Preparation and drug-loading properties of human hair keratin nanoparticles

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Abstract

Human hair keratin (HHK) solution was first prepared by using a modified reduction method. Based on self-assembly and electrostatic interaction of the HHK solution, a novel nano drug delivery system for HHK/mupirocin was also prepared by adjusting the ratio and concentration between mupirocin and HHK. The morphology, particle size, drug-loading capacity and entrapment efficiency of the nanoparticles were also discussed. The results showed that the mutual hydrophobic aggregation between HHK and mupirocin was the main reason for forming nano drug delivery system. The average particle size of the nanoparticles was 74.78 nm. The drug-loading capacity of MPC for the nanoparticles was increased but entrapment efficiency of MPC was decreased with an increase of MPC concentration.

Keywords

Nanoparticles; drug-loading properties; human hair; keratin; electrostatic interaction.

1. Introduction

Human hair keratin-based materials have shown promise for revolutionizing the biomaterial world among commercially available biomacromolecules due to their intrinsic biocompatibility, biodegradability, mechanical durability, and natural abundance [1-3]. extracted keratin proteins have an intrinsic ability to self-assemble and polymerize into porous, fibrous scaffolds [4]. The spontaneous self-assembly of keratin solutions has been studied extensively at both the microscale [5-7] and macroscale levels [8].

In the paper, human hair keratin was first prepared by using a modified reduction method. Then, a novel drug delivery system of HHK/mupirocin nanoparticles was also prepared based on self-assembly and electrostatic interaction of the HHK solution. The drug-loading properties of human hair keratin nanoparticles in vitro were also discussed.

2. Experimental Procedure

2.1 Materials

Human hair was obtained from a local barber. Urea, 2-mercaptoethanol (Shanghai Beier Chemical Ltd, China), thiourea, ethanol, trichloromethane, ammonium hydroxide, hydrogen peroxide, mupirocin (MPC), sodium dodecyl sulfate (SDS) and other chemicals of analytical grade were commercially available and were purchased from Guangzhou chemical reagent factory, china. All other chemicals were of analytical grad and used without further purification.

2.2 Extraction of human hair keratin

The prepared method of HHK extracted from human hair fibers was modified according to the reported method with some modifications [9]. Briefly, human hair from a local barber shop was washed with alkaline detergent for three times, and external lipids were removed with a mixture of chloroform/methanol (2:1, v/v) under reflux at 70 ℃ for 2 h. The clear, dry and delipidized hair was decolorized using a mixture of hydrogen peroxide and ammonia (2:1, v/v). The decolorized hair was refluxed with a mixture of urea (45 wt%), SDS (4 wt %), mercaptoethanol (3 wt %) at 55 ℃ for 12 h. After the mixture was filtered and centrifuged at 4,000 ×g for 20 min at room temperature, the

obtained supernatant was dialyzed against deionized water using dialysis bag (molecular weight cutoff of about 10 kDa) and the outer water was replaced with distilled water four times a day. The dialysis was rapidly concentrated in the dialysis bag embedded into water absorption polyacrylic acid resin, and then the concentrated solutions were diluted to form 10 wt% keratin solution and the extracted keratins were kept sealed dry preservation.

2.3 Preparation of the nanoparticles

The extracted HHK solutions were diluted by using appropriate deionized water to form 0.05%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.7% (wt%) HHK solution, respectively. Mupirocin was dissolved into PBS 7.5 buffer solution to form 0.02%, 0.05%, 0.1%,0.2%, 0.3%, 0.4%, 0.5% (wt%) MPC solution, respectively. The HHK solution and MPC solution were mixed under stirring, and the mixed solution was adjusted with a 0.1 mol/L HCl solution to pH 6.5. The HHK/MPC nanoparticles were obtained after adjusting appropriate amount of HHK solution and MPC solution.

2.4 Characterization of the nanoparticles

HHK/MPC solutions were diluted with distilled water and were examined for size distribution and size, using a dynamic light scattering particle size analyzer (Zetasizer Nano-ZS; Marlvern Instrument, Worcestershire, UK). Values were calculated from measurements performed in triplicate.

The morphology of the nanoparticles was observed using SEM (Ultra 55, Zeiss). The samples were dried at room temperature before observation.

2.5 Determination of drug-loading properties

The entrapment efficiency (EE) and drug-loading (DL) capacity were calculated according to the following equations.

$$\mathrm{EE}\ (\%) = \frac{W_T - W_F}{W_T} \times 100$$

$$\mathrm{DL}\ (ug/mg) = \frac{W_T - W_F}{W_N}$$

where W_T, W_F and W_N represent the total amount of the drug added, the amount of free drug, and the total amount of the nanoparticles, respectively.

3. Results and Disscussion

3.1 Formation Process of the Nanoparticles

Formation of nanoparticles was related to concentration and composition of the matrix materials as well as pH value of solution. Formation feasibility of the nanoparticles was investigated under different concentrations of HHK and MPC and shown in Table 1.

When different concentrations of MPC were added into different concentrations of HHK, two different phenomenon----precipitate and milky suspension were observed. The milky suspension was thought as suspension of nanoparticles and suspension of nanoparticles were formed when sizes of the milky suspension were controlled in range of nanometer.

In addition, it could be seen from the table that formation of the nanoparticles was easy when the concentration of HHK was below 0.4%. When the concentration of HHK or MPC was up to 0.5% formation of the nanoparticles was difficult. Therefore, appropriate concentration and composition of HHK or MPC were essential conditions of the formation of the nanoparticles.

