Characterization of PEDOT:PSS-reduced graphene oxide@Pd composite electrode and its application in voltammetric determination of vitamin K₃

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Abstract

Vitamin K₃ (VK₃), a synthetic blood coagulation vitamin with fat-solubility, easily results in side-effect in animal body due to its toxicity and improper addition of animal feedstuffs. In this study, a novel sensor based on glassy carbon electrode (GCE) modified with a conducting polymer-both nano-noble metals and two-dimensional nano-carbon composite consisting of poly(3,4-ethylenedioxythiophene):poly(styrene sulfonate) (PEDOT:PSS), carboxymethyl cellulose (CMC) and reduced graphene oxide@palladium (rGO@Pd) was successfully used for the voltammetric detection of VK₃ in animal blood and feedstuff samples. Under the optimized conditions, PEDOT:PSS-CMC-rGO@Pd/GCE/GCE displayed the excellent synergistically electrocatalytic ability for the electrochemical oxidation-reduction of VK₃, which exhibited a good voltammetric response for VK₃ in the concentration range from 4×10^{-7} to 9×10^{-5} M, with a limit of detection of 1.4×10^{-8} M. Furthermore, a facile preparation, high sensitivity, good selectivity, superior sensing stability, and satisfactory practicability of PEDOT:PSS-CMC-rGO@Pd/GCE/GCE for sensing VK₃ suggested that the potential applicability of PEDOT:PSS-CMC-rGO@Pd provided a promising electrochemical sensing platform for the determination of VK₃ in pharmaceutical and feed inspection.

Keywords: Sensor, Vitamin K₃, Poly(3,4-ethylenedioxythiophene):poly(styrene sulfonate), Reduced graphene oxide@palladium, Voltammetric determination, Animal feedstuffs

1. Introduction

Vitamin K₃ (VK₃), also called menadione (2-methyl-1, 4-naphthoquinone), a synthetic blood coagulation vitamin with fat-solubility, is mainly used as anti-hemorrhagic agents, but they are also involved in photosynthetic mechanisms, cellular respiration, oxidative phosphorylation and anticancerogenics. Nowadays, in order to promote animal growth in animal husbandry or aquaculture and prevention of disease, many foreign substances are often added improperly or illegally into animal feedstuffs, which cause deficiency of endogenous substance in animals. Therefore, for the sake of preventing VK₃ deficiency of animals, VK₃ was often added irrationally into the feedstuffs eaten by animals. This vitamin can lead to remarkable side-effect owing to the toxicity, especially to the newborn animals. Thus, it is very significant to monitor the content of VK₃ in animal body and feedstuffs. The determination of VK₃ in animal body and animal feedstuffs was achieved by HPLC [1-4]. Subsequently, HPLC coupled with a variety of detectors such as fluorescence [3], UV [5], electrochemistry [6-8] were also reported. Although those methods are generally accurate in the tests of practical samples, they have some defects including time-consuming, high cost and complicated. Besides, other techniques such as flow injection analysis [9], fluorimetric method [10] and colorimetric method [11] were also selected for the detection of VK₃. In addition, the electrochemical technique is a powerful tool owing to its advantages of simple operation, online detection, fast analysis and high sensitivity. In the early stages, the electrochemical behaviors and properties of VK₃ were investigated in organic or aqueous medium due to its complicated mechanism of the oxidation-reduction, and its content was detected by polarographic methods [12-18]. For example, Akman et al determined Plasma VK₃ using differential pulse polarographic assay [15], Alonso et al studied electrochemical behaviors of VK₃ on rotating disk electrode based on glassy carbon, and its content was determined by a chemometric analysis [16], Somer and Doğan detected VK₃ in pharmaceuticals using differential pulse polarography [17], Cannes et al also investigated the electrochemical properties of VK₃ via cyclic voltammetry and scanning electrochemical microscopy using the self-assembled monolayer modified gold electrode [18], Zhu and Li electrochemical studied VK₃ interacting with hemoglobin and determination of hemoglobin [19]. However, these polarographic methods allows determination of VK₃ in the 10⁻⁶ - 10⁻⁵ M range, and signal cannot be used for this purpose as the current does not increase linearly with concentration owing to poor sensitivity. More unfortunately, the mercury was toxic, which limited its applications in biological and medical related fields. Afterwards, other electrochemical techniques were also employed for the determination of VK₃, Vire et al reported square-wave adsorptive stripping voltammetry of menadione [20], Wang et al presented a sensitive method for the determination of VK₃ using cathodic stripping voltammetry [21]. Moreover, chemically modified electrodes based on various functional materials have been developed for the architecture of VK₃ electrochemical sensors. Rizk proposed a new methods for the potentiometric determination of VK₃ [22]. Liu et al fabricated interdigitated array microelectrodes (Pt-IDA and Au-IDA) to detect VK₃, and the sensing performance like limit of detection (LOD), linear range, selectivity and sensitivity obtained the great improvement [23], but the sensing performance for the determination of VK₃ in real samples still needed to be further improved. Consequently, it is quite imperative to develop novel VK₃ electrochemical sensors based on excellent modified materials like conducting polymers (CPs), metal and/or carbon nanomaterials.

