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Electrochemical Oxidation of Ascorbic Acid Mediated by Ru(III) Schiff-base Complex/Multi-Walled Carbon Nanotube/Nafion Modified Carbon Electrode

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Abstract: Carbon electrodes modified with Ru(III) Schiff base complex, multi-walled carbon nanotubes and Nafion[®] were used to develop a low potential sensor for ascorbic acid. Voltammetry measurements showed oxidation of ascorbic acid at the modified glassy carbon electrode at the potential of +0.23 V, and the bare electrode displays no redox peaks in the investigated potential range from 0.00 to +0.35 V. Flow injection amperometric measurements were performed at the operating potential of +0.20 V vs. Ag/AgCl in Britton-Robinson buffer (pH=5.00) with a flow rate of 0.40 mL min⁻¹, and injection volume 100 μL. The modified electrodes shows a fast electric current response for ascorbic acid oxidation. Current response increased with increasing ascorbic acid concentration (dynamic range = 0.50-100 mg L⁻¹) and displayed good linearity (R² = 0.9946). The limit of detection was 0.25 mg L⁻¹, while the limit of quantitation was 0.85 mg L⁻¹. The repeatability was calculated as 2.10 % (n=5 measurement).

Keywords: Ascorbic acid, Ru(III) Schiff base complex, Multi-walled carbon nanotubes, modified carbon electrodes, voltammetry and amperometric analysis.

INTRODUCTION

Ascorbic acid (AA) is a water-soluble organic substance important for a large number of biological processes as formation of skin, blood vessels, ligaments, and healing of wounds. It is known as a strong antioxidant agent that can decrease oxidative stress in the body and is, therefore, may play an important role in the prevention of different illnesses as cancer and cardiovascular diseases^{1, 2}. Deficiency of AA induces gastric irritation, diabetes mellitus, excessive oxidative stress, renal problems and liver disease³. Due to the large importance of AA, numerous analytical methods have been reported in the literature for the determination of AA in different samples. These include chromatography, titrimetric, spectroscopy, photometry, fluorometry and electrochemical method⁴⁻⁹. Among these methods, which require technical training and expensive sample preparation and instrumentation, electrochemical sensors have some benefits such as simplicity, ease of miniaturization, high sensitivity, and relatively low cost compared to other methods. Different electron transfer mediators have been usually applied to decrease the oxidation over-potential and detection of AA at the surface modified carbon electrode. Some inorganic compounds, such Schiff-base copper (II) complex, potassium ferrocyanide and ruthenium complexes were used as electron transfer mediators for determination of AA¹⁰⁻¹³. Ru complexes are the subject of numerous studies for several reasons such as anti-tumor activities, catalytic and electron-transfer mediating properties¹⁴. Ru complexes that are immobilized on the electrode surface transfer electrons between the analyte solution and the substrate electrode, resulting in a reduction in activation over-potential and a decrease in surface fouling and oxide formation in comparison to inert substrate electrodes. Here is described the electrocatalytic study of oxidation AA and development new sensor using glassy carbon (GC) and screen-printed carbon (SPC) electrodes modified with sodium dichloro-*bis*[*N*-phenyl-5-bromo-salicylidenediminato-*N,O*]ruthenat(III) complex (hereinafter referred as Na[RuCl₂(*N*-Ph-5-Br-salim)₂] complex), multi-walled carbon nanotubes (MWCNTs) and Nafion[®].

MATERIAL AND METHODS

Reagents and solution: The synthesis of Na[RuCl₂(*N*-Ph-5-Br-salim)₂] was performed according to the published procedure^{15, 16}. A stock solution of 5 % Nafion[®] in a mixture of lower aliphatic alcohols and water was obtained from Sigma-Aldrich (USA). Multi-walled carbon nanotubes purity of more than 95 % (10-20 nm, 1 μm length) were obtained by Nanolab (Waltham, MA). Britton-Robinson (BR) buffer solutions were prepared by mixing 0.040 M phosphoric acid, 0.040 M boric acid and 0.040 M acetic acid. Ascorbic acid was purchased from Merck (Germany). Standard solutions of AA were freshly prepared using previously deaerated BR buffer (pH 5.00) by bubbling high purity argon gas (99.995%, Messer Griesheim, Gumpoldskirchen, Austria) for 20 minutes prior to the experiments. All solutions were prepared with double distilled water.

