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Improved Adhesion and Performance of Vertically-aligned Mesoporous Silica-nanochannel Film on Reduced Graphene Oxide for Direct Electrochemical Analysis of Human Serum

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Abstract

Vertically-aligned mesoporous silica-nanochannel film (VMSF) has been demonstrated as effective anti-fouling and anti-interference layer for constructing electrochemical sensors. Conductive substrate with high electroactivity and adhesion is very important for improving the performance of VMSF based sensors in terms of stability, sensitivity and discrimination ability. We herein use reduced graphene oxide (rGO) sheets as conductive adhesive and electroactive layer between VMSF and glassy carbon electrode (GCE). VMSF can be stably grown on rGO layer by electrochemically assisted self-assembly method (EASA). Owing to the electroactive properties of rGO, VMSF/rGO/GCE is able to parallel or simultaneously detect small drugs or biomarkers with high sensitivity and distinct oxidation potential for different electroactive organic molecules. Combining the anti-fouling and anti-interference ability of VMSF, direct analysis of small drugs or biomarkers in complex biological (serum) sample is demonstrated.

Keywords

Mesoporous silica-nanochannel, reduced graphene oxide, small drugs, biomarkers, electrochemical detection

1. Introduction

Detection of drugs and biomarkers in complex clinical samples (*e.g.* human serum) are crucial in metabolomics, diagnosis, health monitoring and treatment assessment [1]. For instance, overdose of acetaminophen (APAP, an antipyretic, analgesic agent) poses risk for liver failure [2]. Abnormal serum levels of amino acids, nucleic acid bases, and neurotransmitters are linked to various critical diseases [3-6]. Direct detection of those markers in serum remains challenging because of fouling by biomolecules and interference by electrochemically active compounds [6,7]. Hence current methods for drug or biomarker analysis often suffer from necessity for sophisticated and time-consuming pre-treatments (*e.g.* separation, enrichment).

Vertically-aligned mesoporous silica-nanochannel film (VMSF) has received considerable attention in electrochemical sensors [8-11], molecular separation [12-14], electrocatalysis [15,16], and templated

preparation of nanomaterials [16,17] owing to unique characteristics including presence of highly ordered nanochannels, uniform and tunable pore size, chemical and mechanical stability. More importantly, VMSF exhibits outstanding anti-fouling and anti-interference ability due to permselectivity based on size, charge and lipophilicity. Therefore, VMSF modified electrodes have displayed unique advantage for direct analysis of small substrates in complex samples [18-22].

Many approaches have been developed to grow VMSF on an electrochemical electrode [13, 23-28]. But VMSF adheres poorly onto common electrodes such as glassy carbon electrode (GCE), gold or platinum electrodes. Although VMSF on ITO electrode is stable, VMSF/ITO exhibits high overpotential for electroactive organic molecules, which deprives its ability to analyze a range of important molecules (*e.g.* pharmaceutical compounds, biomarkers, amino acids, nucleic acid bases). To tackle this problem, organosilanes have been used as the molecular glues to modify GCE or metal electrodes to improve the adhesion of VMSF. For instance, Nasir *et al.* have reported that the pretreatment of GCE with 3-amino-propyltriethoxysilane (APTES) can improve the stability of VMSF on GCE [29]. Said *et al.* have also pre-modified gold electrodes with (3-mercaptopropyl)trimethoxysilane before the growth of VMSF [30]. But such adhesion layer unavoidably compromises the electrochemical performance to some extent because these non-conducting molecules reduce the charge transfer from the analytes to the underlying electrode.

In this work, reduced graphene oxide (rGO) sheets were applied as the conductive glue and electroactive layer on GCE. Owing to the abilities to facilitate charge transfer and intimately interact with small organic molecules (*e.g.* through π - π interaction), rGO is able to improve the performance of electrochemical sensors [5,31,32]. Combining the anti-fouling and anti-inference ability of VMSF and the electroactive properties of rGO, we further demonstrate the use of VMSF/rGO/GCE for direct and sensitive analysis of small drugs (acetaminophen) or biomarkers (norepinephrine, tryptophan, and guanine) in human serum.