PEDOT:PSS is Poly(3,4-ethylenedioxythiophene):poly(styrene sulfonate), as one of the most promising conducting polymers (CPs) today, it is successfully employed for the architecture of electrochemical chemo/biosensors because of its excellent environmental and

chemical stability, high electrical conductivity relatively low structure defects from mislinkage and redox potential, the reversible doping/dedoping ability and excellent electrochemical and aqueous stability [24-26]. More importantly, as a water-dispersible form of the intrinsically conducting PEDOT doped with the water-soluble PSS, PPEDOT:PSS is the most successfully solution-processability CPs nowadays that has been commercially produced on a large-scale and sold for fundamental research and practical application in academic and industrial fields, and it has extraordinary advantages over other CPs like the water-dispersibility, mechanical flexibility, optical transparency, electrical conductivity, physical and chemical stability, film-forming ability, miscibility, cost-effectiveness, mass production, and commercial availability [27-29]. Nevertheless, PEDOT:PSS film has also some shortcomings, for example, weak adhesive force between PEDOT:PSS and the interface of substrate electrode [27], swell, and crack in aqueous solutions [28-31], which limits its electrochemical applications in water. Carboxymethyl cellulose (CMC), an excellent water-soluble binder, has many satisfactory properties such as good film forming ability, biocompatibility, nontoxicity, and hydrophilicity, which covered well the above-mentioned problems of PEDOT:PSS film electrode, and the as-prepared PEDOT:PSS composite could enhance synergistically electrocatalytic ability and the long-term electrode stability in water of PEDOT:PSS film electrode for the electrochemical application in sensors [29]. Moreover, inorganic nanomaterials like carbon nanotubes, graphene can improve the performance of sensors based on PEDOT:PSS composite film electrodes [31-33]. Although CMC successfully settled the issue of PEDOT:PSS film electrode, It is difficult to for the electrochemical oxidation and/or reduction of weak electroactive analytes due to its poor electrocatalytic ability. Reduced graphene oxide (rGO) was widely used as electrochemical sensing platform for the determination of various analytes due to its remarkable electrocatalytic ability, high adsorption capacity, excellent mechanical strength, good conductivity, and high thermal stability. Recently, nano noble metals functionalized rGO have been extensively attentions because of combining the merits of both rGO and nano noble metals. Palladium (Pd), one of important catalyst of noble metals, was employed to form diverse Pd functionalized rGO, which was selected for the architecture of high-efficient electrochemical sensors and their application in electrochemical analysis [34-38].

In this work, the conducting polymer-both nano-noble metals and two-dimensional nano-carbon composite consisting of PEDOT:PSS, CMC, and reduced graphene oxide@palladium (rGO@Pd) were selected for forming semiconductor polymer-inorganic nanocomposite electrode PEDOT:PSS-CMC-rGO@Pd/GCE, which was successfully applied for the voltammetric detection of VK₃ in animal blood and feedstuff samples using linear scanning voltammograms (LSV), and its sensing parameters and performances were systematically discussed (Scheme 1).

