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RESEARCH ARTICLE

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Preparation of porcine hemoglobin microcapsules of chitosan-sodium alginate

Abstract Using an emulsification-gelation method, chitosan-sodium alginate-porcine hemoglobin microcapsules were prepared. Results show that these microcapsules have better forms and small granules with 1 μm size of the mean particle size. They possess a relatively narrow and normal Gaussian distribution. The loading efficiency of porcine hemoglobin (pHb) in microcapsules is more than 90%. The pHb released from microcapsules is extended for more than one month. Chitosan-sodium alginate-hemoglobin microcapsules are expected to become an artificial oxygen-carrying therapeutic agent with sustained release for intravenous injection.

Keywords chitosan-sodium alginate, microcapsules, hemo-globin, blood substitute

1 Introduction

It has been the aim of studies on blood substitutes to produce artificial red blood cells by encapsulating hemoglobin (Hb) with biodegradable polymeric material simulating the natural surface and inside of red blood cells. Red blood cells possess characteristics such as good permeability and compatibility similar to biomembranes, prolonged half-life time, and can avoid chemical modification. Red blood cells encapsulate ferrihemoglobin reduction system to cause kidney toxicity by rapid degradation of the Hb tetramer. Hb has normal function to transfer oxygen effectively. Red blood cells encapsulate allo-steric modulator 2, 3-diphosphoglycerate (2, 3-DPG) to lower Hb's affinity to oxygen and increase its oxygen-transferring capacity.

As natural polysaccharides, chitosan and sodium alginate are biocompatible and biodegradable [1,2]. Sodium alginate possesses the characteristics of instant gelation when it touches multivalent cations such as Ca^{2+}. Therefore, protein drugs can be encapsulated in moderate conditions. This simple process avoids high temperature, organic solvents and other hazardous conditions, thus helping to keep the bioactivity of the protein [3,4]. Chitosan, with the characteristics of cations, can form a layer of polyelectrolyte semipermeable membrane on the surface of sodium alginate microcapsules by the electrostatic interaction with polyanionic sodium alginate, improve the stability and drug content of microcapsules, and regulate the release rate of the drug. In addition, chitosan is safer than common polylysine. Chitosan-sodium alginate microcapsule possesses more superiority in the controlled release of proteins and has attracted considerable attention recently [5−8].

At present, Chitosan-sodiun alginate microcapsules are used mainly in oral controlled release preparations. Bioavailability of most oral protein drugs are so low as to not be practical aside from several macromolecular drugs that act in the intestine such as transforming growth factor (TGF) etc. [4]. Special attention has been paid in the use of chitosan-sodium alginate microcapsules in injectable controlled release preparations of peptide and/or protein drugs [3,5,8]. However, microcapsules are mainly produced by traditional methods now and are not fit for injection due to their bigger size with a higher concentration of sodium alginate in particular. What is more, they have a short release time (generally within ten days) for peptide / protein drugs [3,5].

2 Materials and instruments

2.1 Materials

Porcine hemoglobin (pHb): our lab; inositol hexaphosphate (IHP): sigma, sodium alginate, chitosan, calcium chloride, and acetone.

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2.2 Instruments

High-speed tissue smash machine, magnetic stirrer, separatory funnel, microscope.

3 Methods and results

3.1 Preparation of chitosan-sodium alginate microcapsules

Add sodium alginate, pHb, and IHP solution of prescription volume into 50 mL vegetable oil and emulsify at a speed of 8 000 $r \cdot min^{-1}$ for 5 min. Then add calcium chloride solution (30 $g \cdot L^{-1}$, pH5.0) including chitosan of different concentrations and stir for 10 min at a speed of 1 000 $r \cdot min^{-1}$ continuously. Transfer into a separatory funnel, put it aside for 5 h, get Hb microcapsules at the lower layer, wash with acetone and vacuum dry.

3.2 Observation of the form of chitosan-sodium alginate microcapsules

Observe the form of chitosan-sodium alginate microcapsules under a biomicroscope (Fig. 1).

