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A Reduced Graphene Oxide/Gold Nanoparticles Composite Modified Glassy Carbon Electrode as Electrochemical Sensor for Calcium Ions Detection in Bottled Water

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The present study suggested an ultrasensitive reduced graphene oxide (rGO)/gold nanoparticles (AuNPs) modified electrochemical sensor for the detection of trace Ca^{2+} ions in bottled water. The detection was performed by the aid of calcium ligand complexing agent ethylenediaminetetraacetic acid (EDTA). The presence of rGO as a large surface area and supporting material to AuNPs provides a facile electrochemical reduction of Ca^{2+}-EDTA in the presence of ammonia buffer of pH = 10. Under optimized experimental conditions, the fabricated sensor displayed excellent linearity over a concentration range of 2 – 100 µgmL^{-1} (r = 0.999) with calculated detection and quantification limits of 0.01 and 2 µg mL^{-1} for Ca^{2+}, respectively. The fabricated modified sensor was unaffected by the presence of certain possible common interfering species. Using ICH guidelines the proposed method was validated with respect to various analytical parameters including, linearity, detection and quantification limits, precision, accuracy, etc. Moreover, a comparative analytical study for calcium detection using the proposed electrochemical method and other analytical methods was carried out. It revealed good reproducibility, repeatability and success in the detection of trace Ca^{2+} ions in different brands of bottled water.

Keywords: Graphene oxide nanoparticles; Gold nanoparticles; Bottled water, Modified sensor; Ca^{2+}.

1. INTRODUCTION

Calcium is considered one of the most essential micronutrient elements needed for several cellular processes in all living organisms. It plays a vital role in cell physiology through movement in and out of the cytoplasm. It is imperative for the growth of healthy bones and teeth. Therefore, children and pregnant women are encouraged to maximize dietary intake of rich calcium foods such as milk, green vegetables, cereals and nuts [1]. Through the years, it is very necessary for the body to maintain calcium balance to achieve peak bone mass and decrease the risk of bone fracture and osteoporosis. The absolute decrease in the production of vitamin D3 in kidneys, causes the bones to become a source of calcium to other critical extracellular activities [2, 3]. Also, calcium provides a positive protective effect against some neurological disorders and has an inverse correlation with body blood pressure. The increased intake of calcium decreases the smooth muscle contractility and hence, lowers the blood pressure. Therefore, the quantitative detection of calcium levels in drinking water is important for human health [3]. Several methods for the detection of calcium in different matrices have been addressed including, thermal gravimetric analysis of tablets [4], flame atomic absorption spectroscopy [5], spectrophotometry [6, 7], cyclic voltammetry [8, 9] and HPLC-MS [10]. The electrochemical sensors are considered an ultrasensitive technique that is used for the detection of trace elements with various advantages such as low cost, high sensitivity, and fast detection time [11, 12].

Nowadays, the modification of electrochemical sensors using reduced graphene oxide (rGO) and gold nanoparticles (AuNPs) has been explosively developed to avoid some drawbacks of traditional mercury sensors such as high toxicity and handling difficulties [13]. The good conductivity of graphene attracts the attention of many researchers to focus their study on the chemically modified graphene sensors and their applications in analytical chemistry [14-16].

Furthermore, the deposited nanoparticles such as gold nanoparticles are used to improve the performance of the electrochemical sensors [17-22].

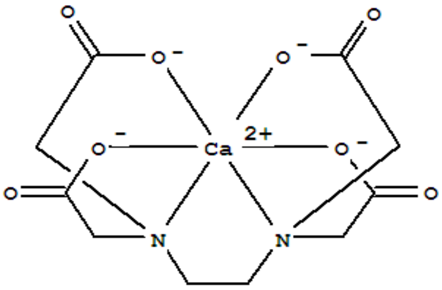

Figure 1. Chemical structure of Ca^{2+}-EDTA complex

The present study focused on the modification of an electrochemical sensor using rGO/AuNPs nanocomposite that can easily be prepared and have an excellent electrochemical activity. The rGO with AuNPs could improve the transfer electrons. Ca^{2+}-EDTA ligand complex (Figure 1) could easily be absorbed at the sensor surface and the fabricated modified sensor was employed for the detection of trace calcium in different brands of bottled water samples with excellent results.

