ABSTRACT: Phosphorous containing chitosan (PCTS) was synthesized by a graft copolymerization technique in order to be used as controlled drug delivery devices. To test this potential, PCTS beads were prepared by using tripolyphosphate, at pH 4.0 and characterized by scanning electron microscopy. The in vitro drug release behavior in various pH solutions was studied using indomethacin (IM) as a model drug at two different concentrations (0.3 and 0.6% w/w). The release percent of IM from PCTS beads was found to be increased with the increasing of pH in the buffer solution. The release rate of IM at pH 7.4 was higher than that at pH 1.4, due to the ionization of phosphorous groups and the high solubility of IM in the alkaline medium. These results indicated that PCTS beads have the potential to be used as controlled drug delivery systems through oral administration by avoiding the drug release in the highly acidic gastric fluid region of the stomach.

KEY WORDS: modified chitosan, chitosan beads, indomethacin, drug delivery, pH sensitive drug delivery, oral drug delivery.

INTRODUCTION

Chitosan (CS) is a biopolymer comprised of D-glucosamine and D-acetyl-glucosamine units that can be readily processed into films, porous scaffold, beads and fibers from aqueous acid solution. CS
beads have been developed for controlled drug release and removal of heavy metal ions from waste water [1–3]. CS displays interesting properties such as biocompatibility, biodegradability [4] and its degradation products are non-toxic, non-immunogenic and non-carcinogenic [5].

Recently, there has been a growing interest in studying the chemical modification of CS to improve its solubility and widen its applications [6–10]. Among the various methods, graft copolymerization is the most attractive because it is a useful technique for modifying chemical and physical properties of natural polymers. CS has both primary amine and alcohol reactive groups that can be grafted. Covalent grafting of molecule entities allows the formation of functional derivatives onto CS backbone with desired chemical groups. It has been shown that, after primary deviation followed by graft modification, CS improved water solubility and bioactivities such as antibacterial and antioxidant properties [11–13], chelating [14] or complexation properties [15], bacteriostatic effect [16] or enhancing adsorption properties [17,18]. Although the grafting of CS modifies its properties, it is possible to maintain some interesting characteristics such as mucoadhesivity [19], biocompatibility [20–22] and biodegradability [23]. Many investigations have been carried out on the graft copolymerization of CS [13–22] with a view to preparing polysaccharide-based advanced materials with unique properties and thus widening their applications in biomedical and environmental fields.

CS is a very promising biomaterial for drug delivery as it readily responds to pH. However, the use of soluble CS polymer in oral administration is restricted by its fast dissolution in the stomach and limited capacity for a controlled drug delivery system [24]. In order to overcome these disadvantages, tripolyphosphate (TPP)/CS polyelectrolyte complexes as gel beads were developed for sustained release performances [25–28]. PCTS has attracted considerable interest because among its advantages, it exhibits anti-inflammatory properties and it forms both metal complexes and anionic polyelectrolyte hydrogels [29–33]. In this work, CS was grafted with TPP to prepare beads in order to improve the sustained release system in a gastric fluid. By using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) mediated coupling reaction, 2-carboxethyl phosphonic acid was covalently grafted onto chitosan. The morphology, swelling properties and in vitro drug profiles of the prepared beads were evaluated to obtain an insight to the potential of this system for pH sensitive controlled release of drugs or other bioactive molecules.
EXPERIMENTAL

Materials

Chitosan (CS) (deacetylation degree 75–85%), tripolyphosphate (TPP) and 2-carboxyethylphosphonic acid were purchased from Aldrich. EDC was obtained from Alfa Aesar Company. IM was purchased from Fluka. All other materials used were of analytical grade.

Synthesis of Phosphorous Containing Chitosan (PCTS)

PCTS was prepared according to the method reported in [32]. Briefly, CS (0.9712 g) was dissolved in 10 mL of 1% w/w acetic acid/methanol solution and the pH solution was then raised to 5.5 by adding 1M NaOH. 2-Carboxyethylphosphonic acid (0.4621 g) was dissolved in 10 mL of water and then added drop by dropwise to the CS solution. To this mixture EDC (0.4552 g) was added. The reaction was carried out at 70°C for 7 h. The reaction mixture was filtered and affluent dialyzed against spectropor membrane tubing (Mw cut-off 3500) for 3 days with 10 changes of water to eliminate the impurities. The resulting solution was precipitated with acetone and dried at room temperature.

