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Research on chitosan-agarose microspheres with berbamine

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ABSTRACT

The preparation of berbamine-loaded chitosan-agarose microspheres was studied. Optimum preparing parameters were determined by orthogonal experiments as follows: ratio of berbamine to chitosan (w/w) is 1:10; percentage of emulsifier (span 80, v/v) is 6%; volume of glutaraldehyde is 2 mL; and reaction temperature is 70°C. Under these optimal conditions, the encapsulation efficiency and loading capacity of microspheres are 84.57% and 8.44%, respectively. The berbamine-loaded microspheres were spherical with smooth surface, uniform size and without aggregation morphology. In vitro release studies showed that berbamine was released from microspheres in a significantly sustained fashion.

Introduction

Microspheres have received great attention because of a variety of applications such as delivery vesicles for drugs, DNA, antigens, and protection proteins and enzymes, especially for controlled or sustained drug-delivering systems[1-2]. Generally, To make drugs encapsulated into microspheres may mask taste and odour, stabilize the quality of the drug, improve gastrointestinal tolerance and provide sustained release after oral administration. There has been considerable interest in developing microspheres prepared from chitosan matrices as effective drug delivery devices. Owing to its nontoxicity, biodegradability, biocompatiblity, mucoadhesion and antibacterial, chitosan, a natural polysaccharide, has been extensively applied in various areas, especially in the pharmaceutical and biomedical fields, such as drug delivery, wound dressing and antimicrobial agents[3-4]. It is reported that chitosan microspheres showed high cytotoxic activity toward tumor cells, while low toxicity against normal human liver cells[5]. The unique cationic character of chitosan microspheres could also provide higher affinity with negatively charged biological membranes and site-specific targets in vivo[6-7]. Agarose obtained from red seaweeds is a natural polysaccharide. It is of unique characteristic of forming a heat-reversible gel in dilute aqueous solution that is hydrophilic and macroporous. Agarose microspheres have been widely used in chromatographic separation, food industry, cell encapsulation and pharmacy as their mechanical properties and size can be readily controlled[8-10].

Berbamine (Fig.1), a natural compound from the plant *Berberis amurensis*, is a *bis*-benzylisoquinoline alkaloid and has been widely used in China for leukopenia treatment over the past decades. Berbamine is a calmodulin antagonist which could influence many functions of mammalian cells by binding with calmodulin. Clinical studies showed that berbamine could stimulate normal hematopoiesis of cancer patients undergoing chemotherapy or radiotherapy and has been used to protect tumor patients from cytotoxic effects of chemotherapeutic agents on bone marrow. Meanwhile, berbamine has anti-inflammatory, anti-arrhythmic effects and antineoplastic activity[11-12]. However, on oral administration, excessive berbamine produces some side effects, such as jaundice, stomach upset, lethargy, nose bleed, skin and eye irritation, kidney irritation, and so on. Therefore, it is essential to deliver berbamine at the intended therapeutic concentrations to the target sites to elicit its activity. Sustained delivery of berbamine may reduce the systemic side effects and provide effective and safe therapy of leukopenia that may reduce the dose and duration of therapy when compared with the conventional treatment.

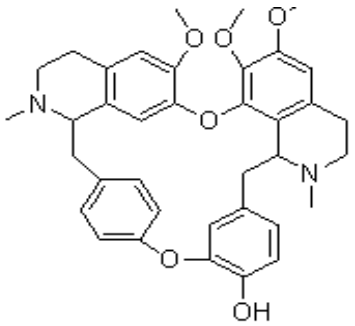

Fig. 1 The structure of berbamine

In this paper, the microspheres were prepared using the composite polymers, chitosan and agarose, and berbamine was chosen as a model drug to test the release behavior of the microspheres.

Materials and methods

Materials

Chitosan with a degree of deacetylation of 90% was purchased from Guoyao Biochemical Co. Ltd, Shanghai, China. Agarose was a gift from Taixing bio-tec Co., Ltd Lianjiang, China. Berbamine was purchased from Sichuan Shikun Medical Raw Materials Co., Ltd. Liquid paraffin, span 80, glutaraldehyde, acetone and acetic acid were of analytical grade.

