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Composite Hydrogel Beads Based on Chitosan and Laponite: Preparation, Swelling, and Drug Release Behaviour

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A B S T R A C T

Biopolymer/layered silicate composites have shown unique advantages and potentials for the passive targeting of drugs because they can overcome the burst drawbacks of organic carrier, increase the drug utilization and enhance the controllable capability of drug release. Furthermore, the biocompatibility and non-toxicity of biopolymers is retained, and therefore it is promising and applicable in pharmaceutical fields. The main research aim of this work was to develop a series of biopolymer/layered silicate composite beads based on chitosan (CTS) and Laponite (LA) by a simple ionic cross-linking reaction using sodium tripolyphosphate as the cross-linker. The resultant beads were characterized by Fourier transform infrared spectroscopy, scanning electronic microscope and X-ray diffraction analysis. The swelling behaviour in physiological pH solutions (7.4 and 1.2), drug encapsulation efficiency and controlled release behaviour were also investigated by using Ofloxacin as the model drug to reveal the effects of introduced LA. The results indicate that the incorporation of LA remarkably improved the swelling behaviour, enhanced the drug entrapment efficiency, and slowed down the drug release behaviour in contrast to the pure organic CTS beads. The exfoliated LA clay could act as a physical cross-linker to facilitate the formation of network structure between the CTS and LA. It is suggested that LA may be developed as an effective additive for fabricating a sustained drug delivery system.

Key Words:

chitosan;
Laponite;
composite;
drug release;
Ofloxacin.

INTRODUCTION

Recently, clay minerals have widely been used as excipients and active agents for the development of new hybrid drug delivery systems. Among them, layered silicates as a new family of drug delivery vehicles have especially been spotlighted because their unique layer structure can accommodate polar organic molecules to form various intercalated compounds [1]. Hence, the layer structure of the silicate can provide a large space to reserve neutral drug molecules via ion-dipole interaction and cationic or biofunctional via ion exchange reaction. The idea is to store the drug in the interlayer region of the lamellar host and allow the drug release as a consequence of diffusion and/or de-intercalation process [2].

Laponite (LA) is a plate-like synthetic hectorite-type clay which

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belongs to a family of phyllosolicates (trimorphic) 2/1 with the empirical chemical formula $Na^{0.7+}[Si_8Mg_{5.5}Li_{0.3}O_{20}(OH)_4]^{0.7-}$. It has a large surface area, anionic surface charges and exchangeable Na^+ cations in hydrated interlayers, and therefore it shows better adsorption properties for cationic drug molecules [3]. Park et al. [4] prepared the inorganic-organic hybrid by intercalating Donepezil molecules into smectite clays (LA, saponite and montmorillonite) and found that the cation exchange capacity of the clay determines the absorption amount of drug molecule and its molecular arrangement in the interlayer. Moreover, many kinds of composites based on LA were prepared and evaluated, e.g., LA-itraconazole nanohybrid [5,6], and modified poly(vinyl alcohol)/LA nanocomposite membrane [7]. It was found that the exfoliated LA particles may act as multifunctional cross-linkers in forming the composite hydrogels, and the polymer chains were anchored to the particles and entangled to form a network [7,8].

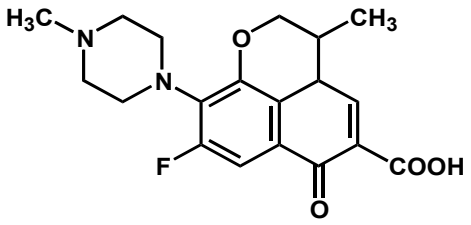
Chitosan (CTS) is a naturally occurring polysaccharide with excellent biodegradable, biocompatible and non-toxic characteristics [9]. Many delivery formulations based on chitosan are usually prepared by chemical cross-linking with glutaraldehyde [10], urea formaldehyde [11], etc., however, the chemical cross-linking agents possibly induce toxicity and other undesirable effects. Due to its unique polymeric cationic characters, CTS can form polyelectrolyte complexes with an anionic polyelectrolyte, such as tripolyphosphate (TPP) [12], dextran sulphate [13,14], poly (methacrylic acid) [15] and alginate [16,17]. Compared with polyanions, using low molecular weight anions such as TPP to cross-link CTS provided an effective approach because of its non-toxicity, much simpler and milder agent. Moreover, it is ideal for maintaining the in-process stability of drugs [12-14]. Furthermore, TPP molecule can freely diffuse into CTS droplets or films to form ionically cross-linked CTS beads or films [18,19]. However, the release profiles of CTS beads prepared by this method showed a high burst effect, which indicates that based on the ionic reactions, the binding properties of CTS may be weak, and the resultant formulation skill is not the optimal excipient as described by other researchers [20].

The biopolymer/layered silicate composites have attracted considerable interest during the past decades. The introduction of layered silicate could not only remedy some flaws of the neat polymer, but also endow them with novel properties which could be further tailored by altering the type and content of silicates [21]. The CTS/montmorillonite (MMT) nanohydrogel was demonstrated to exhibit excellent anti-fatigue behaviour and better pulsatile release compared with neat CTS [22] and the combination of MMT with CTS/TPP beads also proves that the electrostatic interaction between CTS and MMT enhances the stability of the beads and exhibited good potential for the use as drug carriers for sustained release [23]. Compared with MMT, LA has shorter aspect ratio, better dispersibility and colloidal properties which can easily combine with organic matrix to form more homogeneous composite structure.

