Chemiluminescence catalysed by gold nanoparticles for the analysis of arsenic (III) in real water

Mulayam Singh Gaur, Reetu Yadav, Anna N. Berлина, Anatoly V. Zherdev and Boris B. Dzantiev

A gold nanoparticle (AuNPs)-catalysed chemiluminescence (CL) method is developed for the analysis of As$^{3+}$ cations by detecting the enhancement of the luminol–H$_2$O$_2$ reaction by AuNPs. AuNPs of different sizes were prepared by a chemical method. The size and shape of these nanoparticles were determined by transmission electron microscopy. The various parameters of the reaction media such as the pH and the concentration of H$_2$O$_2$ and luminol were optimised. The enhancement of the CL intensity may be as a result of energy transfer by the AuNPs or plasmon-induced enhancement. The interaction of the AuNPs with As$^{3+}$ amplified the CL signal. This amplified CL was used to detect As$^{3+}$ in real water samples. The linear region of the calibration curve from 0.3 to 4 $\mu$g/L shows that this is a suitable method for the detection of low concentrations of arsenic (III).

1. Introduction

Generally, humans are exposed to arsenic by ingestion (drinking or eating) or inhalation (breathing). In ground and surface water, arsenic is generally found in inorganic forms of arsenite (As (III)) and arsenate (As (V)), both of which are acutely toxic if swallowed. Arsenic and lead contamination in water has been prevalent around the world. Many millions of people in South-East Asia and South America have suffered from arsenic exposure [1]. Arsenic contamination in groundwater has been found to adversely affect human health even at very low concentrations (i.e. 10 $\mu$g/L) [2].

A great challenge in the area of heavy metal trace detection is the development of catalytic electrochemical techniques. These techniques are user-friendly, robust and selective, with low detection limits and allowing fast analysis. Chemiluminescence (CL) is the phenomenon when a vibronically excited product of an exoergic reaction relaxes back to its
ground state with emission of photons. CL is a sensitive tool with a wide range of applications in the field of biotechnology, pharmacology, molecular biology and environmental assays [3–5]. At present, diverse chemiluminescence systems are widely used in inorganic, organic, DNA hybridisation, nucleic acid analysis and immunoassays [6–8]. Luminol is one of the most common reagents used in CL reactions. The most frequently used CL reaction is the luminol–hydrogen peroxide system, which can be catalysed by various substances such as metal ions, metal complexes and enzymes [9–12].

Nanoparticles frequently display unusual catalytic properties for a variety of chemical reactions, depending on their size, shape and stabilising agents. AuNPs can be directly used as the catalysts for liquid-phase chemical reactions [13]. Gold and silver nanoparticles are considered to be the best enhancers of chemiluminescence reactions. The use of gold nanoparticles of different size can increase the intensity of the produced light and the duration of the emission. AuNPs can directly induce or catalyse liquid-phase chemiluminescence, which extends the studies on catalysts in liquid-phase chemiluminescence from molecular and ion systems to nanomaterial systems.

AuNPs have been successfully employed as catalysts because of their good chemical stability and high resistance to surface oxidation [14]. The luminol–H$_2$O$_2$ reaction has been widely applied for the detection of metal ions in water samples [15,16]. The properties of AuNP formulations have been modified for the detection of several environmental pollutants. The high ion selectivity of AuNPs and the strong distance-dependent optical properties are ideal attributes for the development of colorimetric sensors for heavy metal ion detection [17,18]. The incorporation of AuNPs in chemiluminescence studies is promising for the construction of inexpensive and stable devices with excellent biocompatibility.

The CL technique has been applied for the detection of vanadium (IV), arsenic (III) and arsenic (V). Vanadium (IV) was used as a catalyst for cinchomeronic hydrazide–H$_2$O$_2$ and purpurogallin–IO$_4^-$ [19] systems. A lucigenin–H$_2$O$_2$–KOH system was used to detect arsenic (III) and arsenic (V) [20,21].

Recently, Attiq-Ur-Rehman et al. [22] reported the detection of arsenic (V) by a flow injection method based on luminol chemiluminescence. The method was applied to freshwater samples and the results were correlated using HGAAS (hydride generation atomic absorption spectrometry). The limit of arsenic (V) detection was 2.8 μg/L. The main drawbacks of the flow injection method are that it is time-consuming and has a complicated experimental arrangement.

