DETERMINATION OF ACRYLAMIDE USING RUTHENIUM DIOXIDE MODIFIED CARBON PASTE ELECTRODE

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A simple indirect method for acrylamide determination using a carbon paste electrode (CPE) modified with powdered ruthenium dioxide is described in the study. The voltammetric response of RuO_2/CPE in a 1.0 M LiCl supporting electrolyte resulted in the reduction current at a potential of 1.03 V vs. Ag/AgCl/ 3.0 M KCl due to the formation of unspecified Ru-Cl species. The addition of acrylamide decreased the reduction current at 1.03 V, giving rise to a linear response in the concentration range from 0.25 μM to 1.5 μM with a limit of detection found to be 0.94 μM. The method developed was successfully applied to analyse potato crisps sample with a recovery rate of 97.9 %.

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Introduction

Acrylamide (AA) is a chemical used in the industry to produce polyacrylamide as a flocculent agent for the treatment of potable water, in processing of pulp for the production of paper, in cosmetics, and for food package materials as additive [1,2]. AA can generate neurotoxicity, neuropathy, and it is considered to act as possible carcinogenic in humans [3,4]. In 2002, the Swedish Agency of Food Security had reported on high concentrations of acrylamide in starch rich food processed at temperature higher than 120 °C, such as fried potato chips, breads or coffee. AA is generated from the reaction of reducing sugars and aminoacids; especially, from asparagin [5,6].

The presence of AA in foods and its harmful effects to human health makes its determination and quantification an important issue. Chromatographic separations including gas chromatography/mass spectrometry, high performance liquid chromatography coupled with mass spectrometry (MS/MS), and capillary electrophoresis are the most widely used analytical techniques [7,8]. The respective methods require an expensive instrumentation, special labor condition (e.g., air-conditioned laboratory), and are rather unfriendly to environment. Therefore, the easy, fast, inexpensive and reliable methods are of great importance. In this context, the electroanalytical techniques offer these desired features and can be employed for detection and quantification of target compounds. Literature search shows two electroanalytical approaches for detection of acrylamide: direct and indirect. Differential pulse polarography was used for direct determination of AA in aqueous solution [9]. Since AA is known for its ability to form complexes with various metals [10], Zargar *et al.* [11] and Veselá and Šucman [12] have developed methods for direct determination of AA complexes using the hanging mercury drop electrode. Among the carbon-based electrodes, only carboxylic group-modified single-walled carbon nanotube screen-printed electrode allowed direct detection and quantification of AA [13]. A formation of an adduct between AA and hemoglobin leads to numerous indirect methods for determination of AA using a single-walled carbon nanotubes glassy carbon electrode [14], hemoglobin-gold-nanoparticles ITO glass electrode [15], and hemoglobin-multiwalled-copper-nanoparticles-polyaniline hybrid film deposited onto the surface of a pencil graphite electrode [16]. Furthermore, a biosensor for AA determination based on the interaction of the analyte and *P. aeruginosa* enzymatic activity has been developed [17]. In this paper, ammonium ion-selective electrode has been used for detectio of NH_4^+ released from AA molecule *via* hydrolysis using amidase.

Carbon paste represents an appealing and widely used electrode material in the field of electroanalysis due to its attractive advantages, such as simple preparation, low-cost implementation, renewability, low background currents, and the ease of modification [18]. Carbon paste electrode (CPE) with various metallic films (Bi, Sb, etc.) represents a suitable substitution for the mercury electrode;

mainly, in stripping analysis of trace heavy metals [19]. Modification of CPE with ruthenium leads to an improvement of the responses toward various organic compounds, such as amino acids [20] or glycerol [21].

Since ruthenium salts were considered to form be strongly coordinated with AA *via* the carbonyl oxygen [10], an attempt to determine AA using ruthenium dioxide-modified CPE has been proposed in this study.

Experimental

Chemicals, Reagents, and Equipment

All the chemicals and reagents were purchased in Sigma-Aldrich (the Czech Republic). Deionized water was used throughout this study ($G \leq 0.055$ µS). Dissolved oxygen was removed from all the solutions by purging with argon for 5 min (purity 99.99 %, Linde Technoplyn, the Czech Republic).

