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Design of a Graphite/Zinc Nanoparticles/1-nitroso-2-naphthol Voltammetric Substrate for the Measurement of Magnesium in Human Serum Samples

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Abstract:

In this research, a voltammetric substrate with a graphite/zinc nanoparticles/1-nitroso-2-naphthol structure has been developed to measure magnesium in human blood serum. Zinc nanoparticles and 1-nitroso-2-naphthol were deposited on the surface of a bare graphite electrode using the cyclic voltammetry technique. The formation of nanostructured layers on the graphite electrode surface was confirmed using scanning electron microscopy. The voltammetric platform created with the graphite/zinc nanoparticles/1-nitroso-2-naphthol structure serves as an electrochemical sensor that exhibits satisfactory electrocatalytic properties, with an electron transfer coefficient (α of 0.75), an apparent charge transfer rate constant (log ks = 3.27), and an active layer concentration on the modified electrode surface of (5.16 ×10-5). Notable features of the proposed sensor include high sensitivity, a low limit of detection (LOD) of 8.31 nM, a limit of quantification (LOQ) of 24.87 nM, and a wide linear range (25 to 250 nM). Additionally, the modified electrode with the graphite/zinc nanoparticles/1-nitroso-2-naphthol configuration demonstrates stability, repeatability, selectivity, and acceptable measurement accuracy in the square wave voltammetric evaluation of magnesium in human blood serum.

Key Words: electrochemistry, voltammetry, modified electrode, zinc nanoparticles, 1-nitroso-2-naphthol, magnesium.

1.Introduction

Magnesium ion (Mg^{2+}), one of the most abundant intracellular ions, plays an essential role in fundamental biological reactions. The reference range of Mg^{2+} in adult blood serum is 0.75–1.0 mmol L^{-1} [1]. Typically, Mg^{2+} deficiency (concentration below 0.75 mmol L^{-1}) provokes biochemical and symptomatic alterations in the human body, such as paresthesia, muscular cramps, irritability, decreased attention span, and mental confusion [2-3]. Therefore, it is crucial to detect Mg^{2+} in serum. Traditional protocols for the detection of Mg^{2+} include flame spectrometry [4], fluorescent methods [5], atomic absorption spectrophotometry [6], enzymatic methods [7-8], and ion chromatography [9]. However, these methods are time-consuming, inconvenient, and require expensive equipment. Consequently, the development of selective and sensitive sensors is desirable. Among various carbon-based electrodes used to fabricate electrochemical sensors, pencil graphite electrodes (PGEs) have garnered widespread attention due to their sp^2 hybridized carbon, which exhibits good adsorption, conductivity, high sensitivity, a smaller background current, and ease of preparation and surface modification [10-11]. Compared to other electrodes, such as glassy carbon electrodes, the renewal of the surface plays a crucial role in subsequent analyses, as the electrochemical reactions of the molecules may alter the surface properties of the electrode [12]. Thus, the continuous renewal of the PGE surface for each trial may facilitate selective and sensitive electrochemical investigations of magnesium. Several studies have utilized 1-nitroso-2-naphthol (Scheme 1). A sensitive procedure has been presented for the voltammetric determination of nickel, which involves the adsorptive accumulation of the nickel-1-nitroso-2-naphthol

