Preparation of gold nanoparticles and carbon dots by hydrothermal reaction of bovine haemoglobin with chloroauric acid and energy band bending in carbon dots

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\textbf{ABSTRACT}

The incubation and hydrothermal reaction under alkaline condition of bovine haemoglobin with chloroauric acid were investigated. The products in the supernatant from the centrifuge were found to be gold nanoparticles and carbon dots. Greigite (Fe\textsubscript{3}S\textsubscript{4}) was found as a byproduct in the precipitation. In the incubation process, the reduction ability of bovine haemoglobin molecules was activated under alkaline condition (pH 12), and Au(III) ions underwent progressive reduction to form Au nanoparticles \textit{in situ}. In the hydrothermal reaction, denatured bovine haemoglobin molecules underwent biomineralisation to form carbon dots and greigite. Photoluminescence was used as a measurement of the energy band bending of as-prepared carbon dots. The effects of the acceptor 4-nitrotoluene and donor \textit{N},\textit{N}-diethylaniline molecules on the photoluminescence intensity imply that oxygen-contained surface groups induce the upward band bending of carbon dots.

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\textbf{1. Introduction}

Monolayer-protected metal and semiconductor nanoparticles \cite{1–4} have gained a great deal of attention because of their unique electronic properties and potential optical-sensing applications. Their unique properties are derived from their composition, nanoparticle size and size distribution. Au nanoparticles, a well-studied type of metal nanoparticle, exhibit extraordinary optical, electronic, molecular recognition and catalytic properties \cite{5–8}. The physical and chemical properties of Au nanoparticles depend not only on their size and size distribution, but also on their shapes \cite{9,10}. A variety of methods have been reported to tailor the size and shape of Au nanoparticles. Conventionally, the \textit{Turkevich} method is widely used to produce water-soluble Au nanoparticles, in which \textit{AuCl\textsubscript{4}}\textsuperscript{-} is reduced by sodium citrate in water \cite{11}. In the last decade, various organic compounds (e.g. amines, polymers and proteins) were found to act as reducing agents for \textit{AuCl\textsubscript{4}}\textsuperscript{-} \cite{12–15}. To date, one of the most common proteins investigated for Au nanoparticles synthesis has been bovine serum albumin \cite{16}. Bovine serum albumin is a ubiquitous protein present in the blood and a commonly used reagent in biological studies. It has long been

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used as a capping agent for preformed nanoparticles [17–20]. In marked contrast to the extensive studies of bovine serum albumin, very few studies of bovine haemoglobin as a reducing agent have been reported.

Currently, iron sulfides are recognised as advanced inorganic materials with nonconventional applications, such as high-energy density batteries, precursors for the synthesis of superconductors, diagnostic materials and materials for photoelectrolysis [21]. Greigite (Fe3S4) was discovered from silt and clay sediments in California in 1964 [22]. Its formation accompanies the decomposition of organic matter in the biogeochemical and bacterial sulfate reduction process [23]. Band structure calculations indicated that Fe3S4 shows a complex Fermi surface with a unique influence of relativistic effects. The existence of sheets of the Fermi surface depends on the direction of the magnetisation. This enables spintronics on the level of a single compound, rather than using traditional heterostructure devices [24]. Considering that greigite is nontoxic and abundant, it is an ideal material for high-performance lithium-ion batteries [25]. Furthermore, greigite also has potential applications in hydrogen storage, cancer hyperthermia, and magnetic-guided delivery of drugs [26–28].

In the present study, we attempted to synthesise Au nanoparticles using bovine haemoglobin without any additional reducing agent or external photoirradiation. The focus of this paper is to determine and characterise the products formed in the incubation and hydrothermal reaction under alkaline condition of bovine haemoglobin with chloroauric acid. The bovine haemoglobin is capable of acting as a reducing agent and forming the carbon dots in the process of hydrothermal reaction. Greigite (Fe3S4) can be obtained by the mineralisation of iron and sulfur elements of bovine haemoglobin. Carbon dots, as a class of ‘zero-dimensional’ carbon nanomaterials, have recently received considerable attention because of their advantageous characteristics. In comparison to conventional semiconductor (such as III–V and II–VI groups compounds) quantum dots, carbon dots are superior, chiefly due to low cytotoxicity, excellent biocompatibility, simple synthesis, economy and remarkable conductivity [29–31]. The coexistence of Au nanoparticles and carbon dots makes them a better candidate for biological applications [31].