Methods: All electrochemical experiments were performed using computer controlled Autolab Potentiostat /Galvanostat (PGSTAT 12) with a three-electrode system. PC ran the system through Autolab software (GPES, Version 4.8). The pH values were measured using pH-meter ISKRA (MA 5740). All potentials given are versus the Ag/AgCl reference electrode. The results obtained were processed and presented using the scientific graphing software SigmaPlot11. Cyclic voltammograms (3 cycles) of AA were recorded in the potential range from 0.00 to + 0.35 V applying scan rate 50 mV s⁻¹ in BR buffer solutions pH 5.00 using bare and modified GC electrode as working, Ag/AgCl reference and the platinum wire as counter electrode. Differential pulse voltammograms were recorded in the potential

range from 0.00 to +0.45 V using 0.12 V step potential, 0.40 V modulation amplitude, 0.006 s modulation time and 0.6 s interval time. Amperometric measurement was carried out using flow injection system that consisted of a high performance liquid chromatographic (HPLC) pump (Model 510, Waters, Milford, MA, USA), a sample injection valve (U6K, Waters), and a thin-layer electrochemical cell (CC5, BAS Bioanalytical Systems Inc., West Lafayette IN, USA). Teflon spacers (MF-1047 and MF-1048, BAS) were used to adjust the thickness of the flow-through cell. Amperometric analysis AA was performed at operating potential of +0.20 V. The flow rate of the carrier buffer solution was 0.40 mL min⁻¹ using BR buffer solution pH 5.00 as carrier with modified screen-printed carbon (SPC) electrode as working, Ag/AgCl (3 M KCl, BAS RE-1) as reference and back plate of the flow cell as counter electrode. The volume of AA solution that was injected was 100 µL. The peak heights of the current response were taken as a measure of the AA concentrations.

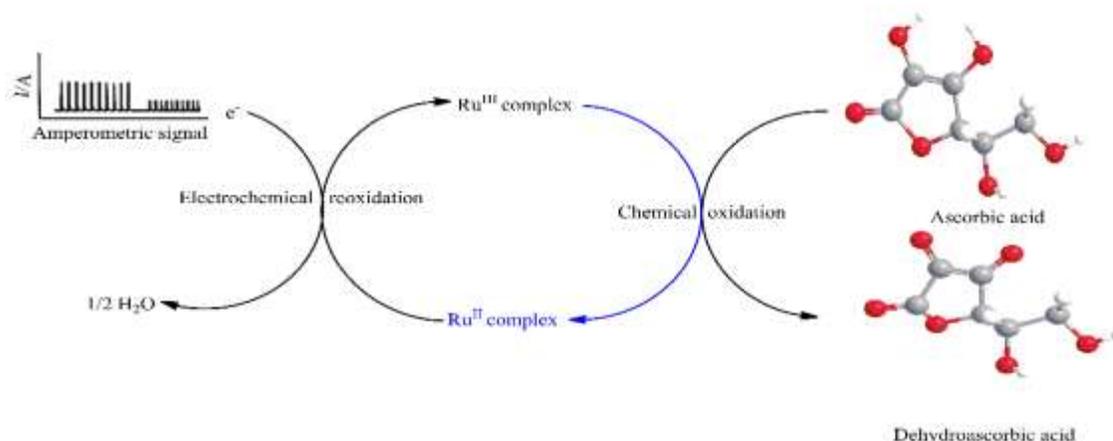
Electrode Preparation: Modifying mixture is prepared by dissolving ruthenium complex (2.50 mg) in 150 µL ethanol, and mixing it with 2.00 mg MWCNTs and Nafion[®] solution (10 µL, 1%). The mixture was homogenized for 30 minutes in an ultrasonic bath (PHYWE Ultraschall-generator, Polytemp POLYSCIENCE) to improve the homogeneity. The homogenous black suspension was immediately used for electrode fabrication, 10 µL being dropped onto the carbon active area (35 x 4 mm) on porcelain plates of SPC electrode (Coors Ceramic GmbH, Chattanooga, TN, USA) and air-dried for 1 hour at room temperature. Prior to modification, the GCE was mechanically polished with 1 µm, 0.3 and 0.05 µm α -alumina powder and cleaned ultrasonically with acetone and water in sequence. Modification mixture, described above, was dropped onto the GCE (0.28 cm² surface area) and then air-dried. Unmodified SPCE and GCE were prepared in the same way, but without modification with Na[RuCl₂(*N*-Ph-5-Br-salim)₂], MWCNTs or Nafion[®].