complex on a bismuth film electrode prepared ex situ by electrodeposition [13]. Gledhill and van den Berg [14], employed competitive ligand exchange–cathodic stripping voltammetry (CLE–CSV) with 1-nitroso-2-naphthol to examine iron speciation in seawater collected from the North Sea. Demir Mülazımoğlu et al [15], used 1-nitroso-2-naphthol for the determination of copper (II) and zinc (II) in drinking water using inductively coupled plasma atomic emission spectroscopy (ICP-AES) after preconcentration. Krzan et al. [16], utilized 1-nitroso-2-naphthol in the potential range of 0 to +1.0 V vs. Ag/AgCl reference electrode using glassy carbon and platinum electrodes, demonstrating a reversible redox couple through cyclic voltammetry. Additionally, 1-nitroso-2-naphthol was employed as a complexing ligand to study iron speciation and the kinetic interaction of Fe^{3+} with excess organic ligands in seawater [17].
Scheme 1. Chemical structure of 1-nitroso-2-naphthol
Furthermore, the synergistic interaction between zinc nanoparticles and 1-nitroso-2-naphthol inspired us to develop an innovative electrochemical sensor. This sensor was created through the electrodeposition of zinc nanoparticles and 1-nitroso-2-naphthol onto the surface of a glassy carbon electrode using the cyclic voltammetry technique. The cyclic voltammetry process resulted in the formation of robust and stable chemical and mechanical films on the electrode surface. The proposed sensor was successfully employed for the voltammetric quantification of magnesium in human blood serum samples, yielding results with high accuracy and precision, while being free from interfering substances.

2. Experimental

2.1. Materials and Equipment

The chemicals used in this study, including 1-nitroso-2-naphthol, zinc nitrate salt, other metal ions, sugars, vitamins, amino acids, hydrochloric acid, acetic acid, phosphoric acid, boric acid, and sodium hydroxide, were obtained in commercial purity from the foreign company "Merck Germany" and did not require repurification. A 300 milimolar (mM) standard solution of magnesium nitrate, the analyte to be measured, was prepared in a 50 mL volumetric flask using deionized water. For substances with low water solubility, separate 100 mM solutions were prepared in 50 mL flasks using a water-ethanol solvent mixture. To prepare the Britton-Robinson buffer, a 300 mL solution was created containing a mixture of acetic acid, phosphoric acid, and boric acid, each at a concentration of 0.04 M. The initial pH of the mixture was 2.7, which was adjusted to a range of 3 to 11 by adding the necessary amounts of 0.2 M sodium hydroxide. A potentiostat-galvanostat from Metrohm Switzerland, model N302, equipped with NOVA 1.11 software, was used to record and perform the voltammetric techniques and the corresponding voltammograms. During the voltammetric experiments, a three-electrode system was employed, consisting of an Ag/AgCl reference electrode, a platinum counter electrode, and a working electrode, which was a modified graphite electrode with a graphite/zinc nanoparticle/1-nitroso-2-naphthol structure. A Metrohm pH meter-ion meter, model 767, was also used to measure different pH values. Electron microscope images of the modified electrode surfaces were recorded using a TESCAN MIRA3 device.

2.2. Graphite Electrode Surface Preparation

To prepare the graphite electrode surface, the electrode was completely covered with Teflon tape. One of the free ends of the graphite electrode was then connected to the potentiostat-galvanostat device using a copper wire. The other free end was immersed in the Britton-Robinson buffer solution with a pH of 11. By applying 14 consecutive cyclic voltammetry scans in the range of -1.2 to +1.2 volts, the graphite electrode surface was cleaned of any chemical and physical impurities in preparation for final modification.

2.3. Fabrication of a Sensor with a Graphite/Zinc Nanoparticles/1-nitroso-2-naphthol Structure

To fabricate the target sensor, the prepared graphite electrode was immersed in a 200 mM zinc nitrate solution. By applying 14 consecutive cycles of cyclic voltammetry in the range of -1.2 to +0.3 V at a rate of 30 mV/s, a graphite/zinc nanoparticle electrode was produced. The graphite/zinc nanoparticle electrode was then immersed in a 100 mM 1-nitroso-2-naphthol solution in 25 mL of a water-ethanol solvent mixture. Subsequently, by applying 12 consecutive cycles of cyclic voltammetry in the range of -1.2 to +1.2 V at a rate of 50 mV/s, a target electrode with a graphite/zinc nanoparticle/1-nitroso-2-naphthol structure was produced for use in the voltammetric measurement of magnesium.