Also, LA has negative surface charges and exchangeable cations which can form strong interfacial interactions with cationic drug Ofloxacin and show relatively higher absorption value than other types of clays (Table 1). Thus, it is expected that the introduction of LA can form new types of composite hydrogels with improved mechanical properties, swelling behaviour, drug loading efficiency and controlled release behaviour in contrast to neat CTS hydrogel. Currently, the nanocomposite matrix based on chitosan/Laponite was successfully utilized to construct a new type of amperometric glucose biosensor [24] and amperometric biosensor for

Table 1. The adsorption capacities of different clays for the ofloxacin.

Clays	Adsorption capacity (mg/g)
Montmorillonite	190.0
Laponite	163.0
Attapulgite	44.5
Rectorite	47.5
Muscovite	26.0
Halloysite	6.5



Scheme I. Chemical structure of Ofloxacin.

phenol [25]. In spite of that there is still a lack of the systematic investigation on CTS/LA composite hydrogel as a drug delivery carrier.

Based on this background, as a part of the systematic work of designing new type of composite drug delivery carrier, a series of CTS/LA composite hydrogel beads were prepared, and the interaction between LA and CTS and the influence of LA on the entrapment efficiency, swelling ratio and drug release properties of the matrices were evaluated. The swelling and in vitro drug release profiles were tested in simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid (SIF, pH 7.4) using Ofloxacin (Scheme I) as a drug candidate. The structure and morphology of the beads were characterized by FTIR, XRD and SEM techniques. In addition, the swelling and in vitro Ofloxacin release profiles were also tested in simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid (SIF, pH 7.4).

EXPERIMENTAL

Materials

Chitosan (CTS, deacetylation degree 81%; weight average molecular weight 9×10^5 Da) derived from shrimp shell was purchased from Yuhuan Ocean Biochemical Co. (Taizhou, China) and passed through a 320-mesh screen before use. Ofloxacin (solubility of about 36.02 mg/mL at pH 1.2 and 1.79 mg/mL at pH 7.4) was obtained from Kunshan Double-crane Pharmaceutical Co. Ltd., China. Laponite (LA, 99%) was obtained from Nuocheng Chemical Co. Ltd., Nanjing, China. Sodium tripolyphosphate (TPP) was purchased from Sinopharm Chemical Reagent Co. Ltd., China. Simulated gastric fluid (SGF, pH 1.2, containing 21.25 mL HCl and 11.18 g KCl in 3000 mL distilled water) and simulated intestinal fluid (SIF, phosphate buffer solutions, PBS, pH 7.4, containing 20.4 g K_2HPO_4 and 4.8 g NaOH in 3000 mL distilled water) were prepared in terms of US Pharmacopoeia 30. All other chemicals were of analytical grade and used as received.

Preparation of the Composite Hydrogel Beads

Ofloxacin loaded hydrogel beads were manufactured according to a modified literature technique using TPP as the gelling counterion [26]. Typically, suitable amounts of Laponite (0, 0.20, 0.40, 0.60 and 0.80 g) were each dispersed in 20 mL of 2% (v/v) acetic acid solution (pH 3.2), and then 0.20 g Ofloxacin was added to each dispersion and all mixtures were stirred at room temperature for 1 h. Subsequently, CTS powder (0.60 g) was dissolved directly into the Ofloxacin/LA mixture solution to a final concentration of 3% (w/v) under continuous stirring for 1 h. The beads were formed by dropping the bubble-free solution or dispersion through a disposable syringe onto a gently agitated TPP solution (50 mL, 5% (w/w), adjusted to pH 5.0 using 1 mol/L HCl solution). After 0.5 h, the obtained beads were separated by filtration and briefly rinsed with distilled water. The obtained gel-like beads was firstly air-dried for 24 h and then oven-dried at 70°C for 6 h.

A similar procedure was used to prepare placebo beads. Hereinafter the beads containing 0, 0.20, 0.40, 0.60 and 0.80 g LA were termed L0, L1, L2, L3, and L4, respectively. The L0 beads without adding the Ofloxacin was designated as L0C.

Viscosity Measurement

The viscosity of LA at different concentrations (1%, 2%, 3%, 4% w/v), CTS (3% w/v) and CTS /LA dispersions at the concentration used for preparing composite hydrogel beads was measured using an Anton Paar rheometer (Physica MCR 301, Germany) at 25±1°C. A rheogram of the samples was plotted using viscosity and shear rate at various revolution rates of the spindle.

Determination of the Entrapment Efficiency

Entrapment efficiency (EE) is one of the critical parameters that have to be considered in the preparation of drug-loaded hydrogel beads. There are

normally two methods available including direct and indirect ways to determine the entrapment efficiency. In this study, the latter method was used to determine entrapment efficiency by loss of drug during the entire manufacturing processes. Such indirect method usually has relatively higher drug concentration when compared with the direct methods. However, both methods could reflect the same tendency.

