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ARTICLE TYPE

Sub-nanometer sized Cu₆(GSH)₃ clusters: one-step synthesis and electrochemical detection of glucose

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We report here a one-pot synthesis of sub-nanometer sized copper clusters capped with water-soluble ligand, L-glutathione (SGH) through a chemical reduction process. The composition of the as-prepared Cu₆(SG)₃ nanoclusters was confirmed by electrospray ionization mass spectrometry (ESI-MS) and matrix-assisted laser desorption ionization time-of-flight mass spectroscopy (MALDI-TOF MS). The FTIR, ¹H NMR and XPS characterizations showed that with the production of Cu₆(SG)₃ clusters and the formation of Cu-S bonds, the surface chemical environment of the clusters exhibited significant change. The produced water-soluble clusters show aggregation-induced fluorescence upon the addition of ethanol into the cluster aqueous solution. By loading on TiO₂ support, the as-prepared copper nanoclusters were successfully applied to the electrochemical detection of glucose. Compared to large Cu nanoparticles, the Cu₆(SG)₃ nanoclusters exhibited higher sensitivity and wider linear range for glucose detection.

1. Introduction

Noble metal nanoclusters consisting of several to hundreds of atoms have attracted intensive attention due to their size-dependent unique physical and chemical properties resulting from the discrete energy levels and band-gap energy structures, and their widely potential applications in biosensors, nanodevices, data storage, and catalysis etc.¹⁻⁶ In recent years, numerous efforts have focused on the composition-controlled synthesis, structure-dependent properties and the practical application of metal nanoclusters.^{3, 7-9} Although there are big challenges in investigating the nanoclusters with tiny core size, much progress has been achieved in this area, especially for gold and silver nanoclusters. Since the two-phase method was reported by Brust et al. for preparation of monolayer-protected gold nanoparticles,¹⁰ various strategies, such as modified Brust-Schiffrin methods, template-based synthesis, ligand-exchange reaction and electrochemical synthesis etc. have been developed for the synthesis of size-tunable metal nanoclusters.^{3, 11, 12} In addition to gold and silver, synthesis and properties of copper nanoclusters have also been investigated extensively recently.¹³⁻¹⁶ Compared to the counterpart, i.e. Au and Ag, the less stability of Cu makes it more difficult to obtain tiny stable copper clusters. Up to now, by using various templates, DNA or organic molecules, stable copper nanoclusters have been successfully prepared. For example, blue emitting copper clusters have been synthesized in the presence of lysozyme.¹⁷ The preparation of DNA-hosted copper nanoclusters has also been successfully realized.¹⁶ In another study, copper quantum clusters were also produced by an electrochemical method and its polypyrrole film was used as

glutathione sensor.¹⁸ To produce monolayer-protected copper clusters, it is important to find proper ligands which can well protect the copper core. As a kind of effective protecting ligand, glutathione (GSH) has been used to prepare metal nanoclusters.¹⁹⁻²¹ Recently, Wang and co-workers synthesized monodisperse GSH-stabilized Cu nanoclusters through the so-called size-focusing etching process from Cu nanocrystals (~ 4.2 nm in diameter).²² However, direct synthesis of GSH-stabilized Cu clusters with small size has not yet been reported. Based on the synthesis of gold and silver nanocluster, we aim to here find a new strategy for the preparation of glutathione-stabilized copper nanoclusters and explore their application in analysis.

Due to the unique photoluminescence properties and high catalytic activities, metal nanoclusters have exhibited promising applications in many fields, such as chemical sensors, catalysis, biomedicine etc.^{23, 24} When metal nanoclusters are used as catalysts, appropriate supports are usually needed to not only improve their dispersity but also enhance the stability. Among the materials, carbon and transition metal oxides are most widely used as supports. For instance, carbon-supported Au₂₅ clusters were used as catalysts for reduction of 4-nitrophenol,²⁴ and CeO₂-supported Au clusters were investigated for CO oxidation.²⁵ By taking advantage of the excellent electrocatalytic properties, copper nanoparticles and nanoclusters have been applied to the oxygen reduction reaction and non-enzyme electrochemical detection of glucose.^{7, 13}

Here, we developed a facile one-step method to prepare water-soluble Cu₆ nanoclusters (Cu NCs) by using L-glutathione(GSH) as protecting ligand. The prepared nanoclusters exhibited aggregation-induced orange fluorescence in the mixed ethanol

and water solution. By dispersing the as-prepared Cu clusters on TiO₂ support, the resulting Cu NCs/TiO₂ composites can be used to fabricate non-enzymatic electrochemical sensor for sensitive and selective detection of glucose.