A stock solution of acrylamide (0.1 M) was prepared freshly using deionized water and kept in dark before the experiment.

Three-electrode system consisting of a CPE (working), Ag/AgCl/3.0 M KCl (reference), and a platinum wire (counter electrode) was used connected to a PalmSens analyser (Ivium Technologies, The Netherlands) for electrochemical measurement. Modified CPE was regenerated by mechanical surface renewal by wipping with a wet filter paper before each measurement.

The Preparation of Ruthenium Dioxide-Modified CPE. Ruthenium(IV) oxide powder (99.9%) and graphite powder 5.5-7.0 µm (CR-5, Maziva Týn n. L., s.r.o., the Czech Republic) were weighed and the total mass was controlled at 0.5 g. The mass ratio of RuO_2 was set to 5, 10 and 15 %. After that, 130 µl mineral oil (M5904, Sigma-Aldrich, Germany) was added to the powder and thoroughly hand mixed together in ceramic mortar with a pestle. The resulting paste was packed into the Teflon piston holder (3.0 mm in diameter) [22]. The bare CPE was used as the control.

Electrochemical Procedure. First of all, the various amount of RuO_2 (5, 10, and 15 %) in the carbon paste electrode material was investigated in a 1.0 M LiCl supporting electrode when using cyclic voltammetry in the potential range from –1.0 to 1.4 V, and at the scan rate of 100 mV s^{-1}. All the measurements were performed using cyclic voltammetric technique using with the RuO_2/CPE and the supporting electrolyte of the same composition.

Sample Preparation. A portion of salted potato crisps (manufactured by Petr Hobža, Strážnice, the Czech Republic) was homogenized and soaked in 50 ml deionized water for 20 min. Acrylamide was extracted by shaking for 60 min at laboratory temperature, followed by centrifugation at 4100 rpm for 10 min. Carezz treatment was selected for purification of the supernatant, followed by the

filtration using Watmann filter paper No. 2. An appropriate amount of the purified sample was added to the supporting electrolyte and the amount of AA determined using cyclic voltammetry specified above. For recovery studies, the same procedure was applied with the sample spiked with the standard AA solution.

Results and Discussion

Reportedly [18], the bare CPE is not suitable for determination of AA in aqueous solutions because of its electrochemical inactivity in the potential window from –1.0 to 1.4 V *vs*. ref. The oxidation of AA occurred at highly negative potential (–1.84 V) when using the hanging mercury drop electrode [9]; however, CPE is not already stable at such a potential. In another study, the formation of the AA-Ni^{2+} complex led to the decrease in the oxidation potential to –0.3 V; again, using the hanging mercury drop electrode [12]. The direct measurement of AA alone or as a complex with ruthenium (due to limited solubility of ruthenium dioxide in aqueous solution) using the bare CPE was not possible in the current study. Nevertheless, after a few cycling of RuO_2/CPE in the supporting electrode containing Cl^- ions, the reduction current appeared at the 1.03 V (Fig. 1).
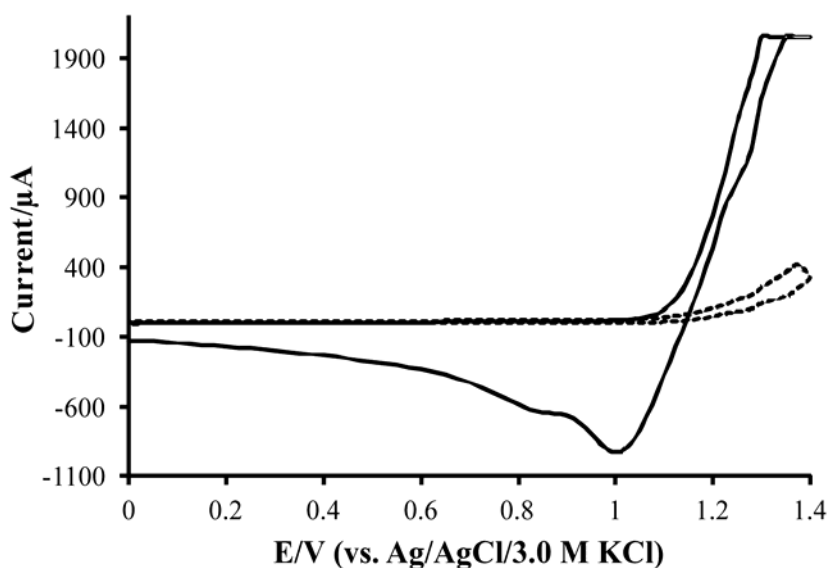