3.2 Particle Size and Morphology of the Nanoparticles

Particle sizes of the nanoparticles formed by using the same volume mixture of 0.03% HHK and 0.2% MPC were examined by using a dynamic light scattering particle size analyzer. The results showed that the average particle size of the nanoparticles was 74.78 nm.

Surface morphology of the nanoparticles formed by using the same volume mixture of 0.03% HHK and 0.2% MPC were observed by SEM and shown in Fig.1. The particle size distribution

of the nanoparticles was not uniform and the particle size was from 10 nm to 200 nm. The surface morphology of the nanoparticles showed irregular shape and obvious concave surface could be observed.

Table 1 Formation feasibility of the nanoparticles under different concentrations of HHK and MPC

MPC concentration (%)	HHK concentration(%)						
	0.05	0.1	0.2	0.3	0.4	0.5	0.7
0.02	■	■	■	■	■	▽	▽
0.05	■	■	■	■	■	▽	▽
0.1	■	■	■	■	■	▽	▽
0.2	■	■	■	■	■	■	▽
0.3	▽	■	■	■	■	■	▽
0.4	▽	▽	■	■	■	▽	▽
0.5	▽	▽	▽	■	■	▽	▽

▽: precipitate; ■: suspension

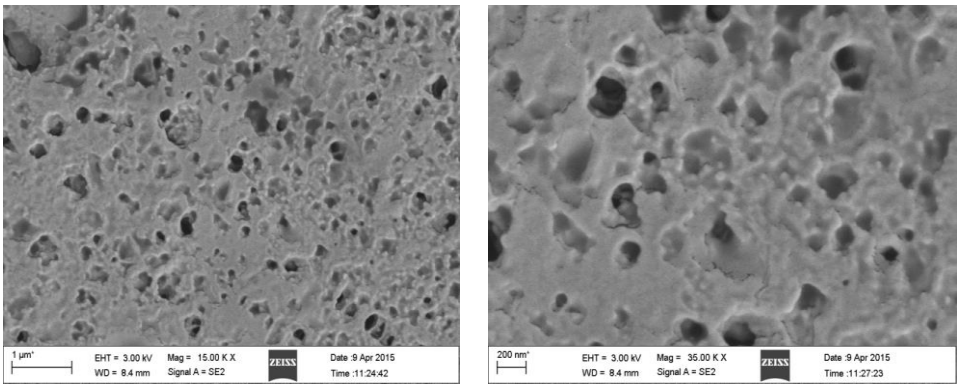

Fig.1 Surface morphology of the nanoparticles

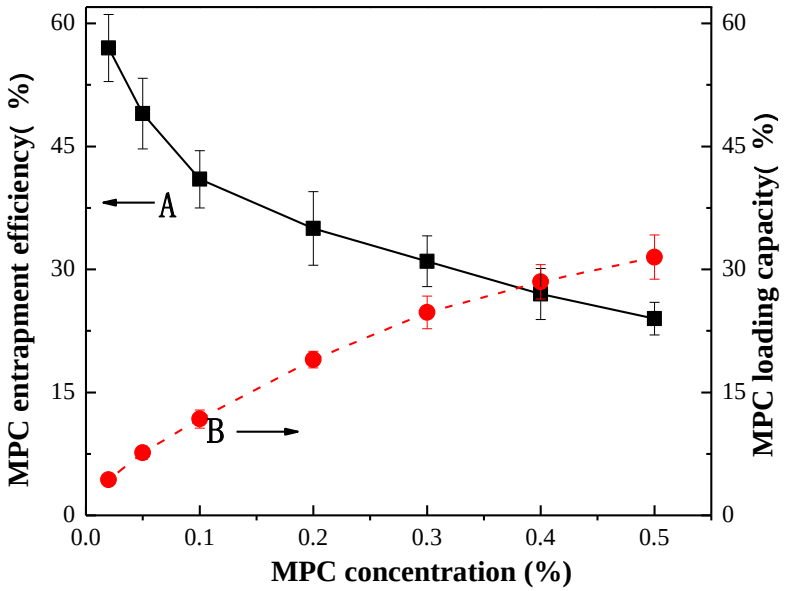

Fig.2 Effect of MPC concentration on the entrapment efficiency and drug-loading capacity for the nanoparticles

3.3 Drug-Loading Properties of the Nanoparticles

Effects of MPC concentration on the entrapment efficiency and drug-loading capacity of the nanoparticles were investigated and shown in Fig.2 when the concentration of HHK was 0.2%. It could be from the figure that drug-loading capacity of MPC for the nanoparticles was increased and but entrapment efficiency of MPC was decreased with an increase of MPC concentration. As MPC concentration was increased, more MPC molecules could interact with HHK (including electrostatic adsorption, hydrophobic aggregation and physical package), which resulted in an increase of the drug-loading capacity of MPC. However, with an increase of MPC concentration, less proportion of MPC could combine with HHK molecules, which resulted in a decrease of the entrapment efficiency of MPC.

4. Conclusion

HHK was first prepared by using a modified reduction method. HHK/MPC nanoparticles were prepared by self-assembly and electrostatic interaction. The formation of the nanoparticles was easy and average particle size of the nanoparticles was 74.78 nm when the concentration of HHK was below 0.4%. The drug-loading capacity of MPC for the nanoparticles was increased and but entrapment efficiency of MPC was decreased with an increase of MPC concentration.

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