Development of Hydroxyapatite-Chitosan Matrix as a Drug Carrier

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Abstract

Polymer-inorganic composites have become important in the development of biomedical applications ranging from diagnostic and therapeutic devices, tissue regeneration and drug delivery systems. In this work, bio-composite consisting of hydroxyapatite (HA) and chitosan (CH) were created by ionic crosslinking with tripolyphosphate (TPP). The polymer matrices were proposed to be used as a drug carrier to bone. Interactions between CH and HA, as well as CH and TPP were monitored by SEM and FTIR for achieving proper structures and sustained release property. Tetracycline hydrochloride (TCH) was encapsulated in the matrices simultaneously during the crosslinking process. The release of drug was sustained for at least 1 week with concentrations above therapeutic range. By increasing content of the entrapped drug, however, burst release was not observed. In specific applications, greater biological information is needed to fully understand the in vitro and in vivo performance of such 3D-structure. Once obtained, it would provide a rationale in designing and optimization of the efficient drug delivery system.

Keywords: hydroxyapatite, drug delivery, encapsulation, release profile, polymer-inorganic hybrid.

1. Introduction

Drug delivery systems have advantages of increasing residence time of a drug within a patient, reducing dosing frequency and toxic effects. These also improve patient compliance and drug efficacy with most dosage requirements. Various natural and synthetic polymers have been adopted for drug delivery system due to their excellent properties such as biocompatibility, biodegradability and long-term safety of drugs [1].

Chitosan (CH) is N-deacetylation product of the polysaccharide chitin, which is the second abundant polysaccharide in the world. CH has unique properties, including cationic nature in acidic medium, good biocompatibility, non-toxicity, biodegradability and mucoadhesivity with absorption enhancing effect [2]. The freely available amino groups of CH can react with many negatively charged molecules [3]. A reversible physical crosslinking by electrostatic interaction is recently applied to avoid possible toxicity of chemical crosslinkers. CH has been examined extensively in pharmaceutical industries for its potential in the development of drug delivery systems [4].

Hydroxyapatite (HA) is a highly biocompatible and biodegradable ceramic material. Applications of HA as drug delivery systems for antibiotics, growth factors, anticancer drugs and orthopedic applications are obvious, due to its similarity to the mineral constituent of hard tissues [5].

Tetracycline is effective against all gram positive and many gram negative bacteria. It exerts antibacterial activity by inhibiting microbial protein synthesis [6].

Tripolyphosphate (TPP) is a polyanion, which can interact with cationic CH by electrostatic forces [4]. TPP-CH microparticles developed by ionotropic gelation method have been reported [2]. Indeed, this complex can be prepared several methods such as dropping, coacervation, freeze-drying etc [4].

In the present work, a number of variables including the ratios of CH, HA, TPP and TCH, morphology and matrix structure, entrapment efficiency, as well as in vitro release study were investigated.

2. Materials and Methods

2.1 Chemicals

CH from crab shells with the deacetylation degree (DD) of 85 %, and sodium tripolyphosphate (TPP) were purchased from Sigma-Aldrich, Japan. HA was obtained from Fluka. Acetic acid was from Guangdong Guanghua Chemical Factory Co., Ltd, China. TCH was a gift from a pharmaceutical industry in Thailand. Other reagents were of analytic grade and used as received.

2.2 Preparation of chitosan beads

CH beads were prepared by ionotropic crosslinking method. CH was dissolved in acetic acid solution (3%v/v) at various concentrations, under magnetic stirring at room temperature. The solution
was dropped through a syringe into a TPP solution dissolved in water. Different concentrations of TPP (Table 1) were used separately for obtaining stable structure of the beads.

HA/CH beads were prepared by dispersing HA powder in CH solution under stirring until smooth paste was generated (Table 1). The beads were formed by dropping the mixture through a syringe into a TPP solution, and soaked overnight to complete the crosslink. After that the beads were washed several with water and freeze-dried.

HA/CH containing TCH beads were prepared mixing HA powder and TCH (Table 2) followed by dispersed in CH solution (1.5 % w/v). The beads were formed by using procedures previously described.

### 2.3 Characterizations

#### 2.3.1 Scanning Electron Microscopy (SEM)

Surface morphology of the beads was examined by SEM (Quanta 400, FEI, Czech Republic) following coated with thin film of gold. The instrument was operated at an acceleration voltage of 20 kV.

#### 2.3.2 Fourier-transform Infrared Spectroscopy (FTIR)

FTIR spectra of the cross-linked beads were obtained by using a Spectrum One FT-IR Spectrometer (Perkin Elmer) within a range of 500 to 4000 cm\(^{-1}\).