2. Experimental

Two solutions were prepared: (a) 50 mg of bovine haemoglobin was dissolved in 5 mL of deionised water and (b) 50 mg of chloroauric acid tetrahydrate (HAuCl4·4H2O) was dissolved in 5 mL of deionised water. Solution b was added to solution a drop by drop with constant stirring. After all of solution b has been added, 0.5 mL 1 M aqueous solution of sodium hydroxide (NaOH) was introduced. Appropriate amount of sodium hydroxide was added in order to adjust the pH value. The liquid mixture was incubated at 37 °C for 12 hours. Then, the golden brown yellow solution was transferred to a 50 mL polytetrafluoroethylene-lined hydrothermal reaction kettle, to which 5 mL of deionised water was added. The hydrothermal reaction mixture was heated to 180 °C and maintained at that temperature for 12 hours. After cooling, the black opaque liquid was centrifuged at 8000 rpm for 20 min. Two products were obtained from the centrifuge: brown yellow supernatant and black precipitate.

X-ray diffraction (XRD) pattern was obtained on a D/MAX-2200 diffractometer equipped with graphite monochromatised Cu Kα (λ = 0.1545 nm) over the 2θ range of
10°–80°. The black precipitate sample was directly used for XRD characterisation. High-resolution transmission electron micrograph (HRTEM) images were recorded on a JEM-2010F microscope operated at 200 kV. The TEM sample was prepared by depositing an aliquot of the brown yellow supernatant on a copper grid coated with holey carbon. The deposited suspension was then dried in air prior to performing the TEM analysis. Fourier transform infrared (FTIR) spectroscopy was performed on an Avatar 370 spectrometer working in the mid-infrared range from 400 to 4000 cm⁻¹. Steady-state fluorescence emission spectra were performed with a RF-5301PC fluorescence spectrometer and Leica DMIRB analysis was conducted on U-LH100HG. The brown yellow supernatant was directly used for fluorescence measurement.

3. Results and discussion

3.1. Structural and morphological characteristics

As seen in the XRD pattern of the black precipitate sample (Figure 1), the observation of Bragg peaks of Au indicates that Au was successfully synthesised. The existence of Au is confirmed by the appearance of strong intensity peaks of (111), (200), (220) and (311) centred at around 2θ = 38.1°, 44.2°, 64.5° and 77.5°, respectively, and is in good agreement with the JCPDS (no. 44-0058) data. The calculated crystallite size (derived from Scherrer’s formula) of Au was about 18 nm. For carbon dots, the XRD pattern shows a broad peak at 22.8° corresponding to the (002) peak. This indicates the interlayer spacing of the carbon dots (0.39 nm) is higher than that of the graphitic interlayer spacing (0.33 nm) along with the broadness character. Besides, the existence of greigite (Fe₃S₄) is confirmed by the appearance of strong intensity peaks of (311), (400), (440) and (533) centred at around 2θ = 30.8°, 35.7°, 51.4° and 61.0°, respectively. Observation of Bragg peaks of Fe₃S₄ indicates that the heme iron in blood protein haemoglobin was oxidised to Fe₃S₄. In nature, greigite forms under anoxic, sulfate-reducing conditions. From the above analysis, it is obviously found that Au nanoparticles and carbon dots are synthesised by the incubation and hydrothermal reaction under alkaline condition of bovine haemoglobin with chloroauric acid. Meanwhile, we found a new method for preparing Fe₃S₄.

![Figure 1. XRD pattern of black precipitate sample.](image-url)
Considering the details of morphology and structure of as-prepared carbon dots and Au nanoparticles, the HRTEM of the brown yellow supernatant sample is employed. As shown in Figure 2(a), the carbon dots with narrow size distribution is in the range of 3–5 nm, and several carbon dots are clustered together. It clearly reveals that the interplanar spacing is 0.21 nm from HRTEM micrograph, indicating a preferential growth along the (002) direction. Figure 2(b) shows the TEM and HRTEM images of the collected Au nanoparticles. It is found that the average particle size of Au nanoparticles is in the range of 15–20 nm. The HRTEM revealed lattice fringe with an interlayer spacing of 0.24 nm, which corresponds to the lattice spacing of the Au (111) planes [32]. Figure 2(c) shows the HRTEM image of the coexistence of carbon dots and Au nanoparticles. The carbon dots and Au nanoparticles with different particle size could aggregate together, as shown in Figure 2(c). The only deficiency of the nanoparticle aggregation is that it was unordered. It is a further research emphasis to construct the carbon dots and Au nanoparticles with ordered nanoaggregates.