2. Experimental

2.1. Chemicals

A commercially available aqueous PEDOT:PSS dispersion (Baytron P, 1.3 wt%) was obtained from Bayer AG. CMC (high viscosity grade 500-800 cPs, USP grade) was obtained from Aldrich Chemical Co., Inc. N,N-dimethylformamide was purchased from Beijing Chemical Industry CO., Ltd. rGO@Pd was obtained from NanoInnovo Technologies. Disodium hydrogen phosphate dodecahydrate (Na₂HPO₄·12H₂O) and sodium dihydrogen phosphate dehydrate (NaH₂PO₄·2H₂O) were purchased from the Sinopharm Chemical Reagent Co., Ltd. 0.1 M phosphate buffer solutions (PBS) with different pH values were prepared from stock solutions of NaH₂PO₄·2H₂O and Na₂HPO₄·12H₂O. 2-Methyl-1,4-naphthoquinone (VK₃) were bought from Beijing lark technology Co., Ltd. A stock

solution of 5 mM VK₃ was obtained in ethanol and kept in dark at 4 °C. The chicken blood and broiler feedstuff were obtained from college of animal science and technology in Jiangxi Agricultural University. The serum of older bovine was purchased from PingRui Biotechnology (Beijing) Company, and the was obtained from chicken house. All reagent were analytical grade and used without further purification. All solutions were prepared by deionized distilled water.

2.2. Apparatus

CHI660B electrochemical workstation (Shanghai Chenhua Instrument Company, China) with a three-electrode system were used for electrochemical measurements in a conventional electrochemical cell at room temperature. PEDOT:PSS, CMC and rGO@Pd modified GCE electrode were selected for the working electrode, a saturated calomel electrode (SCE) was served as the reference electrode and a platinum wire as the auxiliary electrode. A pH meter CT-6023 pH was used for measuring values of solutions. The surface morphology was measured using scanning electron microscopy (SEM) with a Hitachi SU8020. **The chemical composition of the prepared composite was measured by Energy Dispersive X-ray Spectroscopy (EDS) performed in SEM.**

2.3. Preparation of practical samples

At first, the broiler feed were ground into powder, which mixed with deionized distilled water. The dispersion of broiler feed, chicken blood and serum of older bovine were centrifuged at 8000 r/min for 5 minutes. Then, the precipitations were removed and the supernatant liquors were left behind. We made 5 µl supernatant liquors of chicken blood and serum of older bovine into 5 ml PBS because the concentration of supernatant liquors were larger in this test, and the supernatant liquors of broiler feed was adjusted to pH = 6.5 by PBS. Finally, practical samples were stored at 4 °C.

2.4. Fabrication of modified electrode

Prior to modification, the auxiliary electrode platinum wire was polished with abrasive paper (1500 mesh), cleaned successively with doubly-distilled deionized water, acetone, doubly-distilled deionized water, and dried in air. The glassy carbon electrode was polished with chamois leather containing 0.05 µm alumina slurry until a mirror surface was gotten, followed by ultrasonically cleaning in doubly-distilled deionized water, absolute ethanol, and doubly-distilled deionized water each for 5 minutes, respectively, then dried in air. rGO@Pd suspension was separately diluted by DMF, and obtained 1 mg/ml rGO@Pd suspension. CMC was dissolved in distilled water (1 mg.ml⁻¹). The rGO@Pd suspensions was added to 1.3 wt% PEDOT:PSS aqueous dispersion at different volume ratios, then stirred thoroughly for 12 h at room temperature. PEDOT:PSS-rGO@Pd composite was obtained by mixing PEDOT:PSS and rGO@Pd with the 7:3 of a volume optimum ratio, then PEDOT:PSS-CMC-rGO@Pd composite was prepared by blending PEDOT:PSS-rGO@Pd and CMC with a volume ratio of 1:1, and continuously stirring for 12 h at room temperature. Finally, the PEDOT:PSS-CMC-rGO@Pd/GCE was gotten by drop-coating 5 µl PEDOT:PSS-CMC-rGO@Pd suspensions on the surface of bare GCE, and dried in infrared lamp. The procedure of building PEDOT:PSS-CMC-rGO@Pd/ GCE is shown in [Scheme 1](#).

