Sonochemical synthesis of Ag nanoclusters: electrogenerated chemiluminescence determination of dopamine

Tao Liu, Lichun Zhang, Hongjie Song, Zhonghui Wang and Yi Lv

ABSTRACT: We report a facile one-pot sonochemical approach to preparing highly water-soluble Ag nanoclusters (NCs) using bovine serum albumin as a stabilizing agent and reducing agent in aqueous solution. Intensive electrogenerated chemiluminescence (ECL) was observed from the as-prepared Ag (NCs) and successfully applied for the ECL detection of dopamine with high sensitivity and a wide detection range. A possible ECL mechanism is proposed for the preparation of Ag NCs. With this method, the dopamine concentration was determined in the range of $8.3 \times 10^{-9}$ to $8.3 \times 10^{-7}$ mol/L without the obvious interference of uric acid, ascorbic acid and some other neurotransmitters, such as serotonin, epinephrine and norepinephrine, and the detection limit was $9.2 \times 10^{-10}$ mol/L at a signal/noise ratio of 3. Copyright © 2013 John Wiley & Sons, Ltd.

Keywords: sonochemistry; Ag nanoclusters; electrogenerated chemiluminescence; dopamine

Introduction

Noble metal nanoclusters (NCs), as an intermediate stage between atoms and bulk materials, are defined as isolated particles approximately 2 nm in size with several to tens of atoms (1). They have attracted much attention because of their discrete electronic states and size-dependent electronic transitions due to their strong quantum-confinement effect of free electrons in this size regime as compared to nanoparticles (NPs) (2,3). Ag NCs, due to their “molecule-like” properties, such as good physical, electrical and optical properties, have found applications in surface-enhanced Raman scattering (4), chemical sensing (5), optical devices (6), bioimaging (7) and catalysis (8). Ag NCs also show other attractive features (9), including low toxicity, high stability, water solubility and biocompatibility, which also make them promising materials for biological applications.

Electrogenerated chemiluminescence (ECL) technique involves the generation of species at the electrode surface that undergo high-energy electron transfer reactions to form excited states, and light is produced when the excited molecule decays to the ground state (10–12). ECL has recently become important and powerful analytical tool and attracts considerable attention due to its distinct advantages over spectroscopy-based detection systems (13,14), such as simplicity, rapidity, high selectivity, high sensitivity, high stability, easy controllability and flexibility. Although analytical applications of quantum dots, such as CdSe (15–17), CdTe (18,19), CdS (20,21) and CdSe/CdS (22) with their unique electrochemical properties, have been extensively used in ECL-based detection, the intrinsic toxicity of quantum dots (23,24) limit their applications. However, up to now, few noble metal NCs have been developed and applied to the ECL except Au NCs. Fang et al. (25) reported ECL emission from Au NCs using triethylamine as the co-reagent and its application in the determination of Pb$^{2+}$. Li et al. (26) investigated ECL behavior of Au NCs using K$_2$S$_2$O$_8$ as the co-reagent and applied in the detection of dopamine based on Au NCs ECL in aqueous media.

Therefore, more “green” noble metal NCs are desirable for development into novel ECL species.

Dopamine (DA), a neurotransmitter present in the brain, plays crucial roles in the central nervous, cardiovascular and renal systems (27). Deficiency of DA may cause several diseases and neurological disorders such as schizophrenia, Huntington’s disease and Parkinson’s disease (28,29). Uric acid, ascorbic acid (30) and some other neurotransmitters (31), such as serotonin, epinephrine and norepinephrine usually coexist with DA in biological samples. Therefore, it is essential to develop simple and rapid methods for the determination of DA with high selectivity and sensitivity in biodiagnostic applications.

In this paper, Ag NCs were synthesized by a facile one-pot, fast sonochemistry method. ECL emission was observed from the as-prepared Ag NCs. Then we developed a promising Ag NCs-based ECL biosensor for DA detection with higher sensitivity and wider detection range in the presence of interfering components. The Ag NCs were directly immobilized on a 3-aminopropyl-triethoxysilane (APTES) and glutaric dialdehyde (GD) modified indium-tin oxide (ITO) electrode according to a previous report (26). The formation of a charge transfer complex, which is polymer-DA–ITO, between DA and ITO resulted in the increase of ECL intensity, which could be used to detect the DA. This ECL biosensor is simple, environmental friendly, highly selective and highly sensitive. In particular, this approach would open new routes to apply metal NC ECL in bioassays.

