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Research Article

UNSUCCESSFUL DELIVERY OF TETRANDRINE FROM COLON-TARGETED DOSAGE FORMS COMPRISING ALGINATE/HYDROXYPROPYL METHYLCELLULOSE AND ALGINATE–CHITOSAN BEADS

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ABSTRACT

Objective: The objective of this study was to optimize the formulations of antifibrotic tetrandrine beads using alginate and various concentrations of hydroxypropyl methylcellulose (HPMC) and chitosan.

Methods: Beads were formulated with six (F1–F6) concentrations of polymer and were then characterized using scanning electron microscopy, differential scanning calorimetry, and X-ray diffraction; these beads were used for measurements of moisture contents, swelling, and *in vitro* drug release.

Results: Beads with the highest concentrations of HPMC and chitosan produced the highest entrapment efficiencies of 49.83% and 50.71%, respectively. Moreover, drug release under stomach conditions (HCl pH 1.2 medium) was restricted to 75.01%, 61.01%, 51.86%, 74.84%, 66.00%, and 41.63% with increasing HPMC and chitosan concentrations (F1–F6, respectively).

Conclusion: Beads of all formulations showed inadequate retention of tetrandrine under pH conditions of the upper gastrointestinal tract and would likely be unsuccessful as colon-targeted dosage forms.

Keywords: Alginate, Antifibrotic tetrandrine beads, Chitosan, Hydroxypropyl methylcellulose, Ionic gelation.

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INTRODUCTION

Colon-targeted drug delivery was previously used to advance therapy for localized disease and to minimize the side effects of drugs in the gastrointestinal tract [1]. These systems were designed to reach the colon with minimal release of enzyme- and pH-sensitive drugs in the upper gastrointestinal tract [2]. Specifically, these drug delivery systems should achieve long retention times [3], robustness to unique pH conditions [4], decreased doses and side effects, and increase bioavailability, especially for drugs with low absorption [5].

pH-sensitive mechanisms are critical to drug delivery systems for the gastrointestinal tract because pH conditions vary from highly acidic to almost neutral in the colon [4]. Beads are multiparticulate systems that have been considered as pharmaceutical dosage forms for colon-targeted drug delivery [6] and may be used to control and extend drug release [2]. These beads are formed using ionic gelation methods to produce cross-linked complexes of polyelectrolyte polymers. Tetrandrine is an antifibrotic agent for the treatment of intestinal fibrosis, which is characterized by excessive deposition of the extracellular matrix under conditions of ulcerative colitis and Crohn's disease [7]. Intestinal fbrosis is caused by an excess deposition of the extracellular matrix resulting from chronic inflammation and impaired wound healing in the intestine [8]. In addition, patients with fibrosis who were not effectively treated with systemic immunosuppressants benefited from drugs with localized effects [9], and in another study, antifibrotic site-directed effects of tetrandrine were achieved by targeting to the colon [10].

Polymers with crosslinking capacity, such as alginate, may facilitate tetrandrine delivery to the colon by beads because of the resulting cross-linked complexes form three-dimensional networks that can be used to entrap bioactive substances or drugs [11]. Alginate forms biodegradable polymers in which the stability of dosage forms can be manipulated and drug release can be controlled according to pH sensitivity, offering the potential for colon-targeted drug delivery [12,13]. However, alginate has a low drug entrapment efficiency, necessitating the use of combination polymers with polymer agents, such as hydroxypropyl methylcellulose polymer (HPMC) and chitosan, which improve drug entrapment and release profiles [14].

HPMC was chosen due to its stability in the gastrointestinal tract, where pH conditions widely vary. HPMC limits drug release at pH 3–11 [15,16]. HPMC also establishes semi-interpenetrating networks (semi-IPNs) with alginate, leading to the formation of beads when the mixture is dropped into calcium chloride solution. Herein, we performed experiments with chitosan based on a previous study, showing that it can facilitate targeting to the colon [14]. Like HPMC, chitosan is a polysaccharide that forms polymers that are biodegradable by colonic microflora. Specifically, chitosan formed polyelectrolyte cross-linked networks with alginate, and the ensuing electrostatic interactions strengthened the bioadhesive characteristics of alginate and facilitated drug release [5]. Based on these studies, we formulated tetrandrine-loaded beads from alginate/HPMC and calcium alginate–chitosan and compared colon targeting using six formulations of each with varying polymer concentrations.

