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Rapid communication

A novel liposome-encapsulated hemoglobin/silica nanoparticle as an oxygen carrier

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ABSTRACT

A novel liposome-encapsulated hemoglobin/silica nanoparticle (LEHSN) was fabricated by a water-in-oil-in-water (W/O/W) double emulsion approach. Bovine hemoglobin (Hb) was first adsorbed onto the surfaces of silica nanoparticles (SNs), and then the complex of Hb/SNs was encapsulated by liposome to form LEHSN which has a core–shell supramolecular structure. On the one hand, liposomes built a cell membrane-like environment for the controlled release of Hb. On the other hand, SNs which act as rigid core provide a supported framework for lecithin membrane, and enhance the stability of liposomes. In comparison with liposome-encapsulated Hb (LEH), LEHSN shows substantially enhanced stability and improved release property of Hb in vitro. This study highlights the potential of the novel LEHSN as an oxygen carrier for pharmaceutical applications.

Blood substitute is a substance used to simulate and fulfill some physiological function of human blood, especially oxygen-carrying (Agnillo and Alayash, 2000; Patel, 2000). It provides an alternative for blood transfusion, which effectively solves the problems such as limited number of donors and the potential risk brought by unmatched blood or virus infection like human immunodeficiency virus (HIV). Hemoglobin (Hb) is an essential component of red blood cells, the main physiological function of which is responsible for the transport of oxygen from the lungs to the tissue cells and transport of carbon dioxide as a waste product (Xu et al., 2009; Zhao et al., 2007). However, Hb molecule is a tetramer that rapidly dissociates into two dimers, which results in rapid acute tubular necrosis and renal failure (Gao et al., 2011). Liposome-encapsulated Hb (LEH) is one of the most promising oxygen-carrying red cell substitutes, and is beneficial for the treatment of hemorrhagic shock (Huang et al., 1998; Li et al., 2005). The success of the use of LEH as a blood substitute mainly depends on its stability in blood stream and tissues. Unfortunately, the physical and/or chemical stability of liposomes is poor and their elaboration can be hardly reproducible, which causes difficulties in the development of LEH as a pharmaceutically acceptable product (Huang et al., 1998). Therefore, it becomes a very important issue to obtain a stable LEH for finally reaching the point where safe, clinically effective solution becomes a reality (Agashe et al., 2010; Awasthi et al., 2004).

In this paper, we demonstrate a simple strategy to fabricate novel liposome-encapsulated Hb/silica nanoparticle (LEHSN) as an oxygen vehicle. This particular LEHSN has a supramolecular structure with colloidal particles as a core surrounded by a lipid shell. Liposome is characterized by spatial isolation of Hb by an oxygen-permeable lipid bilayer that avoids the toxicity associated with free Hb. Silica nanoparticles (SNs) which serve as core offer a rigid support for lipid membrane, and enhance the physical stability of liposome. Therefore, we believe that the introduction of SNs with an architectural function into liposomes provides the promising prospect for the widespread application of Hb-based oxygen carriers.

LEHSN was prepared by a water-in-oil-in-water (W/O/W) double emulsion approach. Briefly, SNs (~10 nm) was added into bovine Hb aqueous solution under stirring (the concentration of SNs and Hb was 2 mg mL^{-1} and 5–50 μmol L^{-1}, respectively). The mixture was placed into a refrigerator at 4 °C for 24 h, followed by centrifugal filtration and freeze–drying to obtain a complex of Hb/SNs. 0.02 g of Hb/SNs was added into 2.5 g of water in which 0.3 g of cetyltrimethylammonium bromide (CTAB) was dissolved, followed by an ultrasonic dispersion for 5 min. The CTAB aqueous

Abbreviations: LEHSN, liposome-encapsulated hemoglobin/silica nanoparticle; LEH, liposome-encapsulated hemoglobin; Hb, hemoglobin; SNs, silica nanoparticles; HIV, human immunodeficiency virus; W/O/W, water-in-oil-in-water; CTAB, cetyltrimethylammonium bromide; W/O, water-in-oil; FTIR, Fourier transform infrared; TEM, transmission electronic morphology; UV–Vis, ultraviolet–visible.

