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Optimization on preparation conditions of calcium-crosslinked modified chitosan as potential matrix material for theophylline sustained-release beads and its evaluation of release kinetics

Cuimei Chen [a, d], Ming Zhong [b], Guiyin Li [c], Fei Yang [a], Ruixue Huang [a], Wen Xiao [a], Ping Ding [a, *]

[a] School of Public Health, Central South University, Changsha, Hunan, 410078 China
[b] School of Chemistry and Chemical Engineering, Hunan Institute of Science and Technology, Yueyang, Hunan 414006, China
[c] School of Life and Environmental Sciences, Guilin University of Electronic Technology, Guilin, Guangxi 541014, China
[d] Yuelu Center for Disease Control and Prevention, Changsha, Hunan 410013 China

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ABSTRACT

This study aimed to prepare theophylline loaded hydroxamated chitosan alpha-ketoglutaric acid (HKCTS) microspheres by ion crosslinking method, and optimize the process and formulation variables using response surface methodology. The independent variables studied were pH value, concentrations of Ca(II) ion and HKCTS/theophylline ratio. The dependent variables (responses) were drug loading content and encapsulation efficiency. Mathematical equations and response surface plots were used to relate the independent and dependent variables. The release kinetics was evaluated by fitting the experimental data to standard release equations (zero-, first- and Higuchi equation). Results showed that the optimal conditions for microsphere formulation were 9.6 mg/mL Ca(II)ion, 1:1 (g/g) HKCTS/theophylline ratio and pH 9.1, the drug loading content and encapsulation efficiency were 53.40% and 69.61%, respectively. The best fit was found with Higuchi model for the microspheres. The response surface methodology could provide a promising application for calcium cross-linked modified chitosan beads as a new matrix for controlled release of theophylline.

1. Introduction

Theophylline is a widely used methylxanthine drug in the treatment of the patients with moderate to severe reversible bronchospasm. The exploitation of extended release formulation is necessary because of the side effects in clinical practice and associate central nervous system of the fluctuations of serum theophylline level [1]. Using macromolecules to modify the surface of drug particles is a very efficient method to fit their adhesive, wetting, adsorbate and mechanical performance when trying to lessen the difficulty of the production of a medicine [2]. Because of the good reproducibility of sustained and precise releasing of coated dosage forms, polymeric film coatings have been widely used as an active substance from pharmaceutical dosage forms for release controlling [3,4]. Chitosan is the second most luxuriant polysaccharide found on earth in close proximity to cellulose, it is produced from chitin by partly deacetylating its acetamido groups with a strong alkaline liquor. Chitosan is a natural renewable resource, it has attracted interest in many fields such as pharmaceutics, biotechnology, outlet water treatment, food science, cosmetics, agriculture and textile industry because of its characteristic performance such as antimicrobial activity, nontoxicity, biodegradability and biocompatibility [5,6]. Moreover, the use of chitosan and modified chitosan in colon-targeting delivery system and release formulations are widely reported in the literature [7–10], but the latent materials offer is so high that it worth continual research efforts.

Response surface methodology (RSM) is an effective statistical tool for the optimisation of multiple variables for the prediction of best performance conditions with a minimum number of experiments, which has been widely applied to optimize conditions in

* Corresponding author.
E-mail address: pingding2010@163.com (P. Ding).

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synthesis industry [11].

In this study, the polymeric beads of HKCTS-Ca loading oral theophylline medicine was prepared by ion cross-linking method, and optimized the process and formulation variables using response surface methodology. The controlled release behaviour has also been studied in simulated gastric fluid (pH 1.0) and simulated intestinal fluid (pH 7.4) medium. The release kinetics was evaluated by fitting the experimental data to standard release equations (zero-, first- and Higuchi equation).

2. Materials and method

2.1. Materials

Chitosan (CTS) was obtained from Dalian Xindie Chitin Co., Ltd, the degree of deacetylation (DD) was 95% and viscosity averaged molecular weight was 4.9×10^5 g/mol. Alpha-Ketoglutaric acid was of reagent grade and purchased from Shanghai Chemical Industry in China. Theophylline was provided by Sigma–Aldrich. All the other chemicals used in the present study were of analytical grade.

