, which may be due to the increased possibility of drug leaching outside the beads.

However, increasing the concentration of cefaclor from 5 to 10 % (*m/V*) resulted in better drug entrapment in the beads.

The addition of additives during bead preparation resulted in good entrapment efficiency compared to beads with no additive. PEG 400 (2 %) led to a significant increase ($p < 0.05$) in the entrapment efficacy compared to beads prepared with the addition of 1 % PEG 400. This may be attributed to the fact that PEG 400 is a solubilizing agent and freely soluble in water; it therefore enhanced the solubility of cefaclor in sodium alginate solution and thereby improved the entrapment efficiency of the drug inside the beads.

However, increasing the concentration of oleic acid and β-cyclodextrin from 1 to 2 % (*V/V*) did not significantly increase drug entrapment efficiency.

Table III. Particle size and kinetic parameters of cefaclor loaded chitosan-alginate beads

Formula	Particle size (mm)[a]	ln k (%, min^{-1})	n	R^2	Diffusion model
F1	1.05 ± 0.07	4.321	0.069	0.976	Fickian diffusion
F2	1.18 ± 0.13	4.132	0.125	0.977	Fickian diffusion
F3	1.33 ± 0.09	4.123	0.093	0.984	Fickian diffusion
F4	1.37 ± 0.13	4.057	0.105	0.977	Fickian diffusion
F5	1.34 ± 0.12	4.105	0.0942	0.937	Fickian diffusion
F6	1.36 ± 0.21	4.026	0.111	0.972	Fickian diffusion
F7	1.38 ± 0.27	4.049	0.102	0.976	Fickian diffusion
F8	1.62 ± 0.18	3.916	0.121	0.914	Fickian diffusion
F9	1.65 ± 0.23	3.431	0.208	0.891	Fickian diffusion
F10	1.65 ± 0.15	3.278	0.228	0.901	Fickian diffusion
F11	1.95 ± 0.31	1.459	0.561	0.94	Anomalous diffusion
F12	2.04 ± 0.28	1.442	0.528	0.963	Anomalous diffusion
F13	2.06 ± 0.38	1.144	0.574	0.972	Anomalous diffusion
F14	1.68 ± 0.22	1.87	0.498	0.972	Fickian diffusion
F15	1.81 ± 0.36	1.879	0.497	0.956	Fickian diffusion
F16	1.85 ± 0.27	1.762	0.518	0.972	Anomalous diffusion
F17	1.90 ± 0.34	2.119	0.445	0.973	Fickian diffusion
F18	2.06 ± 0.37	2.134	0.369	0.971	Fickian diffusion
F19	2.08 ± 0.31	2.225	0.365	0.972	Fickian diffusion

[a] Mean ± SD, n = 3.

In vitro *drug release*

The release profile of cefaclor from bead formulations F1-F4 showed a rapid burst effect during the first 30 minutes of dissolution (Fig. 2a). This rapid release may be explained as follows. First, when chitosan-alginate loaded beads were placed at a low pH (1.2), the negatively charged carboxylate groups of sodium alginate began to protonate to form uncharged –COOH groups. This reduced the degree of cross linking due to decreased electrostatic interactions among the alginate and chitosan chains within the beads, resulting in increasing fluids uptake and swelling of the beads (12). In addition, cefaclor as a small, hydrophilic molecules at pH 1.2 is highly ionized, resulting in an increase in drug solubility; hence it diffuses out easily.

