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# Electrochemical Behavior and Determination of Matrine by the Reduced Graphene Oxide-Nafion Modified Glassy Carbon Electrode

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Reduced graphene oxide-Nafion composite modified glassy carbon electrode (RGO-Nafion/GCE) was prepared and the electrochemical behavior of matrine at RGO-Nafion/GCE was investigated by cyclic voltammetry (CV) and differential pulse voltammetry (DPV). An irreversible oxidation peak of matrine was observed in 0.2 mol·L$^{-1}$ $Na_2HPO_4$-$NaH_2PO_4$ buffer system (pH 7.2) with 1.05 V of $E_{pa}$ (vs. SCE). A novel method for the determination of matrine was developed based on a well-defined electrochemical response signal of matrine at RGO-Nafion/GCE. Under the optimized conditions, the DPV oxidation peak current and the concentration of matrine showed a good linear relationship in the range from $5.0\times10^{-5}$ to $3.0\times10^{-3}$ mol·L$^{-1}$, the linear regression equation was $I_{pa}$ (μA)= 7.145c+0.3778 (r=0.9990) and the detection limit for matrine could reach $2.5\times10^{-6}$ mol·L$^{-1}$. The recovery of matrine was 99.90-101.82% (RSD=1.3%) in huam serum. The proposed method is quick, sensitive, reliable, and can be used for the determination of matrine.

**Keywords:** Matrine; Reduced graphene oxide; Nafion; Cyclic voltammetry; Differential pulse voltammetry.

## 1. INTRODUCTION

Matrine (matridin-15-one, Fig. 1), a kind of important alkaloid extracted from leguminous plants like sophora flavescens or radix sophorae tonkinensis, has various pharmacological effects such as anti-inflammatory [1], immunomodulatory [2], anti-virus [3], anti-tumor[4] and anti-arrhythmia [5].

It is essential to develop a simple, sensitive, reliable and effective analytical method for the determination of matrine. HPLC has firstly become a typical method to detect matrine in sophora flavescens ait and rat plasma [6-7]. Other analytical methods for this purpose have also been proposed including LC-MS [8-9], GC-MS [10-11], and capillary electrophoresis (CE) [12-13].

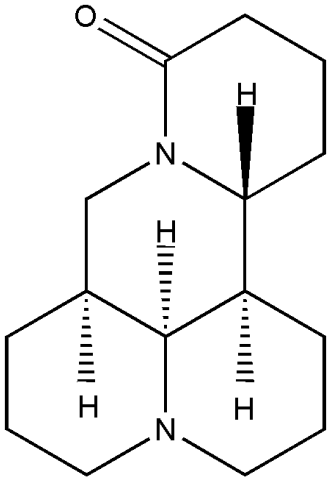


**Figure 1.** The structure of matrine.

In recent ten years, electroanalytical methods have been proposed as an efficient alternatives for matrine analysis due to its simplicity, rapidness, high sensitivity and low cost. Such as cyclic voltammetry and square cyclic voltammetry with a glassy carbon electrode [14]. Chemically modified electrodes were introduced in some other methods using modified electrode [15-18], including multi-wall carbon nanotubes modified electrode [15], the L-cysteine film modified electrode [16], the L-cysteine/graphene oxide-chitosan composite modified electrode[17] and the reduced graphene oxide film modified electrode [18]. The chemical modified electrodes displayed higher sensitivity and selectivity as compared with the conventional electrodes.

Graphene, a new type of carbon nanomaterials, has been widely used in the field of electrochemical analysis due to its unique structure and excellent performance, including a huge specific surface area, high electrical conductivity, strong mechanical properties and stable thermodynamic properties [19-20]. In recent studies, graphene-based electrodes have shown superior performance in terms of electrocatalytic activity and macroscopic scale. However, the poor dispersibility of graphene in solvents limits its further applications. Therefore, the properties of graphene could be better expressed through dissolving it in a particular solution, such as didodecyldimethylammonium bromide (DDDAB) [21], poly-electrolytes [22], chitosan [23-24], and cyclodextrins (CDs) [25]. Nafion, as a cation-exchanging polymer, is also applied to disperse graphene in an ethanol solution [26]. For instance, nafion-graphene nanocomposite film was used as enhanced sensing platform for ultrasensitive determination of cadmium [27]. The combination of Nafion and graphene can not only enhance the dispersive ability and film forming ability, but also reduce the substrate interference [27-28], which greatly improve the sensitivity and selectivity of the modified electrode. Thus, the grapheme-Nafion composite modified electrodes will play an important role in the

field of electrochemical research [29-30], especially in the analysis of cationic alkaloid. However, the electroanalytical methods of cationic alkaloid using the reduced graphene oxide-Nafion composite modified glassy carbon electrode (RGO-Nafion/GCE) is rarely reported.