2. Materials and methods

2.1 Chemicals and materials

GO aqueous solution (1 mg/mL) was purchased from Hangzhou GaoxiTech (China). Tetraethoxysilane (TEOS), cetyltrimethylammonium bromide (CTAB), potassium ferricyanide ($K_3[Fe(CN)_6]$), L-leucine (Leu), L-threonine (Thr), L-alanine (Ala), L-glutamate (Glu), glycine (Gly), glucose, citric acid (CA), oleic acid (OA), potassium hydrogen phthalate (KHP), acetaminophen (APAP), norepinephrine (NE), tryptophan (Trp), bovine serum albumin (BSA) were obtained from Aladdin Chemistry Co. Ltd. (China). Hexaammineruthenium(III) chloride ($Ru(NH_3)_6Cl_3$) was ordered from Sigma. Guanine (G) was bought from Macklin (China). ITO coated glass (surface resistivity $<14.5 \Omega/\text{square}$, ITO thickness: 100

nm) was obtained from Zhuhai Kaivo Optoelectronic Technology (China). Human blood serum (healthy man) was provided by Center for Disease Control and Prevention (Hangzhou, China) for real sample analysis. Ultrapure water (18.2 M Ω ·cm) was used to prepare all aqueous solutions throughout this work.

2.2 Preparation of rGO

rGO was prepared according to the method previously reported with slight modification [33]. Specifically, a mixture of 8 mL GO dispersion solution (1 mg/mL), 32 mL H₂O, 7.0 μ L hydrazine solution (80 wt% in water), and 120 μ L ammonia solution (28 wt%) was incubated in a water bath at 85 °C for 3 h. After centrifugation at 3000 rpm for 30 min, the obtained supernatant was used to prepare rGO modified electrode.

2.3 Preparation of the VMSF/rGO/GCE and VMSF/ITO

10 μ L rGO (0.08 mg/mL) was primarily dropped onto a freshly cleaned GCE, and dried by infrared lamp. The resulting electrode was designated as rGO/GCE. Then the VMSF was prepared on the rGO/GCE by using the Electrochemically assisted self-assembly as previously reported [23]. Briefly, a precursor solution consisting of 20 mL NaNO₃ (0.1M), 20 mL ethanol, 2.833 g TEOS, and 1.585 g CTAB with pH adjusted by HCl to 3.0 was pre-hydrolyzed under stirring for 2.5 h. Subsequently, a constant potential (-2.2 V) was applied to the rGO/GCE for 5 s to obtain VMSF on the rGO/GCE. Here, three electrode system including rGO/GCE as the working electrode, an Ag/AgCl electrode (saturated with KCl) as the reference electrode and a platinum sheet (1 cm $\times$ 1 cm) as the auxiliary electrode, was employed. After the growth of VMSF, the resulting electrode was quickly rinsed with copious amounts of water, dried under a N₂ stream, and finally aged at 130 °C overnight. The electrode thus obtained retains the surfactant micelles (SM) inside the channels, which is denoted as SM@VMSF/rGO/GCE. SM could be removed by treating the SM@VMSF/rGO/GCE with 0.1 M HCl-ethanol solution under stirring for 5 min. To discriminate, the resulting electrode was termed as VMSF/rGO/GCE.

In comparison, VMSF was also prepared on the ITO electrode (designated as VMSF/ITO) using the Stöber-solution growth method [25]. Briefly, the cleaned ITO electrode was immersed in a precursor containing 0.16 g CTAB, 70 mL H₂O, 30 mL ethanol, 10 μ L concentrated ammonia aqueous solution, and 80 μ L TEOS, which was quiescently incubated in the water bath at 60 °C for 24 h. After rinsing with water, aging at 100 °C overnight, and removing SM from the nanochannels, the electrode thus obtained was designated as VMSF/ITO electrode.