In addition, with an emphasis on assessing the stability of drug during the bead preparation process and determining the amount of drug in the prepared beads simultaneously, the HPLC method was selected for entrapment efficiency test. The procedure adopted was as follows: after preparing the beads, the supernatant was collected and diluted with distilled water to 1000 mL, then filtrated through a 0.45 μm membrane filter to remove the floating tiny amount of Laponite particles. The clear superficial solution was analyzed by HPLC (Waters^{TM} 600 Pump, 2998 Photodiode Array Detector, USA) using the C_{18} column. The mobile phase of acetonitrile (a pH 2.2 buffer solution (25/75, v/v) prepared with ammonium acetate and sodium perchlorate according to USP 30) was also filtered by a nylon membrane with the pore size of 0.45 μm. The drug content was determined by comparing with the standard curve of Ofloxacin at the concentration between 0.002 to 0.01 g/L. The drug encapsulation efficiency is expressed as follows:

$$Entrapment\ efficiency\ (\%) = \frac{Practical\ drug\ loading}{Theoretical\ drug\ loading} \times 100 \quad (1)$$

Where "theoretical drug loading" represents the initial amount of drug added in the mixture, and "practical drug loading" is the amount of drug entrapped into the beads.

Swelling and Degradation Studies

The swelling behaviour and in vitro degradation of the beads were carried out at two aqueous media: SGF (pH 1.2) and SIF (phosphate buffer solutions, PBS, pH 7.4). Beads (100 mg) were placed at 37±0.5°C in the basket of dissolution test apparatus (ZRS-8G, Tianjing University Wireless Factory, China) containing 250 mL of respective media at 50 rpm. At regular intervals, the beads were reweighed after carefully wiping off the excess of liquid with a tissue paper (wet weight of the beads) and then the wet beads were re-weighed again after oven-dried at 70°C for 6 h (the dry weight of the beads). The weight change of the beads with respect to time was determined as follows:

$$Q_w = \frac{W_w - W_0}{W_0} \quad (2)$$

$$Q_d = \frac{W_d - W_0}{W_0} \quad (3)$$

Where W_w and W_d are the wet and dry weights of the beads at time t, respectively. W_o is the initial weight of the beads, Q_w and Q_d are the changes of wet and dry weights, respectively.

In Vitro Drug Release Experiment

In the present paper, the in vitro drug release tests were carried out according to the USP 30 NO.2 dissolution test apparatus fixed with six rotating paddles to reveal the effect of LA content on the release profile both in SGF and in SIF. An amount of 50 mg of each sample was dissolved in 500 mL of the SIF or SGF solutions. The speed of rotation was set at 50±1 rpm and the bath temperature was maintained at 37±0.5°C. After the scheduled intervals, 5 mL solution was collected from the release medium and the equivalent medium with the same temperature was added back. The drug-release amount was monitored by a UV-Vis spectrophotometer (SPECORD 200, Analytik Jera AG, Germany) at 294 nm. In the concentration range (2.5-12.5× 10^{-5} mol/L) the UV standard absorbance curve for Ofloxacin was established and the absorbance obeyed the Beer's law.

Characterization

FTIR spectra were recorded on a Thermo Nicolet FTIR spectrophotometer (Nexus, TM, USA) in the range of 4000-400 cm^{-1} using KBr pellets. Surface morphology was visualized by a Jeol scanning electron microscopy (JSM-5600LV, Japan) using an accelerating voltage of 20 kV after coating the sample with gold film. Powder XRD analyses were performed using a diffractometer with Cu anode

(PAN analytical X'pert PRO, The Netherlands) running at 40 kV and 30 mA, scanning from 3° to 40° at 3°/min.

Statistical Analysis

Each experiment was carried out three times to obtain an average result. The effects of various LA contents on the entrapment and release of drug were statistically analyzed by one-way ANOVA. For all statistical calculations, the minimal level of significance was set at $p< 0.05$.

RESULTS AND DISCUSSION

FTIR Spectra

Figure 1 shows the FTIR spectra of CTS, Ofloxacin, LA, L0C and L3 samples. It can be seen that the characteristic bands of CTS at 1651 and 1602 cm^{-1} (vibration bands of amide I and $-NH_2$, respectively) shifted to 1640 and 1557 cm^{-1} after cross-linking with TPP (L0C), indicating that these groups interacted with TPP via ionic bonds and CTS have been cross-linked with TPP in the beads [27].

The absorption band of LA at 3627 cm^{-1} (stretching vibration of the (Si) O-H groups) [5] cannot be quite observed after forming composite beads due to the reduction of LA content, but the Si-O(H) characteristic stretching vibration of LA at 1016 cm^{-1} clearly appears in the spectrum of the composite bead (L3) which has shifted to 1013 cm^{-1} [28]. This indicates that LA existing in the beads has formed a composite structure with CTS and TPP. The characteristic absorption bands of LA have shifted to low wavenumber region, revealing that the electrostatic and hydrogen bonding interactions have taken place among the silanol groups ($-SiO^-$) of the clay and the $-NH_3^+$ groups of CTS [29]. The introduction of LA has led to a change in matrix composition of the beads.