The purpose of this work is to provide a sensitive, selective and rapid electroanalytical method for the determination of matrine. A RGO-Nafion/GCE was fabricated by dispersing graphene in Nafion solutions and then dropping them onto the glassy carbon electrode (GCE) and the electrochemical properties of the electrode for matrine was investigated. What's more, a sensitive electroanalytical method for the determination of matrine in huam serum was proposed.

## 2. EXPERIMENTAL

### *2.1 Chemicals and reagents*

Matrine (purity≥ 98%) was purchased from Xi'an Kailai Biological Engineering Co. Ltd; flake graphite (<20 μm) was purchased from Qingdao Xinghe Graphite Co. Ltd; Nafion (5 wt% ethanol solution, Sigma Chemical Co., Ltd); human serum was obtained from a local hospital.

All the other reagents used were of analytical-reagent grade and all solutions were prepared with double distilled water.

### *2.2 Apparatus*

Zahner Zennium IM6 Electrochemical Workstation (ZAHNER-elektrik GmbH & Co. KG, Kronach, Germany). A three-electrode system consisting of a reduced graphene oxide-Nafion modified glassy carbon electrode (RGO-Nafion/GCE) as working electrode, a platinum counter electrode (213 type) and a saturated calomel reference electrode (SCE).

### *2.3 Preparation of reduced graphene oxide*

Graphene oxide (GO) was prefabricated from flake graphite according to the modified Hummers method [31] and dispersed in 100 mL double distilled water (for 100 mg GO) by ultrasound for 3 h, and then the mixture was reduced with 80% hydrazine hydrate (2.0 mL) [32] as reducing agent under stirring and heating for 24 h at 80 °C until a black cotton-shaped deposition was gained. The resulting mixture was washed with methanol and double distilled water to neutral, respectively, followed by filtration and drying, reduced graphene oxide (RGO) was thus obtained.

### *2.4 Preparation of RGO-Nafion/GCE*

10 mg RGO powder was firstly dispersed in 10 mL of double distilled water by ultrasound, and then RGO-Nafion suspension was gained through dispersing the mixture contains both RGO suspension and 5% Nafion solution (100 μL) under ultrasound for 0.5 h. Prior to modification, a bare

GCE was first mechanically polished to a mirror-like surface stepwise using finer emery-paper and 0.5 μm alumina slurry, and was then washed ultrasonically with 1:1 nitric acid, alcohol and double-distilled water successively. Finally, the reduced graphene oxide-Nafion modified glassy carbon electrode (RGO-Nafion/GCE) was prepared by depositing the above-mentioned suspension (8.0 μL) on a fresh GCE surface using a micro-injector, and then dried naturally.

### *2.5 Analytical procedure*

The sample tested was firstly diluted to 20 mL with 0.2 $mol \cdot L^{-1}$ $Na_2HPO_4$-$NaH_2PO_4$ buffer solution (pH 7.2). Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) analysis were carried out with RGO-Nafion/GCE as working electrode in the potential range from 0.2 to 1.5 V at a stirring speed of 300 rpm and scan rate of 50 $mV \cdot s^{-1}$. The DPV parameter setting were: pulse width of 250 Ms, pulse amplitude of 30 mV and pulse interval of 250 mS.

## 3. RESULTS AND DISCUSSION

### *3.1 Electrochemical behavior of matrine*

An irreversible oxidation peak ($E_{pa}$=1.05 V) of matrine at RGO-Nafion/GCE was observed in 0.2 $mol \cdot L^{-1}$ $Na_2HPO_4$-$NaH_2PO_4$ buffer system (pH 7.2), as shown in Fig. 2.

