Korean J. Chem. Eng., 32(3), 369-372 (2015)
DOI: 10.1007/s11814-015-0007-0

pISSN: 0256-1115
eISSN: 1975-7220

RAPID COMMUNICATION

Novel double-walled microspheres based on chitosan, sodium cellulose sulfate and sodium tripolyphosphate: Preparation, characterization and *in vitro* release study

Li-Ying Zhu*,, Xiao-Qin Yan*, Hong-Man Zhang*, Shan-Jing Yao**, and Ling Jiang***,†**

*College of Sciences, Nanjing University of Technology, Nanjing 211816, P. R. China
**Department of Chemical and Biological Engineering, Zhejiang University, Hangzhou 310027, P. R. China
***College of Food Science and Light Industry, Nanjing University of Technology, Nanjing 211816, P. R. China
(*Received 1 September 2014 • accepted 6 January 2015*)

Abstract−A novel double-walled microsphere composed of chitosan, sodium cellulose sulfate (NaCS) and sodium tripolyphosphate (TPP) was prepared. TPP was used as ionic crosslinker. The morphology of the microspheres was observed by microscope and SEM, and the results showed that the double-walled microsphere was smooth outside, with rough interior surface. FTIR spectra analysis was performed to investigate the PEC formation among chitosan, NaCS, and TPP. In vitro release studies of BSA showed that the double-walled microspheres had regular and sustainable release profiles in simulated colonic fluid (SCF). Our results indicated that the double-walled microspheres prepared could be used as a candidate protein drug carrier for the colon.

Keywords: Chitosan, Sodium Cellulose Sulfate, Sodium Tripolyphosphate, Double-walled Microshperes, *In Vitro* Drug Release

INTRODUCTION

Chitosan is a cationic polymer, chemically a poly-β-(1,4)-D-glucosamine, derived from natural chitin by alkaline deacetylation. Chitosan has been evaluated for conventional and novel gastrointestinal drug delivery systems because of its nontoxic, biocompatible, mucoadhesive, and biodegradable properties [1]. However, chitosan can be easily dissolved in acidic solutions, which makes drug delivery systems based on chitosan difficult to pass through the stomach and small intestine without the dissolution of chitosan. Therefore, this material needs to be modified *via* physical or chemical methods. Sodium cellulose sulfate (NaCS) as a novel hydrophilic cellulose derivative is a polyanionic polymer, which has favorable biological properties as a drug carrier material, such as hydrosolubility, nontoxicity, biodegradability and good film-forming ability [2]. We have found that the polyelectrolyte complex (PEC) composed of chitosan and NaCS has the potential behaviors in the controlled release of drugs, and was used for preparing the colon-specific drug delivery capsules [3], the multilayer microcapsules [4], and the complex films [5]. However, chitosan/NaCS microcapsules tend to collapse, since NaCS with high molecular weight only binds on the surface of chitosan droplets [6].

Sodium tripolyphosphate (TPP) is a low molecular weight crosslinker, which could freely diffuse into chitosan droplets or films to form ionically cross-linked chitosan beads or films [7]. In this study, TPP was introduced to the reaction system to prepare double-walled microspheres by forming a ternary composite. By evaluating the morphology and the complex structure of chitosan/NaCS/TPP microspheres, as well as the drug release profile *in vitro*, a potential protein loaded microsphere system using PEC was developed.

EXPERIMENTAL

1. Materials

Chitosan with 85% deacetylation and Mw of 118.7 kDa was supplied by Jinan Haidebei Co., Ltd. (China). NaCS with degree of substitution (DS) of 0.38 and Mw of 710.8 kDa were prepared by the heterogeneous reaction as described previously [8]. Sodium tripolyphosphate (TPP) was purchased from Wenzhou Dongsheng Chemical Reagent Factory (China). Bovine serum albumin (BSA) was purchased from Sangon Biotech (Shanghai) Co., Ltd. (China). All other chemicals and reagents were of analytical grade and used without further purification.