MATERIALS AND METHODS

Tetrandrine (Shaanxi Ciyuan Biotech, China), tetrandrine standard (Sigma Aldrich, Singapore), sodium alginate (Shandong Jiejing Group Co., China), calcium chloride (Merck, Germany), HPMC (Wuhan Senwayer Century Chemical Co., Ltd., China), chitosan (Bio Chitosan, Indonesia), chloride acid (Brataco, Indonesia), potassium phosphate monobasic (Merck, Germany), sodium hydroxide (Brataco, Indonesia), ethanol (Brataco, Indonesia), Tween 80 (Brataco, Indonesia), and

deionized water (Brataco, Indonesia) were purchased from their respective suppliers.

Preparation of calcium alginate/HPMC beads

Sodium alginate and HPMC were separately dissolved in demineralized water, were mixed and homogenized, and were then slowly cooled to room temperature. Tetrandrine was then dissolved in 0.1 N HCl and was added to the alginate/HPMC solution and stirred until homogeneous. Subsequently, the blended solution was extruded into 2% calcium chloride solution using 21G syringe needle with stirring at 200 rpm (37°C) for 15 min. Beads were then washed with deionized water and dried at room temperature (Table 1).

Preparation of calcium alginate–chitosan beads

Sodium alginate was dissolved in demineralized water and tetrandrine was dissolved in 0.1 N HCl, and the two solutions were then homogeneously mixed. Calcium chloride was dissolved in demineralized water, and chitosan was dissolved separately in 1% glacial acetic acid and was mixed with 1 N NaOH to adjust the pH to about 4.5. The alginate–tetrandrine solution was then extruded into 2% calcium chloride solution using a 26G syringe needle with stirring at 200 rpm (37°C) for 15 min. The resulting beads were finally washed with deionized water and dried at room temperature.

Morphological characterization

Shapes, odors, surface textures, and colors of beads were visually determined.

Scanning electron microscopy

Shapes and morphologies of beads were observed using a scanning electron microscope (SEM; Hitachi SU-3500, Japan) after placing the beads in the sample holder and applying a vacuum [14].

Particle size distributions

The diameters of 300 beads were measured using calipers with 0.02 mm accuracy, and the mean particle sizes and size distributions were calculated [17].

Determination of moisture contents

Moisture contents were measured using a moisture balance (Adam, USA). Briefly, bead samples of about 1 g were placed in an aluminum pan, and percentage water contents were determined when no further weight changes were observed.

Process efficiency

To calculate recovery values, we compared the weights of all materials used during the production of beads with the weights of the resulting beads using the following formula:

$$\text{Process Efficiency}\ (\%) = \frac{\text{Weight of the dried beads (gram)}}{\text{Total weight of material used (gram)}} \times 100\%$$

Determinations of entrapment efficiencies and drug contents

Entrapment efficiency was measured after extracting tetrandrine from beads. To this end, beads were soaked in buffer phosphate-buffered saline (PBS) at pH 6.8 for 24 h and were stirred at 100 rpm until they disintegrated. HCl (pH 1.2) was then added to a volume of 50 ml, and solutions were finally centrifuged at 2500 rpm for 15 min. Supernatants were then collected and diluted again to 50 ml in HCl (pH 1.2), and tetrandrine contents were determined using a ultraviolet–visible (UV-Vis) spectrophotometer (Shimadzu UV-1800, Japan) at 280 nm. Entrapment efficiencies and drug contents were calculated using the following formulas [18]:

$$\text{Entrapment efficiency}\ (\%) = \frac{\text{Practical drug loading (mg)}}{\text{Theoritical drug loading (mg)}} \times 100\%$$

$$\text{Drug content}\ (\%) = \frac{\text{Practical drug loading (mg)}}{\text{Amount of beads (mg)}} \times 100\%$$