Release profiles of the chitosan-alginate multilayer beads (F5-F10) are shown in Fig. 2b. Increasing chitosan concentration in the multilayered bead formulation F8 resulted in a decrease in drug release compared to that of formulation F5. In both formulae, deprotonation of the NH_4^+ group in the outermost chitosan layer to NH_2 groups occurred upon placing the beads in a simulated intestinal fluid, which can form a diffusion barrier for Na^+/Ca^{2+} ion exchange among Ca^{2+} ions in the bulk of the beads and Na^+

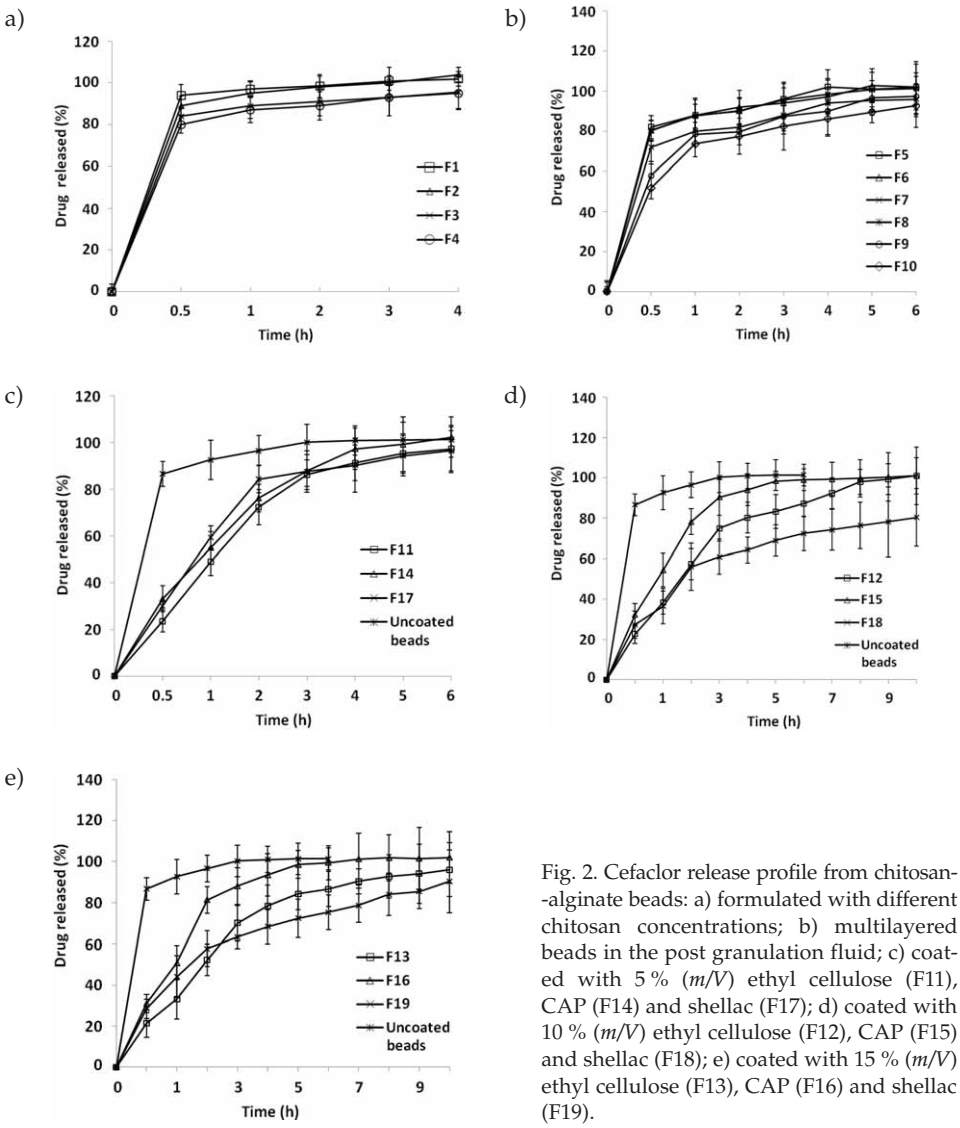

Fig. 2. Cefaclor release profile from chitosan--alginate beads: a) formulated with different chitosan concentrations; b) multilayered beads in the post granulation fluid; c) coated with 5 % (*m/V*) ethyl cellulose (F11), CAP (F14) and shellac (F17); d) coated with 10 % (*m/V*) ethyl cellulose (F12), CAP (F15) and shellac (F18); e) coated with 15 % (*m/V*) ethyl cellulose (F13), CAP (F16) and shellac (F19).

ions in the simulated intestinal fluid. By increasing chitosan concentration in the post coagulation solution (F8), the barrier hindrance also increased and consequently the bead disintegration process was delayed (14).