2. Experimental Section

2.1 Materials

Copper dichloride dihydrate (CuCl₂·2H₂O) was purchased from Tianjin Huadong Reagent. L-glutathione (L-GSH) was obtained from Sigma-Aldrich. Hydrazine hydrate (N₂H₄·H₂O) was obtained from Beijing Yili Chemical Co., Ltd. Sodium hydroxide (NaOH), titanium dioxide (TiO₂, with diameter of 80 nm) and glucose (C₆H₁₂O₆) were bought from Beijing Chemical Reagent. All the reagent in this study are of analytical reagent grade and used as received. Water was supplied by a Water Purifier Ultrapure water system (18.3 MΩ cm)

2.2 Syntheses of Cu nanoclusters (Cu NCs) and the Cu NCs/TiO₂ composites

The GS-protected Cu NCs were prepared by a one-pot method. Briefly, 2.5 ml aqueous solution containing 21.5 mg CuCl₂·2H₂O was added to the solution containing 76.5 mg GSH under vigorous stirring. After the pH value dropped to 2~3, 250 μL, 50 mmol of sodium hydroxide solution was introduced to the reaction mixture with a slow stirring speed. After 10 min, 0.5 ml hydrazine hydrate was then dropwise added to the mixture. The color of the solution turned from colorless to yellow-green, then to gold yellow in half an hour. After three hours of stirring, the reaction flask was moved to fridge and aged overnight in order to obtain stable Cu NCs. The finally obtained nanoclusters were purified by adding ethanol in the cycle way of precipitation and centrifugation and dissolved in water for further use.

The Cu NCs/TiO₂ composites were synthesized by a simple implantation process. Typically, 2 mg TiO₂ powder was dispersed in 1 ml water under ultrasound and then mixed with 0.5 ml 17 mg/ml Cu NCs aqueous solution under vigorous stirring for 20 h at room temperature. The resulting precipitate was collected by centrifugation and dispersed in water for further use. The color of the obtained Cu-NCs/TiO₂ composites is obviously different from that of the pure TiO₂, suggesting the successful dispersion of the Cu NCs on the TiO₂ support.

2.3 Syntheses of Cu nanoparticles (Cu NPs) and the Cu NPs/TiO₂ composites

The large GSH-protected Cu NPs was synthesized following the above procedure for preparing Cu NC with some modification. In brief, 2.5 ml 42.0 mg CuCl₂·2H₂O in aqueous solution was introduced to a flask containing 20 mg glutathione and 2.5 ml water. After 30 min, 1 ml ethanol and 60 mg solid NaBH₄ were added into the above mixture successively. The reaction was allowed to continue 4 h at room temperature. Finally, ethanol/water mixture solvent was used to wash and collect the Cu NPs. The preparation of Cu NPs/TiO₂ composites is similar to that for Cu NCs/TiO₂ composites, just by replacing Cu NCs with Cu NPs.

2.4 Material Characterization

UV-Vis spectra were recorded on a UV-3000PC

Spectrophotometer (Shanghai Manada Instrumenta Co., Ltd.). Photoluminescence spectra were recorded on a Perkin-Elmer LS-55 Luminescence Spectra (Perkin-Elmer Instruments U.K). X-Ray photoelectron spectroscopy (XPS) measurements were performed by using AVG Thermo ESCALAB 250 spectrometer (VG scientific) operated at 120 W. Matrix-assisted laser-desorption ionization time of flight mass spectrometric (MALDI-TOF MS) studies were performed using Bruker autoflexIII smartbeam MALDI-TOF/TOF-MS (Germany) and 2,5-dihydroxy benzoic acid (DHB) was used as the matrix. Electrospray ionization mass spectrometry (ESI-MS) measurements were conducted on a LTQ linear ion trap mass spectrometer (Thermo, San Jose, CA, USA), equipped with a conventional ESI source. ESI-MS spectra in both negative- and positive-mode were collected. Fourier-transformed infrared spectroscopy (FTIR) study was conducted with a VERTTEX 70 FTIR (KBr wafer technique). ¹H NMR was carried out by using Avance III HD 500 (Switzerland, Bruker).

2.5 Polyacrylamide gel electrophoresis (PAGE)

The polyacrylamide gel electrophoresis experiment was following the previous report with a little modification.²⁰ Homemade gels were prepared with 30% concentrations of acrylamide monomer and 4% bis-acrylamide cross-linker for the resolving gel. The stacking gel was made with 4% monomer concentration. The gel dimensions were 20 cm × 20 cm × 1.5 mm. THAM (25 mM) and glycine (192 mM) was used as the eluting buffer. In the stacking gel, the voltage was set as 80 V, and in the resolving gel, the voltage was 120 V. All processes were conducted at room temperature.