2. EXPERIMENTAL

2.1. Instrumentation

The rGO/AuNPs glassy carbon electrode, as a working sensor, was used to accomplish all electrochemical measurements throughout the experiment. 797 VA Computrace connected with 843 pump station (Metrohm, Switzerland), was applied for the samples detection. The system was programmed for data acquisition using software version 2.0. The pH adjustment was performed using HANNA 211 microprocessor pH meter (Cluj, Romania). Scanning Electron microscope (SEM), JEOL JSM-6060 LV model (Akishima, Tokyo, Japan), was used to characterize the surface morphology of the prepared modified glassy carbon (GC) rGO/AuNPs.

2.2. Reagents and solvents

Calcium chloride and sodium sulfate were purchased from Winlab, East Midland, UK. Graphite flakes, ethylenediaminetetraacetic acid (EDTA), aluminum oxide powder (Al_2O_3), ammonium chloride, ammonium hydroxide and chloroauric acid ($HAuCl_4$. $4H_2O$) were provided by Sigma-Aldrich, Hamburg, Germany. Deionized water was used throughout the experiments.

2.3. Preparation of glassy carbon modified rGO/AuNPs sensor

GO suspension was prepared using a modified Hummers method [23]. Briefly, in volumetric flask- 500 mL approximately, 0.25 g of sodium nitrate and 0.5 g of pure graphite were mixed followed by adding 50.0 mL of 96% sulfuric acid. Under vigorous stirring and in cooling ice-bath the previous mixture was kept for 20 min. Then, by maintaining the stirring 1.75 g potassium permanganate was added very slowly and kept stirring for 2 h. Finally, 100.0 mL of deionized water were added followed by 10.0 mL of hydrogen peroxide. To complete the preparation of modified sensor, aqueous 0.3 µmol L^{-1} alumina slurry was used to polish the surface of the glassy carbon sensor. This polished surface was washed thoroughly with deionized water and ultrasonicated for 5 min. The prepared glassy carbon sensor was dipped into 0.2 mol L^{-1} of sodium sulfate solution containing a mixture of 0.5 mg mL^{-1} graphene oxide (GO) and 1.0×10^{-3} mol L^{-1} $HAuCl_4 \cdot 4\ H_2O$. The well dispersed rGO/AuNPs nanocomposite film was obtained electrochemically using constant potential at -1.0 V for approximately 400 s as previously mentioned in the literature [24]. The fabricated modified rGO/AuNPs sensor was washed carefully with deionized water prior to use.

2.4. Water samples

The water samples were obtained from five different brands of bottled water companies in Saudi Arabia, including, Nestle, Hayat, Berain, Aquafina, and Hana water. Square wave adsorptive stripping voltammetry (SW-AdSV) was employed to detect the trace Ca^{2+} in the presence of ammonia buffer of pH = 10 as a supporting electrolyte.

2.5. Method procedure

The electrochemical detection of trace Ca^{2+} was carried out using 10 mL of bottled water in the presence of ammonia buffer of pH = 10 containing 20 µmol L^{-1} EDTA as a complexing ligand. The formed Ca^{2+}-EDTA complex was accumulated on the modified rGO/AuNPs sensor surface under accumulation potential - 0.2 V for 300 s with continuous stirring at 750 rpm. Then SW-AdSV technique was applied. The stripping voltammogram was obtained by applying a scan of a potential range of - 0.8 to 0.4 V using 100 mV pulse amplitude and frequency = 30 Hz.

3. RESULTS AND DISCUSSION

3.1. Characterization of modified rGO/AuNPs sensor

Generaly, the formed GO has many OH^- and COO^- groups and formed a stable suspension in water due to its high oxygen containing groups. However, after chemical reduction, rGO was formed which has a more hydrophobic properties and due to the interlayer π–π conjugate of nano sheets, it can rapidly aggregated and precipitated [25].