2. Preparation of the Microspheres

Chitosan was dissolved in diluted acetic acid solution with the concentration of 30 g/L. Chitosan/NaCS/TPP microspheres were obtained by adding chitosan solution dropwise through a 0.4 mm needle slowly into 20 mL blended solutions of NaCS (15 g/L) and TPP (10 g/L). The microspheres with BSA were prepared by adding the blended solutions of chitosan and BSA into 20 mL solutions of the mixture of NaCS and TPP. The resulting microspheres were allowed to harden for 15 min under gentle stirring (150 r/min) with a small magnetic bar, and filtered, rinsed three times with deionized water and lyophilized. Surface and interior morphology of the microspheres were observed with an optical microscope (Eclipse E200, Nikon, Japan) and TM-1000 scanning electron microscopy (SEM, Hitachi, Japan). Chemical properties of the microspheres and their components were analyzed by Fourier transform infrared spectrometry (FTIR, Nicolet Elmer system 2000, USA).

3. Characterization of the Microshperes

The release study of chitosan/NaCS/TPP microspheres was per-

†To whom correspondence should be addressed.
E-mail: jinagling@njtech.edu.cn

formed in simulated colonic fluid (SCF). About 10 mg microspheres were incubated in SCF, pH 6.4 (20 mL) at 37±0.5 °C, 100 rpm up to 10 h. At predetermined time intervals, 1 mL of medium from the vessel was sampled and replaced with an equal volume of fresh medium. The concentrations of BSA were determined with a UV spectrometer (Ultrospec 3320 pro, GE Healthcare, USA). The calculation of drug release percentage (DR%) was described by Wu and Yao [9].

RESULTS AND DISCUSSION

1. The Morphology of the Microspheres

A simple preparation process was used based on the principle of forming polyelectrolyte complexes (PEC) *via* ionization reaction [4]. In this work, NaCS is used as a polyanion with $-SO_3^-$ groups, while chitosan is a polycationwith $-NH_3^+$ groups. TPP can penetrate the PEC membrane into the chitosan-core, and then consolidate/crosslink with chitosan. As shown in Fig. 1, the chitosan/NaCS/TPP microspheres were spherical with an average diameter of 2.0± 0.1 mm (Fig. 1(a)), which had a typical double-walled structure (Fig. 1(b)) with smooth outside surface (Fig. 1(c)) and rough interior surface (Fig. 1(d)). Similar results were also reported by Liu et al. [10].

2. Interaction of NaCS and TPP with Chitosan

During the preparation process of chitosan-based microspheres, NaCS or TPP binds to chitosan continuously. Investigation of the binding process in quantitative terms will improve our understanding of the interaction between chitosan and other components. Fig. 2 shows the variation of the weights of microspheres with reaction time. As shown, the weight of chitosan/NaCS microspheres gradually increased and reached to a balance at 30 min. At that time, the weight of binding NaCS on the chitosan/NaCS microspheres was about 76% of chitosan's weight, which was much lower than that of chitosan/NaCS films (≥1.5). The results indicated that there was a considerable amount of unreacted chitosan in the microspheres. The formation of both chitosan/TPP microspheres and chitosan/NaCS/TPP double-walled microspheres suffered a decrease of weight between 10-30 min. The results were not in consistence with the kinetic study of chitosan-TPP complex reaction from the reports of Mi et al. [11]. The concentration of TPP may account for the difference. In the work reported by Mi et al., the concentration of TPP was 100 g/L, while TPP concentration was 15 g/L in this work. When TPP concentration is low, TPP would more easily diffuse into chitosan droplets in the beginning. However, excessive TPP would be removed from the microspheres later with the curing process. At the high concentration of TPP, the phenomenon would be not obvious because of the existence of tough competition be-

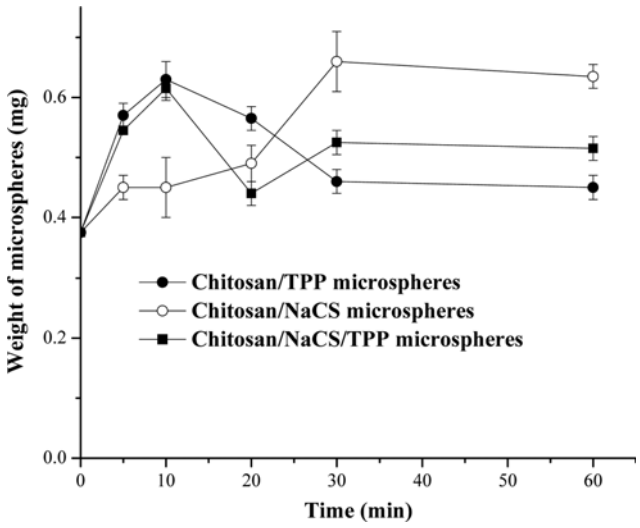

Fig. 2. Variation of the weights of microspheres with reaction time.