The C=O absorption bands at 1714 cm^{-1} (-COOH groups of Oflaxcin) and at 1623 cm^{-1} (C=O group of Ofloxacin) appeared in the spectrum of L3, but shifted to 1707 cm^{-1} and 1625 cm^{-1}, respectively. This result indicates that the Ofloxacin drug was loaded on the composite hydrogel beads and the positively charged Ofloxacin combined with the negatively charged surface of LA like a cationic dye adsorption mechanism [30].

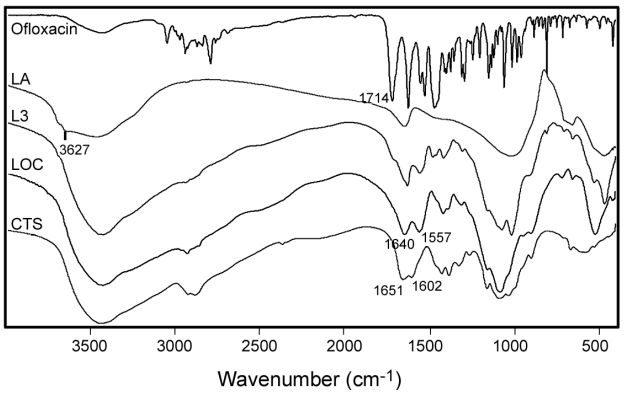


Figure 1. FTIR Spectra of CTS, Ofloxacin, LA, L0C, and L3 samples.

XRD Analysis

The XRD patterns from 3° to 40° exhibit the crystal peaks of LA, Ofloxacin, CTS, L0 samples and the composite beads (Figure 2). One typical peak near 20° can be observed for CTS, but its diffraction intensity has weakened drastically in the placebo bead (L0C) and L0 samples. The diffraction peaks of LA at 19.6° and 27.6° appear in the composite bead (L3), indicating the incorporation of LA and formation of composite structure.

The diffraction peaks of Ofloxacin can be observed in the XRD pattern of L0 with a weak intensity, but no similar diffraction peaks are observed in L3 curve. The weak diffraction peaks can be

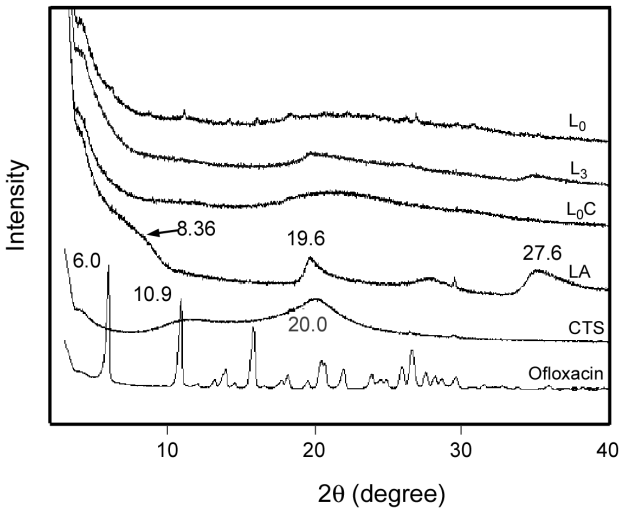


Figure 2. XRD Patterns of Ofloxacin, LA, CTS, L0C, L0 and L3 samples.

attributed to the low content of Ofloxacin in the L0 beads and the appearance of peaks of drug indicates that Ofloxacin shows some crystallinity in the L0 sample.

Although the entrapment of Ofloxacin in L3 has been proved by infrared spectra, UV-Vis measurement and drug release experiment, the diffraction peaks of Ofloxacin cannot be observed in the XRD pattern of L3 sample. This may be ascribed to the fact that Ofloxacin is uniformly distributed in the interlayer of LA and polymer matrix in the form of drug molecules but not in the crystal form. This result also implies that the incorporation of LA improves the dispersion of Ofloxacin drug in the carrier.

Furthermore, the pure LA exhibits a discrete broad XRD pattern at around 8.36°, revealing a low crystallinity and a small particle size [31,32]. However, the broad characteristic diffraction peak of LA at around 8.36° (basal spacing, 1.06 nm) does not appear in the range from 3° to 10° for L3 sample due to the low content and crystallinity of LA.

Morphology

The prepared wet beads were spherically shaped with smooth surfaces (about 5 mm in diameter, Figure 3a). After air drying, the beads show less spherical shape and a noticeable reduced size (about 2 mm in diameter, Figure 3b). There is no significant variation in bead size with changing LA content.