RESULTS AND DISCUSSION

Cyclic voltammetry was used to investigate mediating properties of Na[RuCl₂(*N*-Ph-5-Br-salim)₂] and MWCNTs. **Figure 1** shows cyclic voltammograms of electrochemical oxidation of AA with unmodified/modified GCE. According to this voltammograms, electrochemical oxidation of AA is irreversible process because clearly defined reduction peak is missing. Oxidation process of AA is very slow itself on the surface of unmodified electrode and it requires high values of positive potentials, which indicates slow electron transfer process¹⁷. Therefore, unmodified GC electrode does not show well define anode peaks of AA oxidation in potential range from 0.00 to +0.35 V (**Figure 1**). According to previously published results Pei et al.¹⁸ and Ngai et al.¹⁹ on GC electrode modified by Na[RuCl₂(*N*-Ph-5-Br-salim)₂], anode peak of oxidation of AA appears at +0.20 V (**Figure 1, curve c**), while on GC electrode modified by Na[RuCl₂(*N*-Ph-5-Br-salim)₂], MWCNTs and Nafion[®], oxidation peak is more intense and appears at potential +0.23 V (**Figure 1, curve d**). Sensitivity degree of aforementioned electrodes increases according to the order:

MWCNTs/Ru(III) complex/Nafion[®]/GC electrode > Ru(III) complex/GC electrode > bare GC electrode

Increase in anode peak might be ascribed to good mediators properties of Na[RuCl₂(*N*-Ph-5-Br-salim)₂], as well as to good electrical conductivity and the great surface of MWCNTs. There are a large number of research papers of electrochemical oxidation of AA that showed GC electrode modified by carbon nanotubes has better performance than unmodified GC electrode^{1, 20}. Oxidation of AA on surface of modified electrode is shown in **Scheme 1**.



Scheme 1: Oxidation of ascorbic acid at the surface of modified electrode.

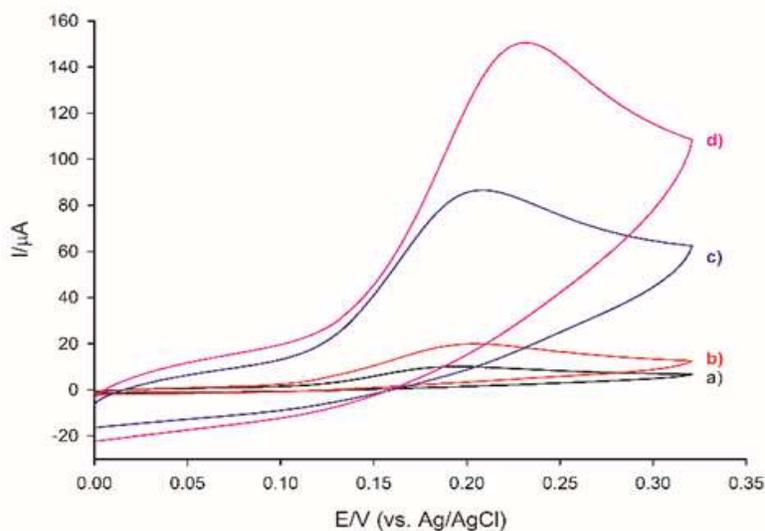


Figure 1: Cyclic voltammograms in the BR buffer (pH 5.00) with (a) an unmodified GC electrode, (b) an unmodified GC electrode with 300 mg L⁻¹ AA, (c) GC electrode modified with Na[RuCl₂(N-Ph-5-Br-salim)₂] with 300 mg L⁻¹ AA (d) GC electrode modified with Na[RuCl₂(N-Ph-5-Br-salim)₂], MWCNTs and Nafion[®] with 300 mg L⁻¹ AA. Scan rate: 50 mV s⁻¹.

Furthermore, cyclic voltammetry was used to investigate dependence of AA concentration versus anodic current response. As shown in **Figure 2A**, increase of AA concentration causes increases of anodic peak current demonstrating a good electrochemical response of the modified electrode. Dependence of AA concentrations versus oxidation current response is given by linear regression: $I (\mu\text{A}) = 0.3303 [\text{AA}] (\text{mg L}^{-1}) + 48.98$, $R^2 = 0.9945$ (**Figure 2B**).

