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Electrochemically Reduced Graphene Oxide Modified Screen-Printed Electrodes for Sensitive Determination of Acetylsalicylic Acid

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In this report, the voltammetric detection of aspirin (ASA) was performed using an electrochemical sensor modified with a graphene–Nafion nanocomposite film. A Nafion graphene oxide-decorated glassy carbon electrode (GCE) was prepared using a facile drop-casting strategy followed by the reduction of graphene oxide on the surface of the GCE via an electrochemical technique. The electrochemically reduced graphene oxide (ER-GO)/Nafion screen-printed electrode (SPE) was finally fabricated and used for the detection of ASA.

Keywords: Reduced graphene oxide; Screen-printed electrode; Aspirin; Electrochemical determination; Human oral fluid

1. INTRODUCTION

Aspirin, or acetylsalicylic acid (ASA), has been applied to the treatment of a range of inflammatory conditions for over 200 years since its introduction in the late 1890s. The active ingredient in aspirin was discovered in willow bark by Edward Stone in 1763 [1]. For the analysis of ASA, a series of analytical techniques have been developed, including high-performance liquid chromatography–mass spectrometry [2-6], gas chromatography–mass spectrometry [7], ultra-performance liquid chromatography tandem mass spectrometry [8] and capillary electrophoresis [9]. Nevertheless, many of the above techniques require specimen pretreatment, complex apparatuses, several time-consuming operation steps, and special training.

Electrochemical sensors are an attractive alternative method for the determination of electroactive species since they are simple, highly sensitive, and cost-effective [10-12]. Due to the low-cost, single-shot disposability with high reproducibility and reliability in the electrochemical analysis of the target analyte, screen-printed electrodes have been extensively used as sensor platforms [13-17].

Recently, a range of techniques have been applied to the electrochemical determination of aspirin, which has gained attention. For example, Srivastava and co-workers [18] reported the detection of ASA in pharmaceutical formulations and blood and urine specimens via voltammetry using a surfactant-modified multi-walled carbon nanotube paste electrode. Tsai and co-workers [19] used a GCE decorated by a multi-walled carbon nanotube alumina-coated silica nanocomposite to study the electrocatalytic oxidation of ASA. Lu and co-workers [20] presented the determination of ASA using an electrochemical sensor modified with a AuNP-modified molecularly imprinted polymer film. Rynkowski and co-workers [21] presented voltammetric investigations of ASA electrooxidation using a platinum electrode. The above works provide desirable sensitivity and limits of detection (LOD), but additional time was needed for the modification of the electrode surface that entailed different steps.

Considering its cost-effectiveness, distinct mechanical features, high electrical and thermal conductivities, and large surface area, graphene has attracted extensive attention since its discovery [22, 23]. In the past few years, graphene has been applied to catalysis, sensor technologies, nanocomposites and capacitors. Graphene sheets possess excellent electrocatalytic activities and electronic transport features [20, 24-26] and have been used as electrode materials in optoelectronic devices [27] and electrochemical supercapacitors [28] and have been used to construct ultrasensitive chemical sensors [29], such as pH sensors [30], gas sensors [31], and biosensors [32].

In this report, a Nafion/ ER-GO-modified SPE was prepared using a facile, cost-effective and eco-friendly technique where a constant cathodic potential was applied to the GO-modified SPE. Our proposed sensor based on the Nafion/ER-GO-decorated electrode exhibited a high selectivity and sensitivity for the detection of ASA.

2. EXPERIMENTS

2.1. Chemicals

All chemicals for the solutions were obtained from Aldrich in the commercially purest forms. The final solutions were prepared using an aqueous stock solution of ASA (10 mM). The standard stock solutions were stored at 40 °C in a refrigerator. All test solutions were prepared by adjusting the concentration to a proper level through dilution using distilled water for further use.