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Experimental

Materials and instrumentation
Bovine serum albumin (BSA) was purchased from Bo Ruite Biochemical Reagent Co. Ltd. (Beijing, China). Silver nitrate (AgNO3) was obtained from Kelong Chemical Reagent Co. Ltd. (Chengdu, China). 3-Hydroxytyramine hydrochloride (DA), uric acid, ascorbic acid were purchased from Adamas (FC Basel, Switzerland), Aladdin (Shanghai, China) and Guoyao Chemical Reagent Co. Ltd. (Chengdu, China), respectively. Norepinephrine, epinephrine and serotonin were all obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). APTES and GD were obtained from Aladdin (Shanghai, China) and Alfa Aesar (Tianjin, China), respectively. Phosphate buffer solution (PBS, 0.1 mol/L) was prepared by mixing stock solutions of NaH2PO4 and Na2HPO4 with 0.1 M KNO3 as the supporting electrolyte. All other reagents were of analytical reagent grade and used without further purification. Ultrapure water with a resistivity 18.24 MΩ/cm obtained from a water purification system (ULUPURE, Chengdu, China) was used for the experiments.

The ECL intensity was detected with chemiluminescence analyzer (XiAn Remax Electronic Science & Technology Co. Ltd., XiAn, China). The emission window was placed in front of the photomultiplier tube biased at 800–1000 V. Electrochemical experiments were performed with a CH660C electrochemical analyzer (ChenHua Instruments in Shanghai, China) at room temperature. A conventional three-electrode setup was used. An ITO was used as working electrode, a platinum sheet was used as counter electrode and an Ag wire was used as quasi-reference electrode. The ITO electrodes with a working area of ~1.5 cm × 0.7 cm were purchased from Huanan Co. Ltd. (Guangzhou, China). Ultraviolet-visible spectra were recorded on a U-2910 spectrophotometer (Hitachi, Tokyo, Japan). Photoluminescence (PL) spectra were obtained on a F-7000 spectrophotometer (Hitachi, Tokyo, Japan). X-ray photoelectron spectroscopy (XPS) was performed with a Kratos XSAM 800 electron spectrometer (Manchester, UK) using monochromatic Mg Kα radiation for analysis of the surface composition and chemical states of the product. High-resolution transmission electron microscopy (HRTEM) images were carried out on a Tecnai G2 F20 S-TWIN transmission electron microscope at an accelerating voltage of 200 kV (FEI Co., Hillsboro, OR, USA). Samples were prepared for analysis by evaporating a drop of aqueous product on a lacey carbon copper TEM grid.

Synthesis of bovine serum albumin-stabilized Ag nanoclusters
The BSA-stabilized Ag NCs were firstly prepared via a simple, rapid, green sonochemical synthesis route. Briefly, 250 mg BSA was dissolved in 9 mL water and mixed with aqueous AgNO3 solution (1 mL, 100 mmol/L), reacting at ambient temperature for 5 min with vigorous stirring, then 0.50 mL 1 mol/L NaOH was added to the solution for adjusting the pH to 12, where BSA acted as stabilizing agent and reducing agent in this condition; finally, the mixture was exposed to ultrasonic irradiation (Kunshan Co. Ltd., China, QK3200DE, 50 W/cm²) under low temperature(15°C) for 4 h (time optimization: the ratio of ultrasonic time and intermittent time is 7:3). During this period, the color of the colloidal solution changed from colorless to yellow, providing clear evidence for the formation of Ag NCs (32). The Ag NCs solution is purified via dialysis using 7000 Da MWCO dialysis bag. The pH value of Ag NCs solution is about 7. The concentration of Ag NCs solution is calculated to be 1.50 × 10⁻⁵ mol/L according to previous work (33).

Preparation of indium-tin oxide electrodes
Before modification, the ITO electrodes were cleaned with acetone, ethanol, and water under sonication for 20 min respectively. After being sonicated in a solution of 1:1 (v/v) ethanol/NaOH (1 mol/L) for 15 min, they were rinsed with water thoroughly, to give ITO electrodes modified with hydroxyl groups.