Differential scanning calorimetry

Tetrandrine, alginate, HPMC, chitosan, calcium chloride, alginate/HPMC beads, alginate–chitosan beads, tetrandrine-loaded alginate/HPMC beads, and tetrandrine-loaded alginate–chitosan beads were analyzed using a differential scanning calorimeter (DSC; Perkin Elmer DSC8000, USA). In these analyses, 5 mg samples were placed in aluminum cylinders under a flow of nitrogen and were heated at 10°C/min from 30°C to 350°C.

X-ray diffraction

To determine whether tetrandrine is amorphous or crystalline in alginate/HPMC and alginate–chitosan beads, we recorded diffraction patterns using an X-ray diffractometer with Cu irradiation at 40 kV and 40 mA.

Swelling index analysis

Bead formulations (1 g) were soaked in 25-mL aliquots of PBS (pH 6.8) at 37°C and were weighed after 5, 10, 15, 30, 45, and 60 min. Swelling indexes were then calculated, as previously described by Pandey *et al.* [19], using the following formula:

$$\text{Percentage swelling}\ (\%) = \frac{W2 - W1}{W1} \times 100$$

Where W_1 is the weight of dried beads and W_2 is the weight of swollen beads.

Fourier-transform infrared (FTIR) analyses

We performed FTIR spectroscopy (Shimadzu FTIR 8400S, Japan) to investigate interactions between the components in prepared formulations [20]. Samples of alginate, HPMC, chitosan, their composite beads, and loaded beads were crushed with dry potassium bromide, and analyses were performed at 400–4000 cm^{-1}.

***In vitro* drug release studies**

Beads were placed in 0.1 N HCl for 2 h, followed by PBS (pH 7.4) for 3 h, and then PBS (pH 6.8) for 2 h. PBS solutions contained Tween 80 (2%, v/v) [2]. 10-ml aliquots of dissolution fluid were withdrawn at regular intervals and were immediately replaced with the same volume of fresh media. Samples were then analyzed using a UV-Vis spectrophotometer at wavelength maxima for each analyte. Sample contents at n min were calculated using the following formula:

Table 1: Composition of the calcium alginate/HPMC and calcium alginate–chitosan beads containing tetrandrine

Formula	Alginate (%, w/v)	Calcium chloride (%, w/v)	HPMC (%, w/v)	Chitosan (%, w/v)	Tetrandrine (%, w/v)
Control	2	2	-	-	1
F1	2	2	0.5	-	1
F2	2	2	1	-	1
F3	2	2	2	-	1
F4	2	2	-	0.25	1.25
F5	2	2	-	0.5	1.25
F6	2	2	-	0.75	1.25

HPMC: Hydroxypropyl methylcellulose

Table 2: Average diameters, moisture contents, process efficiencies, entrapment efficiencies, and drug contents

Formula	Average diameter(μm)	Moisture content±SD (%) (n=3)	Process efficiency (%)	Entrapment efficiency±SD (%) (n=3)	Drug content±SD (%) (n=3)
Control	704.27	10.44±1.02	28.85	22.73±1.42	8.74±0.55
F1	758.70	9.06±0.52	26.62	29.60±1.61	8.46±0.46
F2	852.61	9.46±0.22	19.92	37.58±0.11	9.39±0.03
F3	903.52	9.67±0.06	18.98	49.83±0.46	9.97±0.09
F4	920.27	11.69±0.78	46.95	42.26±1.62	14.09±0.54
F5	994.8	10.76±1.15	42.5	42.93±1.29	15.33±0.46
F6	1054.6	9.06±0.69	51.85	50.71±0.31	16.90±0.10

SD: Standard deviation

$$n\ minute(mg) = \frac{(yn-a)\ x\ fp\ x\ M}{b\ x1000} + \ldots + \frac{(yn-a)\ x\ fp\ x\ S}{b\ x1000},$$

Where yn is tetrandrine absorption at n min, x is the tetrandrine concentration, fp is a dissolution factor, M is the volume of release medium, S is the sample volume, A is the intercept coefficient, and B is the slope.