Fig. 1   Cyclic voltammograms (15th cycle) of 10% RuO_2 /CPE in 1.0 M LiCl (solid line) and 1.0 M KNO_3 (dashed line), scan rate: 100 mV s^{-1} potential range from –1.0 to 1.4 V vs. Ag/AgCl/3.0 M KCl

In a study of Horányi and Rizmayer [23], a significant adsorption of chloride on the surface of ruthenium electrode was noticed. It is evident from Fig. 1 that the process appearing at the potential of 1.03 V corresponds to the presence of chloride salt and Ru. When a solution of 1.0 M KNO_3 was used as the

supporting electrolyte, the reduction current was not apparent. It is important to notice in Fig. 2 that the current response has increased with the number of cycles exhibiting a steady-state response after the 10th cycle. This behaviour can be explained by the slow formation of a specific Ru-Cl film on the surface of the CPE during cycling until all the ruthenium dioxide on the surface of RuO_2/CPE had intereacted.
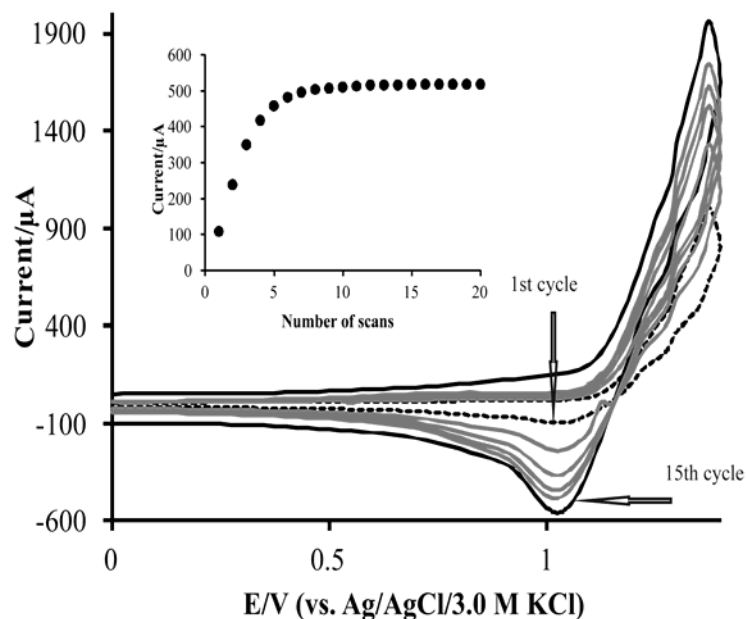

Fig. 2 Cyclic voltammograms (15 cycles) of 10 % RuO_2/CPE in 1.0 M LiCl, scan rate 100 mV s^{-1}, potential range from –1.0 to 1.4 V vs. Ag/AgCl/3.0 M KCl. 1st cycle in dashed line, 15th cycle in solid line. Inlet: the effect of the number of cycles on the reduction peak current at 1.03 V

As previously described, ruthenium(III) Shiff`s base complex proved to be a selective sensor for Cl$^-$ determination [23]. In another study, remaining chloride ions in composite material impaired the electrochemical performance of amorphous RuO_2/carbon electrode [24] by forming an unspecified ruthenium-chloride product. In the study of Martinez–Alvarez and Miranda–Hernandez, the authors found that an oxy-aquacomplex of ruthenium had provided the electroactive species for dissolution of $RuCl_3$ in 1M $HClO_4$ (pH 1.0) [25].