3. Results and discussion

3.1. Characterization of modified electrode

3.1.1. Surface morphology

SEM image of PEDOT:PSS-CMC-rGO@Pd/GCE film were shown in [Fig. 1A](#). There were many small irregular-shaped particles

and small protuberances. As reported in our previous literature [29, 39], the surface morphology of the PEDOT:PSS film was a regular, smooth, compact, and homogeneous structure without any protuberance, and the CMC film was a regular, smooth, compact structure with sodium salt microparticles. Therefore, the SEM of PEDOT:PSS-CMC-rGO@Pd/GCE film with many small irregular-shaped particles and small protuberances was attributed to sodium salt and rGO@Pd. In addition, the corresponding EDS spectrum of PEDOT:PSS-CMC-rGO@Pd/GCE film indicated the presence of different elements in composite materials besides ITO elements (Fig. 1B).

3.1.2. Electrochemical impedance

Fig. 2 shows electrochemical impedance spectroscopy of different electrode at the frequency ranges from 100 mHz to 10 kHz, and 10 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox couple (1:1) containing 0.1 M KCl was used as the supporting electrolyte. The charge transfer resistance (R_{ct}) of the bare GCE was 375 Ω (curve a). It was very obvious that the R_{ct} of CMC/GCE (750 Ω , curve c) was larger than that of the bare GCE when CMC was added to the surface of the bare GCE, implying that CMC impeded the charge transfer between the modified electrode interface and solution due to its nonconductivity. While on the rGO@Pd/GCE, a nearly straight line at all frequencies appeared with the R_{ct} value nearly equal zero, indicating that rGO@Pd has good conductivity. However, The R_{ct} of rGO@Pd-CMC/GCE increased dramatically when CMC was added (590 Ω , curve b), which was ascribed to bad conductivity of CMC. The introduction of non-conducting CMC resulted in the increasing R_{ct} of other inorganic nano-materials, especially semiconductor, these results were in accordance with that in our previous reports [33, 39]. The Nyquist plots of PEDOT:PSS/GCE (curve e) exhibited a nearly straight line, revealing that high electrical conductivity of PEDOT:PSS promoted the electron transfer. Surprisingly, the Nyquist plots of PEDOT:PSS-CMC/GCE (curve f) presented also a nearly straight line, the main difference was that the slope of straight line of PEDOT:PSS-CMC/GCE was larger than that of PEDOT:PSS/GCE, which was assigned to the role of CMC in the PEDOT:PSS film. CMC has bad conductivity, but it could enhance the conductivity of CPs especially PEDOT:PSS when was as a dopant [29, 33]. Moreover, The impedance of PEDOT:PSS-CMC-Pd-rGO/GCE is very close to that of PEDOT:PSS-CMC/GCE. The results manifested that high electrical conductivity of PEDOT:PSS and the synergistic enhanced effect of different components made it easier for fast electron transfer.

3.2. Electrochemical behaviors of VK_3

The electrochemical behaviors of VK_3 on bare GCE (curve a), CMC/GCE (curve b), rGO@Pd/GCE (curve c), PEDOT:PSS/GCE (curve d), CMC-rGO@Pd/GCE (curve e), PEDOT:PSS-CMC/GCE (curve f), PEDOT:PSS-CMC-rGO@Pd/GCE (curve g) were investigated by cyclic voltammetry in 0.1 M PBS (pH 7.0) solution containing 98 μM VK_3 (Fig. 3). A pair of reversible redox peaks were observed, which was imputed to the electrochemical redox of VK_3 [15-17]. Obviously, a pair of well-defined redox peaks were observed when the rGO@Pd was modified the surface of the bare GCE, and the rGO@Pd displayed superior electrocatalytic ability for the electrochemical oxidation-reduction of VK_3 owing to the synergetic electrocatalytic effect of both graphene and nano noble metals. The introduction of CMC had great effect on the electrochemical oxidation-reduction of VK_3 compared with both CMC-rGO@Pd/GCE and PEDOT:PSS-CMC/GCE because the non-conducting CMC hindered the fast electron transfer of rGO@Pd and both CMC synergistically enhanced the electrocatalytic ability of PEDOT:PSS, which was in agreement with our previous reports [29, 33, 39]. In comparison with