It was observed that there was a significant difference in the release profile of F6 and F9 after the first 30 minutes of dissolution. After 30 minutes, F6 released 81 % of cefaclor compared to 57 % released from formula F9. The outermost layer of both F6

and F9 was sodium alginate. In simulated gastric fluid (pH 1.2), the negative $–COO^-$ group of sodium alginate got protonated and acted as a diffusion barrier for the release of the drug. Increasing the thickness of this layer will result in increasing the barrier of drug diffusion and thereby more retardation in drug release. Upon treating the beads with simulated intestinal fluids, the alginic acid will begin to dissolve, leaving chitosan forming the outermost layer. Chitosan will act as a diffusion barrier for Na^+/Ca^{2+} ion exchange, which will result in delayed drug release. This explain why F9 showed more delayed drug release than F6 (15).

Drug release from F10 formulations was retarded, with only 65.6 % of the drug release after 30 minutes of dissolution compared to 80.3 % from formulation F7. This may be attributed to the addition of calcium chloride where the Ca^{2+} ions act as a hardening agent for the outer layer of beads. Therefore, a rigid layer was produced that delayed diffusion and drug release.

The overall results of release profiles for chitosan-alginate and chitosan-alginate multilayer beads demonstrate a burst effect during the first hour of dissolution and no prolonged release of cefaclor was obtained from the prepared formulations (F5–F10).

Chitosan alginate beads coated with 5 % (*m/V*) ethyl cellulose (F11), 5 % CAP (F14) and 5 % (*m/V*) shellac (F17) showed a delay in drug release from the prepared formulations of 23.7, 33.2 and 30.2 %, respectively, after 30 minutes, compared to 86.6 % of drug released from the uncoated formulations (Fig. 2c).

Good sustained drug release was obtained from chitosan-alginate coated formulations using 10 % (*m/V*) of polymeric solutions, especially with 10 % (*m/V*) of EC (F12) and shellac (F18) formulations. For F15 bead formulation coated with 10 % (*m/V*) CAP, drug release was faster with more than 90 % drug released after 4 h, which was similar in pattern to the drug released from 5 % (*m/V*) CAP coated bead formulation. Data are shown in Fig. 2d.

Increasing the concentration of the coating polymeric solution to 15 % (*m/V*) (F13, F16 and F19) showed release patterns dissimilar to those obtained from the 10 % (*m/V*) coated formulations. There was a significant difference ($p < 0.05$) in cefaclor release from chitosan-alginate beads coated with 15 % (*m/V*) of polymeric materials compared to 10 % of the coating polymer (Fig. 2e). F19 (shellac 15 % coated beads) was considered the most successful formula for controlled cefaclor release and was selected for further study.

Differences in the release profile among coated chitosan-alginate bead formulations using different polymers are attributed to the differences in solubility of the polymeric materials used, where CAP solubilizes at pH 5 or more while ethyl cellulose remains intact throughout the GIT and shellac often dissolves too slowly in the intestinal fluid (16). As a result, there is faster solubilization of CAP than EC and shellac while the beads passing through the intestinal fluid (pH 4.6 and 6.8) result in faster drug solubilization and diffusion into the intestinal fluid.