2.6 Thin layer chromatography (TLC)

The TLC process was performed at room temperature. Ethanol and water with different volume ratio used as mobile phase, including ethanol : water = 1:10, 1:9, 1:8, 1:6, 1:1, 1:2, 5:1, 5:2, 2:3, 1:0, and 0:1. The results obtained from the different mobile phase are almost same.

2.7 Electrochemical Measurements

A piece of ITO substrate was cleaned first by water, acetone and ethanol in ultrasound bath and was dried at room temperature. The mixture of the Cu-NCs/TiO₂ composites or TiO₂ dispersing solution and 0.5 % Nafion solution was then dropcast onto the clean ITO substrate and dried at ambient temperature. Control experiments were also performed on blank ITO, ITO modified with only TiO₂, and the Cu-NPs/TiO₂ composites. The electrodes were donated as Cu NC/TiO₂/ITO, ITO, TiO₂-ITO, and Cu NC/TiO₂/ITO respectively. The geometric areas were measured to be 0.7, 0.8, 0.8, and 1.0 cm² for pure ITO, CuNC/TiO₂/ITO, TiO₂/ITO and CuNP/TiO₂/ITO electrodes, respectively.

Electrochemical measurements, including cycle voltammograms and amperometric response, were carried out with a CHI 750D electrochemical workstation in a standard three-electrode cell at room temperature. The ITO, TiO₂/ITO, CuNC/TiO₂/ITO and CuNP/TiO₂/ITO electrodes prepared above were used as working electrodes. An Ag/AgCl (in 3 M NaCl, aq.) and a Pt coil were used as reference and counter electrodes, respectively. All the electrochemical measurements were performed in 0.1 M KOH solution and at room temperature.

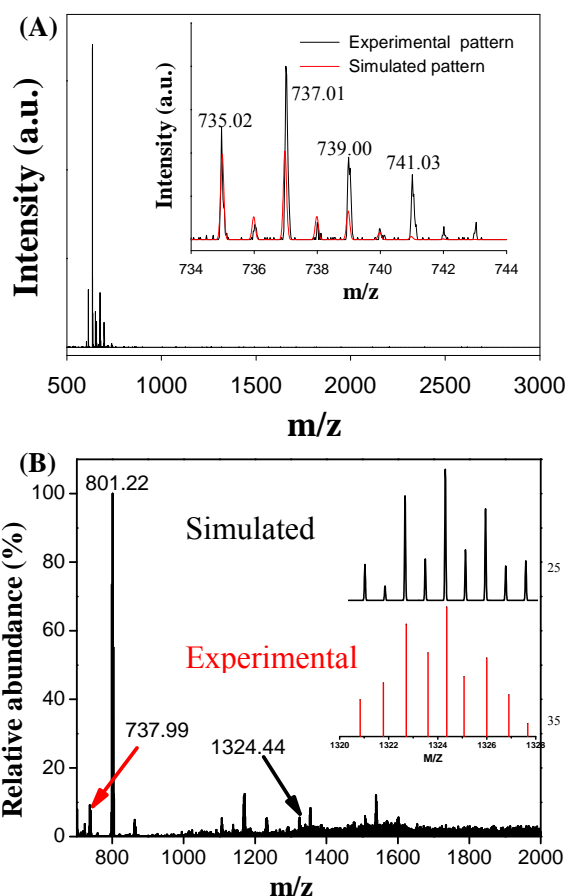


Fig. 1 (A) The negative-mode MALDI-TOF MS of the as-synthesized Cu nanoclusters. Inset shows the experimental (black curve) and simulated (red curve) isotopic patterns of $[\text{Cu}_2(\text{SG})_2\text{-H}]^-$. (B) The positive-mode ESI-MS of the as-synthesized Cu nanoclusters. Inset shows the experimental (red curve) and simulated (black curve) isotopic patterns of $[\text{Cu}_6(\text{SG})_3 + \text{H} + \text{Na}]^+$.

2.8 Catalytic reaction of the methylene blue (MB) to leucomethylene blue (LMB)

The catalytic reduction reaction was performed following the reported procedure.^{26, 27} Typically, under magnetic stirring, 100 μL of 8 mg/mL Cu NC solution was introduced into 2.9 mL aqueous solution with 33 μM MB and 60 mM N_2H_4 . The UV-vis absorption spectra of the solution before and after catalytic reaction were collected for comparison.

