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Optimization of thermosensitive chitosan hydrogels for the sustained delivery of venlafaxine hydrochloride

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ABSTRACT

Chitosan/glycerophosphate disodium (GP) thermosensitive hydrogels were prepared for the sustained delivery of venlafaxine hydrochloride (VH) and optimization of this formulation was mainly studied. Release mechanism was investigated by applying various mathematical models to the in vitro release profiles. Overall, drug release from the hydrogels showed best fit in first-order model and drug release mechanism was diffusion-controlled release. Optimization of VH chitosan/GP thermosensitive hydrogels was conducted by using a three-level three-factorial Box–Behnken experimental design to evaluate the effects of considered variables, the strength of the formulation, chitosan concentration and GP amount, on the selected responses: cumulative percentage drug release in 1 h, 24 h and the rate constant. It presented that higher strength and GP concentration resulted in higher initial release and rate constant, which supported the hypothesis that the kinetic gelation mechanism of this system was nucleation and growth. Drug release profiles illustrated that controlled drug delivery could be obtained over 24 h, which confirmed the validity of optimization. In vivo pharmacokinetic study was investigated and it demonstrated that compared with VH solution, chitosan/GP thermosensitive hydrogels had a better sustained delivery of VH.

1. Introduction

Hydrogels are polymeric networks, which absorb and retain large amounts of water. The research on hydrogels with respect to drug delivery and biomedical devices has attracted much attention because of their biocompatible properties and easy control of solute transport. One of the more recent trends in hydrogel research is in situ hydrogel formation by phase transition (Jeong et al., 2002). In situ hydrogel formation makes it more feasible to apply hydrogels for drug delivery, tissue engineering and cell encapsulation (Ruel-Gariepy and Leroux, 2004). A particularly interesting and important polymeric system is hydrogel forming solutions by a simple phase transition (sol–gel transition) in water without any chemical reaction or external stimulation.

Most natural polymers form a gel phase on lowering the temperature. However, an interesting reverse thermogelation of a combination of chitosan and glycerophosphate disodium salt (GP) was reported by Chenite et al. (2000). Chitosan is a linear polysaccharide, composed of glucosamine and N-acetyl glucosamine linked in a β (1–4) manner. In its crystalline form, chitosan is normally insoluble in aqueous solutions above pH 7, however, in dilute acids, the protonated free amino groups on glucosamine facilitate solubility of the molecule. Depending on the source and preparation procedure, its molecular weight may range from 300 to over 1000 kDa with a degree of deacetylation from 30% to 95% (Martino et al., 2005).

A typical solution was obtained by mixing a chitosan solution and a GP solution. At neutral pH, the formulation was a homogeneous, clear liquid at room temperature and became a gel in the vicinity of 37 °C. The gelation temperature increased with a decrease in the degree of deacetylation of the polymer, but was not significantly influenced by the molecular weight of the chitosan (Chenite et al., 2001). Properties such as biodegradability, low toxicity and good biocompatibility make chitosan suitable for use in biomedical and pharmaceutical formulations. The applications of the chitosan/GP thermosensitive hydrogel as an injectable scaffold in angiogenesis, bone repair and cartilage regeneration were discussed in literature (Hou et al., 2004). Chitosan can be applied onto the nasal epithelium. The positive charge on chitosan polymer gives rise to strong electrostatic interaction with mucus or negatively charged sialic acid residues on the mucosal surface (Sinha et al., 2004). Local delivery of paclitaxel from the chitosan/GP system injected intratumorally was investigated using EMT-6 tumors implanted subcutaneously on Balb/c mice. The results suggested that one intratumoral injection of the thermosensitive hydrogel

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containing paclitaxel was as efficacious as four intravenous injections of Taxol in inhibiting the growth of EMT-6 cancer cells in mice, but in a less toxic manner (Ruel-Gariepy et al., 2004). Liposomes were incorporated with the chitosan/GP hydrogel and their effect on the viscoelastic properties of the system and release kinetics of encapsulated carboxyfluorescein was investigated. The in vitro release profiles demonstrated controlled delivery over at least 2 weeks (Ruel-Gariepy et al., 2002).

In the present study, in order to study the chitosan/GP thermosensitive hydrogels for the sustained delivery of water soluble drugs, venlafaxine hydrochloride (VH), an antidepressant of the serotonin-norepinephrine reuptake inhibitor (SNRI) class was taken as the model drug based on its high water solubility and good liner pharmacokinetics (Wikell et al., 2002), and a nasal or injectable chitosan/GP thermosensitive system was developed. The release dynamics of VH from hydrogels were discussed, for the evaluation of the release mechanism and diffusion coefficients. A three-level three-factorial Box–Behnken experimental design was applied to evaluate the effects of the considered variables and response surface methodology (Nutan et al., 2005) was utilized to optimize the formulation variables.

