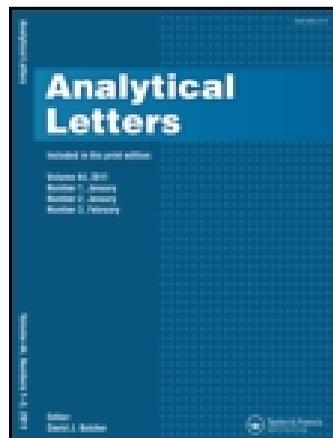


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Jinxiang Yang^a, Yanbin He^a, Xiaoliang Song^a, Lina Lai^a, Junbo Li^a & Buping Guo^a

^a Pharmaceutical Department, Changzhi Medical College, Changzhi, China

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Electrochemistry

A NOVEL SENSITIVE BIOSENSOR FOR Ca^{2+} ION BASED ON GOLD NANOPARTICLES MODIFIED ELECTRODE BY PULSED ELECTRODEPOSITION

Jinxiang Yang, Yanbin He, Xiaoliang Song, Lina Lai,
Junbo Li, and Buping Guo

Pharmaceutical Department, Changzhi Medical College, Changzhi, China

The work demonstrates a simple method for sensitive detection of Ca^{2+} ion by electrochemical response of alizarin red S (ARS) and Ca-ARS at a gold nanoparticle modified glassy carbon electrode (GCE). In the 0.1 M KOH, a sensitive reduction peak was observed at -0.795 V at the gold nanoparticles modified electrode. The peak currents were proportional to the concentrations of Ca^{2+} ion in the range of 2.0×10^{-7} M– 1.2×10^{-4} M. For the different pulse voltammetry (DPV) methods, the detection limit was 2.57×10^{-8} M. The reaction mechanism was primarily determined by cyclic voltammetry, and the experimental results showed that the electrode processes were quasireversible responses of ARS and irreversible responses of ARS-Ca. In addition, the method was simple, fast, precise, and was used in the determination of calcium in blood serum with satisfactory results.

Keywords: Alizarin red S; Ca^{2+} ion; Gold nanoparticles; Pulsed electrodeposition

INTRODUCTION

It is well-known that calcium is essential in living cells and serum samples. Mitochondrial Ca^{2+} homeostasis has a key role in the regulation of aerobic metabolism and cell survival (Szabadkai and Duchon 2008), but the molecular identity of the Ca^{2+} channel, the mitochondrial calcium uniporter, is still unknown. Recent literature reported that mitochondrial calcium uniporter was identified (Baughman et al. 2011). In the human serum, muscle contraction and neuronal activity are obviously related to the concentration of Ca^{2+} . Hence, it is important to know its concentrations in the intracellular media and serum samples. X. T. Chen, Cui, and Hu (2006) and Liu and Song (2005) have determined trace contents of calcium by studying the calcium-alizarin red S (ARS) complex absorptive wave at mercury film electrode and carbon paste electrode. Low limit of detection and high reproducibility

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Address correspondence to Xiaoliang Song, Pharmaceutical Department, Changzhi Medical College, Changzhi 046000, P. R. China. E-mail: hyb_ren@126.com

have been reported in these methods. However, the toxicity of mercury is very high to human bodies, and the fabrication method of carbon paste electrode cannot be easily applied to plenty of analytical tests.

Recently, the applications of gold nanoparticles in sensors have aroused increasing interest due to their surface effect, quantum size effect, electrocatalytic activity, and biocompatibility (Satheesh Babu et al. 2010; Boisselier and Astruc 2009; Wilson 2008). The fabrication of nanoscale structures has attracted much interest recently owing to their potential use in nanoelectrodes for the direct deposition of nanoparticles (Huang and Xiong 2008; X. X. Chen, Li, and Eckhard 2007). In this paper, a simple method for achieving high dispersion and small Au nanoparticles at glassy carbon electrode is presented by pulsed electrodeposition. The electrocatalytic activity of gold nanoparticles can be controlled by varying the concentration of HAuCl_4 solution and adjusting the number of the potential pulse. Trace contents of calcium have been determined by studying the ARS and Ca-ARS complex absorptive wave at gold nanoparticles modified electrodes. This method can be effectively applied to determine the content of calcium in human blood serum. Subsequently, electrochemical behavior and electrode mechanism were also discussed in this paper.