#### 2.3.3 Swelling Index

The beads were weighed initially (Wd) and after immersed in 5 ml PBS at ambient temperature for 7 day. The swollen weight (Ws) was obtained by gently removing the surface water with blotting paper. Swelling percent (%) was calculated using the following formula:

\[
\text{Swelling (\%)} = \left( \frac{W_s - W_d}{W_d} \right) \times 100 \% \quad (1)
\]

#### 2.4 Drug Loading Efficiency

Drug loading efficiency was determined by dissolving the beads in aqueous acetic acid (3\%/v). After centrifugation, the amount of drug entrapped was measured optically at 270 nm (UV-Visible spectrophotometer, Hewlett, HP 8453). The entrapment efficiency (%EE) was calculated according to the following equation:

\[
\text{EE (\%)} = \frac{\text{mass of drug in beads}}{\text{mass of beads}} \times 100 \quad (2)
\]

#### 2.5 In vitro Drug Release Studies

*In vitro* drug release studies were carried out by soaking the beads in 2 ml PBS (pH 7.4) for 7 days. The supernatant was withdrawn at predetermined time interval, and analyzed for drug release at 270 nm. The release experiment was performed in triplicate for each sample.

### Table 1 Formulations of HA/CH beads

<table>
<thead>
<tr>
<th>Formula</th>
<th>CH/TPP (w/w)</th>
<th>Formula</th>
<th>CH/HA/TPP (w/w/w)</th>
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</thead>
<tbody>
<tr>
<td>F1</td>
<td>1 : 4.28</td>
<td>F4</td>
<td>1 : 26.6 : 2</td>
</tr>
<tr>
<td>F2</td>
<td>1 : 2.1</td>
<td>F5</td>
<td>1 : 20 : 2</td>
</tr>
<tr>
<td>F3</td>
<td>1 : 3</td>
<td>F6</td>
<td>1 : 20 : 2.1</td>
</tr>
</tbody>
</table>

### Table 2 Formulations of HA/CH beads containing TCH

<table>
<thead>
<tr>
<th>Formula</th>
<th>CH/HA/TPP/THC (w/w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F7</td>
<td>1 : 26.6 : 2 : 13.3</td>
</tr>
<tr>
<td>F8</td>
<td>1 : 26.6 : 2 : 6.7</td>
</tr>
<tr>
<td>F9</td>
<td>1 : 26.6 : 2 : 3.3</td>
</tr>
</tbody>
</table>

### 3. Results and Discussion

#### 3.1 Morphology of CH- and HA/CH beads

CH with a pK\(_a\) of 6.3 is polycationic when dissolved in acids presenting –NH\(_3\) moieties. Sodium tripolyphosphate (Na\(_3\)P\(_{10}\)O\(_{24}\)) dissolved in water dissociates to give phosphoric ions. The crosslinking of CH is thus dependent on the availability of the cationic sites and the negatively charged species [7]. CH solution dropped into TPP solution can form gel beads instantaneously with the diffusion of tripolyphosphoric ions into CH droplets to further react with –NH\(_3\)\(^+\) functionalities [5]. Fig. 1 showed opened pore structure with interconnectivity of pores. There was higher in crosslink density of F1 than of F2 and F3 (Fig. 1a and b). This was due to the high concentrations of CH and TPP used. The increase of P\(_3\)O\(_8\)\(^-\) ions by additional amounts of TPP also increased the reactive functional groups, resulting in the stronger interaction, and was attributed to the higher density of CH matrix [8].

![Fig. 1 SEM micrographs of CH beads: (a) F3, (b) F2 and (c) F1.](image-url)
However, by the dropping method, only fragile beads were obtained. The method was adopted in our recently study in that HA was included. The HA/CH cross-linked beads had relatively good mechanical strength. The increase of HA content was benefit by keeping uniformly spherical shape of the beads. As a result, F4 were more spherical and stronger than F5 and F6 (Fig.2). This would be that HA promoted a more homogeneous distribution in size during the crosslinking process [5]. In analyzing the outer surfaces and the cross-sectional areas, it revealed that HA particles were homogeneously distributed within the polymer matrices (Fig.2). However, there were boundaries between shells and cores. Both the compactness and the thickness of shells for F4 were higher than those of F5 and F6 (Fig.2). This complex phenomenon occurring between positively and negatively charged molecules, known as complex coacervation, has been utilized in drug delivery applications [9].