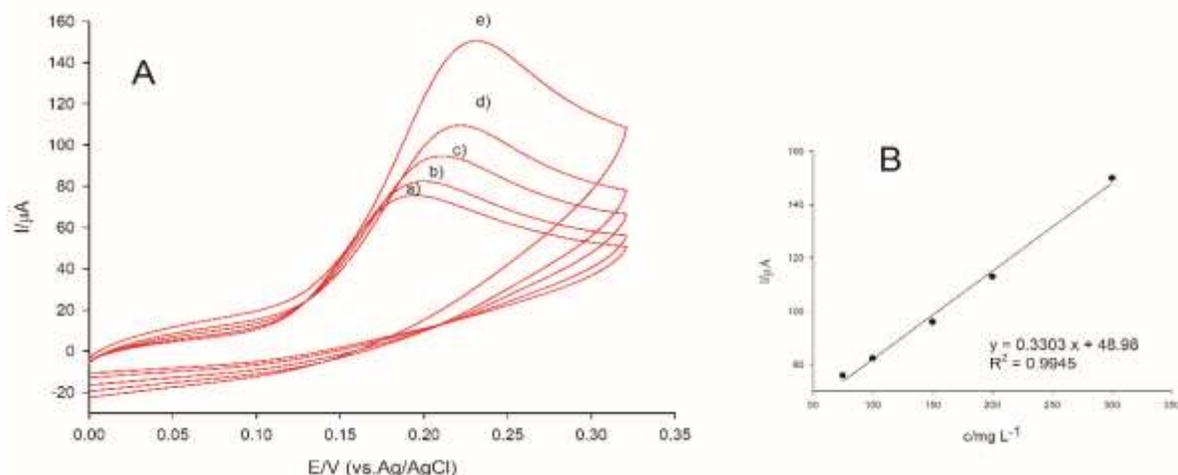


Figure 2: (A) Cyclic voltammograms of AA in BR buffer (pH=5.00) at modified GCE for different AA concentrations (mg L^{-1}): (a) 75; (b) 100; (c) 150; (d) 200; (e) 300. (B) Plot of the peak current as a function of AA concentrations. Scan rate: 50 mV s^{-1} .

Oxidation of AA on modified GC electrode was confirmed by differential pulse voltammetry. **Figure 3** shows a clearly visible oxidation peak at potential +0.23 V attributed to oxidation AA at surface modified electrode. Parameters of FIA measurements for determination of AA were optimized by studying the important factors, which affect the amperometric response. Influence of type buffer solutions, pH value of buffer solutions, the volume of injected solutions, flow rates and operating potential were optimized, an effort to achieve maximum amperometric response due to oxidation of AA at the modified electrode. Optimum values were select by compromising the peak height, reduced time analysis, stability of base line, accessibility and economy, respectively. The optimized parameters, optimum values, and range studied are presented in **Table 1**.

Table 1: Optimized operating parameters for determination of AA by use of a $\text{Na}[\text{RuCl}_2(\text{N-Ph-5-Br-salim})_2]/\text{MWCNTs}/\text{Nafion}^\circledast/\text{SPC}$ electrode in a FIA measurements.

Parameter study	Range studied	Optimum values
pH of BR buffer solution	3.00-9.00	5.00
Injection volume (μL)	50-250	100
Flow rate (mL min^{-1})	0.20-1.90	0.40
Operating potential (V)	-0.20-0.40	0.20