A novel CL immunoassay method of microcystins based on AuNPs was recently developed [23]. This method was applied for the detection of microcystins in drinking water without observing the matrix effect.

The physical properties of AuNPs (i.e. optical and electronic properties) can be modified through control of their size, composition, shape and surface chemistry, to generate highly functional molecular probes [24]. The AuNPs can also be functionalised with molecular capturing agents (i.e. antibodies, aptamers) for very rapid detection of various toxins and pollutants in water [25]. The aim of this work is to develop chemiluminescence nanoassays for the detection of arsenic cations in Agra and Mathura ground water.
2. Experimental

2.1. Apparatus

A round-bottom flask fitted with a reflux condenser (REMI equipment Pvt Ltd., Mumbai, India) was used for the synthesis of AuNPs. The particle size was observed by transmission electron microscopy (JEOL 2100F, Jeol, Japan). All pH measurements were performed using a digital pH meter and the chemiluminescence signal was recorded using a 96-well Luminometer (Wellkang Ltd, London, UK). A schematic diagram of the chemiluminescence measurement system is shown in Figure 1.

2.2. Materials

Gold (III) chloride hydrate (purity 99.999%), luminol and hydrogen peroxide (H$_2$O$_2$) were purchased from Sigma-Aldrich. Sodium hydroxide (NaOH) pellets (97%) were purchased from Qualigens Fine Chemicals Ltd. (Mumbai, India). Deionised water was used during the whole process of the AuNPs synthesis. Other chemicals used in this study were purchased from Sigma-Aldrich.

2.3. Synthesis of AuNPs

AuNPs of different sizes were prepared by a chemical synthesis method. An aqueous solution of HAuCl$_4$ was brought to a vigorous boil while stirring in a round-bottom flask fitted with a reflux condenser. Trisodium citrate was added rapidly to the solution, which was

![Figure 1. Schematic diagram of the chemiluminescence measurement system.](image-url)
then heated for 1–2 h. During this period the colour of the solution changed from pale yellow to deep red. The solution was slowly cooled down from 100 to 25 °C with continuous stirring and then sonicated for 2 min before use.

### 2.4. Solution preparation

A 0.250-mol/L stock solution of luminol (3-aminophthalhydrazide) was prepared by dissolving 0.1326 g luminol in 2.9 mL NaOH (0.1 mol/L) solution and stored in a dark place. A working solution of luminol was prepared by diluting the stock solution, pH 10, using 1 mol/L NaOH solution. A working solution of H2O2 was freshly prepared every day from 30% v/v solution. Arsenic (III) stock solution (0.8 M) was prepared by dissolving 2 g of AsO3³⁻ (LOBA Chemical) in 100 mL of 0.1 M NaOH solution. An arsenic working solution was prepared by diluting the stock solution. The stock solution of As (III) was kept in a sealed container in a refrigerator at 4 °C.

### 2.5. Development of the chemiluminescence nanoassay protocol

The effect of the reaction parameters of CL such as the pH, concentration of luminol, concentration of H2O2, size and quantity of the AuNPs was investigated. Fifty micro litre of luminol solution, 50 μL of AuNPs, 50 μL of As³⁻ solution and 50 μL of H2O2 were added into a 96-well microtitre plate and the chemiluminescence signal was measured using a luminometer.

### 2.6. Collection of water samples and preparation

Water samples were collected from the Agra and Mathura ground water (northern region of India). All the samples were collected into plastic tubes with a total volume of 150 mL and kept frozen at −20 °C prior to analysis. The samples were brought to room temperature (20–25 °C) before the analysis. The samples were passed through a Millex GP filter with a pore diameter of 0.22 μm. Samples with varying content of As³⁻ were prepared by adding the As³⁻ solution to the Milli-Q water to achieve the final concentrations of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15 μg L⁻¹.

### 2.7. Characterisation techniques

TEM images were obtained using a JEOL2100F microscope at 80 kV. The sample was suspended in ethanol and homogenised using a sonicator for 10 min. One drop of the unsetttled suspension was placed on a copper grid and the solvent was allowed to dry at room temperature. The average diameter of the particles was calculated by SIS Soft Imaging GmbH image analyses software.