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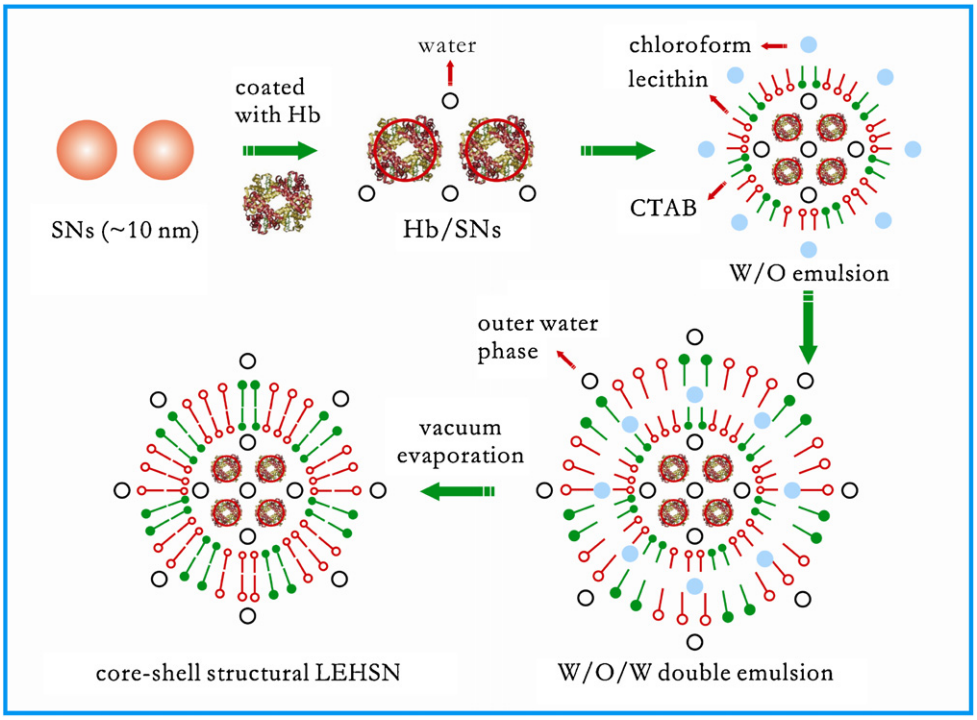

Fig. 1. Schematic formation of core–shell structural LEHSN.

solution containing Hb/SNs served as the water phase for the following emulsion. Under stirring, the water phase was mixed with the oil phase composed of chloroform (3.5 g) and lecithin (0.06 g) to get a water-in-oil (W/O) emulsion. Then additional 100 g of water (acted as the outer water phase) was added into the W/O emulsion to form a W/O/W double emulsion. The final emulsion system was vacuum evaporated to remove the organic solvent and to obtain LEHSN.

Fig. 1 shows the schematic formation of LEHSH, which was basically based on the directional adsorption and hydrophobic interaction of amphiphiles (lecithin and CTAB) at the interface. Due to their hydrophilic properties, Hb/SNs could form a stable dispersion in CTAB solution. This water phase was added into the oil phase to obtain a W/O emulsion in which chloroform acts as the continuous phase and the aqueous solution containing Hb/SNs serves as the discontinuous phase, while the amphiphiles rearrayed at the oil/water interface with the hydrophilic heads gathering in the water phase and the hydrophobic tails facing to the oil phase took place. The primary W/O emulsion was encapsulated by the outer water phase to form a W/O/W emulsion. The tails of amphiphiles moved towards each other through hydrophobic interaction during the removal of chloroform, and LEHSN was finally fabricated with Hb/SNs as a core and liposome as a shell.