3. Results and Discussion

3.1 Synthesis and characterization of Cu nanoclusters

Compared to the recent two-step preparation,²² we present here a one-step method to synthesize Cu_6 nanoclusters without the etching step. In the present preparation, upon addition of CuCl_2 into the GSH aqueous solution, transparent Cu(I)-GS micelles can be formed gradually, which is likely to be the intermediates of Cu NCs. The addition of NaOH could improve the stability of the micelles and promote the growth of specific nanocluster by

changing the pH of the mixture. On the other hand, in order to control the reaction kinetics, a mild reducing reagent, $\text{N}_2\text{H}_4\cdot\text{H}_2\text{O}$, was chosen for the reduction of Cu(I) to Cu(0) to form Cu cluster. The as-synthesized Cu nanoclusters are very stable at room temperature. Fig. S1 displays the solution and solid forms of the as-synthesized copper nanoclusters.

The exact size of metal nanoclusters can be hardly resolved by TEM measurement because of their tiny core size. However, it is well-known that mass spectroscopy is a powerful technique to identify the composition and the exact atom numbers of metal nanoclusters. Here, the core size and the composition of the as-synthesized Cu clusters were first characterized by matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy (MALDI-TOF MS) measurements. Fig. 1A shows the full MALDI-TOF mass spectrum of the as-prepared Cu NCs. The highest mass peak can be observed at m/z 737.01, which is ascribed to $[\text{Cu}_2(\text{SG})_2\text{-H}]^-$. Another pronounced peak at m/z 675.03 could be assigned to the $[\text{Cu}(\text{SG})_2]$ fragments. Fig. 1A inset and Fig. S2 show the experimental and simulated isotopic patterns of the $[\text{Cu}_2(\text{SG})_2\text{-H}]^-$ and $[\text{Cu}(\text{SG})_2]$, respectively. The highly consistency between the experimental and simulated data indicates the successful synthesis of copper cluster. However, during the MALDI-TOF MS analysis, the highly energetic ionization process may lead to the formation of fragments. That is to say the $[\text{Cu}(\text{SG})_2]$ may be the fragments from larger nanoclusters. Therefore, a softer mass spectrometry, electrospray ionization mass spectrometry (ESI-MS), was also performed to further analyze the composition of the formed Cu clusters. As shown in Fig. 1B, positive-mode ESI-MS analysis gives obvious isotope features of copper, and the peak at m/z 1324.44 indicates the $\text{Cu}_6(\text{GS})_3$ composition of the original Cu NCs. It can be seen from Fig. 1B inset, the experimental pattern has a good match with the simulated spectra. It is noteworthy that the most abundant peak located at m/z 801.22 could be assigned to the fragment of $[\text{Cu}_3(\text{SG})_2]^+$. The experimental and simulated isotopic patterns of $[\text{Cu}_3(\text{SG})_2]^+$ are compared in Fig. S3. Interestingly, the isotopic peak centered at m/z 737.99 from $[\text{Cu}(\text{SG})_2 + \text{H}]^+$ also appears in the ESI-MS, which is consistent with the result from the MALDI-TOF MS. Such results suggest that $[\text{Cu}(\text{SG})_2]$ is indeed from the as-prepared $\text{Cu}_6(\text{SG})_3$ clusters. In order to further characterize the as-synthesized Cu NCs, negative-mode ESI-MS was also performed, as displayed in Fig. S4. Surprisingly, we can also see the peak at m/z 800.98 from the $[\text{Cu}_3(\text{SG})_2\text{-H}]^-$, suggesting that $\text{Cu}_3(\text{SG})_2$ may be the solid part of the parent cluster just like the "staple" motif $\text{Au}_x(\text{SR})_y$ in Au clusters.²⁸⁻³¹ In the higher mass region, a fragment peak at m/z 1037.43 attributes to $[\text{Cu}_6(\text{SG})_2 + 2\text{Na}]^+$, while in lower mass region another fragment peak at 368.09 could be from $[\text{Cu}(\text{SG})\text{-H}]^-$. Fig. S5 and S6 show their experimental and simulated isotopic patterns, respectively.

Polyacrylamide gel electrophoresis (PAGE) has been widely used to separate metal nanoclusters and analyze their dispersity.^{12, 20, 32-34} As presented in Fig. 2A, after washing and centrifuging with mixture of water and ethanol, the obtained clusters show only one yellow band in PAGE, indicating the high size homogeneity of the produced copper nanoclusters. On the other hand, thin layer chromatography (TLC) was conducted for the Cu clusters with varied volume ratio of ethanol/water mobile phase.