The effect of RuO_2 content on the response of RuO_2/CPE in 1.0 M LiCl supporting electrolyte was also investigated. As can be seen in Fig. 3, there is no linear relationship between the RuO_2 content and reduction current at the potential of 1.03 V vs. ref.

The most satisfactory response was obtained with 10 % RuO_2 in the composite material. The reduction current is linearly proportional to the scan rate in the range from 20 to 100 mV s^{-1} (Fig. 4) ($I = 5575.3$ Vs^{-1}+ 371.8; $R^2 = 0.998$). These findings have indicated that the electrochemical process at the RuO_2/CPE
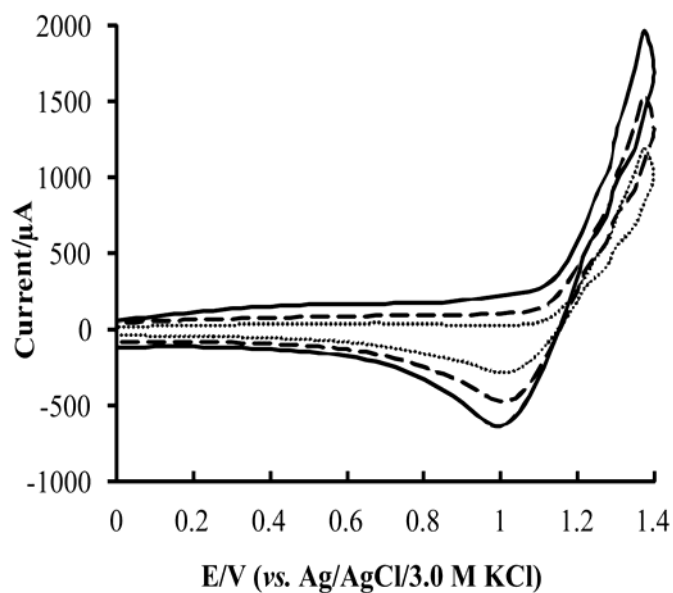
Fig. 3 Cyclic voltammograms (15th cycle) of RuO$_2$/CPE with 5 ($\cdots\cdots$), 10 (——) and 15 % (–––) content of RuO$_2$ in 1.0 M LiCl, scan rate: 100 mV s^{-1}, potential range from –1.0 to 1.4 V

in the 1.0 M LiCl supporting electrolyte is surface-controlled in the limited range of scan rates.

Analytical Application

The effect of the presence of AA in 1.0 M LiCl on the reduction current at the potential of 1.03 V vs. ref. is shown in Fig. 5, where the reduction current has linearly decreased with the increasing concentration of AA in the range of 0.25-1.5 µM, giving the regression equation I (µA) = –28.52×c (10^{-7} mol l^{-1}) + 948.66 (R^2 = 0.979).

The mechanism of decreasing the reduction current in the dependence upon the addition of AA to the LiCl-based electrolyte solution has not been yet successfully explained in the framework of the present study and the respective study has to be made. Herein, we can just deduce that AA may preferentially form a complex with ruthenium on the surface of the RuO$_2$/CPE electrode, so that the lesser amount of free Ru substance has been available for the Ru-Cl formation, giving rise to the reduction current at the potential of 1.03 V.