2.4 Measurements and instrumentations

Transmission electron microscopy (TEM) measurement was carried out on a HT7700 microscope (Hitachi, Japan) at an acceleration voltage of 100 kV. The TEM specimen was prepared by gently peeling off the VMSF from electrode surface. After ultrasonication in ethanol, the dispersion was

dropped onto the copper grids. UV-vis absorption spectra were recorded on a UV-2450 spectrophotometer (Shimadzu Corporation, Japan). X-Ray photoelectron spectroscopy (XPS) analysis was performed on a PHI5300 electron spectrometer using 250 W, 14 kV, Mg K α radiation (PE Ltd, USA). Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) measurements were taken on an Autolab PGSTAT302N electrochemical workstation (Metrohm, Switzerland). A conventional three electrodes system was adopted with a bare or modified GCE as the working electrode, an Ag/AgCl electrode (saturated with KCl) as the reference electrode and a platinum electrode as the auxiliary electrode. The scan rate for CV measurements is 50 mV/s. The DPV parameters used were as follows: step, 0.005 V; modulation amplitude, 0.025 V; modulation time, 0.05 s; interval time, 0.2 s.

3. Results and discussion

3.1 Growth mechanism of VMSF/rGO/GCE

Scheme 1. Illustration of the preparation of VMSF/rGO/GCE.

Scheme 1 illustrates the preparation of VMSF modified electrode (VMSF/rGO/GCE) using rGO sheets as conductive adhesive and electroactive layer between VMSF and GCE. rGO reduced from GO by hydrazine was firstly drop-coated on a freshly cleaned GCE. As shown in Figure S1a (SI), light brown GO solution turns to black rGO solution. As revealed by UV-vis characterization, absorbance peaks of GO significantly changed after reduction (π - π^* transition at 230 nm red-shifts to 260 nm; n - π^* transition at 300 nm disappears; absorption increases over the entire spectrum), indicating the restore of π -conjugated structure of graphene and decrease of O-containing groups [34]. X-Ray photoelectron spectroscopy (XPS) analysis also confirms the remarkable decrease of O-containing groups in rGO (Figure S1b, c in SI). Owing to the strong π - π interaction, rGO sheets can firmly attach onto the surface of GCE. Then VMSF was readily prepared on the rGO/GCE using electrochemically assisted self-assembly (EASA). Specifically, a negative potential was applied to produce hydroxide groups on rGO surface and generated a local pH increase in the vicinity of electrode surface, which in turn not only induced the self-assembly of cationic surfactant (cetyltrimethylammonium bromide, CTAB) into

surfactant micelles (SM), but also catalyzed the hydrolyzation-condensation of the precursor (tetraethylorthosilicate, TEOS) molecules occurring at the interface between micelles and rGO sheets. Finally, vertically-aligned silica nanochannels with SM-template were prepared on the surface of rGO/GCE, designated as SM@VMSF/rGO/GCE. In the growth process of EASA, rGO bearing oxygen-containing groups and hydrophobic π -conjugated structure plays an important role for the creation of VMSF. On the one hand, negatively-charged oxygen-containing groups afforded by rGO favors the electrostatic adsorption of cationic surfactants and promotes the growth of silica nanochannel through co-condensation between oxygen-containing groups on rGO and silanol group. The resulting O-Si-O bonding ensures the stable binding between VMSF and rGO. On the other hand, hydrophobic surface of rGO promotes the formation of cylindrical SM with alkane tails parallel to the rGO surface. VMSF/rGO/GCE with open nanochannels was finally obtained by removing CTAB micelles via solvent extraction in HCl-ethanol solution.