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Preparation of microspheres

The microspheres were prepared by water-in-oil (w/o) emulsion technique and were further utilized to encapsulate berbamine. Chitosan solutions were prepared by dissolving 1.5 g chitosan in 150 mL of 1% acetic acid to form 1 % (w/v) chitosan solutions. Agarose solutions were prepared by dissolving 0.75 g agarose powder in 150 mL distilled water to form 5‰ (w/v) agarose solutions. Chitosan solutions were slowly added into the agarose solutions while stirring to form the aqueous solutions. Known weight of berbamine was completely dispersed in 50 ml of liquid paraffin under sonication as the oil phase. Span 80 was used as an emulsifier. A w/o emulsion was prepared by slowly adding the above-mentioned 30 mL aqueous solutions to the oil phase at a set temperature while being agitated by a homogenizer. Stirring was continued for 1 h until a stable w/o emulsion was obtained. After addition of 25% (w/w) aqueous glutaraldehyde as the crosslinking agent, the mixture was stirred for another 2 h. The emulsion was quickly cooled to 30°C in a water bath allowing the formation of soft solid chitosan-agarose microspheres. The microspheres were collected by repeated washing, centrifugation and finally dried in a vacuum desiccator. The preparation process variables of microspheres were optimized by orthogonal experiment.

Evaluation of drug loading efficiency

50 mg microspheres were accurately weighed to the vial, and treated with ultrasonic at 60°C for 3h in a water bath after the addition of 50 mL, 0.1 mol/L of HCl solution. The suspension was centrifuged, then the supernatant was decanted and diluted appropriately. The amount of free drug in the supernatant was measured with a spectrophotometer at 282 nm. The encapsulation efficiency (EE) and loading capacity (LC) of microspheres were calculated as follows:

$$EE = \frac{M_1 - M_2}{M_1} \times 100 , LC = \frac{M_1 - M_2}{M_3} \times 100$$

Where M1 is the total amount of berbamine; M2 is the amount of free berbamine; M3 is the microspheres weight. All measurements are performed in triplicate.

Morphological characterization

The appearance and morphology were observed by XSZ-107 optical microscope (China). Before the samples were analyzed, an ethereal suspension of microspheres was allowed to dry on a clean slide to form a thin film.

Evaluation of in vitro drug release

Known amounts of the microspheres were suspended in phosphate buffer solution (pH 7.4, 20 mL) and incubated at 37°C under stirring. At varying time points, aliquots of sample were withdrawn from the receiver compartment. The supernatants were isolated by centrifugation and assayed to detect the drug release by UV spectroscopy at 282 nm. After each sampling, the same volume of fresh phosphate buffer was added to the receiver compartment to maintain the constant volume.

Results and discussion

Optimization of microspheres preparation process

Chitosan-agarose microspheres were prepared by w/o emulsification process. The optimum conditions for preparation of microspheres were investigated by independent variation of four parameters: A-ratio of berbamine to chitosan (w/w), B-percentage of emulsifier (span 80, v/v), C-volume of glutaraldehyde (mL) and D-reaction temperature. For each parameter, the three values were investigated. The investigated variables and their test levels were listed in Table 1. Reference to the experimental design theory, the orthogonal array L9 (34) was selected arrange the test program. The encapsulation efficiency (EE) and loading capacity (LC) were designated as the quality indexes (QI). The test results were shown in Table 2.

As shown in Table 2, volume of glutaraldehyde was the most important factor to the microspheres preparation among the four selected factors according to R value. The importance of four factors to the microspheres preparation is in a sequence from high to low as follows: volume of glutaraldehyde, reaction temperature, ratio of berbamine to chitosan, percentage of emulsifier. Obviously, the optimum level of each variable is A-2, B-3, C-2, and D-2 according to the mean value. Thus the optimum reaction conditions were as follows: ratio of berbamine to chitosan (w/w), 1:10; percentage of emulsifier (span 80, v/v), 6%; volume of glutaraldehyde, 2 mL; and reaction temperature, 70°C. Under these optimal conditions, the encapsulation efficiency and loading capacity of microspheres are 84.57% and 8.44%, respectively.