The limit of detection (*LOD*) By using the equation $LOD = 3s_b/m$, where s_b is the standard deviation of the blank response and m the slope of the calibration plot, the limit of detection (*LOD*) has been found to be 0.94 µM whereas the limit of quantification (*LOQ*, signal/noise = 10) being 3.1 µM.
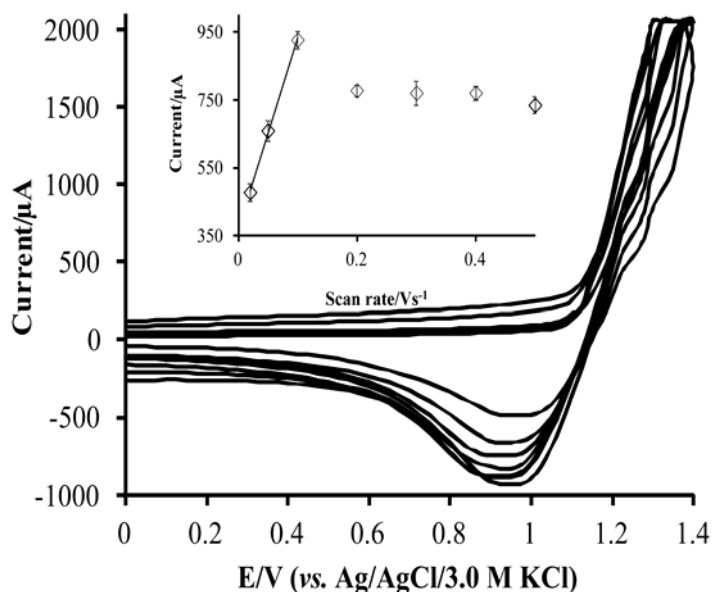
Fig. 4   The effect of the scan rate (20-500 mVs^{-1}) on the cyclic voltammograms (15th cycle) of 10 % RuO$_2$/CPE in 1.0 M LiCl, potential range from –1.0 to 1.4 V vs. Ag/AgCl/3.0 M KCl. Inlet: the effect of the scan rate on the reduction peak current at the potential 1.03 V
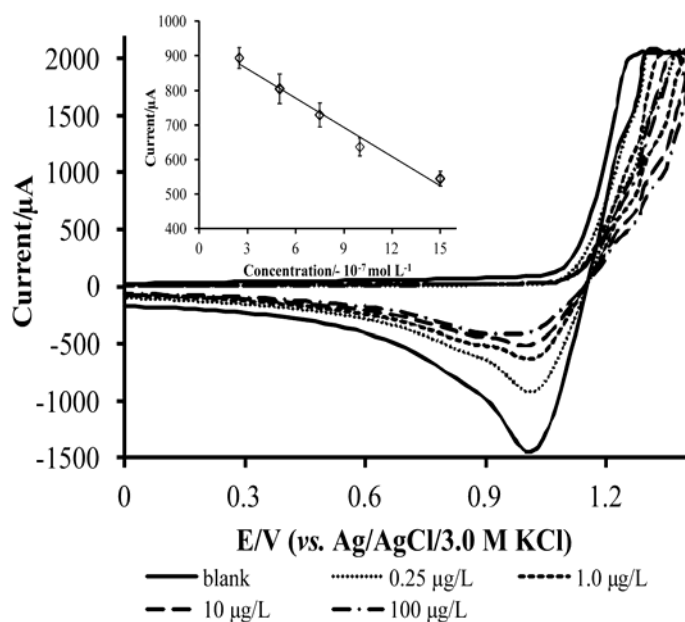

Fig. 5   Cyclic voltammograms of 10 % RuO$_2$/CPE in 1.0 M LiCl in the presence of 0.25 (·····), 1.0 (– – –), 10.0 (——) and 100 µM (–·–·–·) of acrylamide (15th cycle), scan rate: 100 mVs^{-1}, potential range from –1.0 to 1.4 V. Inlet: The calibration plot of reduction current (1.03 V) vs. concentration of acrylamide. Vertical bars represent the standard deviation (for $n = 6$)

The proposed method was applied to the determination of acrylamide in potato crisps and the respective results are shown in Table I. It can be seen that the method is sensitive toward the acrylamide determination with a good recovery of 97.7 %.

Table I   Determination of acrylamide in potato crisps sample and the recovery data ($n = 6$)

Spiked $\mu g\,l^{-1}$	Found $\mu g\,l^{-1}$	Recovery %
0.00	50.5	-
7.12	56.44	97.9

Conclusion

The results of the present study have shown that RuO_2/CPE electrode has some potential to determine acrylamide in potato crisps. A further study has to be made to identify the Ru-Cl electroactive species being reduced on the surface of the electrode. The indicative value of acrylamide for potato crisps was set to 1000 µg kg^{-1} according to the Comission Recommendation 2010/307/EU; therefore, our method is still suitable for AA quantification despite a limited linearity between the reduction current on and the concentration of AA, together with relatively high *LOD* and *LOQ* in comparison with the previous studies cited was determined in this study [9,13,15,16].

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