2.2. Preparation of the graphene modified electrode

The surface was coated after ultrasonically rinsing the bare SPE using 1:1 ethanol:propenol and distilled water, respectively, and drying it at ambient temperature. This was followed by dispersing 1.0

mg/mL graphene oxide (GO) into distilled water via ultrasonication for 0.5 h or until a complete dispersion was obtained. A Nafion–isopropyl-alcohol solution (10 μL, 1.0 wt %) was added into 50 μL of the as-prepared GO solution (1 mg/mL) and ultrasonicated for approximately 0.5 h. Finally, the Nafion/ER-GO-modified SPE was yielded after coating an aliquot of 10 μL of the above mixed solution onto the screen-printed electrode (SPE). The solvent was evaporated for 120 min at ambient temperature. The Nafion/GO/SPE was yielded after air drying. This was followed by immersing the as-prepared Nafion/ER-GO/SPE in a KH_2PO_4 solution (0.02 M), and a cathodic potential (−0.7 V) was applied to the GO-modified GCE for *ca.* 10 min using a potentiostat. The fabricated Nafion/ER-GO/SPE could be used for the electrochemical sensing of ASA.

2.3. Instruments

Cyclic voltammetry (CV) measurements were performed using a Gamry Reference 600 potentiostat. Voltammetric measurements were carried out using an electrochemical geometry, and the working, reference, and counter electrodes were a glassy carbon electrode (diameter, 3 mm), saturated calomel electrode (SCE), and a platinum wire. All the measurements were carried out at ambient temperature. To remove the remaining aluminium oxide particles on the surface of the electrode, the electrode was washed and ultrasonicated using distilled water for 5 min after each treatment. A full pH range of 0-14 was applied to a Hanna HI 221 pH meter to measure the solution pH values.

2.4. Electrochemical Measurements

A range of measurements were performed for the electrochemical characterization of the electrodes, and a heterogeneous electron transfer rate constant of ~1.7×10^{-3} cm/s was obtained using the ferrocyanide redox couple in 0.1 M KCl. CV measurements were carried out at an initial potential of 0 V, a step potential of 5 mV, a vertex potential of 1.5 V, and an end potential of 0 V.

3. RESULTS AND DISCUSSION

The surfaces of the bare SPE and the Nafion/ER-GO-modified SPE were investigated herein. As shown in Fig. 1, CV measurements were performed using an electrochemical probe that contained $[K_3Fe(CN)_6]$ (1.0×10^{-3} M) and KCl (0.1 M) (scan rate, 100 mV/s), and the CV profiles for the original SPE and the Nafion/ER-GO-modified SPE were recorded. The CV shape indicated a reversible one electron transfer process. The peak to peak separation on the original SPE was *ca.* 69 mV, confirming the reversible redox process of $[K_3Fe(CN)_6]$. On the bare SPE, the peak to peak separation observed was approximately 68 mV, which corroborates with the reversible redox process of $[K_3Fe(CN)_6]$ [33, 34]. A voltammogram of $[Fe(CN)_6]^{3-}$ with the peak currents was seen in the curve. Upon the modification of the SPE with ER-GO, an increase in the current was observed. The sharp increase in the peak current can be explained by the more desirable electric conduction of

graphene than that of graphite. The anodic and cathodic peak potentials of graphene were different from that of the original SPE. The anodic peak potential (E_{pa}) and cathodic peak potential (E_{pc}) were 0.113 V and 0.283 V, respectively, for graphene and 0.14 V, and 0.25 V, respectively, for the original SPE.