Fig. 2 shows Fourier transform infrared (FTIR) absorption spectra of SNs, Hb and Hb/SNs. The absorption peaks of 3447 and 1630 cm^{-1} could be ascribed to the asymmetric stretching vibration and bending vibration of O—H. The 952 cm^{-1} peak is assigned to the stretching vibration of Si–OH, and the peaks at 1089, 795 and 475 cm^{-1} correspond to the asymmetric stretching vibration, symmetric stretching vibration and bending vibration of Si–O–Si, respectively, while the 3458 cm^{-1} peak shows the stretching vibration of N—H bond, and 1655, 1542 and 1372 cm^{-1} peaks are I, II and IV absorption bands of amide in the main chain of Hb (Jamil and Mustafa, 2002). The absorption spectrum of Hb/SNs shows combined absorption peaks of Hb and SNs, which indicates that Hb molecules were successfully adsorbed onto the surfaces of SNs.

Fig. 3 shows typical transmission electronic morphology (TEM) images of SNs, LEHSN and LEH, respectively. SNs have a mean diameter of about 10 nm (Fig. 3A). There is agglomeration among SNs due to their surface hydroxyls and the subsequent H-bond interaction. The hydroxyls facilitate the adsorption of Hb molecules onto the surfaces of SNs. LEHSN has an average diameter of about 60 nm with a core of SNs (Fig. 3B). Besides, LEHSN disperses even in the aqueous phase and exhibits higher stability compared with LEH which is easy to be fused or broken (Fig. 3C). In addition, LEHSN is found in our study to be stable for more than three months, while LEH solution appears precipitation in about one month. These results indicate that SNs inside the liposome supply a rigid skeleton for the lipid bilayer to effectively eliminate the fusion, rupture or precipitation phenomena associated with empty liposomes or LEH.

Fig. 4 shows ultraviolet–visible (UV–vis) adsorption spectra of Hb with different concentration. There are two characteristic absorption peaks of Hb which locate at 276 and 406 nm (Sakai et al.,

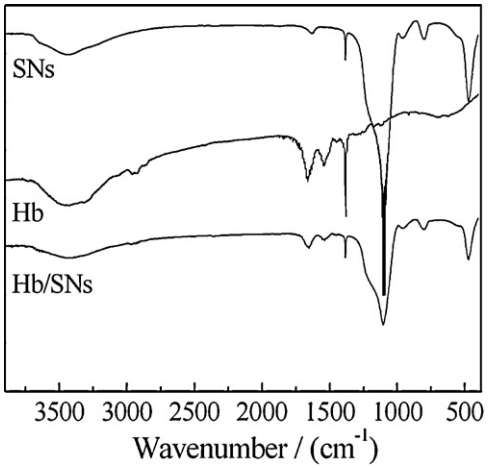

Fig. 2. FTIR adsorption spectra of SNs, Hb and Hb/SNs.