EXPERIMENTAL

Reagents and Chemicals

The alizarin red S (ARS) was purchased from Beijing Chemical Reagents Company. CaCO_3 was obtained from Kelong Chemical Engineering Company (Chengdu, China). The HAuCl_4 , KOH, and other reagents were commercially available. All chemicals were analytical grade and used without any further purification. High-purity deionized water ($18.2 \text{ M}\Omega$) was obtained by passing distilled water through a Milli-Q Plus water purification system. Samples of human serum were obtained from Affiliated Hospital of Changzhi Medical College.

Apparatus

All electrochemical measurements were performed with an Iviumstat (Ivium Technologies—The Netherlands) interfaced to a personal computer. The electrochemical cell consisted of three electrodes, which gold nanoparticles electrode ($d = 3 \text{ mm}$) acted as the working electrode, Pt as the counter electrode, and Ag/AgCl electrode as the reference electrode. All potentials measured in this work were referenced to a Ag/AgCl (3 M KCl) electrode.

Preparation of Gold Nanoparticles Electrode

Prior to modification, the GCE electrode was polished successively with 0.3 and $0.05 \mu\text{m}$ Al_2O_3 powder on micro cloth pads. Then, the electrode was washed using triply distilled water, and subsequently soaking the electrode in an aqueous solution of 6 M HNO_3 for 2–3 min. After being washed ultrasonically by absolute ethanol and water for 3 min, by performing chronoamperometry, the electrode

was scanned in a plating solution containing 1.0×10^{-3} M HAuCl₄ for 120 potential pulses at -0.2 V. Both the pulse duration and the interval were 2 seconds. The surface modification of the prepared electrode was carried out to produce gold nanoparticles coating in golden color.

Electrochemical Detection of Ca²⁺ Ion

The solution containing 2.5×10^{-3} M ARS solution of 1 ml, 1 M KOH solution of 1 ml and a series of concentrations of Ca²⁺ ions were added into colorimetric tube, and then diluted to 10 ml with triply distilled water. Similarly, the solution containing 2.5×10^{-3} M ARS solution of 1 ml, 1 M KOH solution of 1 ml and 0.5 ml serum sample, which was diluted 20 times, were added into colorimetric tube, and then diluted to 10 ml with triply distilled water. The experiments were carried out by measuring the reduction peak current of the ARS that reflected the concentration of Ca²⁺ ion through cyclic voltammetry and differential pulse voltammetry. The CV was carried out in a potential range of -0.6 V– -1.05 V at a scan rate of 100 mV s⁻¹. The DPV experiments were conducted at a scan rate of 100 mV s⁻¹ under 15 mV pulse amplitude and pulse time of 10 ms.

RESULTS AND DISCUSSION

Electrochemical Response of ARS at the Gold Nanoparticles Modified Electrode

The CV is an effective method for probing the feature of surface modified electrode and testing the kinetic barrier of the interface. Figure 1 shows the cyclic

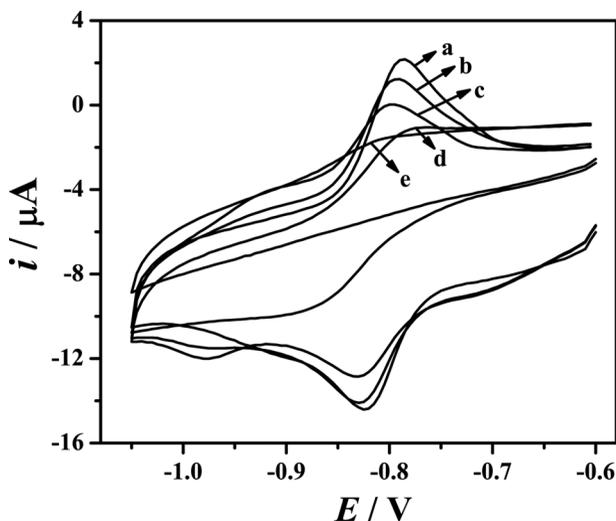


Figure 1. Cyclic voltammograms of 2.5×10^{-4} M ARS solution containing 0.1 M KOH at the gold nanoparticles modified electrodes (a, b, c) and the bare glass carbon electrodes (d, e), in the presence (b, e) of 3.0×10^{-5} M Ca²⁺ and (c) of 7.0×10^{-5} M Ca²⁺. The scan rate was 100 mV · s⁻¹.