Preparation of Phosphorous Containing Chitosan (PCTS) Beads

PCTS beads were prepared using an ionotropic gelation process with the counter polyanion TPP. PCTS (0.7512 g) was dissolved in 10 mL of 2% w/w acetic acid solution and stirred for 24 h to obtain a transparent homogeneous solution. The PCTS solution with or without drug was introduced into a stirred 10% TPP aqueous phase using a syringe needle (0.5 mm in diameter); solid white beads were formed immediately. These beads were allowed to stand for 1 h in the TPP solution with occasional agitation at room temperature and pH 4.0. The gel beads were filtered, washed with distilled water repeatedly and dried under vacuum at room temperature for 2 days. For the preparation of drug loaded PCTS beads, two different amounts of IM (0.3 and 0.6% w/w with respect to PCTS solution) were dispersed in 10 mL of PCTS solution for 24 h. The same procedure was followed for the isolation and purification as described above.
Swelling Evaluation

The dried CS and PCTS beads were carefully weighed and immersed in to 50 mL of buffer solutions of pH 1.4 and 7.4 at 37°C. At predetermined time intervals, swollen beads were taken out, and the excess water was blotted with filter paper from the surface, and then weighed. The following equation was used to determine the swelling degree:

\[
\text{Swelling degree} \[%\] = \left\{ \frac{(X_w - X_d)}{X_d} \right\} \times 100
\]  

(1)

where \(X_d\) and \(X_w\) represent the mass of dry and swollen beads, respectively.

Determination of Drug Loading Efficiency

During the gelation process in TPP aqueous solution, some IM diffused from PCTS droplet into TPP solution. After gelation, the IM concentration in the TPP solution was determined by a UV spectrophotometer at 320 nm. The loading efficiency during the gelation process was calculated by the following formula:

\[
\text{Loading efficiency} \[%\] = \left\{ \frac{(m - c \times v)}{m} \right\} \times 100
\]

(2)

where \(m\), \(c\) and \(v\) represent the initial IM mass in PCTS droplet, the IM concentrations in TPP solution and the volume of TPP solution, respectively.

In Vitro Drug Release Studies

In vitro release tests were carried out on all formulations of the drug-loaded CS beads and PCTS beads. Known amounts of drug-loaded gel beads were suspended in 100 mL of various buffer solutions in different pH media (1.4, 5.8, 7.4 and 9.4, respectively), stirred at 120 rpm and maintained at 37°C in a water bath. At predetermined time intervals, 1 mL aliquots were withdrawn and diluted to determine the concentration of drug released by UV spectrophotometry at 320 nm. The dissolution medium was replaced with a fresh buffer to maintain the total volume. The drug release percent was determined in terms of percentage by the following equation:

\[
\text{Drug release} \[%\] = \frac{R}{L} \times 100
\]

(3)
where, $L$ and $R_t$ represent the initial amount of drug loaded and the cumulative amount of drug released at time $t$.

**Characterization Studies**

The surface morphology of samples was analyzed by scanning electron microscopy (Leica Canbridge S360 microscope). The drug release was monitored by UV spectrophotometry (Shimadzu UV-1601, Japan). The IR spectrum was recorded with a double-beam Perkin-Elmer 1600 FTIR spectrophotometer.

**RESULTS AND DISCUSSION**

**Synthesis of Phosphorous Containing Chitosan (PCTS) Beads**

Chitosan (CS, a polycationic polysaccharide) forms gels with multivalent counter ions through the formation of intermolecular or intramolecular linkages by ionic interactions. The liquid-gel transition of PCTS in TPP aqueous solution is due to the electrostatic interactions between positively charged free amino groups with negatively charged counter anion TPP [24]. A structure of PCTS beads is shown in Figure 1.

The small molecule polyelectrolyte TPP dissociated in water and released OH$^-$/H$^+$ ions, thus providing both OH$^-$/H$^+$ and P$_3$O$_{10}^{5-}$/H$^+$ ions which coexisted in the TPP solution. The OH$^-$ and P$_3$O$_{10}^{5-}$/H$^+$ ions could ionically react with the $-$NH$_3$$^+$ binding site on PCTS by deprotonation and crosslinking, respectively. The CS-TPP complex formation in the gel beads was less time dependant when the outer layer was formed. The CS-phosphoric acid complexation gel beads were found to be time dependant [34]. Consequently, for the PCTS beads, the electrostatic attraction would be dependant on the residual $-$NH$_2$.

![Figure 1. Structure of PCTS.](image-url)
SEM of Beads

The SEM of the CS and PCTS beads is shown in Figure 2; based on these SEM images the size of the CS and PCTS beads varied from 1.5 to 1.75 mm. The CS beads exhibited a smooth surface morphology as well as a more regular spherical geometry and smooth surface than the PCTS beads. This may be due to a more efficient crosslinking in the CS beads. The PCTS beads exhibited some fissures on the surface that may be due to the grafting of phosphorous containing monomer. Crosslinking of PCTS beads was less efficient; some of the amino groups of CS were substituted by phosphorous which led to the formation of a rougher and non-uniform surface structure.

Swelling Behavior of Beads

The swelling behavior of the CS and PCTS beads in pH 1.4 and 7.4 solutions at 37°C are shown in Figures 3 and 4. The PCTS beads at pH 1.4 disintegrated within 3 days and then dissolved while the CS beads dissolved after 4 days. These results showed that the swelling of the CS-TPP beads was different from that of PCTS beads agreement with the difference in their crosslinking density. The less crosslinked PCTS beads swelled quickly and gradually dissolved within 3 days, whereas the highly crosslinked CS beads did not dissolve within the same time period. This was expected behavior, due to the high amount of interchain linkages and masking of the hydrophilic nature of the amino groups in the high ionic crosslinked CS-TPP network [35]. The swelling percentage was found to be much higher at pH 1.4 than pH 7.4. Moreover, the swelling reached a stable equilibrium more rapidly
Figure 3. Swelling behavior of the CS and the PCTS beads at pH 1.4.