2.4. Preparing the Real Sample

A 1.5 mL sample of human blood was collected and mixed with 2 mL of concentrated ethanol, which facilitated the precipitation of proteins in the blood sample through shaking. To further separate the proteins, the solution was centrifuged for 3 minutes at 6000 rpm. The blood serum was then passed through a filter with a pore size of 0.45 μm to ensure complete separation of the precipitated proteins. The filtered volume of blood serum was diluted with 25 mL of distilled deionized water, and 0.5 mL of the diluted blood serum was transferred into the cell of the Autolab device. This setup was optimized under mechanical and chemical conditions, and using the square wave voltammetry technique, the magnesium concentrations in human blood were measured in the presence of a magnesium-sensitive sensor. The external standard addition method was employed at pH 7, optimized using the Britton-Robinson buffer, to validate the response of the magnesium-sensitive sensor.

3. Results and Discussion

3.1 Electrode Surface Morphology

Scanning electron microscopy (SEM) was employed to investigate the morphology of the electrodeposited/electropolymerized layers on the bare graphite electrode surface (Figure 1 A–C). As shown in Figure 1 A, the bare graphite electrode exhibits a layered and unmodified structure, with the graphite flakes clearly visible. The chemical electrodeposition of zinc nanoparticles (Figure 1 B) and the electropolymerization of 1-nitroso-2-naphthol (Figure 1 C) significantly altered the morphology of the graphite electrode surface, demonstrating the successful coating of the electrode surfaces and the creation of a graphite/zinc nanoparticle/1-nitroso-2-naphthol nanostructure sensor.

Scanning electron microscope images of the electrode surfaces: (A) bare graphite, (B) graphite/zinc nanoparticles, (C) graphite/zinc nanoparticles/1-nitroso-2-naphthol.

3.2. Electrochemical Evaluation of Magnesium Response on Modified Electrode Surfaces

Using the cyclic voltammetry technique, cyclic voltammograms were recorded to assess the behavior of 120 μM magnesium ions on the surfaces of the bare graphite, graphite/zinc nanoparticle, and graphite/zinc nanoparticle/1-nitroso-2-naphthol electrodes (Figure 2 A). A weak peak was observed for magnesium on the surface of the unmodified graphite electrode. The increase in the intensity of the magnesium oxidation current peak on the graphite/zinc nanoparticle and graphite/zinc nanoparticle/1-nitroso-2-naphthol electrodes can be attributed to the synergistic effect of the electrodeposited zinc nanoparticles and electropolymerized nitrosonephthol. These surface modifiers not only enhance electronic conductivity but also contribute to a higher received current by imparting electrocatalytic properties [18]. Furthermore, the electrochemical reaction of magnesium and its adsorption on the electrode surface was accompanied by the formation of a complex with nitrosonephthol. During a half-cycle from negative to positive potential, this complex facilitated the oxidation of the magnesium-nitrosonephthol complex at a potential of approximately -0.2 V against the Ag/AgCl reference electrode. The effect of varying magnesium ion concentrations on the behavior of the modified graphite/zinc nanoparticle/nitrosonephthol electrode was also investigated using cyclic voltammetry. The results are presented in Figure 3. As observed, with an increase in magnesium ion concentration in the range of 0-150 μM, the current intensity increased correspondingly. This trend confirms the electrocatalytic behavior of the modified electrode in the voltammetric determination of magnesium.

Cyclic voltammograms of the electrochemical behavior of different electrodes: (A) Bare graphite, (B) Graphite/Zinc nanoparticles, (C) Graphite/Zinc nanoparticles/1-nitroso-2-naphthol versus 120 μM magnesium.

Cyclicvoltammograms of the electrochemical behavior of the graphite/Zinc nanoparticles/Nitrosonephthol electrode versus different micromolar magnesium concentrations.