voltammograms of the bare glassy carbon electrode and the gold nanoparticle modified electrode in 2.5×10^{-4} M ARS solution containing 0.1 M KOH. The reduction peak of ARS at the nanoparticles modified electrode (the potential is at -0.815 V) (Fig. 1a), was higher than the oxidation peak, which shifted to more positive value when scanned back. The results showed that $I_{pc}/I_{pa}=0.96$ and $\Delta E_p=40 \text{ mV} > 58 \text{ mV}/n$ ($n=2$) (X. T. Chen et al. 2006), which indicated a typical quasi-reversible process. After the addition of Ca^{2+} (Fig. 1b and c), the peak currents of ARS, which still indicated a reversible process, was lowered and proportional to the concentration of calcium at the peak potential. Meanwhile there appeared a new reduction peak at a higher negative potential of -0.975 V, with no relative oxidation when scanned back, indicating that the reduction of Ca-ARS complex was an irreversible process. However, there was no obvious reduction peak of ARS at the bare glassy carbon electrode (Fig. 1d and e). It can be seen that the nanoparticle modified electrode showed an obvious electrochemical activity for the reduction of ARS and Ca-ARS complex. Therefore, the nanoparticle modified electrode was better to the reduction of ARS than the bare glassy carbon electrode.

Effect of Number of Pulsed Electrodeposition

Pulsed electrodeposition was used as a suitable and simple method for controlling the size and morphologies of gold nanoparticles electrodeposited on GCE (X. X. Chen et al. 2007; Dai et al. 2004). To get surface information about the electrodeposited Au nanoparticles at glassy carbon electrode, cyclic voltammetric profiles of each electrode under various formation conditions were recorded in 1.5×10^{-4} M ARS solution containing 0.1 M KOH in the potential range from -1.1 to -0.6 V (vs. Ag/AgCl) at the scan rate of 100 mV s^{-1} . The corresponding curves are shown in Figure 2. Different pulses numbers of 60, 90, 120, and 150 were examined using CV. With the increase of number of the potential pulses, the peak heights of ARS increased gradually. This suggested that the increase of the total amount of gold nanoparticles loaded at the glassy carbon surface resulted from the increase in deposition time. The obtained gold nanoparticles deposited on GCE surface showed an excellent electrocatalytic activity toward the reduction of ARS. However, overmuch number of the potential pulses caused a little decrease of the peak heights of ARS, which may be due to excess accumulation of nanoparticles. As a result, the number of the pulses of 120 (optimized) was used for the following experiments.

Effect of Concentration of KOH

Several supporting electrolytes were compared in respect of the response toward ARS at the gold nanoparticle-modified electrodes using different pulse voltammetry. The electrolyte solutions included HCl, HNO_3 , KCl, KNO_3 , and KOH. Among these electrolytes, only KOH provided reduction peaks of ARS and ARS-Ca. As shown in Figure 3, the 0.08 M KOH gave the highest peak current of ARS among the tested electrolytes. But the peak current of ARS-Ca increased with rising of concentration of KOH, and remained steady after 0.1 M KOH. Thus, 0.1 M KOH was selected as the supporting electrolyte in the following electrochemical experiments.