3.2 Characterization of VMSF/rGO/GCE

As shown in **Figure 1**, successful growth of VMSF is evidenced by the appearance of light grey layer fully covered on the dark electrode surface and it remains intact on rGO coated GCE (but not uncoated GCE) after water rinsing and solvent extraction of micelles. VMSF growing on rGO by EASA has a much better stability as compared to VMSF directly grown on GCE. This is mainly because the negative oxygen-containing groups and hydrophobic π -conjugated structure on rGO are beneficial for the adhesion of VMSF [35,36]. As revealed by transmission electron microscopy (TEM), VMSF contains homogeneously distributed mesopores with a diameter ~ 2.7 nm (**Figure 2a**). The thickness of VMSF is determined by TEM to be ~ 95 nm (Figure S2 in SI). The pore density is calculated as 7.5×10^{12} pores/cm², corresponding to a porosity of 44%. The cross-sectional TEM image of VMSF shows perpendicularly aligned nanochannels (**Figure 2b**).

Figure 1. Photographs of bare GCE and rGO/GCE (a), SM@VMSF/GCE and SM@VMSF/rGO/GCE before (b) and after rinsing by water (c) or HCl-ethanol solution (d). VMSF/GCE and VMSF/rGO/GCE were obtained after the removal of SM from nanochannels by using HCl-ethanol solution

Figure 2. (a) Top-view and (b) cross-section view TEM images of VMSF. The insets are corresponding magnified images.

The integrity and permeability of VMSF were investigated by cyclic voltammetry (CV) measurement of the standard charged redox probes, specifically, $\text{Ru}(\text{NH})_6^{3+}$ and $\text{Fe}(\text{CN})_6^{3-}$. As seen in **Figure 3**, the ingress of $\text{Ru}(\text{NH})_6^{3+}$ and $\text{Fe}(\text{CN})_6^{3-}$ was completely blocked and thereby electron transfer between probes in solution and the underlying electrode was inhibited (as exemplified in the left panel of **Figure 3c**). This indicates that the as-prepared VMSF film on the rGO/GCE is intact and nearly defect-free. As previously reported [6], the thickness of VMSF increases with increasing the electro-deposition time. However, too short growth time results in incompletely coating on the electrode surface. On the contrary, too long growth time leads to the formation of heterogenous silica spheres on the surface of VMSF, which hinder the mass diffusion into the nanochannels. TEM characterization (**Figure 2**) and CV results show that VMSF grown with electrodeposition time of 5 s homogeneously cover GCE. After removing SM from nanochannels, VMSF/rGO/GCE gives significantly increased peak current for $\text{Ru}(\text{NH}_3)_6^{3+}$ (as exemplified in the right panel of **Figure 3c**), although the effective electrode area is reduced by ~56% as compared with bare GCE. The much enhanced current signal (1.81 times enhancement per effective electrode area) is attributable to the electrostatic enrichment of the analytes by the negatively charged silanols on the nanochannel surface (pK_a of ~2) and rGO facilitated charge transfer (**Figure 3a**) [37-44]. In contrast, suppressed electrochemical signal for $\text{Fe}(\text{CN})_6^{3-}$ (90% reduction per effective electrode area) was observed on VMSF/rGO/GCE, suggesting the electrostatic repulsion of VMSF nanochannels towards negatively charged molecules.

Figure 3. Cyclic voltammetry (CV) curves obtained from GCE, SM@VMSF/rGO/GCE and VMSF/rGO/GCE electrodes in $\text{Ru}(\text{NH}_3)_6^{3+}$ (a) and $[\text{Fe}(\text{CN})_6]^{3-}$ (b) solution (0.5 mM in 0.05 M KHP). (c) Schematic illustration of transport of $\text{Ru}(\text{NH}_3)_6^{3+}$ and $[\text{Fe}(\text{CN})_6]^{3-}$ probes to SM@VMSF/rGO/GCE and VMSF/rGO/GCE electrodes.