The large size of wet beads suggests high swelling quality and water retention ability of the beads. Detailed examination of the surface structure by SEM (Figures 3b, 3c, 3d, and 3e) reveals severe wrinkles caused by partial collapsing of the polymer network during drying which are in agreement with other research findings [33,34]. Compared to placebo L0C beads (Figure 3c), the addition of drug imparts a high degree of surface roughness (L0 sample, Figure 3d) which may be due to the Ofloxacin crystals leaking from the beads network structure [35]. However, the Ofloxacin crystals have disappeared from the surface of composite beads with the addition of LA content (Figures 3e and 3f), and the surface structure becomes

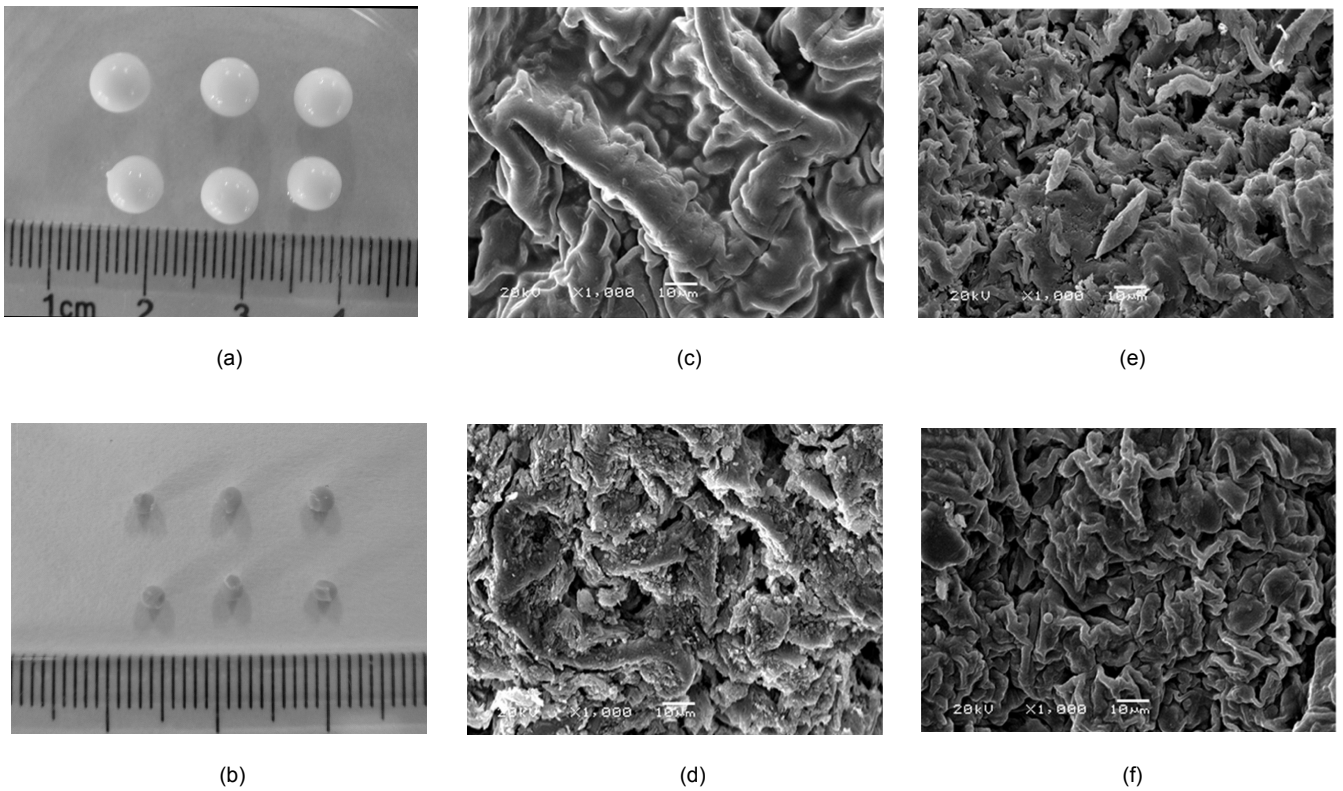


Figure 3. Digital photographs of L3 sample at (a) swollen state and (b) dry state and SEM micrographs of (c) L0C, (d) L0, (e) L1, and (f) L3 samples.

more regular with increasing the content of LA (Figure 3f) which suggests that the incorporation of LA is favourable for drug loading.

Viscosity Determination and Entrapment Efficiency

The CTS solution dropped in TPP aqueous solution may shape immediately owing to the cross-linking of CTS with ionic-cross-linker. In this process, TPP slowly diffused from outside into the core of the gelled bead, and gradually cross-linked the beads from the surface to the center [17,18]. As the curing occurs in TPP solution, part of the drug would leak to the coagulation fluid. The introduction of LA certainly affected the cross-linking process and entrapment of Ofloxacin. In order to reveal the effect of introduced LA in the cross-linking process, the interaction of CTS with LA in dispersion was studied by examining the change in the viscosity of the mixture solutions.

Rheograms of LA dispersions and CTS/LA dispersions prepared using 3% (w/v) CTS added with various concentrations of LA is shown in Figure 4. As can be seen, the viscosities of LA suspension and CTS solution are relatively lower, but the viscosity sharply increased after the incorporation of LA into CTS dispersion ($p < 0.05$), which is an indication of a synergistic effect of CTS and LA on the viscosity of their mixture. The viscosity synergism between CTS and LA is a consequence of electrostatic interactions between the negatively charged silanol groups on the silicate layers of LA and the positively charged amino groups of CTS [29].