Microsphere morphology

Optical microscopic images of chitosan-agarose microspheres and berbamine loaded chitosan-agarose microspheres were showed in Fig. 2. The chitosan-agarose microspheres possessed uniform sizes with smooth surfaces. After berbamine entrapment, the microspheres were still maintained spherical with smooth surfaces and without aggregation. They had even distribution of particle diameters, ranged from 10 to 20 μm.

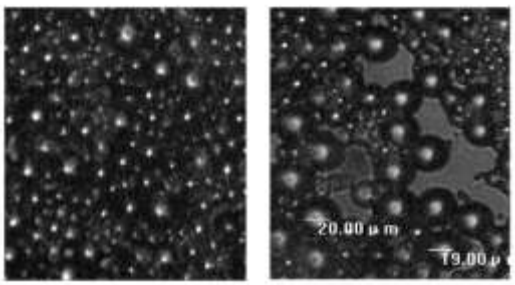

Fig. 2 Morphological features of chitosan-agarose microspheres (Left) and berbamine loaded chitosan-agarose microspheres (right).

In vitro release of berbamine

The cumulative percent of berbamine released from microspheres versus time plots were presented in Fig 3. It is obvious that in the early stages berbamine release in vitro exhibited a burst (≈ 26%) suggesting that few adsorbed drugs were soon released from the surface of microspheres. Then more than 60 % berbamine was released from microspheres by 12 h indicating that most berbamine drugs were included inside the cross-linked shell of microspheres. Apparently, it showed a sustained release pattern indicating the excellent nature of chitosan-agarose microspheres to release the drug

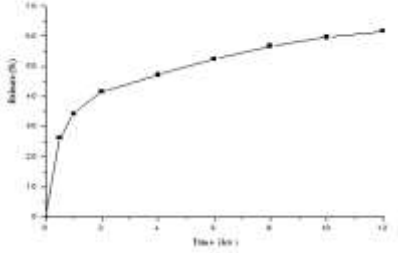

Fig. 3 In vitro release profile of berbamine from microspheres

Conclusions

We have successfully prepared the chitosan-agarose composite microspheres for berbamine delivery using emulsification method by orthogonal design. Encapsulation efficiency and loading capacity of microspheres were 84.57% and 8.44%, respectively. Light microscopic images exhibited that the microspheres were of good morphological features with smooth surfaces, uniform sizes and without aggregation. In vitro studies demonstrated that berbamine was released from microspheres in a significantly sustained fashion.

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Table 1 The investigated variables and their levels

Variables investigated	Levels of each variable		
	1	2	3
A: ratio of berbamine to chitosan (w/w)	1:5	1:10	1:15
B: percentage of emulsifier (span 80, v/v)	2%	4%	6%
C: volume of glutaraldehyde (mL)	1	2	3
D: reaction temperature (°C)	60	70	80

Table 2. Experimental arrangement and test results

Experiment number	Variables investigated				EE %	LC %	OI(EE%+LC%)
	A	B	C	D			
1	1	1	1	1	12.42	3.50	15.92
2	1	2	2	2	83.71	8.24	91.95
3	1	3	3	3	48.33	6.52	54.85
4	2	1	2	3	77.62	7.68	85.30
5	2	2	3	1	53.27	5.52	58.79
6	2	3	1	2	61.36	6.70	68.06
7	3	1	3	2	44.29	4.26	48.55
8	3	2	1	3	37.91	6.26	44.17
9	3	3	2	1	68.27	6.18	74.45
k_1	54.24	49.92	42.72	49.72			
k_2	70.72	64.97	83.90	69.52			
k_3	55.72	65.79	54.06	61.44			
R	16.48	15.87	41.18	19.80			