Fabrication of the electrogenerated chemiluminescence biosensor
Twenty microliters of 4% (v/v) APTES in anhydrous ethanol was put on to the ITO electrodes and dried in air with overnight, followed by adding 20 μL of 4% GD solution at room temperature for 10 min, and then the electrodes were rinsed with water several times. After as-prepared Ag NCs (20 μL) solution was placed on to the ITO and dried in the air with an overnight, all the electrodes were completely rinsed with water to remove uncombined Ag NCs before use.

Electrogenerated chemiluminescence detection
For the experiment, the ECL biosensor was incubated in different concentrations of DA at room temperature for 5 min. Then, the electrodes were scanned from −1.5 to 0 V in 0.1 mol/L PBS (pH 7.4) containing 0.1 mol/L K2S2O8 and 0.1 mol/L KNO3, and Ag NCs ECL signals together with ECL signals related to the DA concentrations could be measured.

Results and discussion

Characterization of the Ag nanocrystals
The HRTEM images (Fig. 1A) showed that the as-prepared Ag NCs had in good monodispersity and about 1.5 nm in diameter. The XPS spectrum in the Ag 3d region is illustrated in Fig. 1(B). The observed peaks of Ag 3d5/2 and Ag 3d3/2, at 367.9 eV and 373.9 eV, respectively confirmed the existence of Ag in its zero valent form, which is in keeping with the result reported previously (34). The Ag NCs in Fig. 2(A) showed a PL peak at about 485 nm and ultraviolet-visible absorption spectra agreed with the reported literature (35), indicating the consequence of quantum confinement on Ag NCs.

Electrogenerated chemiluminescence behaviors of the Ag nanocrystals on the indium-tin oxide electrode
The ECL behavior of Ag NCs was observed in a solution containing K2S2O8 as a coreactant generally used in ECL systems when the potential was set in the range of −1.5–0 V vs. Ag wire quasi-reference electrode. Supplemental Fig. S1 shows the cyclic voltammograms of bare ITO electrode in 0.1 mol/L PBS (pH 7.4) containing 0.1 mol/L K2S2O8 and without 0.1 mol/L K2S2O8 aqueous solution. (Optimization of the Ag NCs ECL conditions, such as pH, scan rate and K2S2O8 concentration, are described in Supporting information, Figs S2 and S3.) Without K2S2O8, the cathodic peak at about −1.3 V represents the reduction of ITO, while in the presence of K2S2O8, the cathodic current began to flow at −0.6 V due to the reduction of peroxydisulfate on ITO (36). As shown in Fig. 1(C), when Ag NCs were modified on ITO electrodes, the ECL signal was observed at 1.27 V, which was...
about seven times higher than that of bare ITO at −1.32 V. Also, as shown in Fig. 1(D), no difference was found on the ECL signal even in the presence of BSA when the concentration was the same as that in Fig. 1(C,b).

Ag nanoparticles (Ag NPs) acted as conductive media facilitating the electron transfer in the ECL reaction and could amplify the ECL signal in some ECL biosensors (37). To confirm that the obtained strong signal at the Ag NCs modified ITO electrode indeed originated from the Ag NCs rather than Ag NPs, we measured the ECL spectra in both the presence and absence of Ag NCs by using a series of optical filters. As can be clearly seen in Fig. 2(B), a distinct ECL peak around 490 nm is observed in the presence of Ag NCs, which is in disagreement with the peak around 450 nm that is too weak to show in the absence of Ag NCs. On the other hand, to compare the ECL potential curves of the Ag NPs modified ITO electrode with the bare ITO electrode, no obvious enhanced signal could be observed in the presence of Ag NPs (see Supplemental Fig. S4). These results all confute the enhancing effect from the Ag NCs. Moreover, the as-prepared Ag NC solution exhibited fluorescence with an emission peak at about 485 nm in Fig. 2(A). Although the PL mechanism of the Ag NCs is not completely clear, the ECL spectrum peak (see Fig. 2B) is consistent with the PL peak, which indicates that Ag NCs are indeed the luminescent species.