### 2.8. Chemiluminescence reaction mechanism

Several approaches have been reported for the enhancement of luminescence using gold nanoparticles [26–32]. However, the most appropriate reaction mechanism was discussed by Zhi-Feng Zhang et al. [33]. They suggested that the O–O bond of H2O2 might be broken up into two HO* radicals by virtue of the catalysis of gold nanoparticles and that the
generated hydroxyl radicals might be stabilised by gold nanoparticles through partial electron exchange interactions. The HO$^*$ radicals reacted with the luminol anion and HO$_2$ to facilitate the formation of luminol radicals and superoxide radical anion (O$_2$$^{•-}$). Further electron-transfer processes between luminol and O$_2$$^{•-}$ radicals on the surface of the gold nanoparticles take place to produce the intermediate hydroxyl hydroperoxide, as indicated in Figure 2, leading to the enhancement of the CL.

3. Results and discussion

3.1. Characterisation of AuNPs

TEM images of the nanoparticles were obtained by passing an accelerating electron beam through the specimen. The diameter of the synthesised AuNPs was characterised by TEM (Figure 3). AuNPs appeared to be nearly monodisperse, with different particle sizes in the nanometre range. TEM images (Figure 3(a)) show different size AuNPs (22, 25, 15, 17, 20 and 16 nm). The diffraction pattern (Figure 3(b)) shows the almost spherical shape of the AuNPs.

3.2. Effect of the size of AuNPs

The effect of the size of the AuNPs was investigated (Figure 4). AuNPs with a diameter of 15 nm showed significant catalytic activity. Figure 4 shows the relationship between CL intensity and reaction time in a luminol–H$_2$O$_2$ system at pH 12.5 with AuNPs of different sizes, 15, 30 and 50 nm. The concentrations of luminol, H$_2$O$_2$ and AuNPs were 1.0 mmol/L,
Figure 3. Transmission electron microscopy image and diffraction pattern of chemically synthesised 15 nm AuNPs.

Figure 4. Effect of AuNPs size on the chemiluminescence intensity (RLU).
0.3 mmol/L and 10 nmol/L, respectively. The 15 nm AuNPs showed stronger CL intensity within 2 min. Therefore, this size of the particles was selected for the nanoassay.

### 3.3. Effect of pH

The effect of the pH of the NaOH solution in the range of 11.0–13.0 was studied. The alkalinity of the system had a great influence on the reaction. It has been reported that luminol could be stabilised by protonation. Therefore, the luminol reaction was carried out under alkaline conditions because the rate of luminol oxidation was extremely low in acidic solutions. The time at which the CL attains its maximum intensity is related to the concentration of the reactants. The CL exhibited flashlight when the concentration was too high, and the intensity decreased dramatically after reaching a maximum. Luminol was dissolved in NaOH solutions of different concentrations and a pH range of 11.0–13.0. When the pH value of the luminol solution was lower than 12.5, the CL intensity increased with increasing pH. However, when the pH value of the luminol solution was higher than 12.5, the CL intensity decreased with increasing pH value. Therefore, luminol prepared in pH 12.5 buffer solution was selected for subsequent experiments. The effect of pH is shown in Figure 5(a).

### 3.4. The effect of luminol concentration

The effect of the luminol concentration on the CL intensity was investigated. The pH of the luminol–H₂O₂ system was 12.5, and the concentrations of H₂O₂ and AuNPs were 0.3 mmol/L and 10 nmol/L, respectively. The concentration of luminol was 1.0 mmol/L. The CL intensity attained its maximum value (Figure 5(b)) when the concentration of luminol was 1 mmol/L. The CL intensity increased linearly with the increase of the luminol concentration in the range 0.01–1.0 mmol/L. When the luminol concentration was 1.0 mmol/L the reaction attained the highest CL intensity. This value did not change a lot when the luminol concentration was increased further, so the optimal concentration of luminol was 1.0 mmol/L.

### 3.5. Effect of H₂O₂ concentration

The effect of the concentration of H₂O₂ on the CL intensity was investigated. The pH of the luminol–H₂O₂ system was 12.5 and the concentrations of luminol and the AuNPs were 1.0 mmol/L and 10 nmol/L, respectively. The concentration range of H₂O₂ was 0.05–0.3 mmol/L. As shown in Figure 5(c), the CL intensity reached its maximum value when the concentration of H₂O₂ was 0.3 mmol/L. The CL intensity increased with increasing H₂O₂ concentration in the range of 0.005–0.3 mmol/L. The CL intensity did not change when the H₂O₂ concentration was between 0.2 and 0.3 mmol/L.