3.3 Electrochemical performance of VMSF/rGO/GCE towards electroactive organic molecules

Owing to the unique 2D π -conjugated structure, rGO has affinity towards small organic molecules through strong π - π interaction and exhibits excellent electrocatalytic effects with reduced overpotentials through facilitating charge transfer [37-44]. We therefore speculate the advantage of VMSF/rGO/GCE sensor for detection of electroactive organic molecules. As the proof-of-concept demonstrations, we employed VMSF/rGO/GCE for detection of four different organic molecules in human serum, including a pharmaceutical compound (acetaminophen-APAP) and three biomarkers (norepinephrine-NE, tryptophan-Trp and guanine-G). **Scheme S1** (in SI) shows molecular structures of these four molecules. APAP (also known as Paracetamol) is a widely used drug for the treatment of fever and pain [2]. However, high concentration of APAP in human plasma ($>200 \mu\text{g/mL}$) will lead to serious liver failure [45]. NE, Trp and G play important roles in various physiological processes. For instance, NE is a critical neurotransmitter and its abnormal concentration ($>17 \text{ nM}$) leads to depression and anxiety [4]. As an essential amino acid for human body, abnormal Trp concentration ($>56.7 \mu\text{M}$) often marks liver dysfunction [46]. G is one of the five nucleotides for DNA and RNA. Therefore, detection of these small molecules is of great significance.

Figure 4. CV curves obtained from GCE, rGO/GCE or VMSF/rGO/GCE in APAP (a), NE (b), Trp (c) and G (d) solution (50 μ M in 0.1 M buffer solution). The corresponding buffer solutions were PBS (pH 6.5), HAc-NaAc (pH 5.0), PBS (pH 5.5), HAc-NaAc (pH 5.0), respectively. Inset in each figure represents the corresponding differential pulse voltammetry (DPV) curves.

As shown in **Figure 4**, decoration of rGO confers GCE excellent electrochemical performance in terms of increased anodic peak currents and negatively-shifted oxidation peak potentials for these four small molecules, suggesting an efficient electrocatalytic activity of rGO. Compared with rGO/GCE, resulted from the reduced effective electrode area, VMSF/rGO/GCE displays slightly decreased peak currents except for Trp. However, in comparison with bare GCE, VMSF/rGO/GCE still gives remarkably increased peak current for the above four molecules (56.0 times for APAP, 11.8 times for NE, 10.2 times for Trp and 6.71 times for G enhancement per effective electrode area). The signal amplification is owing to efficient enrichment by rGO through π - π interaction and electrostatic adsorption by negatively charged VMSF nanochannels towards positively charged analytes ($pK_{a(\text{APAP})}=9.38$; $pK_{a(\text{NE})}=8.85$; $pK_{a(\text{Trp})}=5.89$; $pK_{a1(\text{G})}=3.2$, $pK_{a2(\text{G})}=9.4$). The enhanced current of Trp obtained from VMSF/rGO/GCE is attributable to the electrostatic attraction by VMSF. In addition, VMSF/rGO/GCE gives negatively-shifted oxidation potentials as compared with bare GCE (0.38 V vs 0.41 V for APAP, 0.30 V vs 0.31 V for NE, 0.71 V vs 0.78 V for Trp and 0.79 V vs 0.83 V for G), indicating the electrocatalytic ability of rGO to promote oxidation of these molecules. These results indicate that rGO serving as electroactive substrate plays an important role for improving the sensing performance VMSF based

sensors in terms of signal intensity and distinct oxidation potential for different electroactive organic molecules.

3.4 Individual detection of APAP, NE, Trp and G using VMSF/rGO/GCE

To achieve sensitive detection, pH of supporting electrolyte for the detection of these four molecules was optimized. CV curves obtained from VMSF/rGO/GCE in response to each analyte at different pH values are shown in **Figures S3-S6 (SI)**. For APAP and NE (**Figures S3, S4 in SI**), the linear slopes between peak potential and pH value all follows well to Nernst equation (59 mV/pH), indicating a reversible $2e^-/2H^+$ transfer process [47]. The optimal detection pH for APAP and NE were found as 6.5 and 5.0 respectively with the maximal peak currents. For Trp and G (**Figures S5, S6 in SI**), only oxidative peaks are observed due to the irreversible electrochemical processes. As the increase of pH turns Trp to be negatively charged ($pK_a=5.89$) and decreases the positive charge of G ($pK_{a1}=3.2$, $pK_{a2}=9.4$), the optimal pH for detection of Trp and G was found as 5.5 and 5.0, respectively.