As shown in Fig. 2B, only one yellow band can be observed,

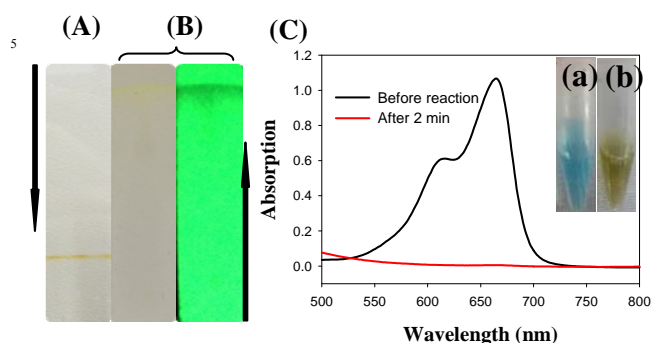


Fig. 2 (A) Polyacrylamide gel electrophoresis (PAGE) result and (B) thin layer chromatography (TLC) result (left: under sunlight; right: under ultra-violet light) of the as-synthesized Cu cluster. (C) UV-vis absorption spectra of the mixture of MB and N_2H_4 before and after the addition of Cu NCs. Inset shows digital photographs of the mixed solution before (a) and after 2 min (b) reaction with Cu NCs.

which is consistent with that of PAGE result. It is worthy to note that the TLC plate even doesn't show other obvious dots or bands under ultraviolet light. The appearance of only one yellow band in PAGE and TLD measurements also support the above MS results, i.e. the as-prepared Cu cluster has the composition of $Cu_6(SG)_3$. On the other hand, Cu clusters with size less than 20 copper atoms are expected to exhibit catalytic activity for the reduction of methylene blue (MB) to leucomethylene blue (LMB) with the presence of hydrazine.²⁶ Fig. 2C shows the UV-vis absorption spectra of MB solution before and after adding Cu nanoclusters. Upon the addition of the as-prepared $Cu_6(SG)_3$ clusters into the MB solution, the absorption between 500 and 800 nm from MB disappeared after 2 min. Fig. 2C inset shows that the original blue color of MB faded within two minutes. Such catalytic reaction further suggests the formation of small Cu nanoclusters.

To analyze the surface chemical bonding of the Cu NCs, Fourier transform infrared spectroscopy (FT-IR) and proton nuclear magnetic resonance (1H -NMR) measurements were performed. Fig. 3A shows the FTIR spectra of free GSH ligand and the $Cu_6(SG)_3$ clusters. Compared to the spectrum of pure GSH ligand, the peak at 2524 cm^{-1} from -S-H stretching vibration disappears in the spectrum of Cu_6 clusters, while peaks located at 3343 and 1557 cm^{-1} that stem from $-NH_2$ and $-COOH$ groups can still be observed. These results indicate that glutathione ligands have been successfully bound onto the surface of Cu_6 clusters through -S-Cu chemical bonds, with the cleavage of S-H bond. Fig. 3B shows the 1H NMR spectra of pure glutathione and $Cu_6(SG)_3$ in the range of 1.5 to 5.0 ppm. Based on the molecular structure of glutathione shown in Fig. 3B inset and the previous reports, the observed NMR peaks from different protons have been assigned (labeled with carbon numbers).^{20, 35} The strong peaks at 4.79 ppm attribute to D_2O and the weak peak at 3.56 ppm (marked with asterisk) could be ascribed to the residual ethanol during the cluster purification. All the rest peaks arise from free GSH or GSH capped on the surface of Cu NCs. Based on the chemical shifts, the signals at 2.16 and 2.54 ppm could be easily assigned to the protons on C3 and C4. Interestingly, it can be seen that the peak from C7 splits into two peaks in the

spectrum of Cu_6 -GSH clusters (labeled with 7 and 7' in Fig. 3B).