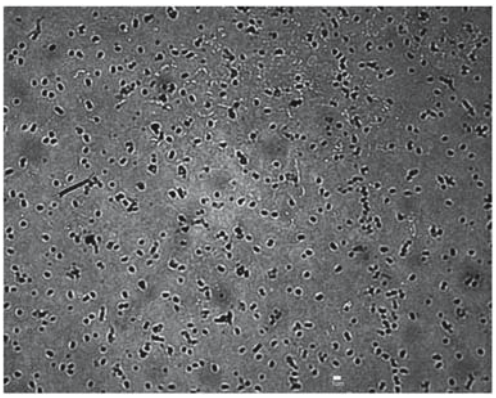

Fig. 1 Micrograph of Hb microcapsules

3.3 Determination of the size of chitosan-sodium alginate microcapsules

Determine the diameter of three groups of Hb microcapsules in leukocyte counting method and count 500 each group. Size distribution of Hb microcapsules is shown in Fig. 2.

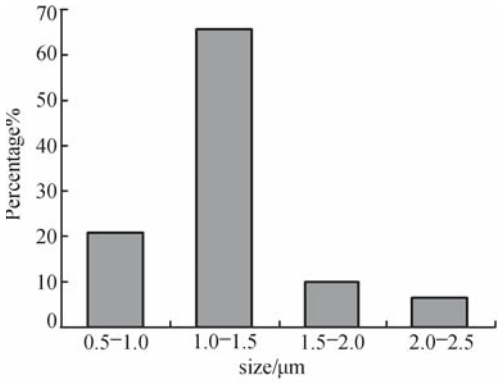

Fig. 2 Typical diameter distribution of Hb microcapsules

3.4 Determination of Hb content and embedding rate

Accurately weigh 100 mg Hb microcapsule sample, grind them into powder in a mortar, mix with adequate buffer PBS (0.1 $mol \cdot L^{-1}$, pH7.2) and put it into a beaker. After the microcapsules fully swell, stir for 30 min to fully dissolve Hb, remove the insoluble substances by filtering, transfer the filtrate into a 50 mL volumetric flask, add water up to the scale, then determine the Hb concentration by HiCN method [9]. Calculate the drug content of the microcapsules

$$DL = \text{drug volume in microcapsules/microcapsules mass} \times 100\%$$

$$DL = C \times 0.05 \div W \times 100\%$$

C is the Hb concentration ($g \cdot L^{-1}$) in the samples, W is the mass of the microcapsules in the formula.

Take 100 mg Hb microcapsules into a 50-mL volumetric flask, add PBS (0.1 $mol \cdot L^{-1}$ pH 7.2) buffer up to the scale. After fully swelling, remove the supernatant by centrifugation, then determine the concentration of Hb(C_1). Transfer the sediment of the Hb microcapsules in the mortar into a beaker, add adequate buffer, stir for 30 min to dissolve the Hb fully, filter, transfer the filtrate into a 50-mL volumetric flask, add PBS buffer up to the scale, then determine the concentration of Hb(C_2) by HiCN method.

Calculate the encapsulation efficiency of microcapsules

$$ER = \text{drug volume in microcapsules/(drug volume in microcapsules + drug volume in media)} \times 100\%$$

$$ER = C_2 \times 0.05/(C_2 \times 0.05 + C_1 \times 0.05) \times 100\%$$

C_1 is the concentration of Hb in media ($g \cdot L^{-1}$), C_2 is the concentration of Hb in microcapsules ($g \cdot L^{-1}$) in the formula.

3.5 Determination of the release of Hb microcapsule

Determine the release of Hb microcapsule by the release determination method in pharmacopoeia [10]. Accurately weigh microcapsules equivalent to 100 mg Hb, place in 1 000 mL normal saline including 0.1 $g \cdot L^{-1}$ sodium azide, stir at the speed of 50 $r \cdot min^{-1}$, separately take 10 mL samples at 0.5, 1, 2, 4, 6, 8, 12 h, 2 to 30 d, determine the concentration of Hb by HiCN [9], and calculate cumulative release. Meanwhile, add an equivalent volume of fresh normal saline. The result of cumulative release is shown in Fig. 3.