For detecting the entrapment efficiency of the test

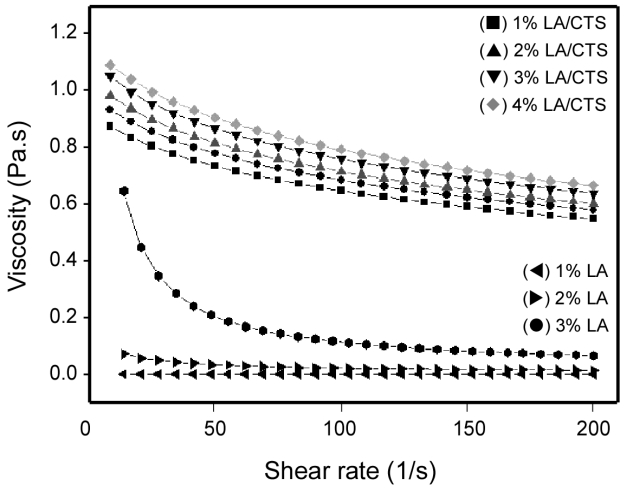


Figure 4. Rheograms of LA dispersions and CTS/LA dispersions prepared using 3% (w/v) CTS at various amounts of LA.

beads, there was a single peak representing Ofloxacin in the chromatogram, suggesting that the molecule was stable during preparation of the beads [36]. The entrapment efficiency of the composite beads for Ofloxacin increases with the increased LA content in the range of 31% to 91% (Table 2). The same property for the beads with different LA contents were evaluated by ANOVA and the F value was found to be 2379 (d f = 14, p = 0). It further shows that the incorporation of LA has an important effect on the beads.

This significant difference is well explained as follows. First, the introduction of LA increased the viscosity of the dispersions (Figure 4) and decreased the diffusion rate of Ofloxacin. Secondly, the large specific area of LA endows it with good adsorption

Table 2. Entrapment efficiency and kinetics analysis data of release at pH 1.2 and pH 7.4.

Sample	Entrapment efficiency and kinetics analysis data						
	EE (%)	pH 1.2			pH 7.4		
		n	k	R	n	k	R
L0	31.23±0.49	0.58	0.94	0.973	0.14	0.79	0.997
L1	49.98±1.58	0.76	0.67	0.999	0.28	0.40	0.993
L2	69.35±0.63	0.96	0.44	0.998	0.45	0.18	0.995
L3	83.44±0.28	1.00	0.31	0.999	0.48	0.11	0.997
L4	91.17±0.76	1.17	0.21	0.999	0.55	0.06	0.998

capacity. This is favourable to the absorption of drug onto LA and this effect is more notable with increasing the interlayer distance and the LA content. It is remarkable that the adsorption may occur on the external surfaces and the interlayer spaces of LA where the latter leads to the intercalation or exfoliation of LA platelets.

Swelling Studies

The release of any encapsulated drug or biological material from dried beads requires a re-hydration process. For this reason, swelling experiments of representative formulations (L0, L1, and L3 samples) were carried out in SGF pH 1.2 and SIF pH 7.4 solutions at physiological temperature of 37°C (Figure 5). It is a general observation that at an acidic pH, the swelling ratio of the beads in SGF medium is higher than that in SIF. The L0 sample was found to dissolve completely at pH 1.2 within 3 h of immersion, whereas it was swollen with its weight increasing by 0.3 times within 30 min in SIF solution. Thus, the CTS beads without any LA (L0) were stable only in SIF solution.

The LA loaded samples (L1 and L3) retained their physical shapes within 4 h of immersion. Furthermore, with higher LA content, the initial swelling rate at pH 1.2 is decreased and the swelling ratio within 4 h is increased significantly ($p < 0.05$). In more detail, the weight of L0 sample increased by 4.68-fold for the initial 1 h, and then sharply reduced until disappeared after the next 1 h of incubation. However, the weight of L1 and L3 samples increased by 8 and 2-fold, respectively for the first 4 h. Subsequently, the bead structure was quickly disaggregated, leading to complete dissolution of the swollen bead.

The swelling of the beads is mainly governed by the net charge of the CTS molecules. At pH 7.4, the net charge of the CTS is low which renders into low electrostatic repulsion of the chains and results in low swelling ratio. However, the beads are highly swollen at gastric pH (1.2). This high swelling ratio is attributed to the electrostatic repulsive force resulting from the positive charge of the protonated amine groups of CTS, an indication that these groups are mainly responsible in pH-sensitive swelling capacity.

Meanwhile, the protonation of phosphate on TPP could break the ionic bond formed by CTS and TPP and lower the cross-linking density of the gel. If the cross-linking density is too low, the interactions are no longer strong enough to avoid disintegration, and therefore the beads disappear completely within 3 h (L0 sample). The stronger gel matrix and lower disintegration resulting from the introduction of LA is due to the dispersion of clay platelets in an aqueous medium which could act as an effective multifunctional cross-linking agent [37]. As shown in Figure 6, the presence of LA increases the degree of cross-linking which it decreases the degradation rate of the beads.