**The mechanism of Ag nanocrystal electrogenerated chemiluminescence on indium-tin oxide electrode**

To date, there are two main mechanisms in the ECL system, one is ion annihilation ECL, the other is coreactant ECL (38). For the Ag NC modified the ITO electrode in the absence of
S2O82−, it was found that the ECL signal was too weak to be distinguished from that of bare ITO electrode, which implied that S2O82− plays a crucial role as coreactant in the process of Ag NC ECL. Moreover, there are also several other coreactants, such as tri-n-propylamine, C6H5O2− to be considered. The strong ECL signal of Ag NCs only could be observed in the presence of S2O82− and no ECL was observed using tri-n-propylamine, C6H5O2− as coreactants (see Supplemental Fig. S5). Meanwhile, as shown in Supplemental Fig. S6, the ECL intensity was slightly reduced between 0 V and −1.5 V in comparison with the range 2.0−1.5 V. It can be concluded that the coreactant ECL mechanism is the primary mechanism in the course of Ag NC ECL. Similar to the ECL of quantum dots (39), the electron transfer between the redox species of Ag NCs also contributes to the overall ECL. In addition, a glassy carbon electrode was used as the working electrode for further investigation, and it was found that even the signal of a bare glassy carbon electrode was much higher than that of the Ag NC modified ITO electrode. The result reveals that ITO plays an important role in this ECL process, which is in agreement with data reported previously (35).

It has been reported that Agn NCs (n = 2−21) exhibits an electronic molecular type HOMO-LUMO band gap from 0.2 to 2.7 eV (40). On the one hand, in the process of cathodic ECL, electrons could be injected into the conduction band of ITO and then transferred to the LUMO of Ag NCs, and then generate Ag+, implying ITO acted as an effective reductant for Ag NCs. On the other hand, the intrinsic, greater ECL activity of Ag NCs is probably due to their small size, which results in reduced crystallinity (41), or even an amorphous state (42). This agrees with the characterization of the selected area electron diffraction (not shown), and then, the distorted, structure of the Ag NCs probably take place in the presence of the potential, thus enhancing its ECL activity. The corresponding ECL mechanisms are as follows, and are similar to previous reports (26):

\[
\begin{align*}
\text{Ag} & \rightarrow \text{Ag}^+ + e^- \\
S_2O_8^{2−} + e^- & \rightarrow S_2O_4^{2−} \\
S_2O_4^{2−} & \rightarrow SO_4^{2−} + SO_4^{2−} \\
Ag^+ + SO_4^{2−} & \rightarrow eAg + + SO_4^{2−} \\
eAg + + hu(ECL) & \rightarrow e(\text{excited state})
\end{align*}
\]

**Electrogenerated chemiluminescence detection of dopamine with the biosensor and its repeatability, stability and selectivity**

When DA was injected into the electrolyte, the cathodic ECL intensity increased linearly with DA concentrations in the range from 8.3 × 10−9 to 8.3 × 10−7 mol/L as shown in Fig. 3(A,B), and the detection limit was estimated to be 9.2 × 10−10 mol/L at a signal/noise ratio of 3. Therefore, a wider detection range and lower detection limit than some label-free methods was obtained (26,43−47) (Table 1). Meanwhile, making comparison with the absence of DA in electrolyte, a higher current in the cyclic voltammogram was achieved in the presence of DA, indicating that DA participated in the electrochemical behavior of this system. The ECL enhancement mechanism of DA here, similar to DA and TiO2 (48), is due to the formation of charge transfer complex polymer-DA–Ag NCs–ITO.

In addition, the repeatability and stability of the DA ECL biosensor model was tested. The relative standard deviation (RSD) value determined at a DA concentration of 0.50 μmol/L from eight different electrodes was 0.9%. The stability of ECL intensity under consecutive potential scans from −1.5 to 0 V for nine cycles with 0.30 μmol/L DA in the ECL testing system is shown in Fig. 3(C). The series of signals with RSD of 1.6% indicated the acceptable reliability and stability for DA detection. There was also no obvious change that could be observed in the solution containing 8.3 × 10−7 mol/L DA (blank) and after the addition of 8.3 × 10−5 mol/L ascorbic acid and 4.15 × 10−5 mol/L uric acid.