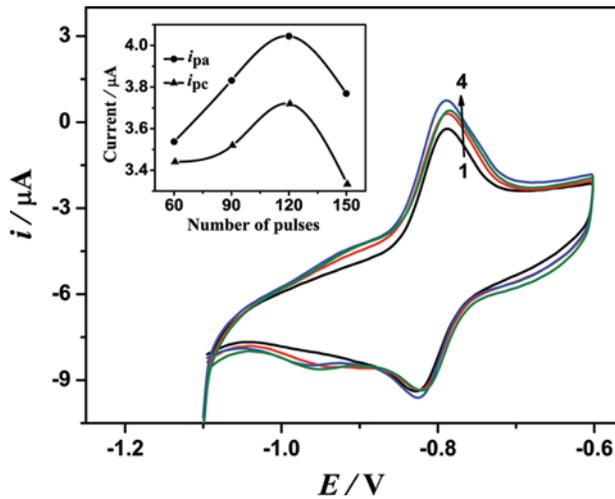


Figure 2. Cyclic voltammograms of different number of pulses in 1.5×10^{-4} M ARS solution containing 0.1 M KOH at the gold nanoparticles modified electrode. Numbers of pulses (from 1 to 4): 60, 90, 120, and 150. The inset shows the peak current of different numbers of pulses. (Figure available in color online.)

Experiments also showed that the peak potential of ARS shifted towards negative direction with increasing of concentration of KOH in Figure 3c, and it presented a good linear relationship, $E = -0.7544 - 0.3312c$ (E : V, c : M), with a correlation coefficient of 0.9973. The result suggested that the reduction of ARS was related

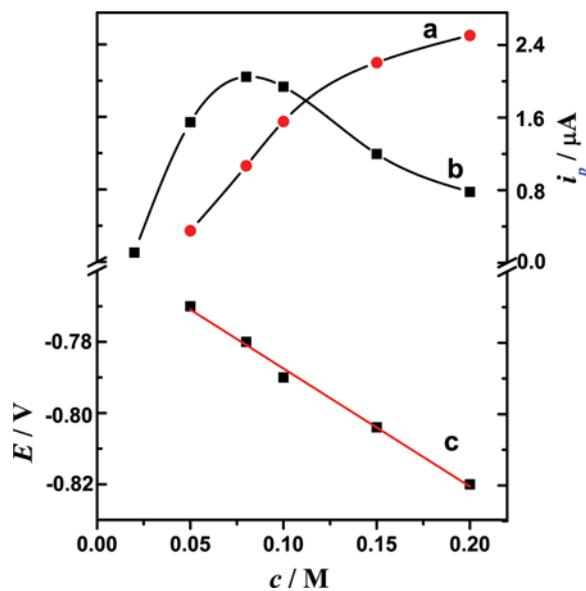


Figure 3. Effect of concentration of KOH: the peak current (a) of ARS-Ca, the peak current (b) and peak potential (c) of ARS in 1.5×10^{-4} M ARS solution containing 2.5×10^{-5} M Ca^{2+} at the gold nanoparticles modified electrode. (Figure available in color online.)

to the acidity of supporting electrolytes, yet the change of peak potential of ARS-Ca was not obvious in different concentrations of KOH solution.

Effect of Accumulating Time

Figure 4 shows the effect of accumulation time at the gold nanoparticles modified electrode. As presented in Fig. 4, the peak currents of ARS and ARS-Ca ascended with increasing in accumulation time and remained steady after 2 minutes. The result suggests that it would take a while to achieve an absorptive equivalence before the maximum peak currents of ARS and ARS-Ca arriving at the gold nanoparticle modified electrode. Therefore, the time of 2 minutes was selected in the following experiments.

Effect of Scan Rate

As shown in Figure 5, in 2.5×10^{-4} M ARS solution containing 7×10^{-5} M Ca^{2+} and 0.1 M KOH, the cathode peak current i_p of ARS increased with the rising of the scan rate at the gold nanoparticle modified electrode and exhibited a linear relation with the square root of the scan rate $v^{1/2}$, with the linear regression equation, $i_p = 1.26 \times 10^{-5} v^{1/2} - 1.90 \times 10^{-6}$ (i_p : A, v : V s^{-1}), and a correlation coefficient of 0.9981. The result indicated that the electron transfer reaction was controlled by the diffusion of ARS. The diffusion coefficient can be calculated according to the following equation (S. J. Dong, Che, and Xie 2003):