To confirm the reduction of GO to rGO, FT-IR spectrum of each was recorded. As shown in Figure 2a, it was noticed that two significant vibrational bands corresponding to carbonyl and carboxylic groups were appeared at 1717 (C=O) and 1568 cm^{-1} (C=C), respectively. Another single band was observed at 1038 cm^{-1} related to C-O which associated with certain functional groups as C=O, COOH and epoxy groups. These results revealed the presence of functional groups containing O and hence explain the successful conversion of pure graphite to GO. In rGO spectrum (Figure 2b) it was noticed that the above recorded band at 1717 cm^{-1} was disappeared indicating the removal of C=O and the reduction of GO to rGO. Moreover, the C=C band was shifted to 1625 cm^{-1}. However, the remaining C-O stretching band after the reduction of GO to rGO was appeared at 1037 cm^{-1} as previously reported [26]. As demonstrated in Figure 2c, a new band was observed at 520 cm^{-1} indicating the formation of rGO/AuNPs.

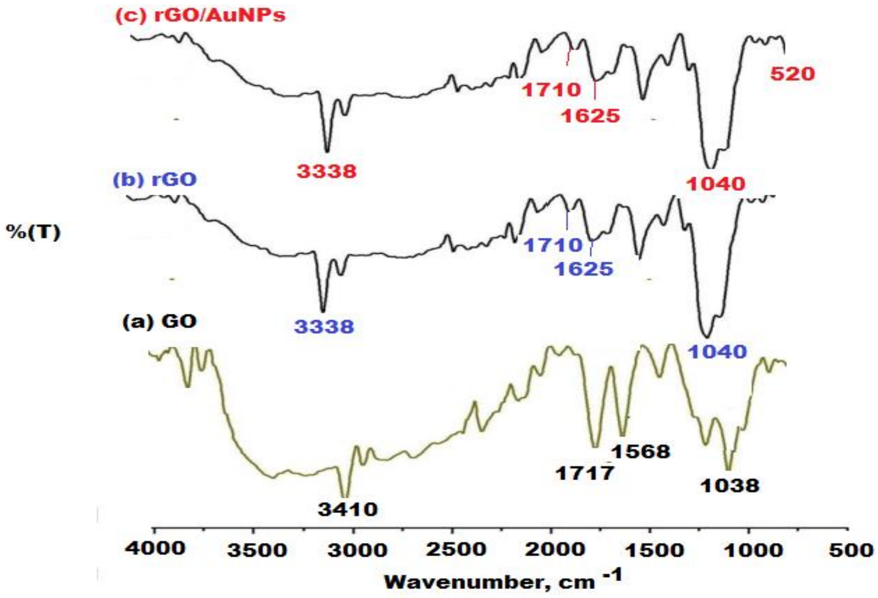

Figure 2. FT-IR spectra of (a) Go, (b) rGO and (c) rGO/AuNPs

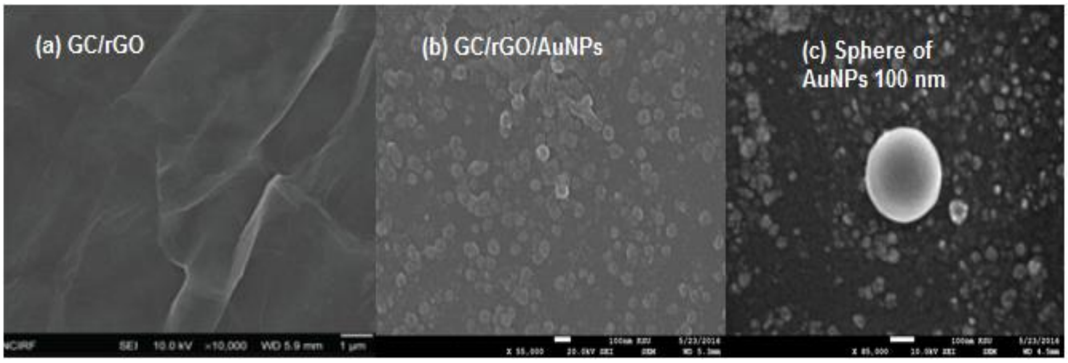

Figure 3. SEM images of (a): surface morphology of GC with projection of rGO sensor, (b): surface morphology of modified GC/rGO/AuNPs (c): surface morphology of GC/rGO/AuNPs with characterized spherical AuNPs of 100 nm diameter.

SEM was used to study the surface morphology of the modified glassy carbon (GC) rGO/AuNPs. Figure 3a, showed the clear homogenous modified surface of GC with slight projection of rGO. Figures 3b and 3c showed excellent formation of rGO/AuNPs nanocomposite with characterized spherical AuNPs of 100 nm diameter on the surface of GC sensor.