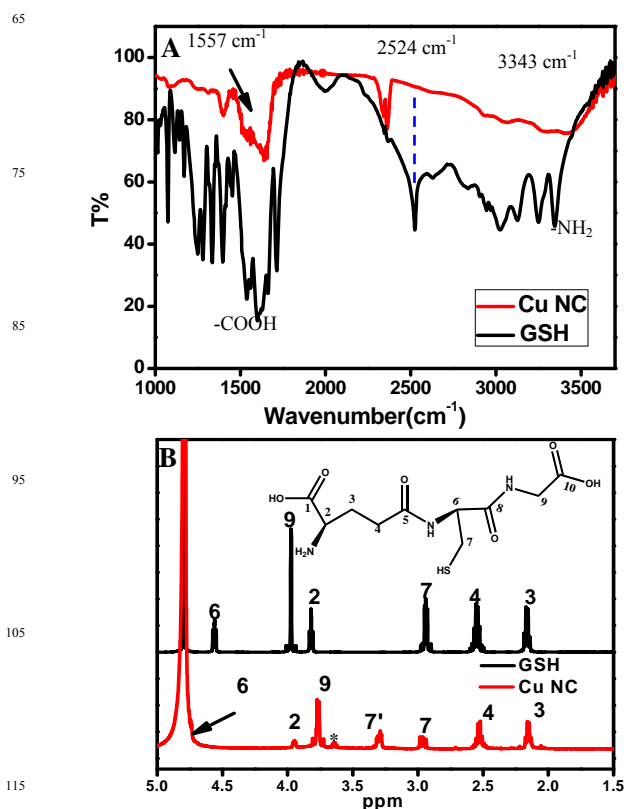


Fig. 3 (A) FT-IR (A) and 1H NMR (B) spectra of glutathione ligand (black curve) and the as-synthesized $Cu_6(SG)_3$ nanoclusters (red curve). Fig. 3B inset shows the molecular structure of glutathione ligand.

According to the previous study,³⁵ such splitting is caused by the adjacent chiral carbon (C6). It should be noted that because C6 and C7 are very close to the copper cores, the corresponding proton signals usually have downfield shift. Therefore, the proton signals from C6 may merge in the strong water peak in the 1H -NMR spectrum, as shown in Fig. 3B. These 1H NMR features further suggest the formation of GSH-protected copper nanoclusters.³⁶⁻⁴⁰

X-ray photoelectron spectroscopy (XPS) analysis was also utilized to characterize the oxidation state of the Cu nanoclusters. The XPS survey spectrum shown in Fig. 4A indicates the presence of Cu, C, N, and S in the product. Fig. 4B shows the Cu 2p XPS spectrum, in which the peaks at 951.5 and 931.5 eV can be assigned to the binding energies of Cu $2p_{1/2}$ and Cu $2p_{3/2}$ from the Cu (0). As described before,¹³ due to the indiscernible binding energies (~ 0.1 eV difference) of Cu(0) and Cu(I) and the possible charge transfer in the Cu-SR bonds, the oxidation states of the prepared Cu NC may involve 0 and +1. It should be pointed out that the binding energy of Cu^{2+} around 942 eV was not observed in the Cu 2p spectrum (Fig. 4B), indicating the formation of pure metallic copper clusters. The S 2p peak corresponds to the binding energy of chemisorbed sulfur (Fig. 4C). The XPS spectra of C, N are displayed in Fig. S7. Fig. 4D shows the UV-Vis absorption of the obtained Cu NCs in water (black curve). The two broad absorption bands around 260 and 305 nm may correspond to the interband transition. Moreover, the surface

plasmonic resonance (SPR) of Cu nanocrystals (~ 570 nm) does

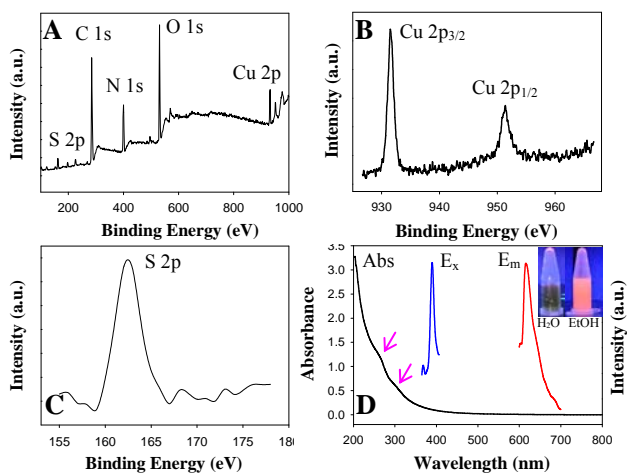


Fig. 4 (A) XPS survey spectrum of the Cu nanoclusters. XPS spectra of Cu 2p (B) and S 2p (C) of Cu nanoclusters. (D) UV-vis absorption (black curve), photoluminescence excitation (blue curve, $\lambda_{\text{ex}} = 617$ nm) and emission (red curve, $\lambda_{\text{em}} = 390$ nm) spectra of the as-synthesized Cu NCs. The absorption was measured in aqueous solution, the excitation and emission spectra were collected in the mixture of water and ethanol (~ 80 vol%). Inset shows the photographs of the Cu NCs in aqueous solution (left) and the mixed solution (right) under UV light.

not appear in the UV-Vis spectrum. The step-like feature and absence of SPR absorption further confirm the successful preparation of molecule-like Cu NCs. Photoluminescence is another intriguing characteristic of metal nanoclusters. Interestingly, the as-synthesized Cu_6 nanoclusters showed very weak fluorescence in water. However, upon addition of ethanol into the cluster aqueous solution, strong orange emission can be observed. As shown in Fig. 4D and inset, under UV irradiation, the Cu_6 nanoclusters in the mixed solution (~ 80 vol% ethanol) exhibit intense emission at 617 nm. As reported previously,⁴¹ such result indicates the obvious aggregation-induced emission (AIE) enhancement effect of the Cu_6 nanoclusters. Similar to the AIE of gold clusters, The observed enhanced fluorescence of the present clusters is probably from the AIE of Cu(I)-thiolate complex on the cluster surface.