$$i_p = 2.69 \times 10^5 A n^{3/2} D^{1/2} C v^{1/2}$$

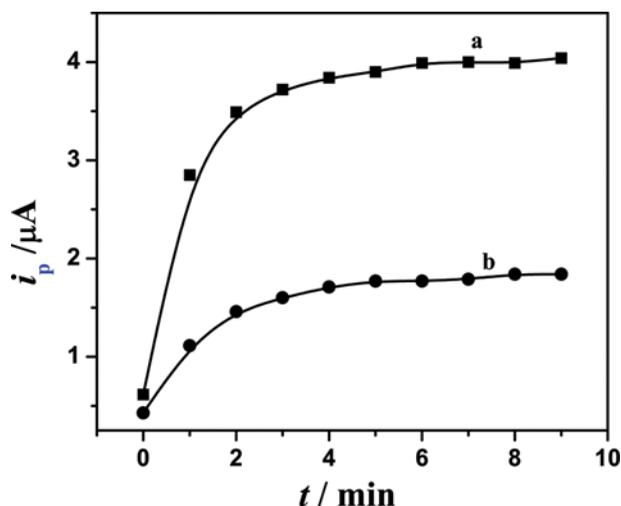


Figure 4. Effect of accumulation time: (a) the peak current of ARS in 1.5×10^{-4} M ARS solution containing 0.1 M KOH at the gold nanoparticles modified electrode; (b) the peak current of ARS-Ca in 1.5×10^{-4} M ARS solution containing 5×10^{-5} M Ca^{2+} and 0.1 M KOH at the gold nanoparticles modified electrode. (Figure available in color online.)

where A is the surface area of the electrode, n is the electron transfer number, D is the diffusion coefficient, and C is the bulk concentration of ARS. From the slope of i_p vs. $v^{1/2}$, D can be obtained if the parameters of A , n , and C are known. In this experiment, there were two electrons transferring in this reaction (S. Y. Dong, Zheng, and Gao 2003). From the slope of the plot between i_p and $v^{1/2}$, D was calculated as $5.45 \times 10^{-10} \text{ cm}^2 \text{ s}^{-1}$.

However, though the cathode peak current i_p of ARS-Ca increased with the increase of the scan rate at the gold nanoparticles modified electrodes, the peak currents were not exhibited a linear relation with the square root of the scan rate (see Figure 5b), which suggested an absorption control process.

Working Plot and Its Limitation

According to the optimum experimental conditions and the procedure described above, DPV was used to examine the electrochemical reduction behaviors of $2.5 \times 10^{-4} \text{ M}$ ARS solution containing 0.1 M KOH and the different Ca^{2+} concentrations, and the peak currents of ARS showed a linear relationship with Ca^{2+} concentration in the range of $2.0 \times 10^{-7} - 1.2 \times 10^{-4} \text{ M}$ with a detection limit of $2.57 \times 10^{-8} \text{ M}$ (3δ). As shown in Figure 6, the linear regression equation of DPV was $i_p = 0.504 c - 7.308$ ($i_p: 10^{-6} \text{ A}$, $c: 10^{-5} \text{ M}$), and the correlation coefficient was 0.9995. In addition, the peak currents of $2.5 \times 10^{-4} \text{ M}$ ARS solution containing 0.1 M KOH and $2.5 \times 10^{-5} \text{ M}$ Ca^{2+} at the gold nanoparticle modified electrode were detected for ten times of successive determinations, and the relative standard deviation was 1.90%.

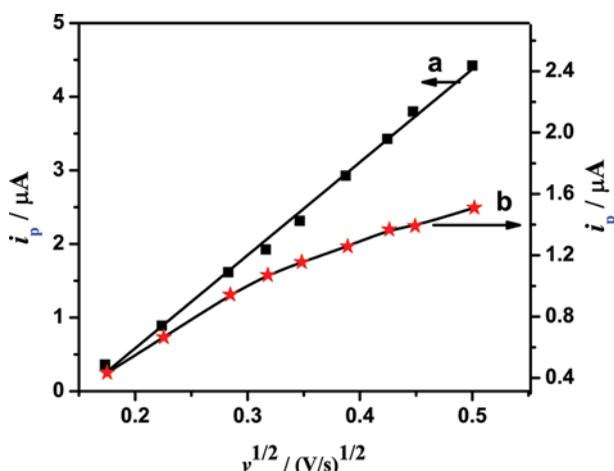


Figure 5. The peak current of ARS (a) and ARS-Ca (b) at the gold nanoparticles modified electrode in $2.5 \times 10^{-4} \text{ M}$ ARS solution containing $7 \times 10^{-5} \text{ M}$ Ca^{2+} and 0.1 M KOH at different scan rates. The scan rate ($\text{mV} \cdot \text{s}^{-1}$): 30, 50, 80, 100, 120, 150, 180, 200, and 250, respectively. (Figure available in color online.)