all other electrodes, the PEDOT:PSS-CMC-rGO@Pd/GCE demonstrated the well-defined peak shape and highest peak height, indicating the significant improvement of the electrocatalytic performance was attributed to synergetic effects of different components.

3.3. Optimization of sensing parameters

3.3.1. Effect of scan rates

The influence of scan rates on the voltammetric response of VK₃ (98 μM) using PEDOT:PSS-CMC-rGO@Pd/GCE was investigated by cyclic voltammetry (Fig. 4). With the increase of scan rates from 0.015 to 0.3 V s⁻¹ (Fig. 4A), the redox peak currents enhanced. For PEDOT:PSS-CMC-rGO@Pd/GCE, the redox peak currents were linearly proportional to scan rates (ν) (Fig. 4B), their regression equations were $I_{pa} (\mu A) = 1267 \nu + 18.85$ ($R^2 = 0.9974$) and $I_{pc} (\mu A) = -1388 \nu - 24.89$ ($R^2 = 0.9936$) (ν in V s⁻¹). Meanwhile, the redox peak currents were linearly proportional to the square root of scan rates ($\nu^{1/2}$) (Fig. 4C), their regression equations were $I_{pa} (\mu A) = 841.1 \nu^{1/2} - 96.33$ ($R^2 = 0.9787$) and $I_{pc} (\mu A) = -926.4 \nu^{1/2} + 102.7$ ($R^2 = 0.986$) (ν in V s⁻¹), indicating that the redox peak currents were linear relation with both $\nu^{1/2}$ and ν . Moreover, their linearities were very good, meaning that the electrodes process might be related by both diffusion and adsorption. In addition, regression equations were $\log I_{pa} (\mu A) = 0.8597 \log \nu + 3.041$ ($R^2 = 0.9999$) and $\log I_{pc} (\mu A) = 0.8583 \log \nu + 3.093$ ($R^2 = 0.9995$) (ν in V s⁻¹) (Fig. 4D). This phenomenon was in accordance with our reports [40], further indicating that the electrode reaction might be a mixed adsorption-diffusion control electrode process. Furthermore, the plot of $\log I_{pa}$ versus $\log \nu$ yielded a slope value in the scan rate range of 0.015 – 0.3 V s⁻¹, which was between the theoretical value of 0.5 and 1. The slope value is greater than the theoretical value of 0.5 for diffusion-controlled process while smaller than the theoretical value of 1 for the adsorption-controlled electrode process. It suggested that the redox peak derives not only from the molecules adsorbed on the surface of PEDOT:PSS-CMC-rGO@Pd/GCE before oxidation-reduction but also from ones reaching the electrode surface by diffusion [41, 42]. In addition, two curves of anodic and cathodic peak currents for PEDOT:PSS-CMC-rGO@Pd/GCE were almost overlapped ($I_{pa}/I_{pc} \approx 1.0$), indicating good reversible redox behavior and high structural stability.