3.2. About mechanism of biomineralisation and carbonisation

At the molecular level, it is not clear how bovine haemoglobin ‘biomineralised’ the Au nanoparticles and how bovine haemoglobin was ‘carbonised’ into carbon dots. For sure, Au(III) ions were reduced in the process of incubation at 37 °C, and carbonisation of bovine haemoglobin was completed during the hydrothermal reaction, as illustrated in Scheme 1. It has been shown that tyrosine (Tyr) or custom peptides containing Tyr residues can reduce Au(III) or Ag(I) ions through their phenolic groups; their reduction capability can be greatly improved by adjusting the reaction pH above the \( pK_a \) of Tyr (~10).
[33]. Functionally, at least, the reducing capability of bovine haemoglobin was anticipated since it contains 21 Tyr residues and possibly other residues with reduction functionality. By a ‘biomineralisation’ reaction at the physiological temperature (37 °C), it has been already proved that upon adding Au(III) ions to the aqueous bovine serum albumin solution, the protein molecules sequester and entrap Au(III) ions; the reduction ability of the protein molecules are then activated by adjusting the reaction pH to ~12; and the entrapped Au(III) ions go through progressive reduction to form Au nanoclusters in situ [34]. During the hydrothermal reaction, bovine haemoglobin molecules underwent ‘carbonisation’ into carbon dots. As a result, the stabilised Au nanoclusters within bovine haemoglobin molecules lost the protection from the protein and further grew into Au-NPs.

3.3. Energy band bending

FTIR spectroscopy was employed to identify the bonding composition and surface groups of as-prepared carbon dots. As shown in the FTIR spectrum (Figure 3), the carbon dots exhibit a C=O, C–O–C and strong C–OH stretching peaks at 1639, 1202 and 1091 cm\(^{-1}\), respectively.

The band bending concept was first developed by Schottky and Mott to explain the rectifying effect of metal–semiconductor contacts [35]. In order to investigate the energy band bending of as-prepared carbon dots, photoluminescence (PL) was used as a

![Scheme 1. Synthesis process of Au nanoparticles, carbon dots and greigite.](image)

![Figure 3. FTIR spectrum of carbon dots.](image)
measurement. The effects of the acceptor 4-nitrotoluene and donor N,N-diethylaniline molecules on carbon dots with oxygen-containing functional groups are shown in Figure 4. It is obvious that the PL of the carbon dots is strongly quenched by 2,4-dinitrotoluene molecules. By contrast, N,N-diethylaniline quenching shows much lower efficiency for the carbon dots. Due to the disruption of N,N-diethylaniline molecules to the radiative recombination on the surface-trapped holes of carbon dots, the PL intensity of the carbon dots may be reduced in donor N,N-diethylaniline solution [36]. Accordingly, oxygen-containing functional groups should induce the upward band bending for the carbon dots. For upward band bending, negative charges exist at the surface and holes accumulate near the surface, causing a decrease of free electron carriers ($n_e$) and an increase of hole carriers ($n_h$), as presented in Scheme 2.

Figure 4. PL spectra (350 nm excitation) of carbon dots in aqueous alcohol without (curve A) and with the electron acceptor 4-nitrotoluene (25 mM, curve C) and donor N,N-diethylaniline (25 mM, curve B) molecules, respectively.

Scheme 2. Illustration of energy band bending near surface for carbon dots.
4. Conclusions

The incubation and hydrothermal reaction under alkaline condition of bovine haemoglobin with chloroauric acid were developed to prepare the coexistence sample of Au nanoparticles and carbon dots. We also first obtained Fe₃S₄ by this route, offering a simple and fast approach for the synthesis of Fe₃S₄. Further works should be performed to separate Fe₃S₄ from Au nanoparticles and carbon dots. The coexistence of Au nanoparticles and carbon dots makes them a better candidate for biological applications, such as electrochemical labels with signal amplification technique. Greigite has potential applications in hydrogen storage, cancer hyperthermia, and magnetic-guided delivery of drugs.

Disclosure statement

No potential conflict of interest was reported by the authors.

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