3.2 Non-enzymatic electrochemical detection of glucose with the Cu_6 nanoclusters

The electrocatalytic activity of the Cu NCs for glucose oxidation and the application in non-enzymatic glucose detection were studied by electrochemistry. Here, in order to enhance the dispersity of Cu NCs and to avoid the degradation and aggregation of Cu NCs in electrochemical measurements, TiO_2 powder was used as the Cu NCs catalyst support. After 20 h stirring of the mixture, the color of TiO_2 changed from white to orange, suggesting the success loading of Cu NCs on the TiO_2 support. For comparison, large Cu nanoparticles (Cu NPs) with average size of 157.4 ± 3.4 nm (DLS data are shown in Fig. S8) were also prepared and their electrocatalytic activity was studied by loading on TiO_2 . Fig. 5A shows the cycle voltammograms (CVs) of different electrodes in 0.1 M KOH with the presence of 5 mM glucose. Clearly, almost no oxidation current can be

observed from the pure ITO and TiO_2/ITO electrodes, indicating the poor electrocatalytic activities of ITO and TiO_2 for glucose oxidation. However, under the same conditions, large oxidation current density was obtained on the Cu NCs/ TiO_2/ITO electrode. By comparison, the current density obtained from the Cu NCs/ TiO_2/ITO is much higher than that of the NPs/ TiO_2/ITO . Moreover, the onset potential of glucose oxidation on the present Cu NCs/ TiO_2/ITO (0.02 V) is more negative than that on the NPs/ TiO_2/ITO (0.09 V) and the previously reported Cu nanoparticles loaded on graphene support.⁷ Such CV comparison strongly demonstrates that the as-synthesized copper nanoclusters have enhanced catalytic activity for glucose oxidation. In order to study the sensitivity of the Cu NCs to the glucose concentration, the CVs of Cu NCs/ TiO_2/ITO in electrolyte with different concentrations of glucose were recorded. As shown in Fig. 5B, the oxidation current density increases gradually with the concentration of glucose increasing from 0 to 8 mM. The CV studies indicate that the as-prepared Cu_6 nanoclusters could be used for the non-enzymatic electrochemical detection of glucose.

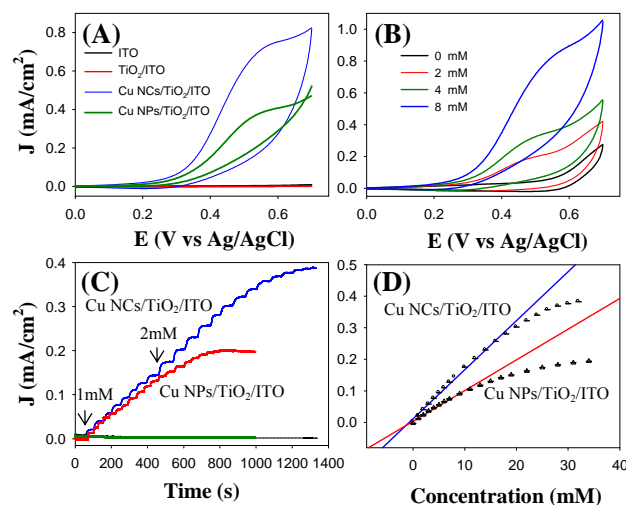


Fig. 5 (A) Cycle voltammograms (CVs) of ITO (black line), TiO_2/ITO (red line), Cu NCs/ TiO_2/ITO (blue line) and Cu NPs/ TiO_2/ITO (dark green line) in 0.1 M KOH with 5 mM glucose. Potential scan rate 0.1 V/s. (B) CVs of Cu NCs/ TiO_2/ITO in 0.1 M KOH with different concentrations of glucose (0, 2, 4, and 8 mM). Potential scan rate 0.1 V/s. (C) Amperometric responses of Cu NCs/ TiO_2/ITO and Cu NPs/ TiO_2/ITO to glucose at 0.5 V in 0.1 M KOH, the black and dark green lines show the J-t curves with no glucose in electrolyte. (D) Relationship of current density and concentration of glucose on the NCs/ TiO_2/ITO and Cu NPs/ TiO_2/ITO electrodes, respectively.