The cathodic peak shifted to more negative values while the anodic peak shifted to more positive values as scan rates increased,. The cathodic peak potential (E_{pc}) and anodic peak potential (E_{pa}) had a linear relationship with the napierian logarithm of scan rates ($\ln \nu$) (Fig. 4E), for PEDOT:PSS-CMC-rGO@Pd/GCE, the regression equations were $E_{pc} (V) = -0.0302 \ln \nu - 0.3646$ ($R^2 = 0.9734$) and $E_{pa} (V) = -0.0302 \ln \nu - 0.0647$ ($R^2 = 0.9182$) (ν in V s⁻¹). According to the Laviron theory [39, 44-45], E_{pa} and E_{pc} were expressed by using following equations:

$$E_{pa} = E^0 + \frac{RT}{\alpha nF} \ln \left(\frac{RTk_s}{\alpha nF} \right) + \frac{RT}{\alpha nF} \ln \nu \quad (1)$$

$$E_{pc} = E^0 + \frac{RT}{(1-\alpha)nF} \ln \left[\frac{RTk_s}{(1-\alpha)nF} \right] - \frac{RT}{(1-\alpha)nF} \ln \nu \quad (2)$$

where α , k_s , ν , E^0 , T , R , and F have their conventional meanings. Generally, the value of α is between 0.3 and 0.7 [43]. Therefore, we could calculate the results that n must be 2, and the value of α is 0.43, which was consistent with previously reported results [18].

3.3.2. Effect of pH

The influence of pH on the detection of VK₃ in the pH range from 5.5 to 8.0 at the PEDOT:PSS-CMC-rGO@Pd/GCE was also studied (Fig 5A). The redox peak currents of VK₃ increased with increasing pH of the solution, and reached maximum value at pH 6.5,

after that, pH decreased. So, the optimal pH (6.5) of PBS was used for the detection of VK₃ in the subsequent experiments. Plots of E_{pa} vs. pH showed good linearity with slopes of 0.053 at PEDOT:PSS-CMC-rGO@Pd/GCE (Fig. 5B). A formula $dE_{pa}/dpH = (-2.303mRT)/nF$ was easily obtained according to the Nernst equation, and the m/n value was calculated from the slope of a plot of E_{pa} vs. pH. The m/n ratio at PEDOT:PSS-CMC-rGO@Pd/GCE was approximately 1, implying that the numbers of electrons and protons were equal. Thus, the electrochemical redox of VK₃ was a two-electron and two-proton process, which was in accordance with previously reported results [18]. The electrochemical reaction mechanism of VK₃ was expressed as follow:

3.4 Sensing performance

3.4.1 Detection of VK₃

The linear sweep voltammograms of VK₃ with different concentration at PEDOT:PSS-CMC-rGO@Pd/GCE were recorded under the optimal experimental conditions (Fig. 6A). The peak height of VK₃ oxidation increased linearly with the square root of VK₃ concentration in the range from 4×10^{-7} to 9×10^{-5} M, and the corresponding linear regression equation can be expressed as $I_{pa} = 4.568 C^{1/2} - 3.711$ ($R_2 = 0.9945$) with the LOD of 1.4×10^{-8} M (Fig 6B). Comparing with VK₃ electrochemical sensors in previous reports especially chemically modified electrodes (Table 1), it can be seen that PEDOT:PSS-CMC-rGO@Pd/GCE offered wider linear range for the detection of VK₃ and lower LOD, indicating that PEDOT:PSS-CMC-rGO@Pd is an outstanding electrochemical sensing material for the fabrication of VK₃ sensors.

3.4.2. Sensing stability

Fig 7A presents the repeatability of PEDOT:PSS-CMC-rGO@Pd/GCE. 30 successive assays indicated that a relative standard deviation (RSD) was 0.4%, showing a great repeatability of the PEDOT:PSS-CMC-rGO@Pd/GCE. The reproducibility of the prepared seven electrodes was also investigated in the same conditions (Fig 7B), and the several electrodes were evaluated independently, and its values of RSD was 1.44%, indicating the PEDOT:PSS-CMC-rGO@Pd/GCE had good reproducibility. The excellent reproducibility and repeatability revealed the high sensing stability of PEDOT:PSS-rGO@Pd/GCE/GCE, This is mainly because CMC extensively enhanced the adhesive force of electrode interface and the electrode stability of sensing element [29, 33, 39].