3.2. The behavior of Ca^{2+} on the modified rGO/AuNPs sensor

The electrochemical behavior of 10 µg mL^{-1} Ca^{2+} in the presence and absence of 20 µmol L^{-1} EDTA on the modified rGO/AuNPs sensor was recorded using SW-AdSV mode in the presence of ammonia buffer of pH = 10. As shown in Figure 4, the recorded voltammogram described that no cathodic peak of Ca^{2+} was detected at the modified rGO/AuNPs sensor (Curve a), and a well defined cathodic reduction peak for free EDTA was detected at - 0.3 V (Curve b). A current peak of free Ca^{2+} reduction was observed at - 0.5 V (Curve c). Furthermore, in the presence of Ca^{2+}- EDTA complex, the recorded voltammogram displayed two significant peaks at - 0.3 V and - 0.5 V for EDTA and Ca^{2+}, respectively (Curve d).

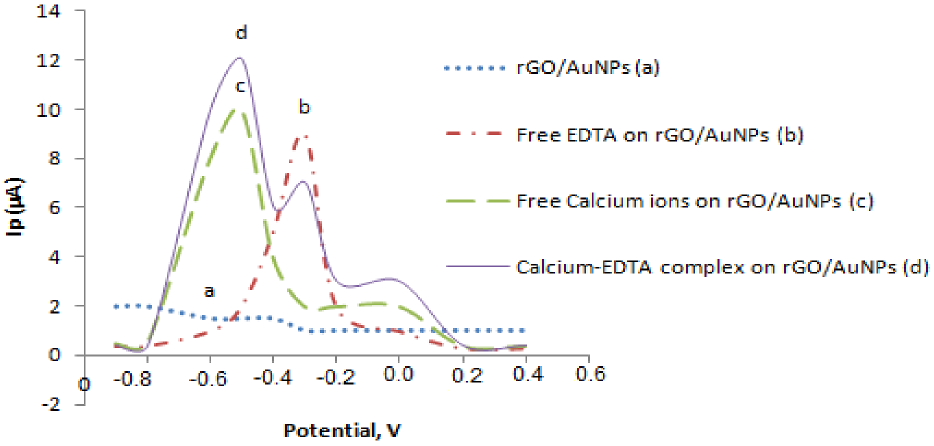

Figure 4. SW-AdSV voltammograms of free EDTA, Ca^{2+} and Ca^{2+}-EDTA complex on the surface of modified rGO/AuNPs sensor

3.3. Optimization of conditions for Ca^{2+} detection on rGO/AuNPs modified sensor

3.3.1. Effect of rGO/AuNPs deposition time

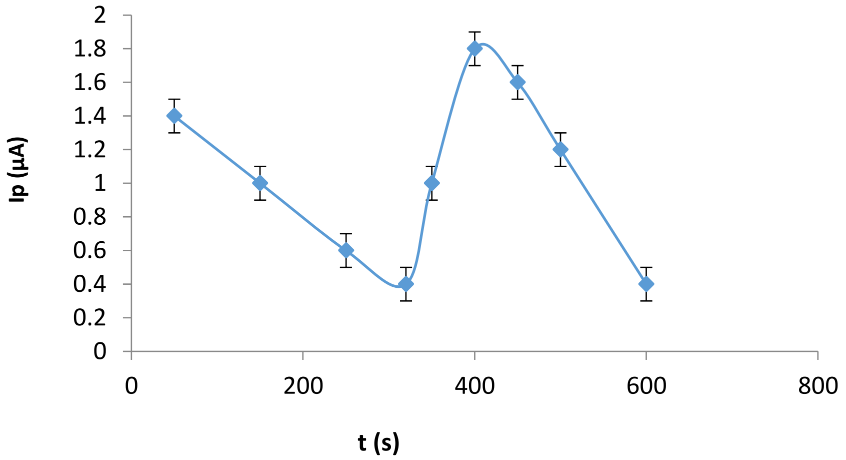

Figure 5. Optimization of deposition time of rGO/AuNPs on the surface of GC sensor

The deposition time of both rGO and AuNPs on the GC surface was tested over time ranging from 50 - 600 s. As described in Figure 5, the optimum deposition time was recorded after approximately 400 s. At the time range from 50 to 320, gradual decrease in the peak current was observed which could be attributed to the little amount of AuNPs that deposited on the sensor surface. When the time reached to 400 s and due to the gradual increase of AuNPs amount a significant sharp peak current was obtained. Therefore, we approved 400 s as the optimum deposition time.