3.3 pH Effect

The effect of pH changes in the range of 3 to 11 (stabilized by Britton-Robinson buffer) on the electrochemical behavior of 120 μM magnesium on the surface of the graphite/zinc nanoparticle/1-nitroso-2-naphthol electrode was investigated (Figure 4A). The results indicate that as the pH values increase up to 7, the intensity of the measured currents also increases, after which the signals begin to decrease. Therefore, the maximum current signal associated with magnesium oxidation occurs at pH 7. Additionally, the changes in (Ep) versus pH (Figure 4B) produced a slope of 0.0375, which deviates from the slope predicted by the theoretical case of the Nernst equation. This deviation suggests an unequal exchange of electrons and protons during the magnesium reaction on the surface of the modified electrode [19].

(A) Cyclic voltammograms of the response of the graphite/zinc nanoparticle/1-nitroso-2-naphthol electrode to 120 μM magnesium at different pH values. (B) Linear relationship between potential and

3.4 Effect of scsn Rate on Electrocatalytic Parameter

To investigate the effect of potential sweep rate on electrocatalytic parameters, the potential sweep rate was increased from 25 to 250 mV/s (Figure 5A). The cyclic voltammograms of the response of the graphite/zinc nanoparticle/1-nitroso-2-naphthol electrode to 120 μM magnesium at pH 7 were recorded (Figure 5A). As observed, with an increasing potential sweep rate, the catalytic oxidation current of magnesium also increases linearly, indicating a significant enhancement in magnesium adsorption on the graphite/zinc nanoparticle/1-nitroso-2-naphthol electrode surface. Furthermore, the linear relationship between the oxidation current and potential sweep rate suggests the presence of a magnesium adsorption mechanism on the modified electrode surface. The electron transfers coefficient (α) was determined using the slope of Ep versus log v (Figure 5C). Consequently, the value of the electron transfers coefficient (α) was reported to be 0.75.

According to the equations (1-2) [20]:

The apparent charge transfer rate constant (log ks = 3.27) for electron transfer between magnesium and the electrode surface was reported. Additionally, the concentration of the active layer deposited on the modified electrode surface Γ was calculated to be 5.16×10-5. The parameters indicate the high electrocatalytic capability of the graphite/zinc nanoparticle/1-nitroso-2-naphthol electrode in the voltammetric measurement of magnesium.

(A) Cyclic voltammograms of 120 μM magnesium on the surface of a graphite/zinc nanoparticle/1-nitroso-2-naphthol electrode at different scan rates. (B) Linear relationship between current and scan rate. (C) Linear relationship between potential and the logarithm of scan rate.

3.5 Calibration Curve

To establish a linear standard calibration curve for the electrochemical behavior of the graphite/zinc nanoparticle/1-nitroso-2-naphthol electrode in the quantitative determination of magnesium, the square wave voltammetry technique was employed at pH 7 under optimal conditions: a potential intensity of 55 mV, a potential amplitude of 55 mV, and a frequency of 60 Hz. In this context, square wave voltammograms were recorded in response to different concentrations of magnesium, ranging from 25 to 250 nanomolar (Figure 6 A). The linear relationship between the oxidation peak current and magnesium concentration is illustrated as a linear standard calibration equation in Figure 6 B. The linear calibration range of the sensor for the voltammetric detection of magnesium was determined to be 25-250 nM, with a detection limit of 8.31 nM and a quantification limit of 24.87 nM. The repeatability for a concentration of 120 nM, using the modified graphite/zinc nanoparticle/1-nitroso-2-naphthol electrode with square wave voltammetry, yielded a standard deviation of 3.55. The reproducibility of the response from three separate electrodes designed with the graphite/zinc nanoparticle/1-nitroso-2-naphthol structure to a concentration of 120 nM of magnesium, also measured by square wave voltammetry, resulted in a relative standard deviation of 3.88.

(A) Square wave voltammograms of the response of the graphite/zinc nanoparticle/1-nitroso-2-naphthol electrode to different concentrations of magnesium in nanomolar amounts. (B) Linear relationship between current and magnesium concentration in nanomolar amounts.

