Synthesis of Modified Chitosan as Medicine Sustained Releasing beads and Properties Researching

Ping Ding, Ke-Long Huang * (for supervisor), **Gui-Ying Li**

College of Chemistry and Chemical Engineering, Central South University, Changsha, 410083 P. R. China
E-mail: ping--989@163.com

Introduction

Chitosan, the deacetylated derivative of chitin, is the second most abundant polysaccharide found on earth next to cellulose. As a natural renewable resource, chitosan has a number of unique properties such as antimicrobial activity, nontoxicity, biocompatibility and biodegradability, which attract scientific and industrial interest in such fields as biotechnology, pharmaceutics, wastewater treatment, cosmetics, agriculture, food science, and textiles[1-2]. Furthermore, many recent reports discussed the use of chitosan and modified chitosan in sustained release formulations and colon-targeting delivery systems[3-7]. However, in the thrust of our interest in this natural polymer we came across some contradicting reports regarding the pH-dependency of drug release from inter matrices[8]. Accordingly, we were prompted to search for novel chitosan-based matrices that allow pH-independent drug release. An ideal sustained-released matrix should release the embedded drug at a constant rate regardless to the pH of the surrounding medium[9]. The aim of this paper is to report the preparation of a novel Chitosan a-ketoglutaric acid(KCTS) and Hydroxamated chitosan a-ketoglutaric acid (HKCTS), and the polymeric beads of KCTS-Fe and HKCTS-Fe loading oral theophylline medicine are prepared as the sustained release of oral theophylline medicine by iron（Ⅲ）crosslinking. The generated beads were then investigated in vitro for their capacity to achieve prolonged theophylline release.

2. Materials and methods

2.1. Chemicals

Chitosan (CTS, MW4.9x10^5, degree of deacetylation: 95%) was procured from Dalian Xindie Chitin Co., Ltd. Theophylline was provided by Qingdao Medicine Institute. α-ketoglutaric acid was purchased from Qianshan Science and Technology Development Company, Zhuhai of China. The other reagents were of analytical grade and used without further purification.

2.2. Synthesis of KCTS and HKCTS

KCTS was prepared as described reference [10]. KCTS (4.5 g) was dissolved in water (100 ml). Subsequently, the pH of the polymeric solution was adjusted to 4.0–4.5 using hydrochloric acid solution (1.0N). Afterwards, dicyclohexylcarbodiimide(DCCI, 0.74 g) was added to the stirred mixture. Two hours later, hydroxylamine hydrochloride (4.5 g,) was added and the reaction was further stirred for 1 h, then the pH of the reaction was raised to 6.0 using sodium hydroxide solution (1.0 N), and the mixture was stirred for 2 h. Thereafter, the pH was raised again to pH 9.0, and the reaction mixture was stirred over 24 h at room temperature. The reaction was terminated by precipitation with concentrated HCl (20 ml) and acetone (200 ml). The precipitated polymer

was filtered, washed 3-4 times with ethanol, followed by acetone and diethyl ether, respectively. The polymer was dried in an infrared drier.

2.3 Preparation of the loaded polymeric beads and crosslinking with Fe

The particular polymer (HKCTS or KCTS, 1.0 g) was dissolved in 0.1 N sodium hydroxide solution (20 ml).Theophylline (1.0 g) was added to the viscous polymeric solution and the mixture was stirred for 40 min. The resulting viscous suspension was carefully dropped, using glass dropper, into a stagnant aqueous solution of ferric chloride (0.5%, w/v, 100 ml). The viscous droplets were left to cure in the ferric chloride solution over 2 h to generate dark yellow beads. Bead preparation and curing was carried out under ambient room temperature. The beads were then collected and washed twice with deionized water (50 ml) and left to dry at room temperature over 24–48 h. The beads were evaluated using microscopy.

2.4. characterization

The IR spectra of KCTS, HKCTS, KCTS-Fe and HKCTS -Fe matrices were recorded on a Hitachi 270-50 IR spectrophotometer using KBr discs. The particle size distribution of the microspheres was analysed using a laser-based particle size analyzer (Galai, CIS-1, Israe) as well as using standard test sieves(Filterwel, Bombay, India). Fractions that passed through each sieve were collected, weighed on an analytical balance and the distribution calculated.