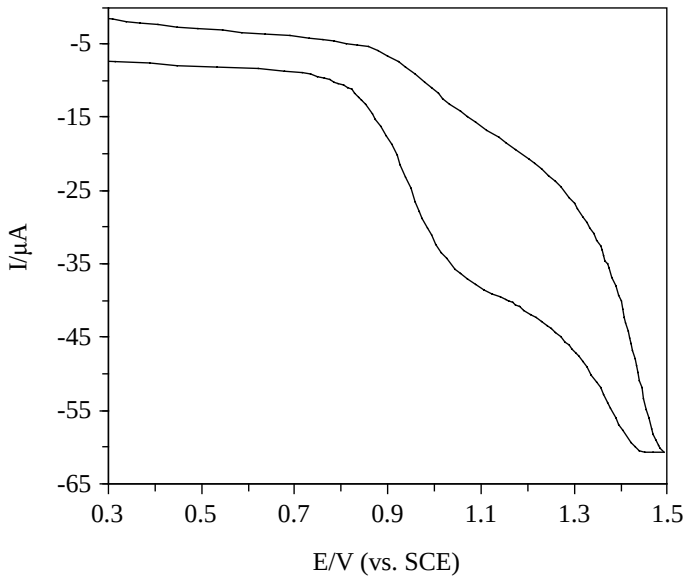


**Figure 2.** The cyclic voltammetry curve of matrine at RGO-Nafion/GCE.

Fig. 3 exhibits the DPV curves of 0.1 mM matrine in 0.2 $mol \cdot L^{-1}$ $Na_2HPO_4$-$NaH_2PO_4$ buffer solution (pH 7.2) at GCE (a), RGO/GCE (b), Nafion/GCE (c), and RGO-Nafion/GCE (d). The oxidation peak currents of matrine at RGO/GCE (b) and Nafion/GCE (c) are much higher than that at GCE (a), indicating that both RGO and Nafion have a sensitizing effect on the surface of electrode for

the determination of matrine. Furthermore, the highest oxidation peak current of matrine is obtained at RGO-Nafion/GCE (d), which means that the combination of RGO and Nafion has a synergistic sensitizing effect on electrode, and the RGO-Nafion composite film can greatly improve the sensitivity and analytical performance of the electrode.

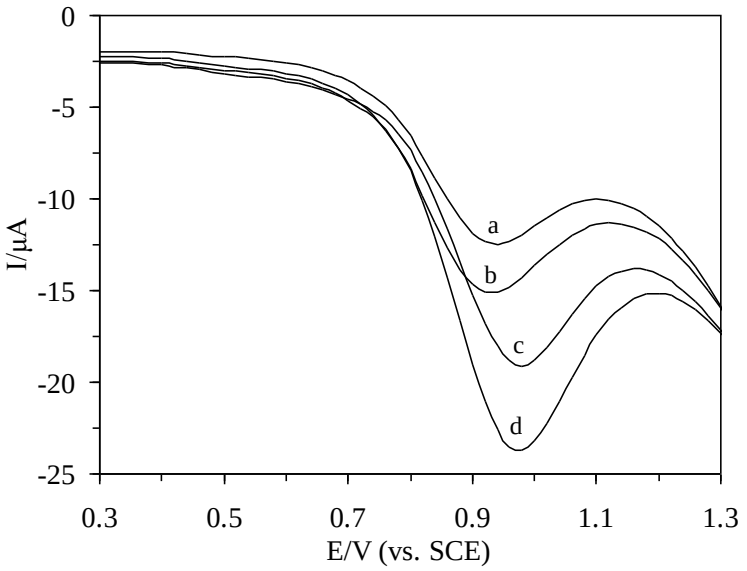


**Figure 3.** The differential pulse voltammetry curves of matrine at GCE (a), RGO/GCE (b), Nafion/GCE (c) and RGO-Nafion/GCE (d).

*3.2 Influence of supporting electrolyte and pH*

Different supporting electrolytes such as 0.2 mol·$L^{-1}$ NaAc-HAc buffer solution, 0.2 mol·$L^{-1}$ $Na_2HPO_4$-$NaH_2PO_4$ buffer solution and 0.2 mol·$L^{-1}$ $Na_2HPO_4$-$KH_2PO_4$ buffer solution were investigated at RGO-Nafion/GCE. Well-defined CV response with stable oxidation peak and high peak current of matrine was obtained in 0.2 mol·$L^{-1}$ $Na_2HPO_4$-$NaH_2PO_4$ buffer solution.

The influence of pH on the DPV oxidation peak of matrine at RGO-Nafion/GCE was then investigated in 0.2 mol·$L^{-1}$$Na_2HPO_4$-$NaH_2PO_4$ buffer solution (pH 6.0 to 7.5). The oxidation peak current of matrine increases with the increase of pH from 6.0 to 7.2, and then tends to a steady value as the increase of pH above 7.2, as shown in Fig. 4. Because the oxidation of matrine is a process with the deprotonation and the contribution of oxidation peak current is mainly from the deprotonation of matrine [16], the performance of electrochemical oxidation reaction is more suitable under the near-neutral or slightly alkaline conditions rather than acidic. Therefore, the 0.2 mol·$L^{-1}$ $Na_2HPO_4$-$NaH_2PO_4$ (pH 7.2) was selected as supporting electrolyte in this study.