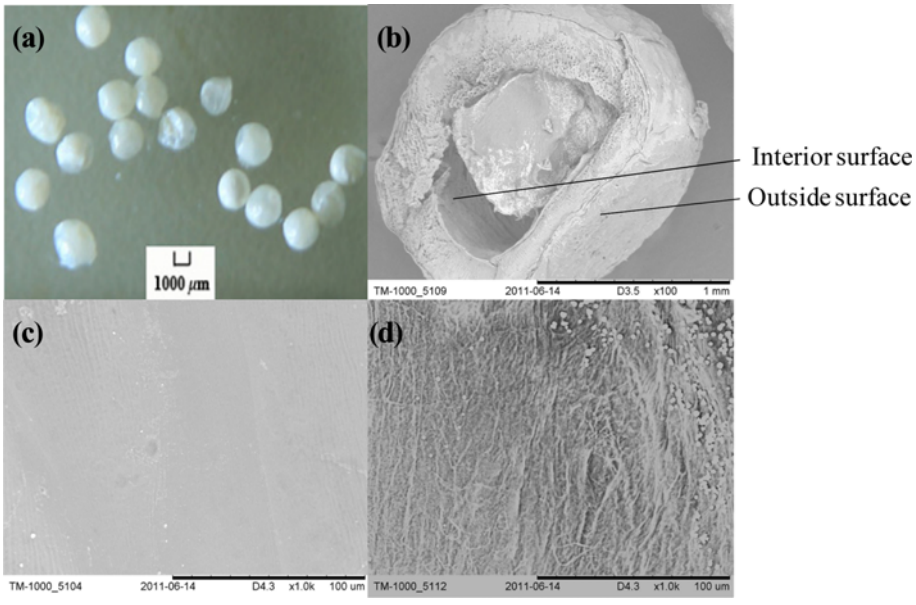

Fig. 1. Microscope (a) and SEM (b), (c), (d) photographs of chitosan/NaCS/TPP microspheres. (b) The cross-section of the microsphere, (c) the outside surface of the microsphere, (d) the interior surface of the microsphere.