3.3.2. Effect of pH

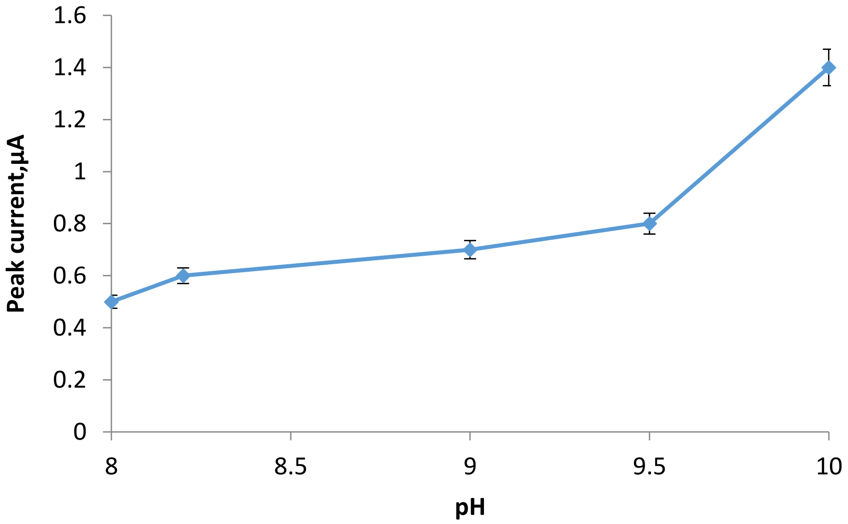

Figure 6. Effect of pH value of the supporting electrolyte on the reduction of Ca^{2+}-EDTA complex on the surface of modified rGO/AuNPs sensor

The formation of Ca^{2+}- EDTA complex and its stability is greatly affected by the pH value of the supporting electrolyte. Therefore, the pH value of ammonia buffer was investigated and optimized. The peak current of the ammonia buffer was recorded using pH values ranging from 8 to 10. The high peak current was found at pH = 10 (Figure 6).

3.3.3. Effect of accumulation potential

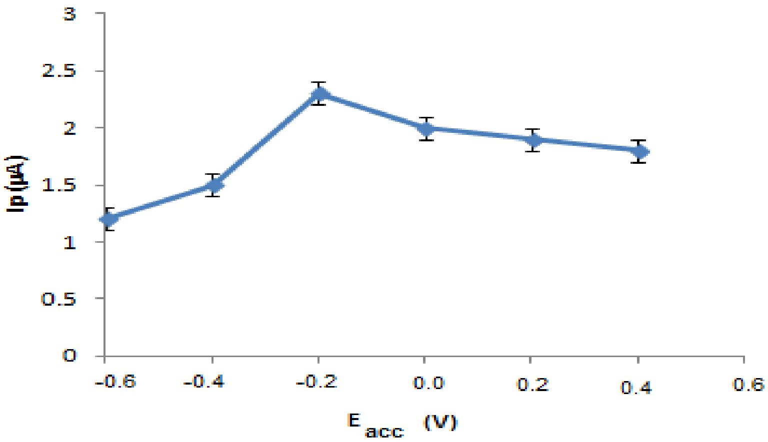

Figure 7. Optimization of accumulation potential to accumulate the Ca^{2+}-EDTA complex on the surface of modified rGO/AuNPs sensor

To study the accumulation potential of Ca^{2+}- EDTA complex on the surface of the modified rGO/AuNPs sensor, the maximum peak current was selected after investigating the previously mentioned complex using an accumulation potential in the range of - 0.6 to 0.4 V. As shown in Figure 7, due to the strong adsorption of the positively charged complex on the negative charge on the modified sensor surface, it was noticed that the peak current was gradually increased to reach maximum accumulation potential at - 0.2 V.