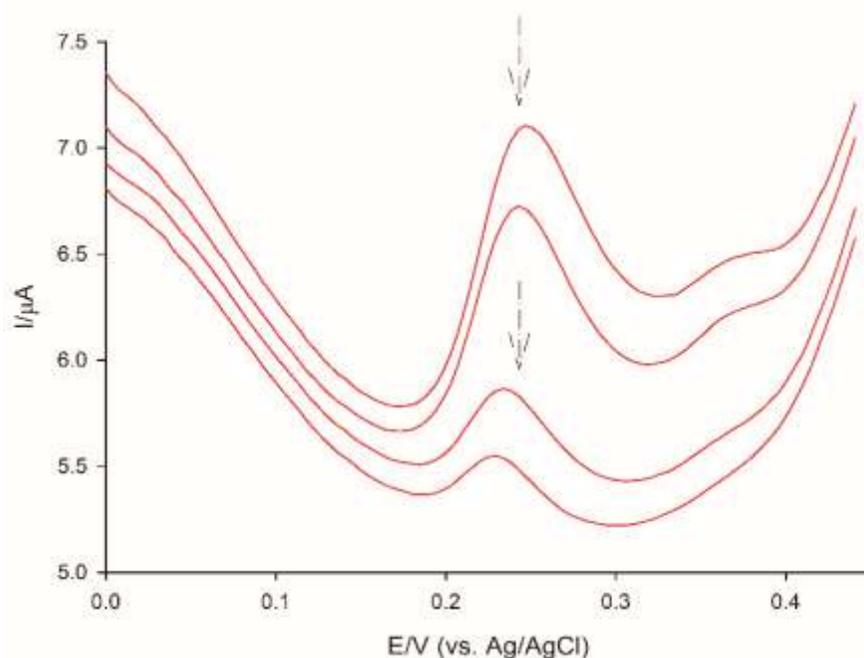


Figure 3: Differential pulse voltammograms of AA in BR buffer (pH = 5.00) at modified GCE for AA concentrations (mg L⁻¹): 1; 3; 7 and 10.

Linearity, limit of detection and reproducibility: Figure 4 shows a typical amperogram for different concentration of AA using Na[RuCl₂(*N*-Ph-5-Br-salim)₂]/MWCNT/Nafion[®]/SPC electrode as detector. The linearity of the relationship between peak heights (current signals) versus varying AA concentrations was studied by injecting standard solutions into FIA system under the optimal parameters (Table 1). The linear relation between peak heights and the AA concentrations was found in 0.50-100 mg L⁻¹ AA range: ($I (\mu\text{A}) = 0.009911 [\text{AA}] (\text{mg L}^{-1}) + 0.008636$; $R^2=0.9946$ (Figure 5). The limit of detection (LOD) and limit of quantification (LOQ) was calculated from equations²¹:

$$LOD = \frac{3 \cdot s}{m}; \quad LOQ = \frac{10 \cdot s}{m}$$

where s is the standard deviation of FIA peaks ($n=3$) at 1 mg L⁻¹ AA and m is the slope of the calibration curve (Figure 5). LOD were calculated as 0.25 mg L⁻¹, and LOQ of 0.85 mg L⁻¹. The repeatability ($n=5$ measurements, 10 mg L⁻¹ AA) was calculated 2.10 %.