2.2. Synthesis of hydroxamated chitosan alpha-ketoglutaric acid (HKCTS)

Hydroxamated chitosan a-ketoglutaric acid (HKCTS) was prepared as the previous reference [12]. 4.5 g HKCTS was first swelling in 100 mL of 1 wt% acetic acid, and the pH of the solution was adjusted to 4.0–4.5 with hydrochloric acid. Dicyclohexylcarbodiimide (0.74 g) and hydroxylamine hydrochloride (4.5 g) were added to the above solution, and stirred for 3 h. Then, the pH value was adjusted to 9.0 with sodium hydroxide solution, and stirred over 24 h. At last, the reaction was terminated by adding 20 mL concentrated hydrochloric acid and 200 mL acetone. The reaction mixture was precipitated, followed by the filtration and washed several times with ethanol, acetone and diethyl ether, respectively. The product was purified in Soxhlet with 95% ethanol, and dried in an infrared drier.

2.3. Preparation of the loaded polymeric microspheres and crosslinking with Ca

1.0 g HKCTS was dissolved in 20 mL, 0.1N sodium hydroxide solution. Theophylline was added to the above viscous solution and stirred for 40 min. The resulting solution was dropped slowly with glass dropper into a calcium chloride aqueous solution. The adhesive droplets became white microspheres in the calcium chloride solution. The HKCTS-Ca-T microspheres were gathered and repeatedly washed in deionized water and dried at room temperature for 48 h. The microspheres were evaluated by scanning electron microscopy (SEM).

HKCTS-Ca-T microspheres was dissolved in 10 mL of 2% acetic acid aqueous solution and centrifuged to remove the polymeric debris. The clear supernatant solution was measured by UV Spectroscop at 272 nm wavelength and the weight of drug was calculated by using the calibration curve. The drug loading content and encapsulation efficiency were calculated using the following equations:

$$\text{drug loading content}(\%) = \frac{\text{drug weight in the microparticles}}{\text{weight of the microparticles}} \times 100\% \quad (1)$$

$$\text{encapsulation efficiency}(\%) = \frac{\text{drug weight in the microparticles}}{\text{initial feeding amount of the drug}} \times 100\% \quad (2)$$

2.4. Experimental design for selection of synthesis parameters

The theophylline-loaded polymeric microspheres were prepared by concentrations of Ca(II) ion (5–15 mg/mL), various temperatures (20–50 °C) and stirring rate (100–1200 rpm), with a HKCTS/theophylline ratio (g/g) (ranging from 1:1 to3:1 g/g), for a given time (from 60 to 180 min), while the pH value of deionized water ranged from 8 to 10. The study aimed to evaluate the effects of different parameters on the drug loading content (Y_1) and encapsulation efficiency (Y_2).

2.5. Experimental design and optimization by RSM

The Box-Behnken design was employed to optimize the significant factors. Regression analysis was performed to fit the response function with the experimental data. Statistical analysis of the model was used to evaluate the analysis of variance (ANOVA). Three-level design (Box-Behnken design) consisted of 17 experiments. The non-linear quadratic model generated by the design is of the form:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 \quad (3)$$

Where The coefficients of the polynomial equation were depicted by b_0 (intercept), b_1, b_2 and b_3 (linear effects), b_{11}, b_{22}, and b_{33} (quadratic effects), and b_{12}, b_{13} and b_{23} (interaction effects).The terms X_i and $X_i{}^2$ ($i = 1, 2$ or 3) depict the interaction and quadratic terms, respectively [13,14], X_1, X_2 and X_3 are the independent variables, and Y is the predicted response value. The optimum values of these process parameters were obtained using Design Expert software. 17 experiments were designed and performed in a random order (Table 1).

2.6. Statistical analysis

All the data were analysed with Design Expert software, and Second-order coefficients were obtained by regression analysis with backward elimination. The fit of the multiple regression model was evaluated by coefficients of determination and a lack-of-fit test, which was executed by comparing mean square lack of fit to mean square experimental error, from the analysis of variance (ANOVA).

2.7. In vitro drug release studies

Theophylline loaded microparticles (100 mg) were dispersed in buffer solutions (5 mL, 0.1M HCl, pH 1.0 or PBS, pH 7.4, 0.1M). The solution was placed into a dialysis membrane. The dialysis bag was then sealed and put into a glass bottle with 95 mL of PBS solution (or HCl solution). The system was stirred at 100 rpm and the temperature was 37 °C. The concentration of released theophylline in sample was determined by using UV spectrophotometer method. The concentrations of the released drug were transformed into percent-release by dividing the released quantities over the total loaded drug.