Kinetic analysis of in vitro *drug release*

Fractions of the drug released from all developed formulae were fitted using an empirical equation. Straight lines were obtained from plotting ln M_t/M_∞ *vs*. ln t and the kinetic parameter (n) was calculated, with the correlation coefficients (R) and 95 % confidence intervals for all formulations (Table II). The values of n obtained for most

Table IV. Inhibition zone diameter (mm) of cefaclor discs incubated in two culture media

Disc[a]	*Staphylococcus aureus*[b]	*Klebsiella pneumoniae*[b]
Cefaclor	24 ± 1	15 ± 0
F19 formula	33 ± 0	17 ± 1
F19 formula	35 ± 1	19 ± 0

[a] 15 μg per disc
[b] Mean ± SD, $n = 3$.

of the formulations are less than 0.5, which suggests drug release from the prepared formulations *via* Fickian release mechanism. However, the values of n for the drug released from CAP coated chitosan-alginate beads were greater than 0.5, indicating anomalous drug release from the beads by both diffusion and polymer erosion mechanisms.

Microbiological assay

The amount of cefaclor released from F19 after 7 and 8 h was 15 μg and 20 μg, respectively. The growth inhibition zones of both *Staphylococcus aureus* and *Klebsiella pneumoniae* were measured and significantly higher antibacterial activity ($p < 0.05$) was observed for formula F19 compared to the standard antibiotic disc (Table IV). These results demonstrate the efficiency of the new formulation (F19) as a sustained antimicrobial oral dosage form.

Convolution

Simulation was done to predict the drug plasma concentration from the various formulae administered *in vivo*. Results are presented in Fig. 3. Simulated data showed that

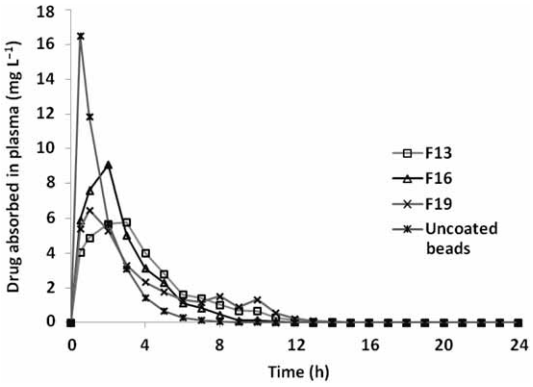

Fig. 3. Simulated Cefaclor plasma concentration time profiles after the administration of 500 mg per body mass oral dose of selected beads formulations, F13, F16, F19 and the uncoated beads formulations.

uncoated beads produced the highest concentration (γ_{max}) in the blood stream, with a concentration of 16.5 mg L^{-1} reached within 30 minutes, and cefaclor plasma level was lower than 0.1 mg L^{-1} after 8 h. γ_{max} obtained from the uncoated bead formulations was comparable to that reported for cefaclor immediate release dosage form at a dose of 500 mg per body mass (17.3 ± 3.6 mg L^{-1}). For formula F13, at the simulated drug plasma profile was maximum (5.77 mg L^{-1}) after 3 h and cefaclor concentration was less than 0.1 mg L^{-1} after 12 h, which proves the sustained release action of cefaclor from coated beads formulations. For F16 and F19, the maximum concentration of 9.07 mg L^{-1} was reached after 2 h and 6.5 mg L^{-1} after one hour, respectively. Cefaclor concentration reached less than 0.1 mg L^{-1} after 11 h and 13 h from F16 and F19 formulae, respectively.

The simulated plasma profile for the selected coated formulations showed the superiority of F19 and F13 in sustaining drug release for more than 12 h.

CONCLUSIONS

Cefaclor loaded chitosan-alginate coated beads were developed successfully for modified oral delivery of cefaclor. The desired entrapment efficiency of cefaclor in the prepared beads was optimized by adjusting different parameters such as polymer type concentration of the solubilizing agent and stirring time.

The best *in vitro* sustained release pattern of cefaclor was obtained from bead formulations coated with shellac 15 % (*m/V*) (F19). In addition, the microbiological activities demonstrated that F19 is effective against *Staphylococcus aureus* and *Klebsiella pneumoniae*. There was a significant difference in its activity against ($p < 0.05$) *Staphylococcus aureus* and *Klebsiella pneumoniae* compared to the standard. Convolution of the *in vitro* dissolution data showed a good sustained release action from both the F19 and F13 formulae over 12 h.

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