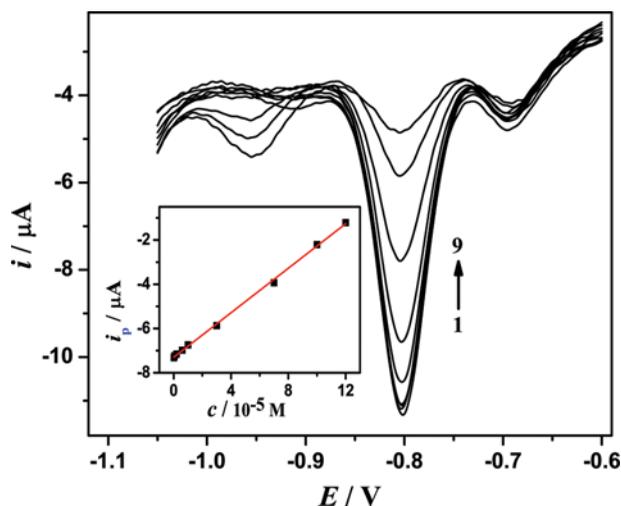


Figure 6. Differential pulse voltammograms of different concentrations of Ca²⁺ solution containing 2.5×10^{-4} M ARS and 0.1 M KOH. Concentration of Ca²⁺ (from 1 to 9, M): 2×10^{-7} , 6×10^{-7} , 2×10^{-6} , 6×10^{-6} , 1×10^{-5} , 3×10^{-5} , 7×10^{-5} , 1×10^{-4} , and 1.2×10^{-4} , respectively. Inset: the calibration curve of Ca²⁺ at the gold nanoparticles modified electrode by DPV method. (Figure available in color online.)

Tolerance of Foreign Substances

The influence of various foreign species on the determination of 2.5×10^{-4} M ARS solution containing 0.1 M KOH and 3×10^{-5} M Ca²⁺ was investigated. The tolerance limit was taken as the maximum concentration of the foreign substances that caused an approximately $\pm 5\%$ relative error in the determination. The tolerated ratios of foreign substances to 3×10^{-5} M Ca²⁺ were 1000 for Na⁺, Cl⁻, K⁺, Fe³⁺, SO₄²⁻, NO₃⁻, HPO₄²⁻, and H₂PO₄⁻; 50 for alanine, cystine; 5 for Zn²⁺, Mg²⁺, and Al³⁺, respectively.

Analytical Application

The contents of Ca²⁺ in human serum were determined by standard addition method under optimum conditions, and the results were shown in Table 1. The

Table 1. Analysis of Ca²⁺ in human serum

Amount of diluted serum (ml)	Added amount (10 ⁻⁵ M)	Found amount ^b (10 ⁻⁵ M)	Amount of Ca ²⁺ in human serum ^a (10 ⁻³ M)	Recovery (%)
0.5	—	0.610	2.44	103.4
0.5	1.0	1.542	2.16	91.8
0.5	2.5	3.045	2.18	92.4
0.5	5.0	5.553	2.21	93.7

^aThe concentration of Ca²⁺ in human serum was 2.36×10^{-3} M by ion selective electrode method in affiliated hospital of Changzhi Medical College.

^bMean of the three determinations.

average determination results of Ca^{2+} in human serum was 2.25×10^{-3} M by the DPV method, which was in good correspondence to the value that was given by affiliated hospital (2.36×10^{-3} M). The recoveries were between 91.8% and 103.4% by DPV.

CONCLUSIONS

This study demonstrates a fast and easy way to detect Ca^{2+} at gold nanoparticles modified GC electrode. A limit of detection of 2.57×10^{-8} M using DPV was achieved. These provided the possibility of applying this method for the detection of Ca^{2+} in serum samples. The method offers considerable simplicity and economy of electrode preparation compared to those of the present state-of-the-art electrochemical methods for the detection of Ca^{2+} with similar rapidity.

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