Figure 4. Swelling behavior of the CS and the PCTS beads at pH 7.4.
at pH 7.4 than at pH 1.4. This was due to the lack of protonation of amino groups at pH 7.4, which prevents swelling in an alkaline medium [36].

**Entrapment of IM in PCTS Beads**

IM is a non-steroid anti-inflammatory drug used for the treatment of arthritis [33]. It was efficiently entrapped into both CS and PCTS beads during the ionotropic process (Table 1). The relatively high efficiency of loading may be due to the low water-solubility of IM as well as the ionic binding between negatively charged carboxyl groups in IM and positively charged free amino groups in CS. The carboxyl groups in IM and the phosphorous groups in PCTS are ionized at pH 4. In this work, the drug was not chemically attached to the polymer. Thus, the drug in the gel was only held by the electrostatic and hydrophobic interactions, and physical entrapment within the polymer matrix. Consequently, the drug remained in a biologically active form and therapeutically effective in the body as soon as it was released from the polymeric matrix.

**The In Vitro Release Profiles of IM**

The release profiles for 0.3% IM-PCTS beads at various pH’s and at 37°C in a buffer solution are shown in Figure 5. In general, the percent of drug released increased with increasing pH of medium. Within 3h, 30% of the IM was released from the PCTS beads at pH 1.4 and 50% at pH 7.4. This behavior indicates that drug release profiles are pH-sensitive. There are two factors to be considered in the noticeably higher release rate of IM at pH 7.4 than at pH 1.4. The first factor is the high solubility of IM in alkaline medium and the other is the electrostatic repulsion between the negatively ionized carboxyl group of IM and the phosphate groups in PCTS at pH 7.4 [36–38]. Similar drug release profiles were observed for the CS beads. However, the release percentage

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**Table 1. Loading efficiencies for IM-loaded CS and PCTS beads.**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Initial Drug Content (mg/g-beads)</th>
<th>Loading Level (mg/g-beads)</th>
<th>Loading Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3% IM-CS</td>
<td>28.9</td>
<td>27.11 ± 0.5</td>
<td>93.81 ± 1.9</td>
</tr>
<tr>
<td>0.6% IM-CS</td>
<td>58.2</td>
<td>53.21 ± 2.0</td>
<td>91.43 ± 3.8</td>
</tr>
<tr>
<td>0.3% IM-PCTS</td>
<td>28.9</td>
<td>25.17 ± 0.3</td>
<td>87.09 ± 1.4</td>
</tr>
<tr>
<td>0.6%IM-PCTS</td>
<td>58.2</td>
<td>49.34 ± 5.1</td>
<td>84.78 ± 5.3</td>
</tr>
</tbody>
</table>
of IM at pH 1.4 was found to be less than that for the PCTS beads. The reason may be due to the formation of complexes between the carboxyl group of IM and the amino group of CS and poor solubility of the drug at pH 1.4 [36–38]. The diffusion rate of the drug molecules was higher in the alkaline medium, effectively contributing to the controlled drug release. The polymer matrix probably combines two types of mechanisms for drug release: controlled diffusion and swelling. The *in vitro* release profiles of 0.6% IM-loaded CS and PCTS beads at pH 1.4 and 7.4 are shown in Figure 6; similar release profiles were observed.

The influence of the initial drug loading on the release profiles of IM from PCTS beads at pH 1.4 and 7.4 is shown in Figure 7. Surprisingly, there was no significant difference between the release of IM for 0.3 and 0.6% loaded PCTS beads. For initial drug loadings, the amounts of IM released per unit mass of PCTS beads at pH 7.4, are shown in Figure 8. As expected, the amount of drug released from highly loaded beads was higher than that from slightly loaded beads. Increasing the initial drug content provided the larger equilibrium amount of drug released at each pH.

![Figure 5](image_url). Release profiles of IM from 0.3% loaded PCTS beads in various pH media.
Figure 6. Drug release from IM-loaded PCTS beads with different initial drug contents (0.3 and 0.6%) in simulated gastric fluid, pH 1.4, and simulated intestinal fluid, pH 7.4.

Figure 7. Time course of IM amount released from PCTS beads and IM concentration in dissolution buffer at different initial drug contents (0.3 and 0.6%) at pH 7.4.
CONCLUSIONS

In this work, PCTS beads were developed in order to be used as a pH sensitive and gastric fluid-resistant drug carriers. The beads were prepared under mild conditions at room temperature and pH 4.0, and the loading efficiency of the model drug, IM, was over 85%. The water solubility of the drug, the swelling degrees of beads and the ionization of phosphate groups were found to have a significant influence on the release profiles of IM at various pHs. For example, the release rate from the PCTS beads in simulated intestinal fluid (pH 7.4) was higher than that in simulated gastric fluid (pH 1.4). These factors suggest that the PCTS beads could be better than chitosan beads as a controlled drug delivery system for oral administration by avoiding drug release in the highly acidic gastric fluid region of the stomach.

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