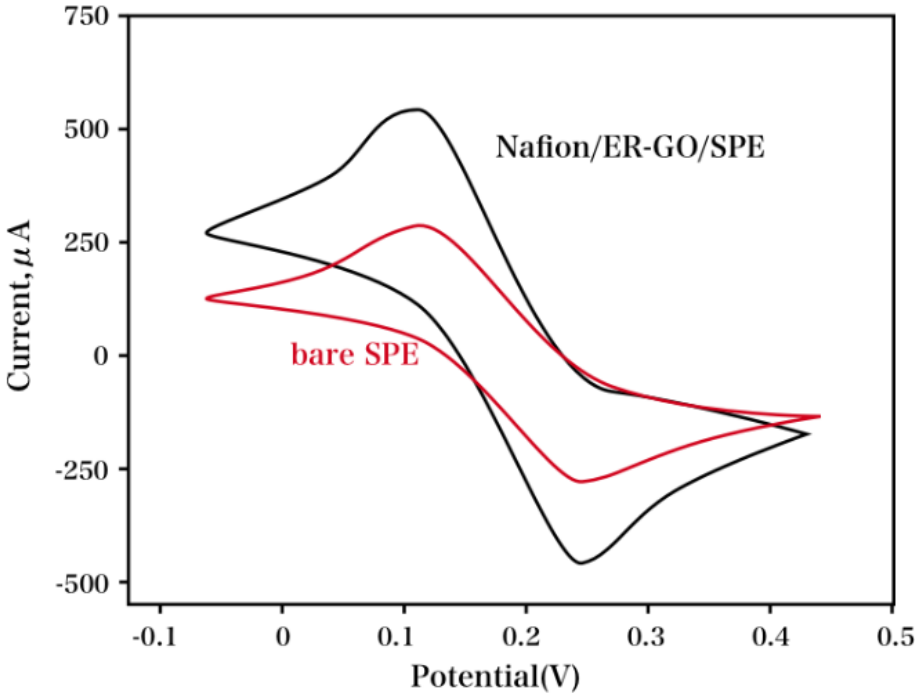

Figure 1. CV responses for the original SPE and the Nafion/ER-GO-modified SPE in $[Fe(CN)^6]^{3-}$ (1 mM) with KCl (0.1 M) at a scan rate of 100 mV/s.

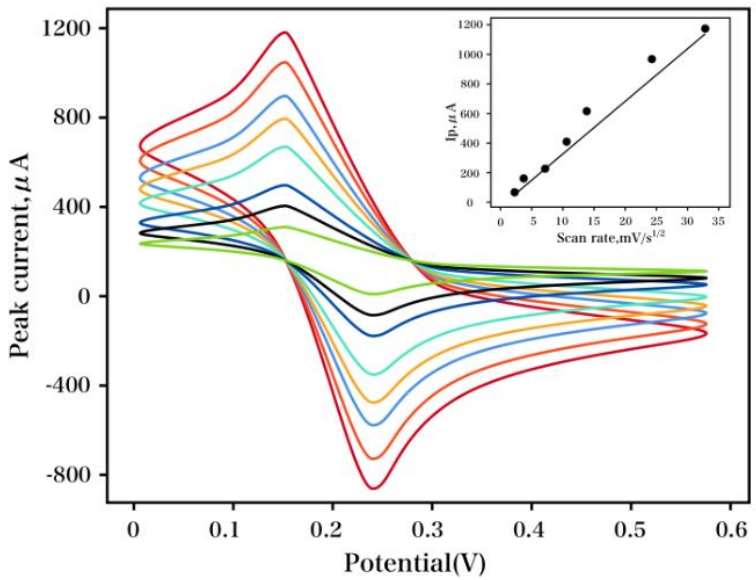

Figure 2. CV response for the Nafion/ER-GO-modified SPE in $[Fe(CN)_6]^{3-}$ (0.1 M) with KCl (0.1 M) at different scan rates.

A comparison of the electroactive area of the SPE and the Nafion/ER-GO-modified SPE is shown by the CV measurement. The peak potential was also affected by the modification of the electrode surface with graphene [35]. The Randles–Sevcik equation is expressed as $Ip = 2.69 \times 10^5 n^{3/2} A D_0 S C_0 v^{1/2}$, and the electroactive area was calculated from the slope of a plot of the voltammetric peak current (I_p) *vs.* the square root of the scan rate (Fig. 2).

The redox peak currents on the graphene-modified SPE showed a linear increase with the scan rate (5 - 1000 mV/s), as indicated in the Randles–Sevcik equation, suggesting the surface confined property of the modified electrode reaction. A CV measurement was performed using the Nafion/ER-GO-modified SPE in a phosphate buffer solution (PBS) (pH 4) to study the electrochemical performance of ASA. The Nafion/ER-GO-modified SPE before and after adding ASA (10 μM) in PBS was characterized via two CVs in Fig. 3. If the pH values are higher than 8, the *E*p and $E1_{/2}$ values are independent of the pH. This means that the protons are no longer involved in the ASA electrooxidation. Most likely this results from the fact that at higher pH values, the hydrolysed form of ASA is already chemically deprotonated [36]. At pH 4, ASA is easily oxidized compared to the more basic pH values.

The Nafion/ER-GO-modified SPE exhibited no redox peaks in the potential range of 0 - 1.5 V in the absence of aspirin as an electroactive species, suggesting the non-electroactive property of graphene in the scanned potential window. However, the Nafion/ER-GO-modified SPE exhibited an exceptionally sensitive anodic peak (current, 780 mV) after adding ASA (10 μM) to the PBS (pH 4).