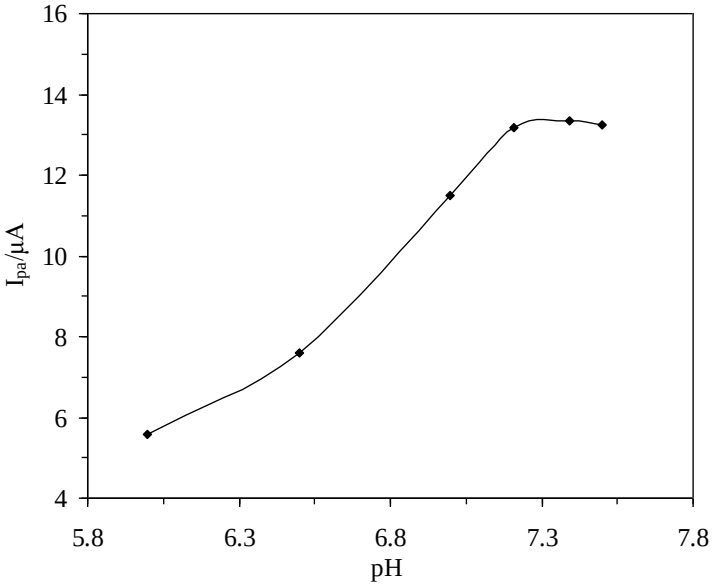


**Figure 4** The influence of pH on the DPV oxidation peak current of matrine.

*3.3 Influence of scan rate*

Fig. 5 displays the effect of scan rate (from 40 to 120 $mV \cdot s^{-1}$) on the oxidation peak potential and current of 0.1 mM matrine at RGO-Nafion/GCE. The oxidation peak potential slowly moves to positive direction and the oxidation peak current increases significantly as the increase of the scan rate. Moreover, the oxidation peak current and the square root of the scan rate showed a good linear relationship in the scan rate range from 40 to 120 $mV \cdot s^{-1}$, and the linear regression equation was $I_{pa}$ (μA)= $0.6808v^{1/2}$+0.09831 (r=0.9969), which means that the electrode reaction process of matrine at RGO-Nafion/GCE was diffusion-controlled [14]. A high and stable peak current for matrine was obtained at the scan rate of 50 $mV \cdot s^{-1}$, the scan rate of 50 $mV \cdot s^{-1}$ was hence selected in this study.

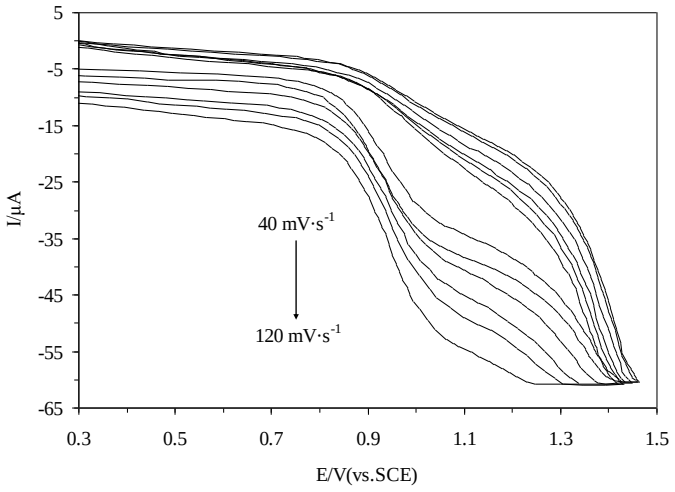


**Figure 5** The Cyclic voltammetry curves of matrine at different scan rates.

The relationship between the peak current (I) and electron transfer number (n) to comply with Equation (1) in the electrode reaction according to the Laviron theory [33].

$$I = \frac{n^2F^2A\Gamma_T v}{4RT} = \frac{nFQv}{4RT} \qquad (1)$$

Where F (96485 $C \cdot mol^{-1}$) is the Faraday constant, A ($cm^2$) is the electrode surface area, $\Gamma_T$ ($mol \cdot cm^{-2}$) is the adsorption quantity, v ($mV \cdot s^{-1}$) is the scan rate, T=298 K, R=8.3145 $J \cdot K \cdot mol^{-1}$, $Q=nFA\Gamma_T$, Q is the peak area of a single process of cyclic voltammetry (with quantity of electricity). In this study, the electron transfer number (n) was calculated to be 1.7 at 50 $mV \cdot s^{-1}$ of scan rate.