RESULTS

Particle size distributions

Beads sizes were determined for 300 beads from each formula using calipers with 0.02 mm accuracy. From subsequent calculations of mean sizes, 39% of control beads were distributed between 589 and 671 μm, whereas 19% of F1 beads were 772–809 μm. F2 bead sizes were distributed in 16% portions at 802–835 μm and 836–869 μm, 15% of F3 beads were 888–914 μm, 30% of F4 beads were 1058–1100 μm, and 20.67% of F6 beads were 1088–1125 μm. These particle size distributions (Table 2) showed that increasing polymer concentrations are associated with increased bead sizes. Higher concentrations of chitosan and HPMC polymers also contributed to higher retention of mixtures during the gelation process, likely explaining the production of larger beads.

Moisture content determination

In evaluations of process efficiency (Table 2), water contents of beads indicated hygroscopic properties, similar to those of calcium chloride. In agreement, HPMC is an ether cellulose that acts as a hydrophilic carrier [21].

Process efficiency

Process efficiencies of F1, F2, F3, F4, F5, and F6 and control beads were 28.85%, 26.62%, 19.92%, 18.98%, 46.95%, and 42.5% and 51.85%, respectively.

Entrapment efficiency and drug content determinations

The present formulas varied in their capacities to entrap drug substances (Table 2). In experiments with control beads, complexes were not strong enough and had porous surfaces that allowed drug diffusion during the gelation process. We also found that entrapment efficiency increased with polymer concentrations. Foremost, interactions between alginate carboxyl groups and amine protons of chitosan limited the drug diffusion into the medium during gelation, indicating increased solidity of the polymer layers in beads containing alginate or chitosan [22].

Morphological characterization

Wet alginate/HPMC beads were spherical and were whitish, whereas alginate–chitosan wet beads were spherical and yellowish. None of the beads had strong odors, although alginate–chitosan beads had a weak acidic odor. On drying, beads turned into a yellowish color due to changes in density (Fig. 1).

SEM analyses

All formulations were observed to be vaguely spherical at 100× magnification, and rough, wavy, porous, and creviced surfaces were visible at 500× magnification (Fig. 2).

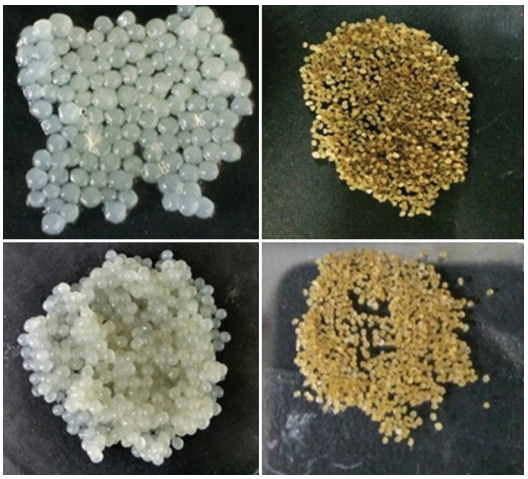

Fig. 1: Beads changed color into a yellowish

DSC analyses

In thermograms, melting points of the present bead-forming materials differed from those of the formed beads, reflecting chemical interactions between components. In particular, exothermic peaks disappeared in unloaded beads, suggesting that alginate forms a more solid bead structure [23], indicating the establishment of interactions between alginate and HPMC in the formed beads.

Unloaded alginate–chitosan beads showed two endothermic peaks at 75.22°C and 205.46°C, with corresponding melting energies of 142.38 J/g and 20.12 J/g and an exothermic peak at 274.86°C (117.462 J/g). Loaded alginate–chitosan beads had an endothermic peak at 75.71°C (181.52 J/g) and an exothermic peak at 272.91°C (41.21 J/g).