### 3.6. Effect of AuNPs concentration

The effect of the concentration of the AuNPs on the CL intensity was investigated (Figure 6). According to the optimal conditions, the pH of the luminol–H₂O₂ system was 12.5, and the concentrations of luminol and H₂O₂ were 1.0 and 0.3 mmol/L, respectively.
Figure 5. The development of a chemiluminescence nanoassay: (a) effect of pH on RLU, (b) effect of luminol concentrations on RLU and (c) effect of H$_2$O$_2$ concentrations on RLU.

Figure 6. Calibration curve of the registered chemiluminescence for different concentrations of AuNPs.
The concentration range of the AuNPs was 0–10 nmol/L. Above 10 nmol/L the CL intensity decreases significantly. The regression coefficient was very close to 1. Therefore, the calibration curve shows that a AuNPs concentration range between 0 and 10 nmol/L is suitable for nanoassays.

3.7. Effect of interfering elements

The effect of interfering elements in the nanoassays was studied by using a similar protocol for CL detection in the presence of Mg$^{2+}$, Ni$^{2+}$, Fe$^{3+}$, Zn$^{2+}$, Ca$^{2+}$ and Na$^+$, as shown in Figure 7. We observed that the CL intensity was maximal for As$^{3+}$ and very weak when these interfering elements were used. Therefore, our nanoassay protocol for the detection of As$^{3+}$ is free from interfering elements in the water samples.

3.8. Determination of As$^{3+}$ in real water samples

Samples of As$^{3+}$ in distilled water, Agra and Mathura ground water were prepared with different concentrations of As$^{3+}$. The initial concentrations of the metals in Agra ground water and Mathura ground water were observed by AAS. The initial concentrations of the metals in Agra ground water, Mathura ground water and Chambal river water were observed by AAS. The concentrations of different metals by AAS analysis were found to be As (0.17–7.25 μg/L), Cd (0.01–3.24 μg/L), Cr (0.06–21.86 μg/L), Cu (0.31–43.41 μg/L), Hg (0.01–0.58 μg/L), Pb (0.06–8.5 μg/L), Ni (0.21–8.66 μg/L), Zn (0.008–0.089 mg/L) and Fe (0.020–0.33 mg/L. All the metals were under the acceptable limit.

We investigated the effect of different concentration of As$^{3+}$ on the CL intensity. The calibration curve of relative luminescent units (RLU) versus concentration of As$^{3+}$ is shown in Figure 8. Several observations were performed by adding different concentrations of As$^{3+}$. We observed a significant change in the RLU characteristics with different concentration of As$^{3+}$. The calibration curve was not linear for lower concentrations of
arsenic (III) (<0.3 μg/L); however, it was linear for higher concentrations. The linear region of the calibration curve from 0.3 to 4 μg/L shows that this method is suitable for the detection of low concentrations of arsenic (III). Therefore, the detection limit of As$^{3+}$ was 0.3 to 4 μg/L.

3.9. Analytical parameters

The calibration curve was plotted using a series of 20 standard solutions under the selected experimental condition. The plot was linear over the As$^{3+}$ concentration range from 0.3 to 4.0 μg/L. The corresponding linear regression coefficient ($r^2$) was 0.99. This shows the linear correlation between RLU and As$^{3+}$ concentration.

4. Conclusions

Colloidal AuNPs were synthesised in the presence of trisodium citrate and used in a homogeneous assay. The CL intensity of the luminol–H$_2$O$_2$ system increased in the presence of AuNPs, proving that the AuNPs catalysed the luminol CL reaction. AuNPs with a diameter of 15 nm showed significant catalytic activity in the CL system. Different concentrations of As$^{3+}$ caused a change in the CL intensity owing to the interaction between the As$^{3+}$ cations and the citrate-covered AuNPs. An increase in the CL intensity was accompanied by a change in the number of photons owing to a chemical reaction. This chemiluminescence system based on a AuNPs nanoassay is a rapid and simple tool for the detection of As$^{3+}$ cations.

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**Disclosure statement**

No potential conflict of interest was reported by the authors.

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**ORCID**

Mulayam Singh Gaur http://orcid.org/0000-0003-0905-9781

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