Figure 5. DPV curves obtained from VMSF/rGO/GCE in response to different concentrations of APAP (a), NE (b), Trp (c) and G (d). Insets depict the dose response curve (top) and the enlarged view of the DPV curves in the low-concentration region. The error bars represent the standard deviation (SD) of three measurements.

Figure 5 shows the DPV responses of VMSF/rGO/GCE upon successive addition of the analytes. As seen, an increase of the oxidation peak currents is observed with increasing analyte concentration. As shown in the insets of **Figure 5**, high sensitivity (616.4 nA/ μ M for APAP, 422.4 nA/ μ M for NE, 170.5 nA/ μ M for Trp and 62.4 nA/ μ M for G) in the low concentration range and wide linear response range (0.2–100 μ M for APAP, 0.1–30 μ M for NE, 0.8–200 μ M for Trp and 0.2–200 μ M for G) are obtained. The corresponding limit of detection (LOD) is 9.7 nM for APAP, 14.0 nM for NE, 36.0 nM for Trp and

96.0 nM for G (S/N = 3) respectively. Attributable to the analyte enrichment through π - π interaction with rGO and electrostatic attraction by negatively charged VMSF nanochannels as well as rGO facilitated charge transfer, LODs from VMSF/rGO/GCE sensor are much lower than those obtained from poly(3,4-ethylenedioxythiophene)/graphene oxide modified electrode (0.57 μ M for APAP) [48], nanogold modified electrode (87 nM for NE) [49], nano-TiO₂/ferrocene carboxylic acid modified electrode (0.124 μ M for Trp) [50], and Cu@Ni/multiwalled carbon nanotubes modified electrode (0.17 μ M for G) [51].

3.5 Simultaneous detection of APAP, NE and Trp using VMSF/rGO/GCE

The oxidation potentials of Trp and G (0.75 V for Trp, 0.82 V for at pH 5) are well separated from that of APAP (0.48 V) and NE (0.31 V), promising for simultaneous detection. We choose APAP (drug), NE (hormone) and Trp (amino acid) to evaluate the ability of VMSF/RGO/GCE for simultaneous detection at the optimal pH for NE (pH 5.0), because they represent three different classes of molecules in blood. As shown in **Figure 6**, VMSF/rGO/GCE sensor is able to simultaneously detect APAP, NE, and Trp (for APAP, for NE, 0.75 V for Trp, **Figure 6a**). In contrast, VMSF/ITO electrode is not able to distinguish their overlapping oxidation peaks (**Figure 6b**) due to much high overpotentials (1.20 V for APAP, 0.85 V for NE, 0.92 V for Trp, **Figure S7 in SI**). With increasing the concentration of each molecules in their mixture solution, the anodic peak currents of them increase and good linear correlations are found between the peak current and the concentration from 1 μ M to 10 μ M (**Figure 7**). Comparing to the individual analysis, the coexisting molecules did not disturb the quantitative detection of each molecule and the obtained sensitivities for NE and Trp (+3% for NE, -4% for Trp) were almost same. However, reduction in sensitivity is observed for APAP (-10%). This is mainly ascribed to the solution pH (5.0) for simultaneous detection is the same as that for the optimal detection pH for NE and Trp, but not the optimal detection pH (6.5) for APAP. These results demonstrate the potential of our sensor for simultaneous analysis of multiple analytes.

Figure 6. CV curves obtained from VMSF/rGO/GCE (a) or VMSF/ITO (b) electrode in 0.1 M HAc-NaAc solution (pH 5.0) containing APAP (50 μ M), NE (50 μ M) and Trp (50 μ M). Insets represent the corresponding DPV curves.

Figure 7. (a) DPV curves obtained from VMSF/rGO/GCE in a 0.1 M HAc-NaAc solution (pH 5.0) containing APAP, NE and Trp ranging from 1 μM to 10 μM . The dose response curves of APAP (b), NE (c) and Trp (d). The error bars represent the SD of three measurements.