3.3.4. Effect of accumulated time

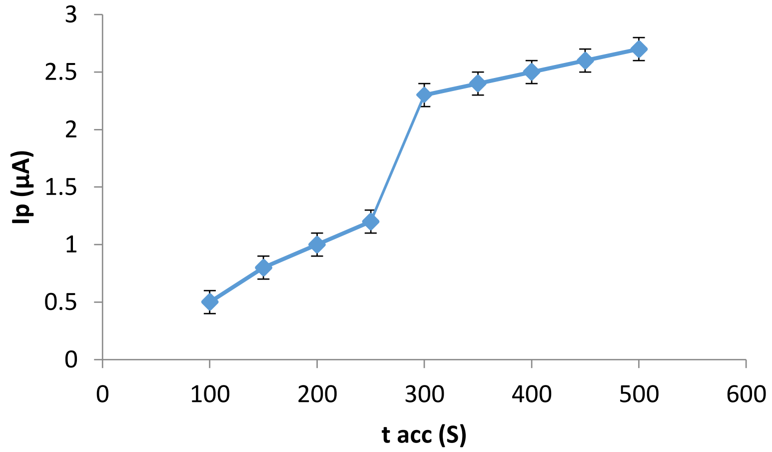

Figure 8. Optimization of accumulation time to accumulate Ca^{2+}-EDTA complex on the surface of modified rGO/AuNPs sensor

The effect of accumulation time on the peak current of the modified rGO/AuNPs sensor was studied using time ranging from 100 – 500 s. The peak current of Ca^{2+}- EDTA complex increased rapidly and reached its maximum at 300 s (Figure 8). Due to the energy consumption and time of analysis, problem the peak current was increased slowly after 300 s. Therefore, the optimal accumulation time was considered as 300 s.

3.3.5. Effect of EDTA concentration

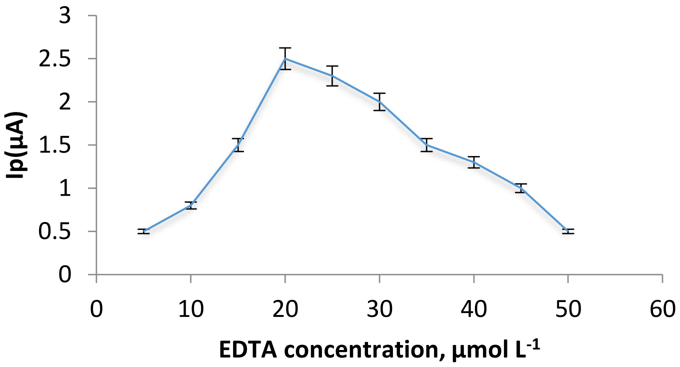

Figure 9. Optimization of the concentration of EDTA for the formation of Ca^{2+}-EDTA complex on the surface of modified rGO/AuNPs sensor

The investigation was performed in the presence of the supporting electrolyte ammonia buffer of pH = 10 containing 10 µg mL^{-1} Ca^{2+}. The EDTA concentration was ranged from 10 – 50 µmol L^{-1}. As recorded in Figure 9, the maximum peak current was achieved at an EDTA concentration of 20 µmol L^{-1}. By increasing the concentration above 20 µmol L^{-1}, a significant decrease of the peak current was recorded, which can be attributed to the competitive effect of Ca^{2+}-EDTA complex. Therefore, 20 µmol L^{-1} EDTA was selected to be used in the study.

3.3.6. Effect of chelating reaction time

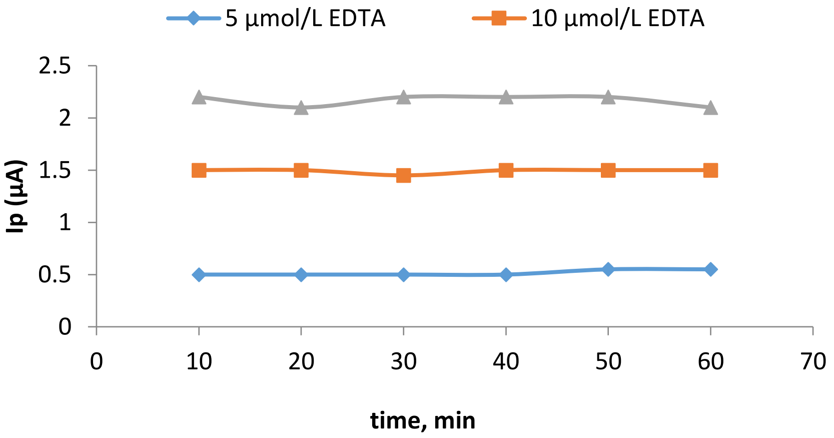

Figure 10. Optimization of the chelating time for the formation of Ca^{2+}-EDTA complex on the surface of modified RGO/AuNPs sensor using 5, 10 and 20 µmol L^{-1} EDTA