3.4.3. Anti-interferential ability

To evaluate the selectivity of the prepared sensor, various components in animal blood and feeds are tested. There was no observable interference towards many substances due to relatively far away from the peak potential of VK₃ or no electrochemical activity. Furthermore, oxidation potentials of substances like Glycine (Gly), Cd²⁺, glucose (Glu), Cu²⁺, vitamin B₃ (VB₃), vitamin D₂ (VD₂), niclosamide (NA) and methyl parathion (MP) were usually close to that of VK₃. The voltammetric responses of these substances with or without VK₃ were listed in Fig. 8. the voltammetric responses of the PEDOT:PSS-CMC-rGO@Pd/GCE towards VK₃ (5 μ M) were not influenced by the introduction of Cd²⁺, Glu, VB₃ or VD₂ with 100-fold concentrations, 10-fold concentrations of Gly and 1-fold concentrations of NA, MP or Cu²⁺. Thus, the results demonstrated that the sensor could some anti-interference ability.

3.5. Practical application

The practical samples such as the broiler feed, chicken blood and serum of older bovine were used to testify the feasibility and practicability of the PEDOT:PSS-CMC-rGO@Pd/GCE for determination of VK₃, and the results were presented in Table 2. The prepared diluted sample solutions were determined by the standard addition method, and each sample solution was hand over to three parallel detections. The RSD of VK₃ in three samples for the different concentration levels was no more than 5%, indicating that the as-fabricated sensor is acceptable for practical use. The recovery values (99%) of VK₃ are satisfactory for broiler feed, 94% for chicken blood, and 95% for serum of older bovine, respectively. Thus, it can be said that PEDOT:PSS-CMC-rGO@Pd/GCE is applicable for the detection of VK₃ in animal blood and feedstuff samples.

4. Conclusions

In this study, PEDOT:PSS-reduced graphene oxide@Pd composite was prepared by simple sonication and agitation and used as a novel sensing platform for electrochemical detection of VK₃. Although the non-conductivity of CMC decreased the conductivity of rGO@Pd, it enhanced the conductivity of PEDOT:PSS as a dopant. Meanwhile, CMC extremely improved the sensing performance such as electrode stability and adhesion. Due to excellent properties of PEDOT:PSS (high conductivity) and rGO@Pd (excellent electrocatalytic activity), PEDOT:PSS-CMC-rGO@Pd/GCE displayed the improved conductivity and electrode stability as well as the enhanced synergistically electrocatalytic ability for the electrochemical oxidation-reduction of VK₃ compared with the bare GCE and GCE modified with other materials. These results implied that the PEDOT:PSS-CMC-rGO@Pd modified electrode can provide an excellent sensing platform for electroanalysis and potential applicability for the electrochemical determination of VK₃ in pharmaceutical and feed inspection. In addition, the further improvement on the selectivity or anti-interference of the VK₃ sensor for promoting accuracy and reliability in practical application is also in progress.

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Figure captions

Scheme 1. The fabrication procedure of PEDOT:PSS-CMC-rGO@Pd/GCE for the voltammetric determination of vitamin K₃ in real samples.

Fig. 1. SEM (A) and EDS (B) images of PEDOT:PSS-CMC-rGO@Pd

Fig. 2 Nyquist plots of different electrodes in 10 mM [Fe(CN)₆]^{4-/3-} containing 0.1 M KCl: bare GCE(a), CMC-rGO@Pd/GCE(b), CMC/GCE(c), rGO@Pd/GCE(d), PEDOT:PSS/GCE(e), PEDOT:PSS-CMC/GE(f), PEDOT:PSS-CMC-rGO@Pd/GCE(g).

Fig. 3 CVs of VK₃ in 0.1 M PBS (pH 7.0): (A) bare GCE (a), CMC/GCE (b), CMC-rGO@Pd/GCE (e); (B) bare GCE (a), CMC/GCE (b), rGO@Pd/GCE(c), PEDOT:PSS/GCE (d), CMC-rGO@Pd/GCE(e), PEDOT:PSS-CMC/GCE(f), PEDOT:PSS-CMC-rGO@Pd/GCE (g), scan rate: 50 mVs⁻¹.

