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Physicochemical properties and drug release behavior of biguanidino and *O*-carboxymethyl chitosan microcapsules

Weiqiang Huo[a], Weixin Zhang[b], Wei Wang[a], Xiaohua Zhou[a,c,*]

[a] College of Science, South China Agricultural University, Guangzhou, China
[b] College of Life Science, South China Agricultural University, Guangzhou, China
[c] Institute of Biomaterial, South China Agricultural University, Guangzhou, China

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ABSTRACT

Two types of microcapsules (MCs) were prepared by the emulsion cross-linking method, where biguanidino chitosan (BGCS)and *O*-carboxymethyl chitosan (*O*-CMCS) served as the wall materials, and the antibacterial agent 2,4-diamino-6-(2-pyridyl)-1,3,5-triazine (PyTNH) served as a model water-soluble drug. The physicochemical performance of the MCs and their drug release behavior were investigated by Fourier transform infrared spectroscopy, thermogravimetric analysis/derivative thermogravimetric analysis, scanning electron microscopy, and swelling and in vitro drug release studies of the two MCs with unmodified chitosan-MCs (CS-MCs) used as the control. The results indicated that the degree of cross-linking, encapsulation efficiency, and thermal stability of the shell wall of the BGCS-microcapsules (BGCS-MCs) were much higher than those of the control and the *O*-CMCS-microcapsules (CMCS-MCs), owing to the reduction of steric hindrance and development of the conjugation effect in the cross-linking process. Studies on the swelling and in vitro drug-release behavior revealed a sustained release effect of the BGCS-MCs. Moreover, the CMCS-MCs were found to exhibit a pH-dependent drug release behavior, which can be attributed to the successive formation of H-bonds and repulsive forces with the change in the pH of the medium. Based on these results, the swelling-release models and the drug release kinetics of BGCS-MCs and CMCS-MCs are proposed.

1. Introduction

It is believed that chitosan (CS), a cationic linear polysaccharide obtained from the extensive deacetylation of chitin, is suitable to be a shell wall material for microcapsules (MCs) owing to its non-toxic nature, biodegradability, and good biocompatibility [1–3]. However, the applications of CS-MCs have been limited by some shortcomings: the low encapsulation efficiency (*EE*, %) for the encapsulation of water-soluble core material because of the poor aqueous solubility of CS [4] and the significant burst effect [5,6]. Several methods, including CS modification, have been investigated for overcoming the selimitations. For instance, a quaternized CS derivative has been proven to increase the *EE* effectively as the solubility of CS in a neutral medium was enhanced after modification [7], while a PEG-grafted CS has been widely used as a wall material to reduce the initial burst release effect [8,9].

In the present study, we modified CS with biguanidino (BG) or carboxymethyl (CM) groups, and prepared two types of modified MCs, BGCS-MCs and CMCS-MCs, by the emulsion cross-linking method. After the introduction of the BG groups, the water-solubility [10] of the wall material and the *EE* of the BGCS-MCs were greatly enhanced. The Schiff base reaction was expected to occur selectively between the cross-linker and the amino group of BG in the cross-linking process because of the reduction in steric hindrance and the conjugation effect, thus, prolonging the sustained release effect.

Only a few examples of the preparation of CMCS-MCs by the emulsion cross-linking method have been reported in the literature [11,12]. They are usually obtained by the ionic cross-linking method with CM as the cross-linking group, and have been widely used in pharmaceutical applications [13]. However, their drug release is only time dependent, which limits their application in drug delivery systems [14]. According to previously reported studies [15,16], CM groups are still available when the MCs are prepared by the emulsion cross-linking method. They can actively participate in the swelling behavior, where the pH adjustment of the releasing medium acts as a regulator: in a basic medium, CM is ionized

* Corresponding author: College of Science & Institute of Biomaterial, South China Agricultural University, Guangzhou, China. Tel.: +86 13268013393.
E-mail address: xhzhou@scau.edu.cn (X. Zhou).

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(—$CH_2COOH \rightarrow -CH_3COO^-$), while in a neutral (or acidic) medium, H-bonds are easily formed between —CH_2COOH and —OH groups in the matrix. Therefore, exploring the pH sensitivity of the CMCS-MCs was also one of the aims of this study.

Through abacteriostatic evaluation, 2,4-diamino-6-(2-pyridyl)-1,3,5-triazine (PyTNH) was found to possess excellent antibacterial activity and was chosen as the model water-soluble drug in the preparation of MCs. The effect of the modification of the walls on the physicochemical performance and the drug release behavior of the two MCs was the focus of our investigation. The characteristics and the performance of the two MCs were investigated by Fourier transform infrared spectroscopy (FT-IR), thermogravimetric analysis/derivative thermogravimetric analysis (TGA/DTG), scanning electron microscopy (SEM), in vitro drug release and release kinetics studies, with unmodified CS-MCs used as the control.

2. Materials and methods

2.1. Materials and characterization

PyTNH was prepared according to the literature method [17]. *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) were obtained from the college of life science, South China Agricultural University. Chitosan (degree of deacetylation, DD = 89.2%, $M_w = 2.8 \times 10^5$ amu) was provide by Shandong Aokang biotech Ltd. Dicyandiamide, Mueller-Hinton agar, Mueller-Hinton Borth, monochloroacetic acid, paraffin liquid and glutaraldehyde (GA, 25% water solution) were purchased from Aladdin Industrial Inc. All of the chemicals and reagents were of analytical grade.