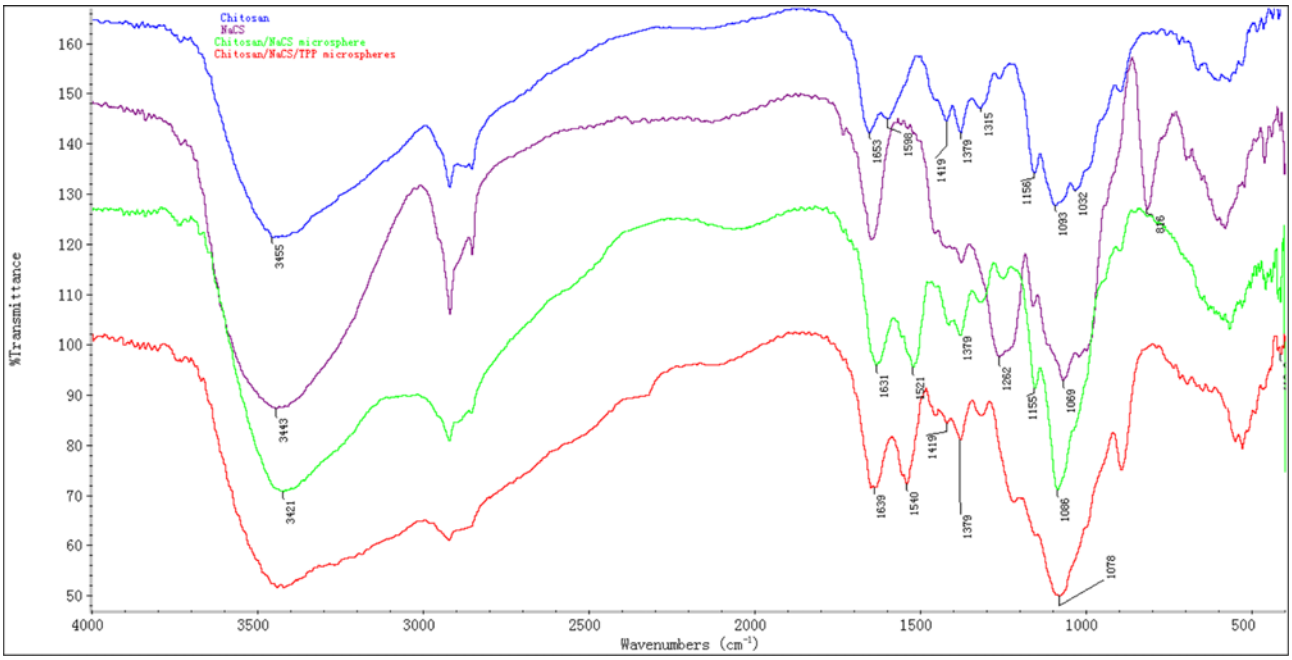

Fig. 3. FTIR spectra of chitosan, NaCS, chitosan/NaCS microspheres and chitosan/NaCS/TPP microspheres.

tween TPP molecules. In addition, the weight of chitosan/NaCS/TPP microspheres had a value between that of chitosan/NaCS microspheres and chitosan/TPP microspheres after 30 min, indicating that both NaCS and TPP took part in the formation of chitosan/NaCS/TPP microspheres.

FTIR studies were conducted to investigate the PEC formation among chitosan, NaCS, and TPP (Fig. 3). As can be seen in Fig. 3, the FTIR spectrum of chitosan shows an absorption of the stretching vibration band of C=O-NHR at 1,653 cm^{-1}, and the stretching vibration band of N-H in amino-group at 1,598 cm^{-1}. NaCS shows strong absorption bands of S=O at 1,262 cm^{-1} and C-O-S at 816 cm^{-1}, which was similar to our previous results [12]. Compared with the FTIR spectrum of chitosan, the stretching vibration band of -NH_2 and -OH of chitosan/NaCS microspheres moved from 3,455 cm^{-1} to 3,421 cm^{-1}, and the peaks at 1,653 cm^{-1} and 1,598 cm^{-1} of chitosan moved to a lower field, indicating that the amino group of chitosan took part in the reaction. Whereas, the FTIR spectrum of chitosan/TPP microspheres was almost the same as that of chitosan/NaCS microspheres. The FTIR spectrum of chitosan/NaCS/TPP microspheres revealed that the peak areas of N-H bending vibration became big and wide, which possibly indicated that -NH^{3+} of chitosan, -SO^{3-} of NaCS and -[$P_2O_5^{4-}$]- of TPP might react and formed PEC.

3. *In Vitro* Release of Protein

The amount of BSA released from the chitosan/NaCS/TPP microcapsules was determined in the SCF with the chitosan/NaCS microcapsules as the control. As shown in Fig. 4, under the weak acidic condition of pH 6.4, chitosan/NaCS/TPP microcapsules showed a regular drug release behavior with a trend of slowly increasing, and the BSA was released sustainably and almost completely during 10 h. However, the control microcapsules had a burst drug release (more than 63.1±0.7%) effect during the first hour and the drug