3.6 Determination of the oxygen-carrying capacity of chitosan-sodium alginate Hb microcapsules

Determine the oxygen-carrying capacity of the microcapsules by Ref. [11]. Weigh a certain amount of the microcapsules and put in a conical flask. Place the flask in a thermostatic bath pot. When it reaches a constant temperature, close the flask mouth. Pour potassium ferricyanide into the flask.

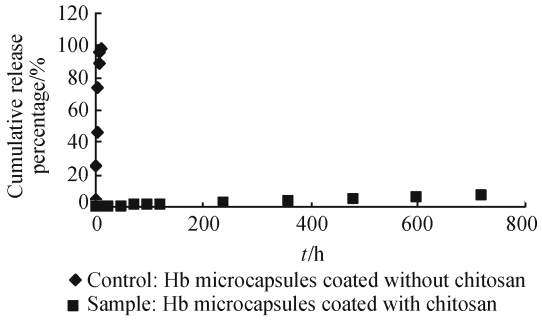

Fig. 3 Release of Hb microcapsules

Observe the scale of the level and record time and volume changes of the declining level, e.g. the volume of oxygen released from Hb microcapsules. The release curve of Hb microcapsules is shown in Fig. 4. The greatest release rate of oxygen is 50%.

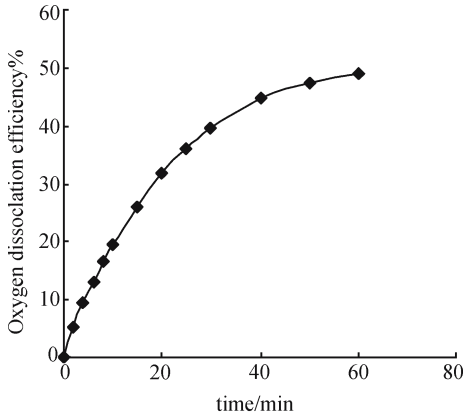

Fig. 4 Oxygen dissociation curve of Hb microcapsules

4 Discussion

Chitosan is a weak polybase with pKa = 6.3; Alginate is a weak polyacid of which guluronic acid has pKa = 3.5 and mannuronic acid has pKa = 4.0. At pH5.0, 70%−80% carboxyl of chitosan and alginate ionize. Chitosan and alginate form a compact polyelectrolyte semipermeable membrane. Sodium alginate is a hydrophilic gel, which helps keep the activity of protein. The release of chitosan-sodium alginate microcapsule is related to the relative molecular mass (RMM). If RMM is great, the capacity of release is good; if RMM is small, the capacity of release is bad. According to the need for release, we select different RMM chitosan or certain ratio of different RMM chitosan in order to realize the regulation of release. This research selected chitosan of RMM 1 million to improve the controlled release capacity of microcapsules and prevent the rapid release of Hb from the microcapsules and lose oxygen-carrying capacity. Whether patients of serious hemorrhagic shock can be resuscitated is one of the important issues of artificial oxygen-carrier evaluation. Primary research results show that SD rats enter hemorrhagic shock period when relative hemorrhagic volume reaches 30% with pulse pressure (36.2 ± 2.1) mmHg and heart rate (312 ± 12.3) per min. At 60 min after infusing 6% chitosan-sodium alginate coated pHb, the pulse pressure is (120.6 ± 4.5) mmHg and heart rate (405 ± 9.3) per min. Meanwhile, the tested rats appeared to have no hematuria. At present, the pharmacodynamical research of artificial oxygen-carrier chitosan-sodium alginate coated pHb is being carried out deeply.

The chitosan-sodium alginate coated pHb microcapsules have better form and smaller size, with an average size of 1 μm, and a relatively narrow Gaussian distribution. The encapsulation rate of pHb is beyond 90%. The release of pHb from microcapsules lasts over a month. Therefore, a chitosan-sodium alginate Hb microcapsule can be an artificial oxygen-carrier with delayed release by intravenous injection.

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