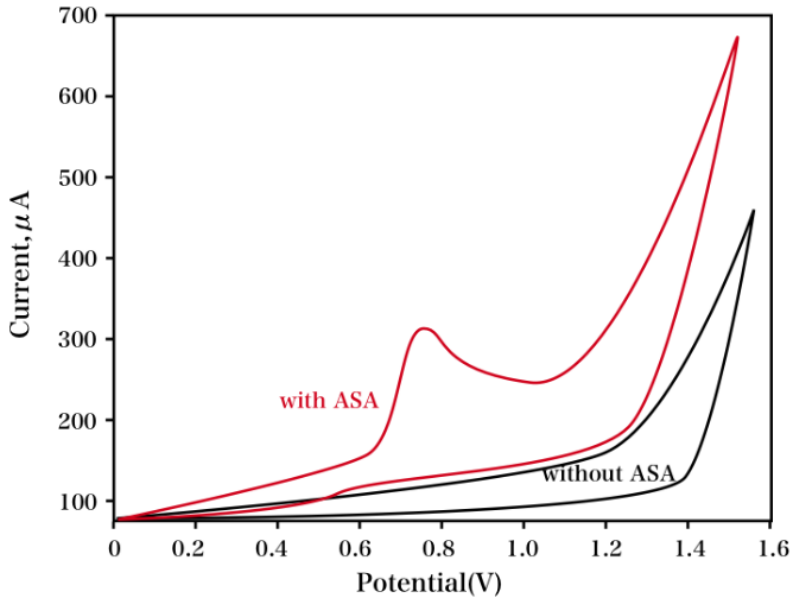

Figure 3. CV response obtained on a Nafion/ER-GO-modified SPE before and after adding ASA (10 μM) to PBS (pH 4).

Acetic acid and salicylic acid can be yielded after the oxidization of ASA in aqueous media. The first step of the ASA electrooxidation was visible in the voltammograms as a peak (Fig. 4) and involves an exchange of one electron. This step determines the rate. The next step, which is invisible in the voltammograms because of overlapping with the oxygen evolution, involves an exchange of the second electron. Considering the above two steps require an exchange of two electrons, it is possible

that three protons are exchanged. The pKa of ASA, i.e., the dissociation constant, which is a significant parameter, was 3.51. Hence, ASA was prepared in a pH 4 PBS that contributed to more desirable electrooxidation of the ASA. The lowest peak height value was observed at a basic pH value of 10, indicating that the electrooxidation process of ASA is less favourable [37].

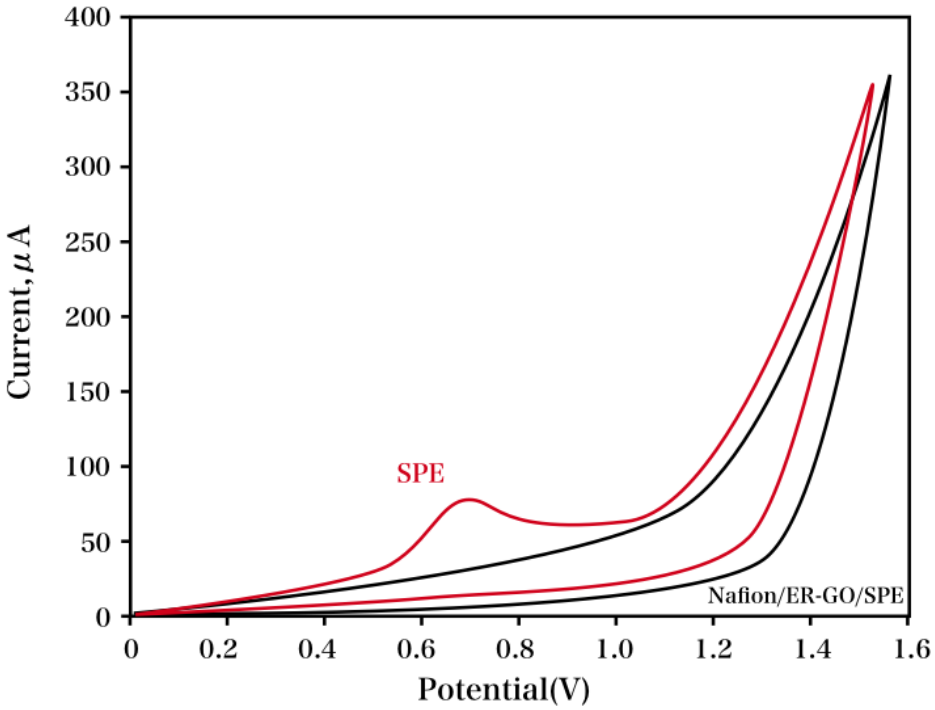

Figure 4. CV response obtained for the original SPE and the Nafion/ER-GO-modified SPE after adding ASA (10 μM) to the PBS (pH 4).