Fig. 5C presents the amperometric responses of the Cu NCs/ TiO_2/ITO and Cu NPs/ TiO_2/ITO electrodes against the glucose concentration. At the Cu NCs/ TiO_2/ITO electrode (blue curve), with a successive addition of glucose into the 0.1 M KOH electrolyte at + 0.5 V, the oxidation current density continues increase until the glucose concentration reaches 32 mM after which steady-state current density appears. The black baseline in Figure 5C demonstrates that with the absence of glucose in electrolyte, the Cu NC/ TiO_2/ITO electrode can only give low and almost constant current density during the whole tested time (1400 s), which again confirms that the current responses are

from the glucose oxidation on the Cu NCs. Similarly, the Cu NPs/TiO₂/ITO (red curve) also shows amperometric response to the glucose concentration, but with much lower sensitivity compared to the Cu NCs. The linear relationships (J-c curves) between current density (J) and the glucose concentration (c) on the Cu NCs/TiO₂/ITO and Cu NPs/TiO₂/ITO electrodes are presented in Fig. 5D. From the fitting line obtained from the Cu NCs/TiO₂/ITO electrode, the linear range locates between 4.07 μM to 20 mM, which is much wider than those obtained from the Cu₂O and Cu nanomaterials reported in the literature.^{7, 42, 43} The linear calibration equation can be expressed as: $J = 0.0151 * C + 0.0145$ and the limit of the detection is evaluated to be 4.07 μM based on the equation of $LOD = 3\sigma/s_d$. Here, s_d is the slope of the current-concentration curve and σ is the standard deviation of any five current points without the presence of glucose in electrolyte. Meanwhile, from Figure 5D, a linear calibration equation of $J = 0.0113 * C + 0.0021$ with linear range of 0~13 mM and LOD of 13.6 μM were obtained from the NPs/TiO₂/ITO electrode. Obviously, the Cu NPs/TiO₂/ITO electrode exhibits much lower sensing sensitivity and narrower linear range compared to the Cu NCs. Based on these electrochemical results, it can be seen that the Cu₆ clusters exhibit much higher sensing performance than large Cu nanoparticles. Such size-dependent electrocatalytic activities of metal nanoclusters have also been observed before.^{23, 44, 45} As is known, ascorbic acid (AA), dopamine (DA) and uric acid (UA) generally co-exist with glucose in human serum. The interference of the co-existences to the glucose detection was also investigated. Fig. S9 and S10 show the amperometric responses upon addition of different analytes in 0.1 M KOH at 0.5 V. It should be noted the normal physical level of the above analytes is only 1/4~1/6 of glucose.⁴² Here, much higher concentration of the analytes (10 mM) than that reported previously was used in the measurements. It can be seen that 10 mM NaCl and UA have little effect on the current response, while much lower current responses were obtained from 10 mM ascorbic acid and dopamine compared to that from glucose oxidation. The results indicate the high selectivity of the Cu₆ nanoclusters to the electrochemical detection of glucose.

4. Conclusion

In summary, GSH-protected Cu₆ nanoclusters were synthesized successfully by a one-step method. ESI-MS and MALDI-TOF MS measurements confirmed the Cu₆(SG)₃ composition of the formed nanoclusters. FTIR, ¹H NMR and XPS characterizations showed the surface bonding of Cu-S and the metallic state of Cu. Moreover, the prepared Cu nanoclusters exhibited aggregation-induced emission enhancement effect. By loading the Cu NCs on TiO₂ support, the resultant composites can be used as novel electrochemical sensing materials for glucose detection. The sensor based on Cu₆ nanoclusters displayed a wider linear range and higher detection sensitivity compared with large Cu nanoparticles. We hope this work could open a new route for the synthesis of Cu nanocluster and provide a new application of Cu nanoclusters.

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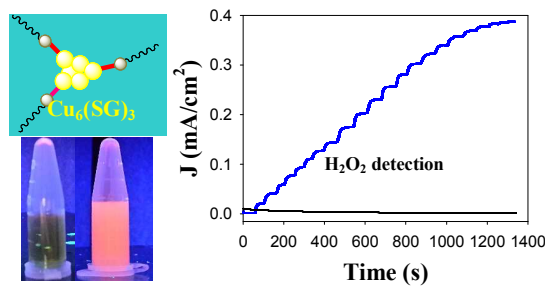
Notes and references

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Sub-nanometer sized $\text{Cu}_6(\text{GSH})_3$ clusters: one-step synthesis and electrochemical detection of glucose

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L-glutathione-protected Cu nanoclusters were synthesized and they exhibit aggregation-induced emission enhancement effect and high performance for electrochemical detection of glucose.