The chelating time required for the reaction between 10 µg mL^{-1} Ca^{2+} and (5, 10 and 20) µmol L^{-1} EDTA was investigated at the time ranging from 10 – 60 min in the presence of supporting ammonia buffer electrolyte of pH = 10. Figure 10, showed the peak current of the chelating reactions. The quick chelating reaction between Ca^{2+} and EDTA was recorded within 10 min.

3.4. Calibration graph

The calibration graph for Ca^{2+} detection was derived using SW-AdSV on the surface of modified rGO/AuNPs sensor under the above optimized conditions (Figure 11). The recorded peak current displayed linearity over Ca^{2+} concentration range of 2-100 µg mL^{-1}. The evaluated regression equation was $I_p = 0.1385\ c + 0.0561$, (r = 0.9995). The LOD and LQD were determined using S/N=3 and they were found to be 0.01 and 2 µg mL^{-1} respectively. Table 1, presented the comparative analytical data between the proposed method and other previously published analytical methods for the detection of Ca^{2+}.

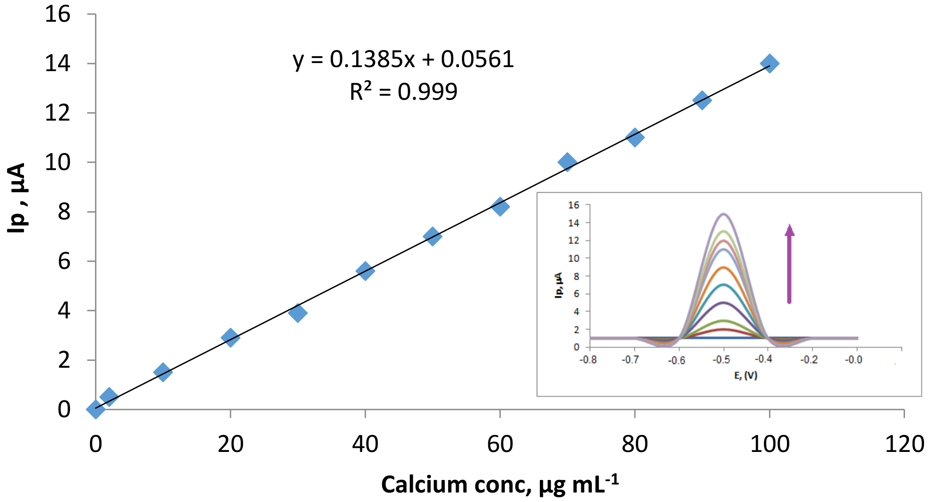

Figure 11. Linear range of Ca^{2+} at the modified rGO/AuNPs sensor using Ca^{2+} concentrations 2-10 µg mL^{-1} in ammonia buffer of pH = 10 containing 20 µmol $L^{-1}$of EDTA

Table 1. Comparative analytical data for the detection of calcium using the recent electrochemical technique and other analytical methods

Methods	Conditions	Sample	LOD	Reference
Spectrophotometry	Calcium-Alizarin complex in the presence of cationic surfactant cetyltrimethylammonium bromide with detection at 630 nm	Water	0.035 µg mL^{-1}	[7]
Thermogravimetry-differential thermal analysis	Flow rate of purge gas 50 mL min^{-1} heating rate 10ºC min^{-1}	Tablets	17.7%	[4]
Adsorptive-stripping	Reduction of calcium- alizarin red s complex at carbon paste electrode. Peak	Tap water	0.37 µg mL^{-1}	[9]

voltammetry	potential -0.89 V, accumulation potential -0.1 V, accumulation time 90 s and scan rate 100 mV s^{-1}.			
Proposed method	Reduction of Ca^{2+}-EDTA in the presence of ammonia buffer of pH = 10 at a modified rGO/AuNPs sensor. Peak potential -0.5 V, 100 mV pulse amplitude, accumulation potential -0.2 V and accumulation time 300 s.	Bottled water	0.01 µg mL^{-1}	Present work