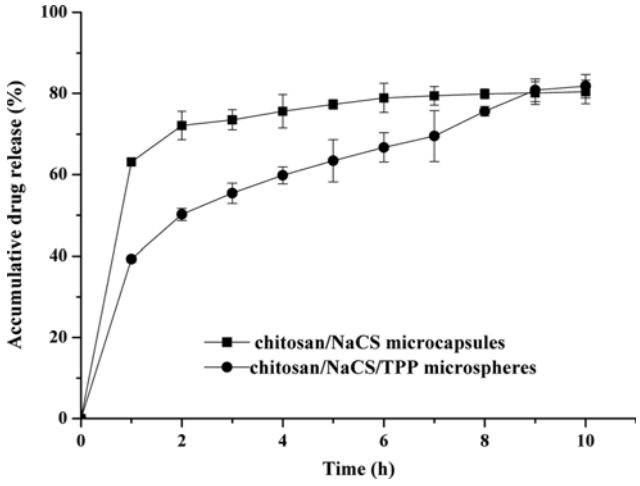

Fig. 4. BSA release profiles of different microcapsules in the simulated colonic fluid (SCF) at pH 6.4. Each point is expressed as mean±SD from n=3, significant difference from drug release of chitosan/NaCS/TPP microspheres at the first hour ($P<0.05$).

released irregularly during the 10 h study, which was not suitable for use as a sustained drug carrier according to the Ritger-Peppas model [13] (less than 60.0%). Since PEC formed with chitosan and NaCS was a hydrophilic and swellable system, and can be degraded by the enzyme in SCF [3,5], the drug release would be a combination of diffusion and macromolecular relaxation processes followed by the erosion procedures of the system [14]. The chitosan/NaCS/TPP microcapsules prepared here could be used as a protein carrier in a further appropriate formulation for controllable release of drug in colon.

CONCLUSIONS

A novel ternary composite microsphere based on chitosan, NaCS and TPP was prepared, with chitosan/NaCS microspheres in comparison. The results showed that the ternary composite microspheres were rather round with apparent double-walled structure. It is speculated that double-walled microspheres were composed of chitosan, NaCS and TPP by the FTIR results. *In vitro* release studies showed that the chitosan/NaCS/TPP microcapsules had regular release behavior in SCF and were able to release the drug sustainably and almost completely. The results indicated that the chitosan/NaCS/TPP microcapsules in the form of PEC could be used as a protein carrier for drug release in the colon.

ACKNOWLEDGEMENT

The project was supported by the Natural Science Foundation of Jiangsu Province (Nos. BK20131406, BK20130917), the Natural Science Foundation for Colleges and Universities in Jiangsu Province (No. 14KJB530003), and the Ministry of Education Research Fundation for the Doctoral Program (No. 20123221120011).

REFERENCES

1. W. S. Xia, P. Liu and J. Liu, *Bioresour. Technol.*, **99**, 6751 (2008).
2. D. A. Fluri, C. Kemmer, M. D. E. Baba and M. Fussenegger, *J. Controlled Release*, **131**, 211 (2008).
3. M. J. Wang, Y. L. Xie, Q. D. Zheng and S. J. Yao, *Ind. Eng. Chem. Res.*, **48**, 5276 (2009).
4. Y. L. Xie, M. J. Wang and S. J. Yao, *Langmuir*, **25**, 8999 (2009).
5. L. Y. Zhu, D. Q. Lin and S. J. Yao, *Carbohyd. Polym.*, **82**, 323 (2010).
6. L. Y. Wang, Y. H. Gu, Z. G. Su and G. H. Ma, *Int. J. Pharmaceut.*, **311**, 187 (2006).
7. X. Z. Shu and K. J. Zhu, *Int. J. Pharmaceut.*, **233**, 217 (2002).
8. S. J. Yao, *Chem. Eng. J.*, **78**, 199 (2000).
9. Q. X. Wu and S. J. Yao, *Colloids Surf. B.*, **109**, 147 (2013).
10. F. X. Liu, L. R. Liu, X. M. Li and Q. Q. Zhang, *J. Mater. Sci. Mater. Med.*, **11**, 2215 (2007).
11. F. L. Mi, S. S. Shyu, S. T. Lee and T. B. Wong, *J. Polym. Sci.: Part B: Polym. Phys.*, **37**, 1551 (1999).
12. Q. X. Wu, Q. L. Zhang, D. Q. Lin and S. J. Yao, *Int. J. Pharmaceut.*, **455**, 124 (2013).
13. P. L. Ritger and N. A. Peppas, *J. Controlled Release*, **5**, 37 (1987).
14. S. K. Lanjhiyana, P. Bajpayee, K. Kesavan, S. Lanjhiyana and M. S. Muthu, *Expert. Opin. Drug Deliv.*, **10**, 5 (2013).