As indicated in Fig. 5A, the peak height current of the ASA solution (10 μM; pH 2 - 10) in the presence of the Nafion/ER-GO-modified SPE was recorded to study the effect of the peak height current *vs.* the pH. When the pH was 4, the peak height current for ASA on the Nafion/ER-GO-modified SPE reached its maximum value that corresponded to the pKa value, suggesting the most desirable electro-oxidation process of ASA occurred at pH 4. Fig. 5B shows the effect of the peak potential *vs.* the pH. Therefore, the optimized pH value for the electrooxidation of ASA was 4, as evidenced by the potential decrease in Fig. 5B. A further pH increase led to a less desirable electrooxidation process of ASA. The plot of the peak potential (*E*p) *vs.* the pH (4 - 10) was linear and depended on the anodic peak potential of the analyte. These results suggested the effect of the solution pH on the peak potential and the peak current. Therefore, a pH of 4 was used throughout the analytical measurements to achieve the most desirable electrooxidation of ASA. We also investigated the effect of the ASA concentration on the Nafion/ER-GO-modified SPE. CV measurements were performed for ASA in a range of 0.1 - 100 μM in a pH 4 PBS using the Nafion/ER-GO-modified SPE (Figure 6). After ASA was added, an increase (in magnitude) in the peak height current (I_{pa}) was observed. The concentration of the ASA (0.1 - 100 μM) *vs.* the peak height current was also investigated. To allow

for a comparison to previous reports, the characteristics of the different electrochemical sensors for ASA are summarized in Table 2.

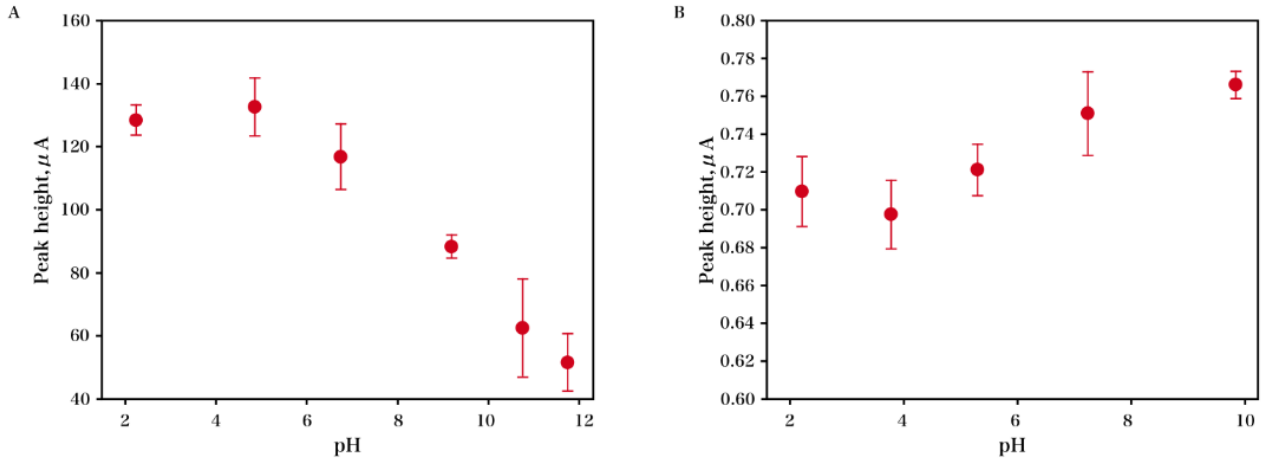

Figure 5. (A) Plot of the peak height as a function of the pH for the electrochemical oxidation of ASA (10 μM) on the Nafion/ER-GO-modified SPE. (B) Plot of the peak potential (E_p) as a function of the pH for the electrochemical oxidation of ASA (10 μM) on the Nafion/ER-GO-modified SPE.