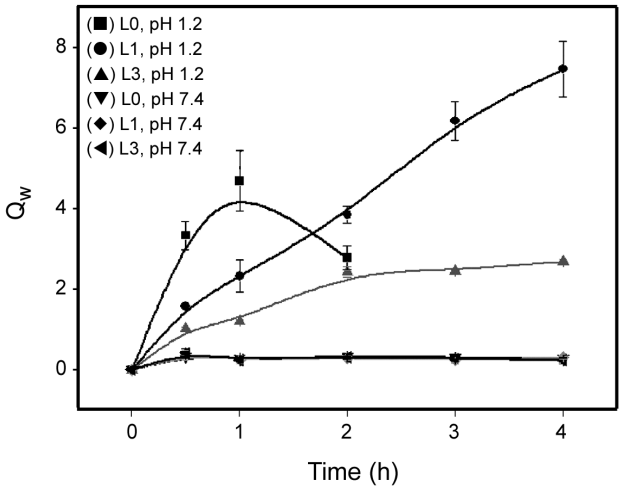


Figure 5. Weight changes of wet beads (L0, L1, and L3 samples) at pH 7.4 and pH 1.2.

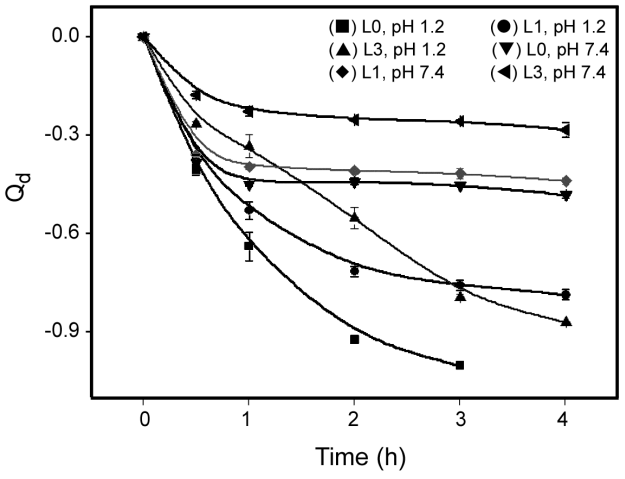


Figure 6. Weight changes of dry beads (L0, L1 and L3) at pH 7.4 and pH 1.2.

The dry weight of the beads at pH 7.4 was lower than that at pH 1.2. No significant disintegration was visually observed at pH 7.4 and the spherical shape of the beads was well retained even after 10 h of incubation. However, the dry weights of the beads decreased greatly at pH 1.2 and a notable disintegration could be clearly seen. This stronger gel matrix and lower disintegration due to the introduction of LA would respond well with the wet weight change described above. The disintegration of the pure CTS beads at pH 1.2 was overcome by the introduction of LA which makes this system more useful in targeting lower intestine system.

In Vitro Release Studies

The in vitro release of Ofloxacin from the beads was measured in both SGF (pH 1.2, Figure 7) and SIF (pH 7.4, Figure 8) solutions at 37±0.5°C. As shown in Figure 8, almost the total Ofloxacin loaded in L0 sample was released within the initial 2 h following its incubation in gastric simulated fluid. This may be attributed to the low matrix stability and the higher drug solubility at this pH environment [38].

As it is mentioned already, the size of CTS beads increased when the particles are incubated in solutions of pH 1.2 owing to the reduction in TPP ions present, which in turn reduced the extent of interaction within the beads. The effect of incorporation of LA layers can be significantly observed in reduced rate of release ($p < 0.05$). The explanation of this

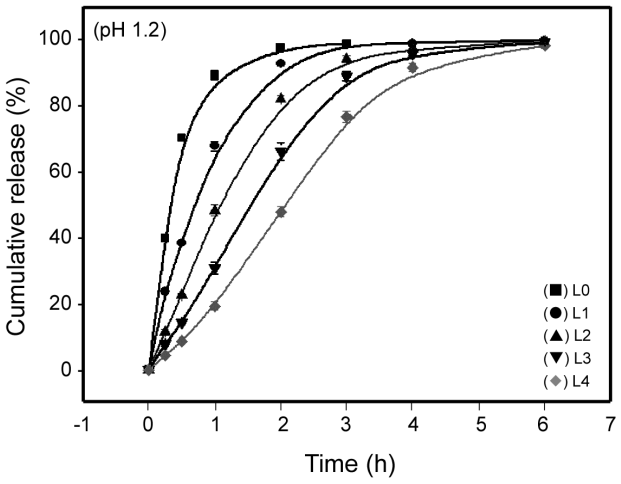


Figure 7. Effect of LA content on the release profile of the prepared beads in pH 1.2.

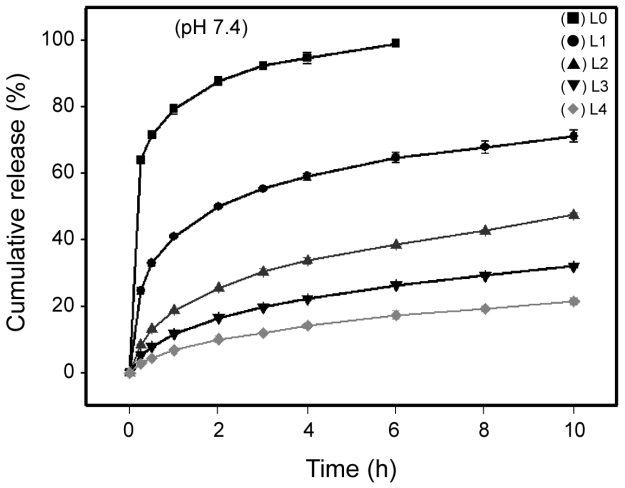


Figure 8. Effect of LA content on the release profile of the prepared beads in pH 7.4.

behaviour is the greater cross-linking density with greater LA content as well as the existence of strong electrostatic interaction between the protonated amino groups of Ofloxacin cations and the anionic groups of the layers of LA which are not overcome by the interaction with the solvent.