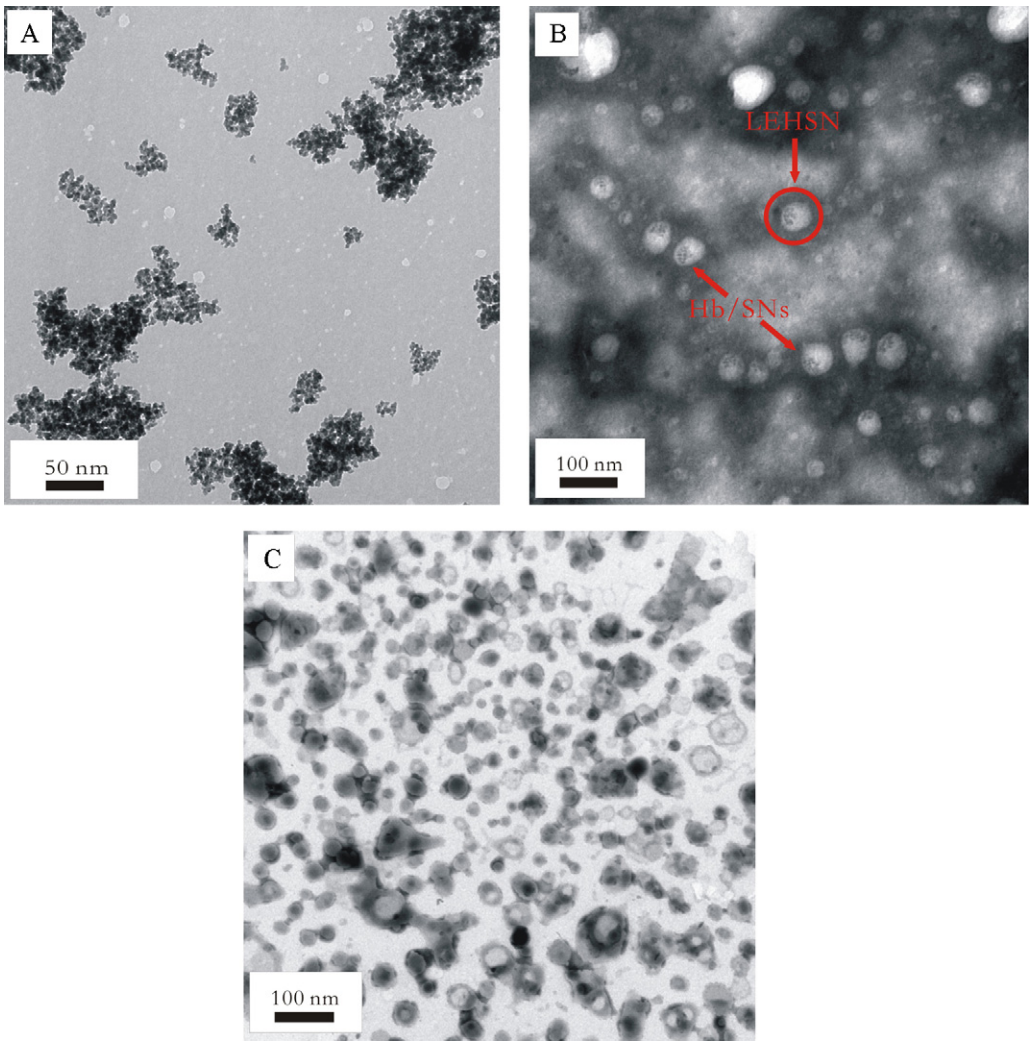

Fig. 3. Representative TEM images of SNs (A), LEHSN (B) and LEH (C).

2004). With the increasing concentration of Hb, the intensity of the absorption peak (406 nm) increases. The inset of Fig. 4 shows a good linear relationship between the absorbance at 406 nm and the concentration of Hb. According to this relationship, we can calculate the adsorption efficiency of SNs in Hb solution. Table 1 shows the effect of Hb concentration on the adsorption ratio and adsorption amount of Hb on SNs. One gram of SNs could adsorb 0.12 g of Hb molecules with an adsorption ratio of 75.0% when the concentration of Hb is 5.0 μmol L^{-1}. With the initial concentration of Hb increases, the adsorption ratio of Hb on SNs increases with a decreasing adsorption amount. The maximum adsorption amount of Hb is 0.47 g g^{-1} on SNs with an adsorption ratio of 73.1% when the concentration of Hb was increased to 20 μmol L^{-1}.

Table 2 shows the effects of the concentration of Hb/SNs and lecithin on the encapsulation efficiency of Hb in liposomes.

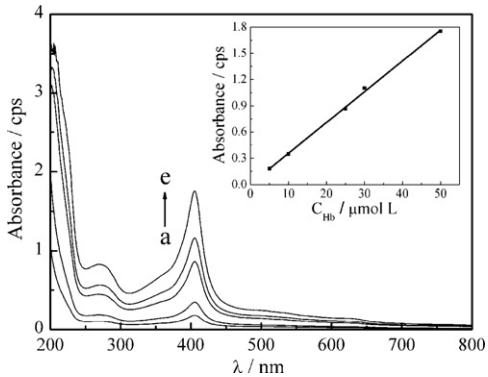

Fig. 4. UV–vis adsorption spectra of Hb with a concentration of 5.0 μmol L^{-1} (a), 10.0 μmol L^{-1} (b), 25.0 μmol L^{-1} (c), 30.0 μmol L^{-1} (d), and 50.0 μmol L^{-1} (e).