*3.4 Influence of rotation rate*

Sufficient agitation is necessary in the electrode reaction process because the oxidation of matrine at RGO-Nafion/GCE was diffusion-controlled. Accordingly, the impact of rotation rate (from 0 to 1000 rpm) on the DPV oxidation peak of 0.1 mM matrine at RGO-Nafion/GCE was investigated. The oxidation peak current of matrine increases firstly with the increase of rotation rate, and then tends to a steady value as the increase of rotation rate above 300 rpm, as shown in Fig. 6. Furthermore, the oxidation peak potential of matrine moves slightly toward the positive direction as the increase of rotation rate. Therefore, the rotation rate of 300 rpm was identified in this study.

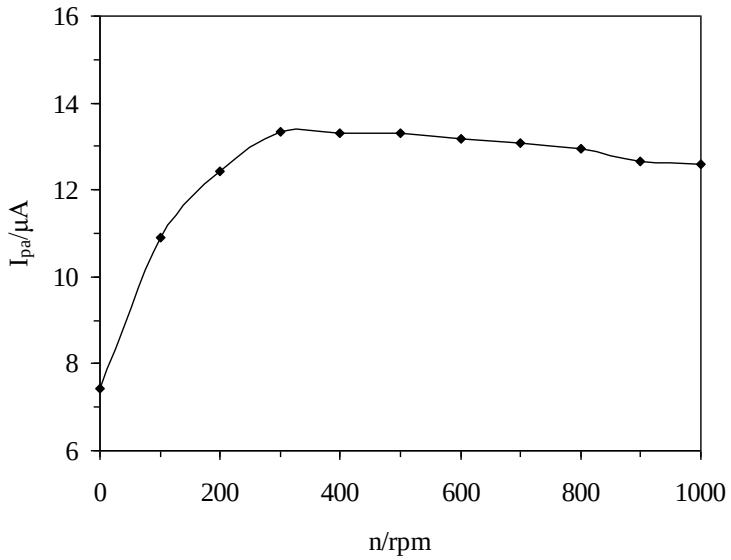


**Figure 6** The influence of rotation rate on the DPV oxidation peak current of matrine.

*3.5 Analytical performance of proposed method*

The DPV analysis was performed for the different concentrations of matrine at RGO-Nafion/GCE in 0.2 $mol \cdot L^{-1}$ $Na_2HPO_4$-$NaH_2PO_4$ buffer system (pH 7.2), as shown in Fig. 7 a. The DPV oxidation peak current and the concentration of matrine showed a good linear relationship in the range from $5.0\times10^{-5}$ to $3.0\times10^{-3}$ $mol \cdot L^{-1}$ (Fig. 7 b), the linear regression equation was $I_{pa}$ (μA)=

7.145c+0.3778 (r=0.9990). According to the method recommended by IUPAC, detection limit (CL)=$3S_b/S_x$, where, 3 is confidence factor, $S_b$ is background noise standard deviation, $S_x$ is the measurement sensitivity (The slope of the standard curve), as the result, the detection limit for matrine could reach $2.5\times10^{-6}$ mol·$L^{-1}$.