Fig. 4 CVs of VK₃ in 0.1 M PBS (pH 6.5): (A) Scan rates from a to k with 0.015, 0.025, 0.035, 0.05, 0.075, 0.1, 0.15, 0.2, 0.3 V s⁻¹ respectively; (B) the plots of I_{pa} and I_{pc} vs. v ; (C) the plots of $\log I_{pa}$ and I_{pc} vs. the square root of scan rates; (D) the plots of $\log I_{pa}$ and I_{pc} vs. $\log v$; (E) the plots of E_{pa} and E_{pc} vs. $\ln v$ for PEDOT:PSS-CMC-rGO@Pd/GCE.

Fig. 5 (A) CVs of 192 μ M VK₃ at PEDOT:PSS-CMC-rGO@Pd/GCE with different pH values; (B) Effect of pH on the anodic peak currents (solid line) and the anodic peak potentials (dash line) for VK₃ in 0.1 M PBS. Scan rate: 50 mV s⁻¹.

Fig. 6 LSVs of VK₃ with different concentrations using PEDOT:PSS-CMC-rGO@Pd/GCE. (A) The concentrations of VK₃ from a to n with 0.4, 0.6, 0.8, 1, 3, 5, 7, 10, 13, 16, 19, 22, 25, 33, 38, 48 and 54 μ M, respectively. (B) Linear relationship between anodic peak current and the concentration of VK₃.

Fig. 7 The repeatability (A) and reproducibility (B) of PEDOT:PSS-CMC-rGO@Pd/GCE in 0.1 M PBS (pH 6.5) containing 98 μ M VK₃, scan rate: 50 mV s⁻¹.

Fig. 8 The interference study of VK₃ containing various substances with different concentrations, and effects of (A) Cd²⁺, (B) Glu, (C) VB₂, (D) VD₂, (E) MP, (F) NA, (G) Cu²⁺, and (H) Gly on voltammetric responses of VK₃.

Table captions

Table 1 Performances comparison of some electrochemical methods previously used for the determination of VK₃.

Table 2 Determination of VK₃ in diluted chicken blood, broiler feed and serum of older bovine using PEDOT:PSS-CMC-rGO@Pd/GCE.

Scheme 1

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig.5

Fig. 6

Fig. 7

Fig. 8

Table 1 Performances comparison of some electrochemical methods previously used for the determination of VK₃.

Electrode	Buffer solution	Linear range (M)	LOD (M)	Real samples	Ref.
Mercury Drop	0.1 M HClO ₄	2×10 ⁻¹⁰ -5×10 ⁻⁷	1.3×10 ⁻¹⁰	-	20
Poly(vinyl chloride complexes	Acetate	10 ⁻⁵ -10 ⁻¹	2 × 10 ⁻⁵	Human plasma	22
Au-IDA/Pt-IDA	PBS	10 ⁻⁶ -10 ⁻³ /10 ⁻⁵ -10 ⁻³	-	-	23
Mercury Drop	CHCl ₃	-	5×10 ⁻⁷	Pharmaceutic product and food additives	46
Mercury Drop	Acetate	5×10 ⁻⁷ -3×10 ⁻⁵	1.5×10 ⁻⁷	Clinical injection and blood serum	47
PEDOT:PSS-CMC-Pd-rGO/GCE	PBS	4×10 ⁻⁷ -9×10 ⁻⁵	1.3 × 10 ⁻⁷	Animal blood and feedstuff samples	This work

Table 2 Determination of VK₃ in diluted chicken blood, broiler feed and serum of older bovine using PEDOT:PSS-CMC-Pd-rGO/GCE.

Samples	PEDOT:PSS-CMC-Pd-rGO/GCE			
	Added (μM)	Found (μM)	RSD (%)	Recovery (%)
Chicken blood	-	<DL	-	-
	25	23.14±0.39	2.14	93
Serum of older bovine	-	<DL	-	-
	25	23.54±0.09	0.51	94
Broiler feed	-	<DL	-	-
	25	24.41±0.72	3.81	98

^a Average of three determination.
^b Less than limit of detection.