3.5. Method suitability

To investigate the reproducibility of the fabricated modified rGO/AuNPs sensor, three different calcium concentrations (10, 50 and 100 µg mL^{-1}) were tested in terms of percentage relative standard deviations (%RSD) using five modified sensors fabricated using the same procedure. The obtained results were 1.2%, 0.9% and 1.5%, respectively. Additionally, the repeatability of the current method was tested by detecting the above three concentrations at the same sensor for six measurements. The calculated %RSD was 1.0%, 1.3% and 0.8%, respectively. Therefore, these results revealed good reproducibility and repeatability. Also, the response of the modified rGO/AuNPs sensor was tested after storing it in air conditions using three different calcium concentrations (50, 70 and 100 µg mL^{-1}). It was found that no significant decrease in peak current within 10 days and approximately 85 % of its initial response was retained in more than ten days.

The effect of possible interfering species for Ca^{2+} detection was investigated by testing 100-fold Mg^{2+}, Fe^{3+}, K^+ and Na^+, 20-fold HCO_3^-, SO_4^{2-}, NO_3^- and F^- in the presence of ammonia buffer of pH = 10 containing 50 µg mL^{-1} Ca^{2+}. No significant effect of the added species on the current peak was found in the detection of Ca^{2+} (< 5% of response current change).

3.6. Analytical applications

To evaluate the calcium content of the different bottled water samples, the modified rGO/AuNPs was applied under the previously mentioned, optimal conditions for the detection of Ca^{2+} using the standard addition method.

Table 2. Comparative analytical data for the detection of calcium using the current electrochemical technique and a reference spectrophotometric method [7]

	Modified rGO/AuNPs sensor			Spectrophotometric method [7]	
Sample	Added (µg mL^{-1})	Detected (µg mL^{-1})	Recovery %	Detected (µg mL^{-1})	Recovery %
Nestle water	40	39.5±0.1	98.8	38.9±0.2	97.3
	80	78.1±2.3	97.6	79.2±0.6	99.0
	100	97.3±0.9	97.3	98.9±0.1	98.9
Berain water	20	19.2±0.6	96.0	19.6±0.8	98.0
	40	39.9±0.1	99.8	39.0±1.2	97.5
	50	48.9±1.2	97.8	49.2±0.4	98.4

Aquafina water	5	4.9±0.8	98.0	4.8±1.1	96.0
	10	9.7±1.0	97.0	9.8±0.8	98.0
	20	19.6±0.3	98.0	19.3±0.5	96.5
Hayat water	10	9.9±0.4	99.0	9.8±0.7	98.0
	20	20±0.1	100.0	19.9±0.2	99.5
	30	28.9±1.4	96.3	28.8±0.6	96.0
Hana water	6	5.9±1.5	98.3	6.0±0.4	100.0
	12	12.0±0.3	100.0	11.9±0.6	99.2
	18	17.8±1.1	98.8	17.9±0.7	99.4

Table 2, described the comparative analytical data obtained from the analysis of five different kinds of bottled water by the suggested sensor and another reported spectrophotometric method [7] which described the detection of calcium-alizarin complex in the presence of cationic surfactant cetyltrimethylammonium bromide at 630 nm. The obtained results indicated that this technique is reliable and suitable for the quantification of calcium in bottled water.

4. CONCLUSION

The suggested modified rGO/AuNPs was fabricated and employed for the detection of Ca^{2+} in Saudi Arabia bottled water by the reduction of Ca^{2+}-EDTA complex using SW-AdSV. The excellent sensitivity, repeatability and reliability of the new sensor can be associated with the fast detection of the calcium content in the bottled water. The obtained results gave excellent agreement with those obtained from the other recommended methods. Therefore, the fabricated modified rGO/AuNPs has a great potential for calcium detection.

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CONFLICT OF INTEREST
All Authors declare that no conflict of interest in this study

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