3.6 Anti-interference ability, repeatability, reproducibility, stability of VMSF/rGO/GCE

We further demonstrate in Figure S8 (SI) that the detection of each analyte is not disturbed by the presence of some possible interfering species including protein (BSA), biomolecules (glucose, heme, citric acid, amino acids, oleic acid) and metal ions may co-exist in biological samples. This is ascribed to the size exclusion and charge selectivity of VMSF. The relative standard deviations (RSDs) for 10 repeated measurements for each analyte (20 μM) are 2.7% for APAP, 2.8% for NE, 4.7% for Trp and 3.4% for G. The reproducibility was evaluated using three different electrodes prepared under the same condition independently and a RSD of 4.2% (response to 20 μM APAP, $n=3$) was obtained. These results indicated that the sensor exhibited good repeatability and reproducibility. VMSF/rGO/GCE is also very stable because its response level (to 20 μM APAP, $n=5$) retains 98.4% even after 10-day storage at ambient condition.

3.7 Detection in human serum

Making use of the anti-fouling and anti-interference abilities of VMSF, VMSF/rGO/GCE sensor was directly employed to analyze human serum. Serum samples were diluted by a factor of 50 with buffer solution without any further pretreatment. Figure S9 (SI) presents eight consecutive detections (within 14 min) on VMSF/rGO/GCE or rGO/GCE towards APAP (20 μM) in serum. The response from rGO/GCE largely decreased (25% of the initial signal at the eighth detection) presumably because of

electrode fouling caused by absorbed proteins from serum. In contrast, the response of VMSF/rGO/GCE remains stable (94% of its initial signal), suggesting high anti-fouling ability afforded by VMSF nanochannels. APAP, NE, Trp or G with different concentrations was added in the diluted serum for electrochemical detection. By comparing the measured results and the pre-defined concentrations, the satisfactory recoveries (between 93.3% to 106.5%) and relative standard deviation (RSD, between 1.1% to 4.1%, n=3) (**Table 1**) indicate the reliability of VMSF/rGO/GCE sensor for analyzing complex samples. As shown in Table S1, the spiked experiments also present satisfactory recoveries (between 90.6% to 109.7%) for simultaneous analysis of APAP, NE and Trp in serum. This result indicates simultaneous analysis of multiple analytes in serum is feasible by the sensor. In comparison with the commonly applied methods for plasma analysis (such as liquid chromatography mass spectrometry and high-performance of liquid chromatography) which require complicated and time-consuming separation technologies [52,53], the present electrochemical method is simple, convenient and of low-cost.

Table 1. Detection of artificially added APAP, NE, Trp and G in human serum samples.

Analyte ^a	Added (μM)	Found ^b (μM)	RSD (%)	Recovery (%)
APAP	3.00	2.80	1.1	93.3
	50.00	51.62	1.2	103.2
NE	2.00	2.13	1.7	106.5
	25.00	24.87	2.4	99.5
Trp	2.00	1.99	1.9	99.5
	50.00	50.31	4.1	100.6
G	3.00	2.92	1.1	97.3
	25.00	25.31	3.7	101.2

^aHuman serum samples were diluted by a factor of 50 using corresponding buffer solution before a series of APAP, NE, Trp or G with standard concentration were added.

^bAverage of three determinations

Conclusions

In summary, we demonstrated that rGO sheets can be employed as efficient conductive adhesive and electroactive layer for the fabrication of stable and sensitive VMSF-based sensor. Owing to the excellent anti-fouling and anti-interference ability of VMSF and electrocatalytic performance of rGO, the VMSF/rGO/GCE sensor is able to directly analyze small molecules in serum with low oxidation potential and superior sensitivity. Moreover, the obtained sensor is capable of simultaneously detecting APAP, NE and Trp without mutual interference. The present approach could be easily extended for detecting a scope of analytes in complex biological, environmental, clinical or food samples.

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Supporting Information

Supplementary data associated with this article can be found in the online version.

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