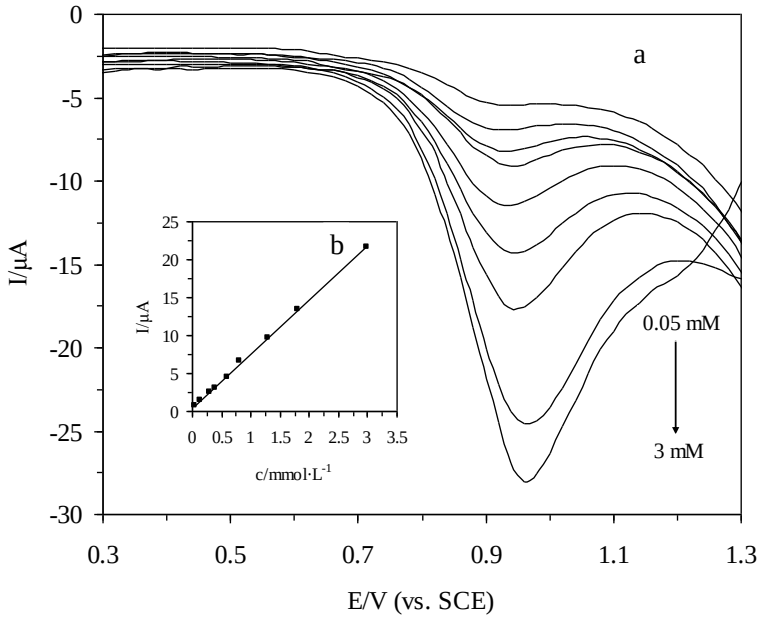


**Figure 7.** The differential pulse voltammetry curves of different concentrations of matrine at the RGO-Nafion/GCE (a). The linear relationship between DPV oxidation peak current and the concentration of matrine (b).

*3.6 Interference experiment*

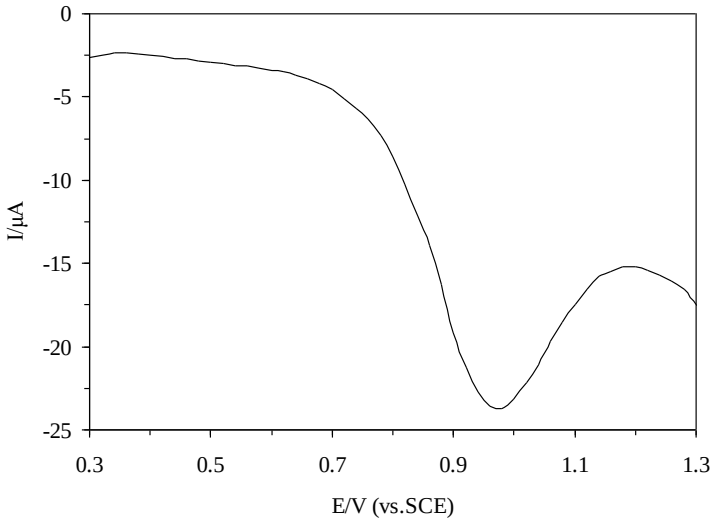


**Figure 8.** The differential pulse voltammetry curve of matrine at RGO-Nafion/GCE in the mixture solution.

Fig. 8 exhibits the DPV curve of 0.1 mM matrine at RGO-Nafion/GCE in the mixture solution formed by 20 mM sodium chloride, glucose and lysine, and 10 mM ascorbic acid and uric acid. The oxidation peak potential and shape of matrine have no significant change as compared with Fig. 3 (d), the oxidation peak current only has a very small change (<5%, n=5), indicating that the RGO-Nafion composite film can effectively improve selectivity and anti-jamming performance of electrode.

### *3.7 The application analysis*

The proposed method was applied to determination of matrine in 5% human serum by standard addition experiment. The recovery of matrine was 99.90-101.82% (RSD=1.3%) in human serum as shown in Table 1, which means that the proposed method is feasible for the determination of matrine in human serum which is a complex system that may produce interference. This novel method to detect matrine based on the RGO-Nafion/GCE is as simple, quick and sensitive as other electrochemical methods [15-18]. Moreover, the determination results suggest that the method has an excellent analysis performence with a high selectivity and a potential application value for the determination of practical alkaloid samples.

**Table 1.** The measurement results of matrine in 5% human serum (n=5).

| Samples | Added ($\mu M \cdot L^{-1}$) | Found ($\mu M \cdot L^{-1}$) | RSD (%) | Recovery (%) |
|---|---|---|---|---|
| 1 | 100.00 | 101.82 | 1.8 | 101.82 |
| 2 | 200.00 | 200.10 | 1.0 | 100.05 |
| 3 | 400.00 | 399.63 | 1.0 | 99.90 |

## 4. CONCLUSIONS

In this paper, the reduced graphene oxide-Nafion composite modified glassy carbon electrode (RGO-Nafion/GCE) was prepared and it was applied to the electrochemical behavior research and determination of matrine. A well-defined electrochemical response signal of matrine at RGO-Nafion/GCE was obtained and the proposed method based on this showed a good linear relationship and high sensitivity for the determination of matrine. What's more, the test results of matrine at RGO-Nafion/GCE in mixture solution formed by distractors and human serum sample demonstrated the high selectivity and strong anti-jamming ability of electrode. Therefore, the developed method is feasible to detect matrine in practical samples.

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