3. Results and discussion

3.1 Infrared characterization of KCTS, HKCTS, KCTS-Fe and HKCTS -Fe matrices

As we have known from Fig.2. The IR spectrum of KCTS shows a carboxyl carbonyl band at 1730 cm^{-1} and an amide carbonyl band at 1645 cm^{-1}; The IR spectrum of HKCTS shows the presence of a new pronounced sharp carbonyl band at 1636 cm^{-1} corresponding to the newly formed hydroxamic carbonyl groups; these indicate that both KCTS and HKCTS were modified successfully. In the IR spectrum of KCTS-Fe and HKCTS-Fe, both carboxyl and amide carbonyls were shifted to lower frequencies upon complexation to iron, i.e., from 1645 to 1560 cm^{-1} for amide carbonyls and from 1730 to 1654 cm for carboxylic carbonyls, and the newly formed hydroxamic carbonyl groups at 1636 cm^{-1} in HKCTS-Fe were also shifted to lower frequencies upon complexation to iron. Such shifts indicate that both carboxylic and amide groups are coordinated to ferric ion as shown is Scheme 1 and Scheme 2.

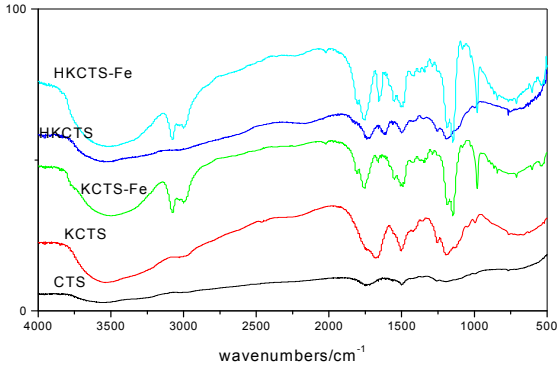

Fig.2 FT-IR spectra for KCTS、KCTS-Fe、HKCTS、HKCTS-Fe and CTS

3.2 Morphology of Fe crosslinked polymeric beads

Upon qualitative visual inspection using microscopy, the prepared beads were dark brown in color, spherical biconcave in shape. It could be found that the surface of *polymeric beads* was smooth. They underwent approximately 5-fold reduction in their size upon drying. A photograph illustrating the generated beads is shown in Fig. 3. Detailed particle size analysis, for these beads as well as others from related newly developed polymers, is to be published in the near future.

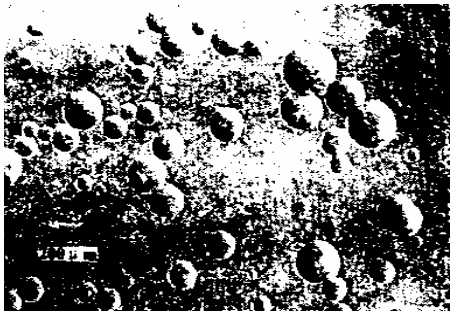

Fig. 3 The optical microscope image for polymer beads

3.3 particle size analysis

The particle size distribution curve for KCTS-Fe microspheres is shown in Fig.4. About 50% of the measured particles were found to have a mean particle size under 89.41 μm. About 10% of the microspheres were below 14.59 μm in size range and 90% were below 250.70 μm. In the case of HKCTS-Fe microspheres, as shown in Fig.5 the particle size of 50% of the spheres was below 35.78 μm and 10% was below 6.73 μm. About 90% of the spheres were below 79.24 μm. The range of HKCTS-Fe microspheres was found to be smaller than KCTS-Fe microspheres. Aggregation of smaller spheres could be attributed to the very small amounts of very large particles in the size distribution curve of HKCTS-Fe microspheres. Therefore, the properties of theophylline-loaded HKCTS-Fe are superior to that of theophylline-loaded KCTS-Fe obviously.