The content of the drug was confirmed by using a UV–vis spectrophotometer (UV-2550, Shimadzu, Japan) and the *EE* was calculated by using the reported method [18]. The chemical structures of the polysaccharides (CS and modified CS), PyTNH, shell walls, and the MCs were determined by FT-IR spectroscopy (Avatar 360, Nicolet, America,). The TGA/DTG analysis was conducted by using a DTA-TG thermal analyzer (DTG-60, Shimadzu, Japan) by heating from 50 to 400 °C at the rate of 10 °C/min under a constant N_2 flow. The surface morphology of the MCs was analyzed by SEM (FEI-XL30, Philips Electron Optics, Netherlands) at 10 kV with the samples being sputter coated with gold after drying overnight.

2.2. Evaluation on bacteriostasis effect of PyTNH

Antibacterial activity of PyTNH was determined against gram negative *E. coli* and gram positive *S. aureus* bacteria by disc diffusion method [19] and agar dilution method [20]. The diameters of the inhibition zone were measured with an image detection technique based on morphological edge detection [21].

2.3. Modification of CS

Four types of *O*-CMCS with different degrees of substitution (*DS*) were obtained by the reported method [22]. The *DS* of *O*-CMCS (DS_1), defined as the relative number of carboxymethylated groups in chitosan, was measured by a potentiometric titration method [23], and the concentration of the free amino (c_{amino}) groups was determined by the ninhydrin method [24]. The DS_1 and c_{amino} of the four types of *O*-CMCS were 21.58% (c_{amino} = 94.52%), 35.69% (c_{amino} = 87.25%), 47.69% (c_{amino} = 84.78%), and 68.71% (c_{amino} = 81.23%).

Four types of BGCS were prepared as described in the literature [10] with minor changes. The *DS* of the BGCS (DS_2) was calculated according to the following equation:

$$C/N\% = \frac{M_C \times 6 + 2 \times M_C \times (1 - DD) + 2 \times DS_2 \times M_C}{M_N + 4 \times DS_2 \times M_N} \quad (1)$$

where C/N, based on the result of the elemental analysis (CHN), is the mass ratio of C to N. M_C and M_N are the relative molecular weights of C and N, respectively. The corresponding DS_2 of the four types of BGCS were 10.13%, 20.30%, 35.72%, and 47.69%.

2.4. Preparation of PyTNH-loaded microcapsules

Three types of PyTNH-loaded MCs (Scheme 1) were prepared via the emulsion cross-linking method. First, 100 mg of the polysaccharides (CS, *O*-CMCS or BGCS) were dissolved in 10 mL of 1.0% (v/v) acetic acid solution; 50 mg of PyTNH was dispersed in the CS solution, and then stirred overnight to for man aqueous phase. Second, 6 mL of the aqueous phase was added slowly to an oil phase containing a paraffin liquid and a 6% solution (v/v) of Span 80, and the mixture was stirred at 1000 rpm to form an emulsion. After 30 min of homogenization, the cross-linker GA was added drop wise into the emulsion at 40 °C with continuous stirring. Afterwards, the MCs were obtained by centrifugal separation, washed by petroleum ether followed by acetone, and finally dried at 50 °C for 24 h.

There was no chemical reaction between GA and PyTNH in the cross-linking process though PyTNH possesses two primary amine groups. This could be verified by the unchanged characteristic peaks in the UV spectra of PyTNH released from the MCs compared with those of free PyTNH.

2.5. Swelling and drug releasing in vitro

The swelling behaviors of CS-MCs, CMCS-MCs, and BGCS-MCs were studied in different buffer mediums of pH 3.5, 5.5, 7, and 9. 100 mg of each sample was immersed in the buffer medium, and placed in the water bath at 37 ± 0.5 °C for swelling. A specific amount of the sample was withdrawn at predetermined time intervals (4, 12, and 24 h), and weighed after removing the surface-adhered water. Subsequently, these samples were dried to a constant weight in an oven at 60 °C for 24 h. The degree of swelling (S_d) was calculated by Eq. (2).

$$S_d\,(\%) = \left[\frac{(W_s - W_d)}{W_d}\right] \times 100 \quad (2)$$

where W_s and W_d represent the weight of the swollen and dry drug-loaded microcapsules, respectively.

For the in vitro drug release studies, 50 mg of the MCs (CS-MCs, CMCS-MCs or BGCS-MCs) containing a known amount of the drug were placed in a dialysis bag, suspended in phosphate buffer solutions of different pH, and then incubated at 37 ± 0.5 °C in an incubator shaker at 100 rpm. Aliquots of 1.5 mL of the buffer medium were withdrawn at predetermined time intervals and replaced by a fresh buffer medium. The contents of these samples were analyzed using the UV–vis. spectrophotometer at 273 nm. All experiments were performed in triplicate. The amounts of the PyTNH released were quantified by Eq. (3).

$$\text{Drug release}\,(\%) = \frac{\text{Amount of released drug}}{\text{Amounts of drug encapsulated within microcapsules}} \times 100 \quad (3)$$

2.6. Drug release kinetics studies

To obtain more information about the drug release mechanism, further investigation was carried out by fitting the first 60% of the drug release data to the Korsmeyer–Peppas equation [25], a semi-empirical equation that describes an important model for analyzing