2. Materials and methods

2.1. Materials

VH was purchased from Dingkang Corp. (Shanghai, China). Chitosan with the deacetylation of 95% was obtained from Haidebei Corp. (Jinan, China). α,β-GP was provided by Ziguang Corp. (Beijing, China). All other chemicals were reagent grade and used as received.

Rabbits were provided by Animal Center of Shenyang Pharmaceutical University (Shenyang, China).

2.2. Preparation of thermosensitive hydrogels

Chitosan (1–3%, w/w) was slowly added into 0.2 M hydrochloric acid under stirring (300 rpm), and the mixture was continuously stirred for 24 h to make a solution (4.5%, w/w). Various amounts of GP (8–16%, w/w) and VH (25–75% mg/g) were dissolved in deionized water separately. Droplets of GP solution were added to the chitosan solution in an ice bath and then the obtained solution was mixed in this ice bath for 5 min. The final formulation was prepared by adding the VH solution droplets into the GP–chitosan solution at 4 °C under stirring (200 rpm) for 10 min. The flow chart of this preparation procedure was shown in Fig. 1.

2.3. Release experiments

Samples of 3 g of the preparation were carefully distributed into glass vials (diameter 25 mm) and kept warm in a water bath at 37 °C for 1 h so that they were transformed into gel sufficiently. Then 15 ml phosphate buffered saline (pH 7.4) were added into the dissolution vials, which were placed in a shaking incubator at 37 °C and 50 rpm. At the allotted times, all the release medium were collected for analysis and the dissolution vials were replenished with fresh buffer. The concentration of VH in the medium was spectrophotometrically measured at 274 nm.

2.4. Experimental design

A three-level three-factorial Box–Behnken experimental design (Design Expert, Version 7.0.2, Stat-Ease Inc., Minneapolis, MN) was used to evaluate the effects of selected variables, the strength of the formulation, chitosan concentration and GP amount, on the responses, to characterize the drug release process and to optimize the formulation parameters. This design is suitable for exploring quadratic response surfaces and constructing second order polynomial models. The design consists of replicated center points and the set of points lying at the midpoint of each edge of the multidimensional cube that defines the region of interest. The nonlinear quadratic model generated by the design is of the form:

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

where Y represents the response associated with each factor level combination, b_0 an intercept and b_1–b_{33} are the regression coefficients of the factors (Box and Behnken, 1960; Chopra et al., 2007b). The factors chosen and settings of factor levels were presented in Table 1.

Table 1
Variables in Box–Behnken experimental design. X_1 is factor of strength of the formulation, X_2 is factor of chitosan concentration, X_3 is factor of glycerophosphate disodium (GP) amount, Y_1 is response of cumulative percentage drug release in 1 h, Y_2 is response of cumulative percentage drug release in 24 h, Y_3 is response of the rate constant.

Factor	Level		
	−1	0	+1
X_1 Strength (mg/g)	25	50	75
X_2 Chitosan % (w/w)	1.4	1.8	2.2
X_3 GP % (w/w)	8	12	16

Response	Constraints
Y_1% Released VH in 1 h	Minimize
Y_2% Released VH in 24 h	Maximize
Y_3 Rate constant k	In range

2.5. In vivo pharmacokinetic study

Eight healthy rabbits (female or male, mean body weight 2.0–2.5 kg) provided by the Animal Center of Shenyang Pharmaceutical University were used to perform in vivo pharmacokinetic study. The experimental procedures complied with the University Animal Ethics Committee Guidelines. The study was approved by the University Animal Ethics Committee.

The rabbits were randomly divided into two groups, including VH solution group and VH hydrogels group. Four rabbits in VH solution group were administered 25 mg doses of VH saline solution via subcutaneous while rabbits in the other group were administered 25 mg doses of VH chitosan/GP thermosensitive hydrogels via subcutaneous. After administration, blood samples (2 ml) were withdrawn from ear marginal vein at predetermined time intervals with heparinized tubes and centrifuged to get plasma. Subsequently, 500 μl of plasma was added into a test tube and mixed with 100 μl of the internal standard (600 ng/ml levamisole hydrochloride aqueous solution) and 200 μl sodium hydroxide solution (0.2 mol/l). The sample was diluted with 3 ml of ether, and the mixture was vortexed for 5 min. The mixture was then centrifuged for 10 min, and the organic phase was withdrawn into another test tube. 100 μl hydrochloride acid solution (0.01 mol/l) was added into this test tube and then the obtained mixture was vortexed for 5 min followed by centrifuged for 10 min. The obtained organic phase was withdrawn and dried under a nitrogen stream to evaporate ether. 20 μl of this sample was injected into the HPLC system. Pharmacokinetic parameters were obtained by using the Kinetica Pharmacokinetic Software Version 4.4.1.