X-ray diffraction

The present diffractograms showed that tetrandrine is in the crystalline phase (Fig. 3), but only small, short, and tight peaks were identified for alginate/HPMC beads. In contrast, alginate–chitosan beads decreased peak heights compared with those of tetrandrine. Some peaks also indicated decreases in drug crystallinity. These data further indicated that tetrandrine was dispersed inside these beads [24] and is compatible with their polymeric matrixes [25]. Peak disappearances in diffractograms indicated the formation of amorphous materials, and differences in peaks reflect the sizes of vestigial tetrandrine crystals [26].

Swelling index

F1, F2, F3, F4, F5, and F6 formulas swelled by 561.08%, 1021.36%, 1116.53%, 937.68%, 646.3%, and 622.33%, respectively, over 1 h.

FTIR spectroscopy analysis

FTIR spectra of alginate showed an asymmetric carboxyl group at 1608 cm^{-1} and a symmetrical carboxyl group at 1429 cm^{-1}. G and M uronic acid were also detected at 1030 cm^{-1} and 1050 cm^{-1}, respectively.

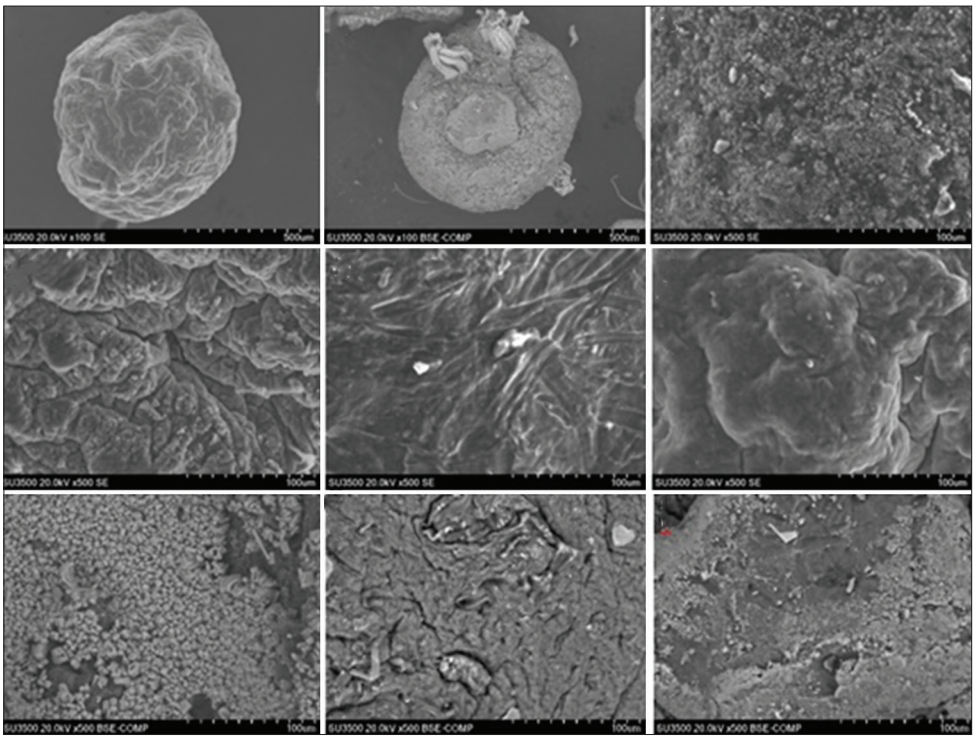

Fig. 2: Scanning electron micrograph under 100x and 500x magnification

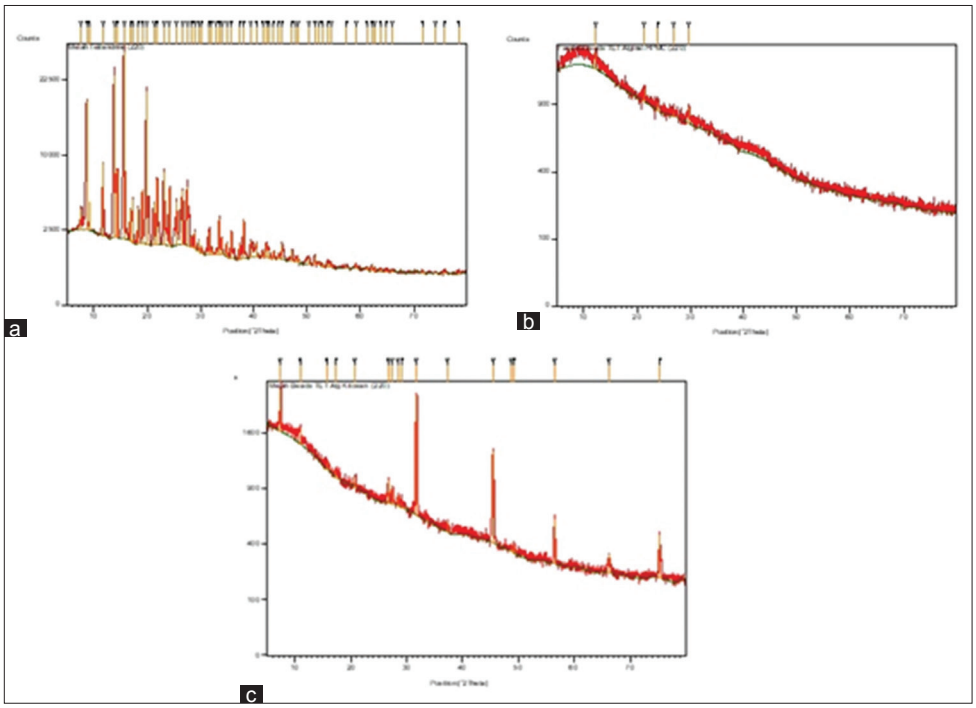