![Figure 3](https://example.com/image.png)

**Figure 3.** (A) ECL profiles of the Ag nanocrystal electrode in different concentrations of dopamine (DA) in pH 7.4 phosphate-buffered saline containing 0.1 mol/L KNO3 and 0.1 mol/L K2S2O8, DA concentration (mol/L): (a) 8.3 × 10−9, (b) 4.2 × 10−7, (c) 8.3 × 10−6, (d) 1.7 × 10−5, (e) 3.3 × 10−5, (f) 5.0 × 10−5, (g) 5.8 × 10−5, (h) 6.7 × 10−5, (i) 8.3 × 10−5. (B) Linear plots of ECL intensity vs. DA concentrations. Every point was an average value of 11 independent measurements. (C) Consecutive ECL intensity in the presence of 0.3 μmol/L under continuous cyclic voltammetry for nine cycles. Scanning rate: 100 mV/s. ECL, electrogenerated chemiluminescence.
acid, respectively. In addition, other neurotransmitters, such as serotonin, epinephrine and norepinephrine (the concentration is 8.3 \times 10^{-6} \text{ mol/L} for each of the three agents) showed minimal use interference, indicating an acceptable selectivity for DA detection (see Supplemental Fig. S7). Thus, this method can be used to detect DA concentration qualitatively in some biological fluids.

### Determination of dopamine in human blood serum samples

The application of the proposed method in real samples has been investigated by direct analysis of DA in human blood serum samples (obtained from West China Hospital of Sichuan University). All the serum samples were diluted 100-fold with 0.1 mol/L PBS (pH 7.4) before measurement. No other pretreatment process was performed. The results are listed in Table 2. It can be concluded that for sample analysis, the presence of uric acid, ascorbic acid and some other interferences did not interfere with the determination of DA. The acceptable recovery and RSD indicate that the method based on Ag NCs could be potentially used for determining DA in real samples.

### Conclusions

In summary, ECL was observed from BSA-stabilized Ag NCs, which were synthesized by a facile one-pot, green sonochemistry method. Based on the ECL behavior, a mechanism was proposed, and the potential application as an ECL biosensor for DA detection was demonstrated with a wider detection range and lower detection limit than some label-free methods. Owing to the advantages of easier fabrication method, higher stability, lower toxicity, the Ag NCs could become promising candidates, which could be widely applied in ECL biological analysis and stimulate more interest in some other metal NCs in different aspects of biochemistry.

### SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

### Acknowledgments

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### References


### Table 1. Comparison of some label-free methods for the detection of dopamine

<table>
<thead>
<tr>
<th>Detection system</th>
<th>Linear range (µM)</th>
<th>Detection limit (µM)</th>
<th>Reference</th>
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</thead>
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<tr>
<td>ECL</td>
<td>2.5–47.5</td>
<td>No</td>
<td>26</td>
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<tr>
<td>FI-CL</td>
<td>0.01–0.2</td>
<td>0.002</td>
<td>43</td>
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<tr>
<td>EC</td>
<td>0.05–1</td>
<td>0.015</td>
<td>44</td>
</tr>
<tr>
<td>FL</td>
<td>0.1–50</td>
<td>0.04</td>
<td>45</td>
</tr>
<tr>
<td>CE</td>
<td>20–100</td>
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<td>46</td>
</tr>
<tr>
<td>CL-CE</td>
<td>0.08–5</td>
<td>0.023</td>
<td>47</td>
</tr>
<tr>
<td>ECL</td>
<td>0.0083–0.83</td>
<td>0.00092</td>
<td>This work</td>
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</table>

CE, capillary electrophoresis; CL-CE, chemiluminescence-capillary electrophoresis; EC, electrochemistry; ECL, electrogenerated chemiluminescence; FI-CL, flow-injection chemiluminescence; FL, fluorescence.

### Table 2. Determination of dopamine in human blood serum samples (n = 3)

<table>
<thead>
<tr>
<th>Samples</th>
<th>Added (nmol/L)</th>
<th>Found (nmol/L)</th>
<th>RSD (%)</th>
<th>Recovery (%)</th>
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<td>800.0</td>
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<td>100.7</td>
</tr>
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</table>

Table 1. Comparison of some label-free methods for the detection of dopamine

Table 2. Determination of dopamine in human blood serum samples (n = 3)