The drug release pattern at pH 7.4 showed a distinctive biphasic release pattern: initial burst and slow release which is followed by sustained release (Figure 8). This behaviour was clearly affected by the LA content. The initial burst release might be the result of the rapid dissolution of the drugs located at or close to the surface of the beads (Figure 3). The subsequent sustained release period might be the dominant release mechanism, which changed to drug diffusion through the CTS matrix or the exchange of drug ions absorbed on the clay platelets. Comparing with the release of Ofloxacin from CTS beads incubated at pH 1.2 and at pH 7.4, the composite beads show correspondingly slower release rate where the initial release rate tends to drop with greater LA proportion in the beads.

In addition, the release mechanism of the drug from the hybrids was also examined on the basis of the experimental data plotted in Figures 7 and 8, and the results were analyzed using a semi-empirical equation as follows:

$$\frac{M_t}{M_\infty} = kt^n \qquad (4)$$

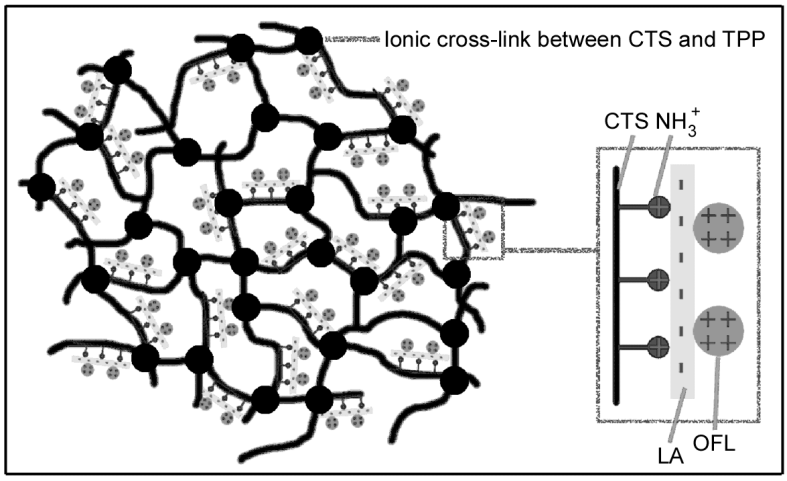


Figure 9. Schematic illustration of interactions among CTS, Ofloxacin and LA samples.

where, M_t/M_∞, is the fractional release of the drug at time t, k is the constant related to the structural and geometrical shapes of the device, and the swelling exponent, n, is an indicative of the drug release mechanism. These data along with the values of the correlation coefficient r are included in Table 2.

For spheres, the value of n between 0.43 and 0.85 is an indication of both diffusion controlled drug release and swelling controlled drug release (anomalous transport). The values below 0.43 indicate that the release of drug from polymer is due to Fickian diffusion. The values above 0.85 indicate case-II transport which relate to polymer relaxation during gel swelling [39]. As shown in Table 2, by increasing LA content in the beads, the values of k decrease but the values of n increase which reveal a change in drug release mechanism from a diffusion-controlled to a swelling-controlled mode.

From the characterization results of the swelling and in vitro release studies, a possible model for the composite beads is shown in Figure 9, where the cross-linking between CTS and TPP and electrostatic interactions between CTS and LA as well as Ofloxacin are indicated. It is worth to note that the addition of a certain amount of LA not only improves the drug entrapment efficiency of the composite beads, but also provides a slower and sustained drug release. Thus, the inclusion of LA clay into biopolymers may be used for the sustained delivery of drug and other bioactive molecules.

CONCLUSION

In summary, the intercalated CTS/LA composite samples were developed as a novel biomaterial which combined the structure, physical and chemical properties of both inorganic and organic materials. The preparation of the beads is facile, non-toxic, much simpler and milder. Molecular interaction of CTS with LA caused a change of the characteristics of the composite beads. The synergistic effect of biopolymer and inorganic material as well as the strong interfacial interactions between them via electrostatic interaction could improve the mechanical properties, swelling behaviour, drug loading efficiency and controlled release behaviour of the pristine biopolymer matrices. The disintegration of the pure CTS beads at pH 1.2 was overcome by introduction of LA which made this system more useful in targeting lower intestine environment. The swelling and degradation of the beads were influenced by the pH of test medium and the LA content, and the prepared beads swelled and drug was released quickly in SGF solution while in SIF medium they remained at a shrinkage state and drug was released slowly. Moreover, the release rate of Ofloxacin was obviously lowered with increased LA content both in SIF and SGF solutions. Therefore, the introduced LA endowed the composite beads with a controllable degradation rate, and a sustained release multiparticulate system can be obtained by the introduction of LA into the composite beads except

that the high cost of LA is disadvantageous for its applications on a large scale.

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