Table 1
The effect of the concentration of Hb (C_{Hb}) on the adsorption ratio (AR) and the adsorption amount (AA) of Hb on SNs.

C_{Hb} (μmol L^{-1})	AR (%)	AA (g g^{-1})
5.0	75.0	0.12
10.0	74.5	0.24
20.0	73.1	0.47

Table 2
The effects of the concentration of Hb/SNs ($C_{Hb/SNs}$) and lecithin ($C_{lecthin}$) on the encapsulation efficiency of Hb in liposomes (EE).

$C_{Hb/SNs}$ (mg mL^{-1})	$C_{lecthin}$ (mg mL^{-1})	EE (%)
0.20	0.54	70.2
0.20	0.79	79.3
0.25	0.79	75.4
0.15	0.79	83.2
0.10	0.79	87.9

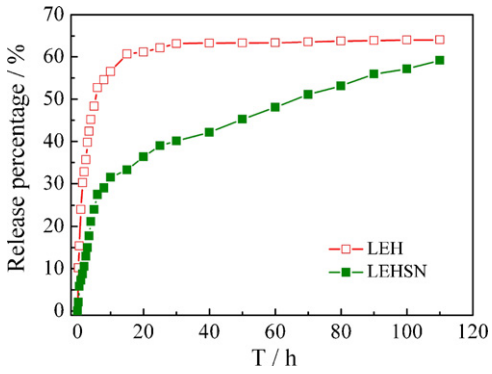

Fig. 5. Cumulative release curves of Hb from LEH and LEHSN in vitro.

Liposomes exhibit a good encapsulated ability to Hb/SNs, which reaches above 70% or above. The encapsulation efficiency of Hb by liposomes increases with the increasing of lecithin concentration when the concentration of Hb/SNs is 0.20 mg mL^{-1}. This is because more lecithin molecules result in the formation of more liposomes to encapsulate Hb. The encapsulation efficiency of Hb by liposomes reaches 79.3% when lecithin concentration is 0.79 mmol L^{-1}. Further increase the concentration of lecithin would reduce the encapsulation efficiency of Hb, which could be ascribed to the limited encapsulated ability of liposomes. Besides, decrease of the concentration of Hb/SNs to 0.10 mg mL^{-1} would enhance the encapsulation efficiency of Hb (87.9%). However, the practical encapsulated amount of Hb decreases in this case.

Fig. 5 shows the cumulative release curves of Hb from LEH and LEHSN in vitro. LEH shows a burst release phenomenon at the beginning of 3 h with a cumulative release amount of about 40% and the release of Hb was basically finished in 30 h with a total release percentage of 60.7%. The rapid release of Hb from LEH should be ascribed to the instability of liposomes which are easy to break or fuse without the presence of a rigid support. By contrast, the release of Hb from core–shell structural LEHSN becomes much slower with a release amount of 15.0% in 3 h, and 59.2% in 110 h, respectively. Thereafter, there are still Hb molecules to be released continually from LEHSN. This result indicates that the introduction of SNs with an architectural function into liposomes effectively enhances the stability of liposomes, and substantially improves the release performance of Hb. The controlled release of Hb is very important for pharmaceutical application because Hb is a tetrameric protein which fast breaks down into two dimers composed of an alpha and a beta subunit without a red blood cell environment. The resultant dimers are then filtered by the kidney, leading to rapid acute tubular necrosis and renal failure. Therefore, we believe that as-prepared LEHSN overcomes the drawbacks of raw Hb or common LEH and provides the promising prospect for the widespread pharmaceutical application of Hb-based oxygen carriers.

In summary, a novel LEHSN was fabricated by a W/O/W double emulsion approach. A cell membrane-like environment of liposomes facilitates the isolation and controlled release of Hb, and SNs offer a rigid framework for lipid membrane, and enhance the stability of liposomes. Compared with LEH, core–shell structural LEHSN shows effectively enhanced stability and substantially improved release performance of Hb in vitro. The present finding provides us a new example for Hb-based liposome to be a promising oxygen carrier for pharmaceutical applications.

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