![Fig. 2 SEM micrographs of the beads and their cross-sections: (a, b) F4, (c, b) F5 and (e, f) F6.](image)

### 3.2 FTIR analysis

Fig. 3 was FTIR spectra of crosslinked CH. The characteristic peak at 3466 cm\(^{-1}\) was attributed to the stretching vibration of –NH\(_2\) and –OH groups. In addition, the bands for amide I at 1636 cm\(^{-1}\) and amide III at 1321 cm\(^{-1}\) of CH were observed. The peak at 1150 cm\(^{-1}\) was assigned to P=O groups of TPP, which being attributed to the linkages between phosphoric and ammonium ions [7]. The spectra of HA/CH beads were shown as sample1-3. By using the spectrum of for comparison, the incorporation of HA led to the emergence of peaks assigned to the \(\nu_1\) PO\(_4^{3-}\) peak at 961 cm\(^{-1}\), the \(\nu_3\) PO\(_4^{3-}\) peak at 1034 cm\(^{-1}\), the \(\nu_4\) PO\(_4^{3-}\) peak at 602 and 565 cm\(^{-1}\), and O-H peak at 632 cm\(^{-1}\). As the amount of HA increased, overlapping of the CH bands was occurred.

![Fig.3 FTIR spectra of HA/CH beads: sample1, F5; sample2, F4 and sample3, F6.](image)

### 3.3 Swelling behavior

Swelling is mainly influenced by ionic interactions between CH chains, and dependent on crosslinking density set during the formation of the polymer network [10]. In Fig.4, the swelling curves were not greatly changed with times of the immersion in PBS. The strongest interaction of TPP and CH caused insensitivity of beads to swelling [11]. F6 was more swollen than F7. At high HA contents, the network became densely packed, resulting in reduction of mesh size and thus a decrease in % swelling. Because of narrower mesh sizes of the matrix, diffusion of water and subsequent relaxation of polymer chains would be restricted [6]. The crosslinked beads were found to be not dissociated. Therefore, the swelling of the beads in medium would be attributed to hydration or ionization of unreacted–NH\(_2\) sites in CH polymer. The increase of ionic crosslinking reduced the unreacted –NH\(_2\) site in chitosan, resulting in a decrease of swelling of the beads.

### 3.4 Drug entrapment efficiency

To verify the effect of initial concentration of TCH, drug entrapment was carried by varying its concentrations. The results showed that %EE of F7, F8 and F9 were of 3.33, 2.87 and 1.77%, respectively. Increasing in TCH concentrations led to a slight increase of encapsulation efficiency. Many factors have reported to affect the entrapment efficiency of drugs in CH beads, including nature of the drugs, drug polymer ratio, etc [12]. In these systems, drugs are
physically embedded in the matrices. However, the increase in entrapment efficiency for increased TCH would be due to the formed structures that facilitated the penetration and entrapment of the drug inside the polymer matrices [13]. The low TCH incorporated increased the drug loading efficiency, which was attributed to the soluble nature of TCH in acid solution leading to its rapid migration into the medium [14].

Fig.4 The swelling curves of HA/CH beads as a function of time

3.5 In vitro drug release

The release profiles of TCH from the beads in PBS were shown in Fig.5. The release pattern of the highly drug-loaded beads was found to be similar to that of the beads containing lower drug loading. These suggested that the percentage of drug releasing from the beads decreased with the increase of TCH concentration. However, Total amount of drug released was found to be higher for the highly loaded beads compared to those loaded with lower drug concentrations. In addition, the release pattern was of sustained drug release within 7 days. The drug was not chemically attached to the polymer. The only likely interaction happening was electrostatic attraction between TCH and Ca²⁺ ions of HA that retained the drug stably in the beads. However, at much greater drug loading, the polymer networks were shrank due to the disruption of the polymer networks by drug molecules impregnated, causing the diffusion of PBS into the beads, which simultaneously increased drug release.

4. Conclusion

The ionic crosslinking method could be modified to form HA/CH composite beads. The concentrations of TPP and CH played major roles in controlling matrix density, i.e., as the TPP and CH concentrations increased, these attributed to an increase of polymer matrix density. Increasing of HA content also promoted the strength of beads with spherical shape. Water absorption decreased with the increased HA. The drug solubility, the bead swelling property, and ionic functionality were found to influence the release pattern of TCH. A sustained release of TCH was achieved by the increment of drug loading. All of results provided a rationale in designing and optimization of the efficient drug delivery system.

Fig.5 The release curves of TCH from the beads in PBS

Acknowledgments

The authors gratefully acknowledged the Graduate School of Prince of Songkla University (PSU) and Discipline of Excellence (DoE) in Chemical Engineering, Department of Chemical Engineering, Faculty of Engineering, PSU for financial supports.

References