3.6 Interference Effect

To evaluate the interference effect of various organic and inorganic species associated with magnesium in human blood on the response behavior of the graphite/zinc nanoparticle/1-nitroso-2-naphthol electrode towards magnesium, the magnesium concentration was fixed at a constant value of 120 nM. The concentrations of individual interfering species were varied until a 5% change in the current signal of the recorded square wave voltammograms was observed. The ratio of the interfering concentration to the magnesium concentration for each interferent is reported as the interference threshold in Table 1. As shown, at the reported ratio and below, no significant interference from the interfering species on the behavior of the graphite/zinc nanoparticle/1-nitroso-2-naphthol electrode towards magnesium was observed. This indicates the high capability of the modified electrode in the voltammetric determination of magnesium in real complex samples containing it.

Table 1: Investigation of the interference effect of various organic and inorganic materials on the square wave voltammetric response of the graphite/zinc nanoparticle/1-nitroso-2-naphthol electrode towards 120 nanomolar magnesium ions (triplicate).

Interferer	Tolerated Ratio [Interference] / [Mg^{2+}]	Interferer	Tolerated Ratio [Interference] / [Mg^{2+}]
Co^{2+}	38	Mn^{2+}	46
Cu^{2+}	36	Fe^{3+}	28
Zn^{2+}	42	Fe^{2+}	32
K^{+}	93	Glucose	53
Vitamin A	125	Ammonia	66
Vitamin B12, B6, B5	≥ 45	Vitamin C	31
L-cyctein	43	Cholestrol	52

3.7 Evaluation of the Electrolytic Performance of the Modified Electrode

To evaluate the electroanalytical performance of the proposed sensor, which consists of a graphite electrode/zinc nanoparticle/1-nitroso-2-naphthol structure, the direct determination of magnesium concentration in human blood serum samples was conducted using the square wave voltammetry technique. In this study, different amounts of magnesium, expressed in nanomolar terms, were injected into human blood serum samples using the external standard addition method. The results obtained and recovered by the proposed sensor, in comparison with the injected amounts, are presented in Table 2. These results indicate that the graphite electrode/zinc nanoparticle/1-nitroso-2-naphthol structure exhibits high accuracy and precision in the voltammetric measurement of magnesium in complex

Evaluation of the electrolytic efficiency of the modified electrode with a graphite/zinc nanoparticle/1-nitroso-2-naphthol structure in the analysis of magnesium content in human blood plasma using the square wave voltammetry technique and the standard addition method (5 repetitions).

Sample	Spiked value (nM)	Found value (nM)	Recovery %
Sample 1	40	41.98±2.08 (RSD%=4.95)	104.95
Sample 2	80	83.63±3.79 (RSD%=4.53)	104.53
Sample 3	120	115.30±4.51 (RSD%=3.91)	96.08
Sample 4	160	165.64±5.85	

4. Conclusion

In this study, zinc nanoparticles were deposited by electroprecipitation, and 1-nitroso-2-naphthol was deposited by electropolymerization on the surface of a bare graphite pen electrode using the cyclic voltammetry technique. The voltammetric platform created with the graphite/zinc nanoparticle/1-nitroso-2-naphthol structure served as an electrochemical sensor that demonstrated satisfactory electrocatalytic properties, with an electron transfer coefficient α of 0.75, an apparent charge transfer rate constant log ks = 3.27, and an active layer concentration deposited on the modified electrode surface Γ of 5.16 ×10-5. These parameters indicate the high electrocatalytic capability of the graphite/zinc nanoparticle/1-nitroso-2-naphthol electrode in the voltammetric measurement of magnesium. Notable features of the proposed sensor include high sensitivity, a low detection limit (8.31 nM), a quantification limit (24.87 nM), and a wide linear range (30–300 nM). Furthermore, the modified electrode with the graphite/zinc nanoparticle/1-nitroso-2-naphthol configuration demonstrates acceptable stability, repeatability, selectivity, and measurement accuracy in the square wave voltammetric assessment of magnesium in real biological blood serum samples.

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