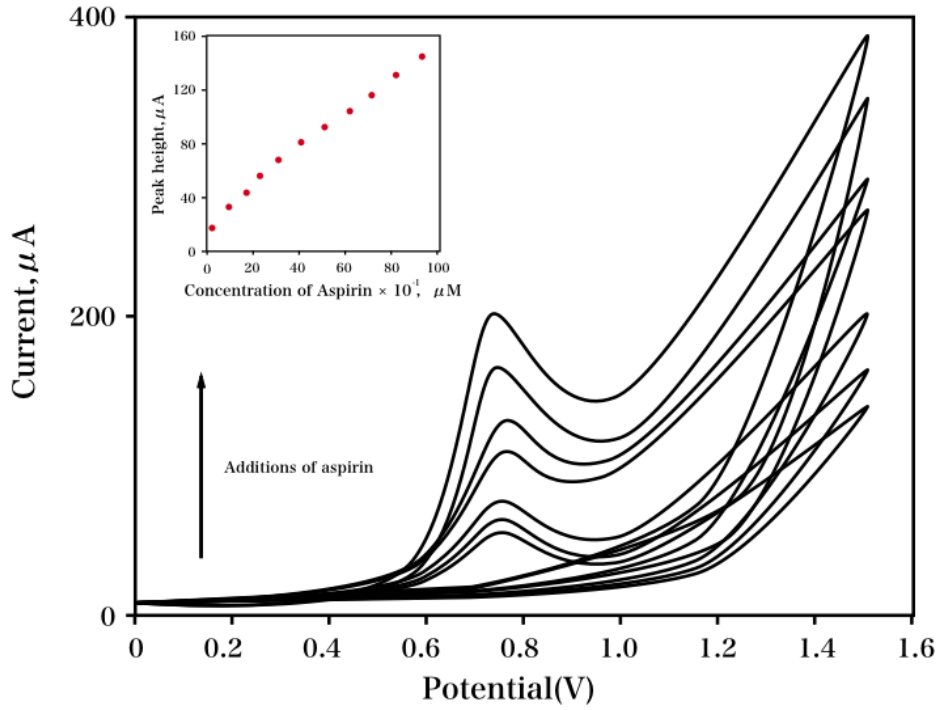

Figure 6. Cyclic voltammogram response observed in a phosphate buffer solution at a pH of 4 on the Nafion/ER-GO-modified SPE for a range of ASA concentrations from 0.1 to 100 mM. Inset: a plot of the peak height (Ip) as a function of the ASA concentration using the Nafion/ER-GO-modified SPE.

Table 1. Comparison of the major characteristics of the electrochemical sensors used for the detection of ASA.

Electrode	Linear detection range	Detection limit	Reference
Graphene	1–100 μM	0.03 ng/mL	[38]
ZnO nanoparticle ionic liquid composite	0.7–950 μM	0.3 μM	[39]
Edge plane pyrolytic graphite electrode	0.02–100 μM	0.032 μM	[40]
Nafion/ER-GO、SPE	0.1 - 100 μM	0.05μM	This work

The viability of the analytical protocol was investigated in relation to detection within analytically relevant media and confirmed that ASA could be successfully determined under ideal conditions using a standard PBS at a pH of 4. The determination of the concentration of ASA in human saliva is important because of the clinical use of ASA for antipyretic and analgesic effects, and ASA can be taken orally. Human saliva specimens were collected from three healthy individuals, and ASA was added to the specimens (10 - 100 μM). Each concentration was repeated three times and plotted with the appropriate error bars (Table 3). The response was linearly related to the ASA concentration in the range of 10 - 150 μM, and the LOD was 8.4 μM. Before use, the human saliva solution was used without modification.

Table 3. Determination of ASA in human oral fluid.

Sample	Found (μM)	Added (μM)	Found (μM)	RSD (%)
1	None	10	9.88	0.87
2	None	50	51.22	4.81
3	None	100	103.65	2.05

4. CONCLUSIONS

In this report, the ER-GO-modified SPE provided a selective and sensitive performance for the detection of ASA in an ideal buffer solution and in a biological matrix, including human saliva specimens. Our proposed sensor was exceptionally reproducible and stable for the routine detection of ASA without a pretreatment or maintenance. A linear response was observed in a buffered solution (0.1 - 100 μM) with an LOD of 0.09 μM. In a biological matrix, the ASA detection was also linear over the concentration range of 10 to 150 μM.

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