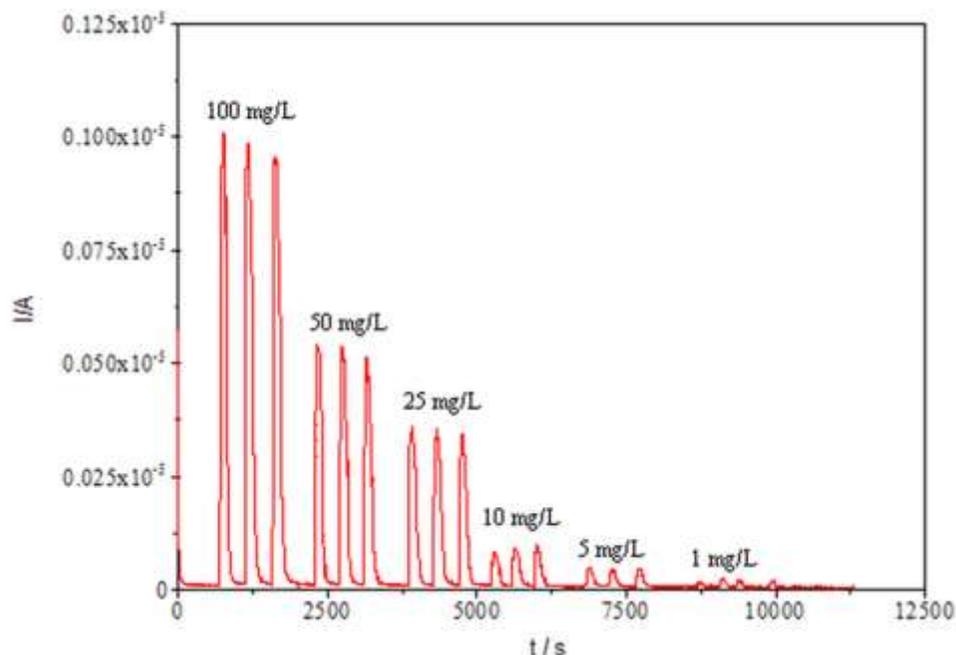


Figure 4: Amperometric FI response of AA at a $\text{Na}[\text{RuCl}_2(\text{N-Ph-5-Br-salim})_2]/\text{MWCNT}/\text{Nafion}^\circledast/\text{SPC}$ electrode, operating potential +0.20 V vs. Ag/AgCl, BR buffer (pH=5.00), flow rate 0.40 mL min^{-1} , injection volume $100 \mu\text{L}$.

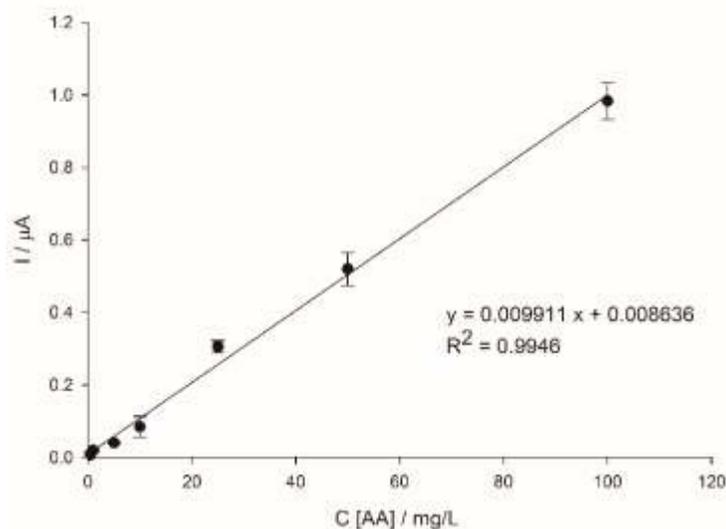


Figure 5: Calibration curve of the amperometric current response as a function of different concentrations (from 0.50 to 100 mg L^{-1}) of AA. $\text{Na}[\text{RuCl}_2(\text{N-Ph-5-Br-salim})_2]/\text{MWCNT}/\text{Nafion}^\circledast/\text{SPC}$ electrode, operating potential +0.20 V vs. Ag/AgCl, BR buffer (pH=5.00), flow rate 0.40 mL min^{-1} , injection volume $100 \mu\text{L}$.

The **Table 2** shows the comparison of the proposed sensor with those previously reported in the literature. The amperometric sensor presented in this work has a low limit of detection and good linearity range for AA which are due to the excellent electrocatalytic activity of $\text{Na}[\text{RuCl}_2(\text{N-Ph-5-Br-salim})_2]$ and MWCNTs in combination with Nafion[®].

Table 2: Comparison of the proposed sensor for determination AA with others described in the literature

Electrode	Modifiers	Method	Limit of Detection (mg L ⁻¹)	Linearity Range(mg L ⁻¹)	Ref.
NC/ME ¹	Carbon fibers/ZnO ¹¹	Voltammetric	27.60	105.7-317	Gu et al. ¹¹
GCE	Graphene-Polyaniline	Amperometric	4.75	8.80-88	Norfun et al. ⁹
GCE	Poly(aniline- <i>co-m</i> -ferrocenylniline)	Amperometric	0.35	8.80-1004	Chairam et al. ²²
GCE	Multi-walled carbon nanotube-polycation-	Amperometric	0.09	0.18-17.6	Su et al. ¹
SPCE	$\text{Na}[\text{RuCl}_2(\text{N-Ph-5-Br-salim})_2]$ /MWCNT/Nafion [®]	Amperometric	0.25	1-100	This work

CONCLUSION

In this work, the preparation and electrochemical characterization of modified glassy carbon and screen-printed carbon electrode were reported. Low-potential sensor for ascorbic acid based on carbon electrodes modified with water-insoluble Ru(III) complex, multi-walled carbon nanotubes and Nafion[®] shows fast current response and sensitivity. The results showed that multi walled carbon nanotubes and Ru(III) complex in combination with Nafion[®] improve oxidation ascorbic acid also and amperometric and voltammetric behavior of ascorbic acid at the carbon electrodes. Further research will be focused on sensor testing with real samples.

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