Fig. 3: Diffractograms of (a) tetrandrine (b) tetrandrine loaded alginate/HPMC beads, and (c) tetrandrine loaded alginate-chitosan beads

HPMC in beads had spectra in the range 3500–3400 cm^{-1}, with stretching vibrations of methyl and propyl groups at 2900 cm^{-1}, cyclic groups at 1650–1600 cm^{-1}, broad peaks for cyclic anhydride at 1400–1350 cm^{-1}, and a pyranose ring at 1000–950 cm^{-1} [12]. Tetrandrine spectra showed the presence of C–N groups at 1125 cm^{-1}, the main spectra of benzene at 1455 and 1637 cm^{-1}, and ether spectra at 1025 and 1315 cm^{-1}.

Chitosan showed N-–H groups at 1655 cm^{-1}, C–N groups at 1315 cm^{-1}, and ether groups at 1151 and 1180 cm^{-1} (Fig. 4) [14,27].

***In vitro* release study**

In this study, drug release from beads was shown to be high under acidic conditions (HCl at pH 1.2), which represent the stomach environment, with the release of >50% of tetrandrine after 120 min. These observations are consistent with the known acid solubility of tetrandrine [28], but this release may also be affected by the porous and rough surfaces of beads. Hence, under these conditions, semi-IPNs of alginate and HPMC were not strong enough to protect the drugs. Colon-targeted drugs require protection until arrival at the colon, which we modeled using PBS at pH 6.8 (Fig. 5) [29].