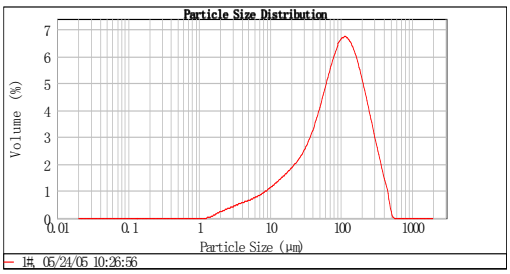

Fig.4 The particle size distribution of KCTS-Fe microspheres

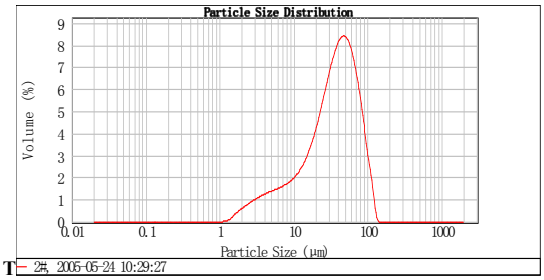

Fig.5 The particle size distribution of HKCTS-Fe microspheres

3.4 Drug dissolution profiles from KCTS-Fe and HKCTS-Fe beads

Fig. 6 illustrates the dissolution profiles of theophylline from HKCTS-Fe and KCTS-Fe beads. By comparing the two polymeric matrices, it is clearly evident that the initial release rate of theophylline from HKCTS-Fe was higher than that of KCTS-Fe, which is probably related to the higher amount of loaded theophylline in HKCTS-Fe (202.2±5.2 mg per 1 g KCTS-Fe and 282.3 ±6.4mg per 1 g HKCTS-Fe). Higher levels of loaded drug are anticipated to lead to a wider concentration gap between the polymeric beads and the dissolution medium, leading to initially higher dissolution rate, and thus causing the observed initial difference between HKCTS-Fe and

KCTS-Fe. However, as the amounts of theophylline within the polymeric beads gradually decrease, the degree of cross-linking becomes the major factor controlling the release. After 2 h, the released amounts from KCTS-Fe were higher than that of HKCTS-Fe, which is probably related to the higher degree of crosslinking within the hydroxamated polymeric matrix resulting from the superior iron–hydroxamate affinity. Moreover, the probable involvement of hydroxamate–iron and amide/ carboxylate–iron complexes in Fe-HKCTS is expected to further contribute to its extensive crosslinking.

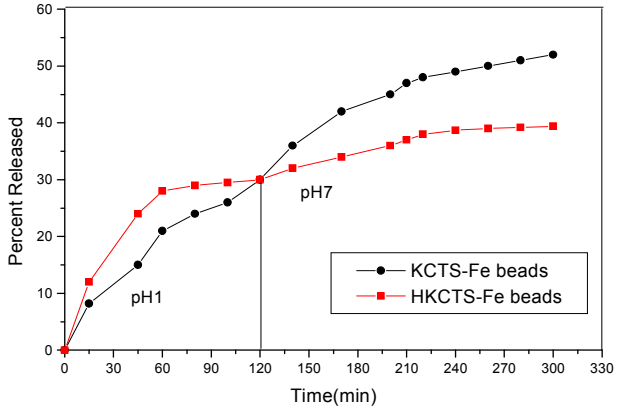

Fig.6 The curve of average theophylline released (%)

Conclusions

In this study, KCTS and HKCTS were prepared successfully, theophylline-loaded KCTS-Fe and HKCTS-Fe beads were prepared, and it was well shown to retard drug release under physiologically simulated pH conditions. By comparing with the two polymeric matrices, the properties of theophylline-loaded HKCTS-Fe are superior to that of theophylline-loaded KCTS-Fe obviously. Therefore, preparation of theophylline-loaded HKCTS-Fe and KCTS-Fe polymeric beads via such a chemical crosslinking process may improve their usage for biomedical applications.

References

1 Cristiani, K., Daniela, V., Silvana, S., Clovis, A.R. J. International Journal of Pharmaceutics, 2001, 223:29-33

2 Gupta, K.C., Kumar, M.N.V.R.. J. Sci. Ind. Res. , 2000, 59: 201–213

3 Tokumitsu H, Ichikawa H, Fukumori Y. Pharm Res 1999;16:1830.

4 Seferian PG, Martinez ML. Vaccine 2001;19:661.

5 Takeuchi H, Yasuji T, Yamamoto H, Kawashima Y. Pharm Res 2000; 17:94.

6 Kofuji K, Ito T, Murata Y, Kawashima S. Chem Pharm Bull 2000;48: 579.

7 Kato Y, Onishi H, Machida Y. J Controlled Release 2001;70:295.

8 Aiedeh, K., Taha, M.O... J. Arch. Pharm, 1999, 332: 103–107

9 Taha, M.O., Aiedeh, K.. J. International Journal of Pharmaceutics, 2000, 55: 663–667

10 Quanhong Ma, Yonghong Gao, Zongbai Zou. J. Chemical Reagent, 2000, 22(6), 327-330