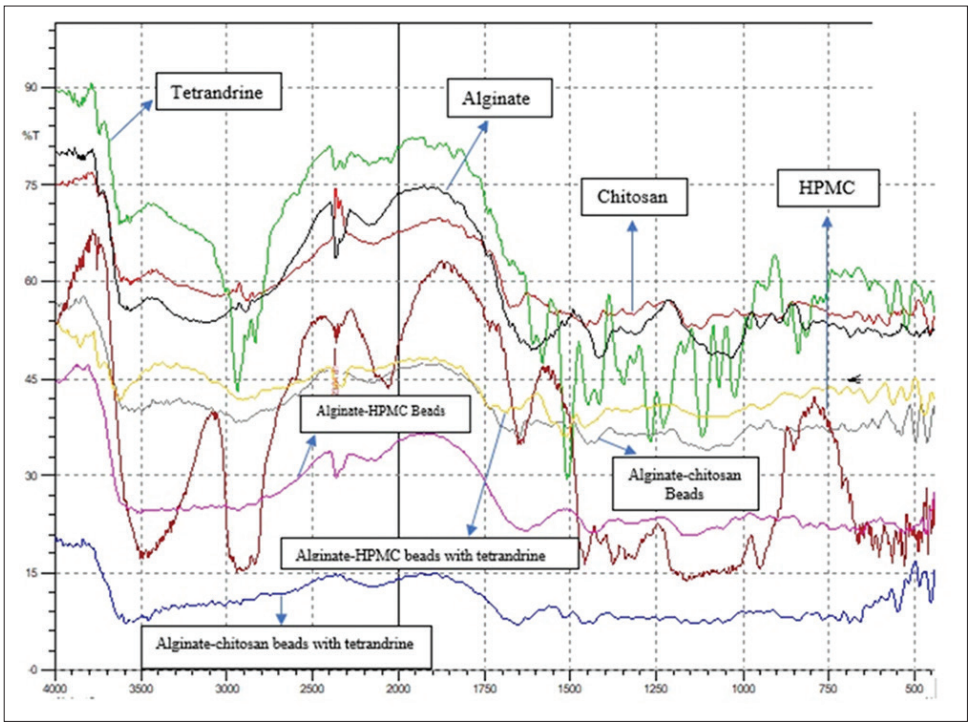

Fig. 4: FTIR spectra

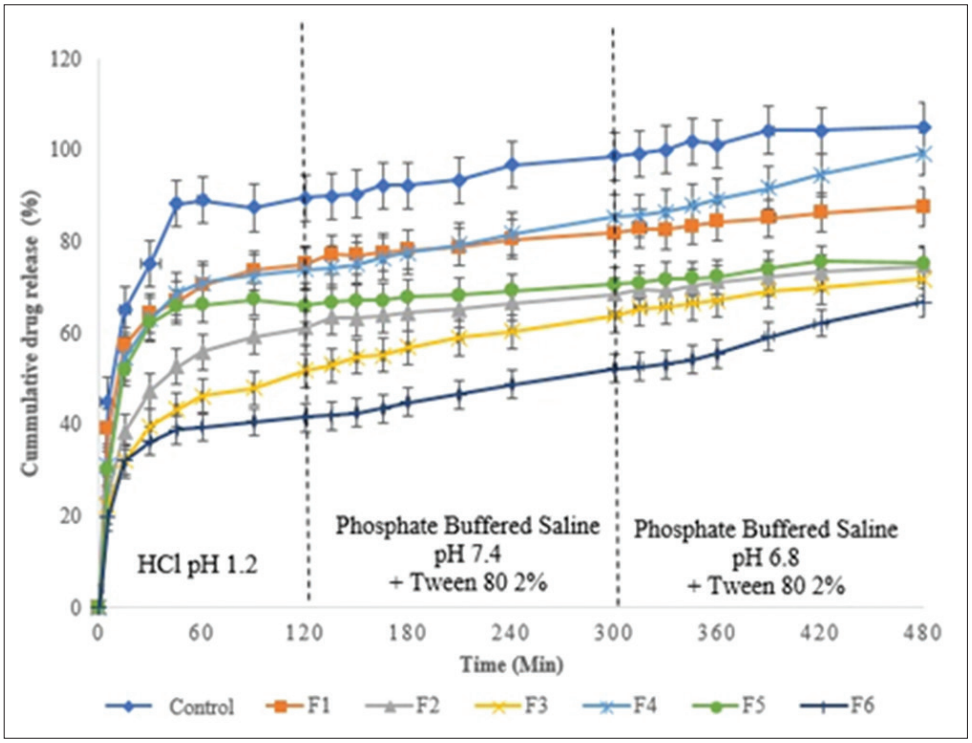

Fig. 5: Cumulative drug release

DISCUSSION

Alginate beads with lower HPMC concentrations produced wavier surfaces than those with higher concentrations. Rough and porous surfaces were also related to the viscosity of polymer matrix, with lower viscosities decreasing the density of dried beads and causing more wavy surfaces and smaller sizes and shapes [30]. Alginate–chitosan beads had more varying shapes than control beads, reflecting interactions between alginate and chitosan and production of more solid structures. Surface wrinkles were also fewer than on control beads, and a solid structure followed the formation of electrostatic interactions between alginate and chitosan. pH adjustments may also influence interactions between carboxylate groups in alginate and chitosan [14].

The viscosity of alginate/HPMC mixtures affected the diameters and densities of beads and the volumes of entrapped drug substance.

Alginate–chitosan beads increased in diameter with increasing chitosan concentrations, reflecting the formation of polyelectrolyte complexes between carboxyl groups of alginate and amine groups of chitosan [31-34]. Previous studies have shown that bead sizes are normally distributed over 50–2000 µm [35]. The alginate–chitosan beads in the present study had greater average diameters and entrapped more water than alginate/HPMC beads, as indicated by higher moisture contents; however, this led to suboptimal process efficiency due to shrinking during drying phases and manual processing.

Swelling indexes of alginate/HPMC beads increased with increasing concentrations of HPMC, and this likely relates to its hydrophilic properties. In contrast, swelling indexes of alginate–chitosan beads decreased with increasing chitosan concentrations, leading to a stronger structure with increased stability under basic conditions. These structures also became more rigid with increasing chitosan concentrations and with the formation of inter- and intra-polymer linkages on beads [14]. Swelling of beads is caused by differences in osmotic pressure between beads and their surrounding media, as shown in the determination of swelling indexes, in which penetration of solution caused polymer relaxation and swelling of beads [12,25].

Reactions between alginate and chitosan were confirmed by the disappearance of exothermic alginate peaks at 249.08°C, and exothermic chitosan peaks at 306.96°C were replaced with new exothermic peaks at 274.86°C. Furthermore, an acquired endothermic peak at 205.46°C indicated the formation of a linkage between carboxylic ion on alginate and an amine on chitosan. The disappearance of tetrandrine peaks indicated that tetrandrine molecules were dispersed on the beads [27,36]. These observations also showed that tetrandrine is entrapped on beads and is chemically altered by heat.

Spectral displacements of alginate carboxylic groups were observed after the formation of complexes with chitosan, with peak shifts from 1608 and 1429 cm^{-1} to 1616 and 1447 cm^{-1}, respectively (Fig. 5). A new peak was also observed at 2886 cm^{-1}, indicating a CH_2 vibration of chitosan [14]. A crosslinking interaction between alginate and calcium ions was evident from a shift of the carboxylic group wavenumber from 1622 to 1634 cm^{-1} (Fig. 4). No interactions between the drug substance and polymers were evident in these studies, with no significant spectral differences between unloaded and loaded beads.

In a previous study, beads with pores and crevices were associated with diffusion of drugs during gelation [14]. Alginate/HPMC and alginate–chitosan beads were porous and had crevices, although drug release from alginate–chitosan beads was lower than from alginate/HPMC beads, likely reflecting the density of alginate–chitosan beads and inter- and intra-polymer interactions of alginate, chitosan, and calcium chloride. Swelling indexes were also related to the release of drugs, and beads with lower swelling indexes restrained drugs from the premature release [14].

CONCLUSION

Beads from the present formulations prematurely released tetrandrine in HCl at pH 1.2. Therefore, calcium alginate–chitosan and alginate/HPMC beads containing tetrandrine may fail as colon-targeted dosage forms.

CONFLICTS OF INTEREST

All authors have none to declare.

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