

Article

Voltammetric Detection of Urea on an Ag-Modified Zeolite-Expanded Graphite-Epoxy Composite Electrode

Florica Manea ^{1,*}, Aniela Pop ¹, Ciprian Radovan ², Plamen Malchev ³, Adriana Bebeselea ¹, Georgeta Burtica ¹, Stephen Picken ³ and Joop Schoonman ³

¹ “Politehnica” University of Timișoara, P-ta Victoriei, nr.2, 300006, Timișoara, Romania; E-mails: aniela.pop@chim.upt.ro (A.P.); adriana.bebeselea@chim.upt.ro (A.B.); georgeta.burtica@chim.upt.ro (G.B.)

² West University of Timișoara, Laboratory of Electrochemistry, Str. Pestalozzi nr.16, 300115 Timișoara, Romania; E-Mail: radovan@cbg.uvt.ro (C. R.)

³ Delft University of Technology, 2600 GA Delft, The Netherlands; E-mails: p.g.malchev@tudelft.nl (P.M.); s.j.picken@tudelft.nl (S.P.); j.schoonman@tudelft.nl (J.S.)

* Author to whom correspondence should be addressed; E-mail: florica.manea@chim.upt.ro (F.M.); Tel.: +40256403070; Fax: +40256403069

Received: 23 Aug 2008; in revised form: 13 Sep 2008 / Accepted: 18 Sep 2008 /

Published: 22 September 2008

Abstract: In this paper, a modified expanded graphite composite electrode based on natural zeolitic volcanic tuff modified with silver (EG-Ag-Z-Epoxy) was developed. Cyclic voltammetry measurements revealed a reasonably fast electron transfer and a good stability of the electrode in 0.1 M NaOH supporting electrolyte. This modified electrode exhibited moderate electrocatalytic effect towards urea oxidation, allowing its determination in aqueous solution. The linear dependence of the current versus urea concentration was reached using square-wave voltammetry in the concentrations range of urea between 0.2 to 1.4 mM, with a relatively low limit of detection of 0.05 mM. A moderate enhancement of electroanalytical sensitivity for the determination of urea at EG-Ag-Z-Epoxy electrode was reached by applying a chemical preconcentration step prior to voltammetric/amperometric quantification.

Keywords: Urea determination, expanded graphite-Ag-zeolite-epoxy composite electrode, electrocatalytic effect

1. Introduction

The determination of urea is essential for a number of applications, including clinical diagnosis, food processing, agricultural process and environmental protection [1-5]. Because of very fast and high expansion of industrial and farming activity, the presence of urea in water is increasing. Also, high quantities of urea are present in municipal wastewaters because about 80% the nitrogen in fresh urine is bound in urea [6].

Several analytical procedures have been developed for the analysis of urea in aqueous samples. Most of these investigations on the determination of urea have been based on measuring the changes of ammonia enzymatically released during the hydrolysis of urea. There are several analytical procedure based on the optical methods [7, 8] and electrochemical techniques. In general, the assay of urea by electrochemical techniques means the use of enzyme-modified electrodes by potentiometric and amperometric methods [1, 3, 4]. Though, a non-enzimatic amperometric detection of urea in aqueous solution at poly(Ni-cyclam) film-modified glassy carbon electrode was reported by Ferrer [2].

Zeolite-modified electrodes (ZMEs) have been drawing attention as chemically modified electrodes (CMEs) because of synergistic combination of zeolite features with electrochemical interfaces [9-12]. ZME can be exploited as electrochemical sensors in relation with zeolite's properties, e.g., ion-exchange capacity, molecular selectivity, and catalyst-assisted reactivity. Moreover, metal ion-doped zeolites allow exploitation of ion-exchange capacity of zeolite for the development of electrochemical sensors for the sensing non-electroactive inorganic or organic species [10, 13]. Also, the zeolite-supported electrocatalyst can be exploited to improve the performance of analytical sensing device. For the electrochemical determination of urea, the clinoptilolite has been exploited for the preparation of biosensors by covalent binding of enzymes to the surface of an ion-sensitive membrane made up of a zeolite-polymer matrix [14]. In this situation, the electrochemical strategy was based on the clinoptilolite affinity for ammonium to determine urea using urease. The main disadvantages of the ammonium sensor were its long response time and the limited stability of the urease layer.

In the present study, an expanded graphite-Ag-modified zeolite-epoxy composite (EG-Ag-Z-Epoxy) electrode was prepared from Romanian natural zeolitic volcanic tuff modified with silver, and electrochemically characterized for urea detection. The catalytic electrochemical oxidation of urea on (EG-Ag-Z-Epoxy) electrode was investigated. The kinetic parameters for the heterogeneous electron transfer at EG-Ag-Z-Epoxy electrode (the rate constant and the transfer coefficient) were estimated using Laviron's treatment [15]. The electrode was examined for its electrocatalytic activity toward urea oxidation by cyclic voltammetry (CV), liner-scan voltammetry (LSV), differential-pulse voltammetry (DPV), square-wave voltammetry (SWV), and chronoamperometry (CA).

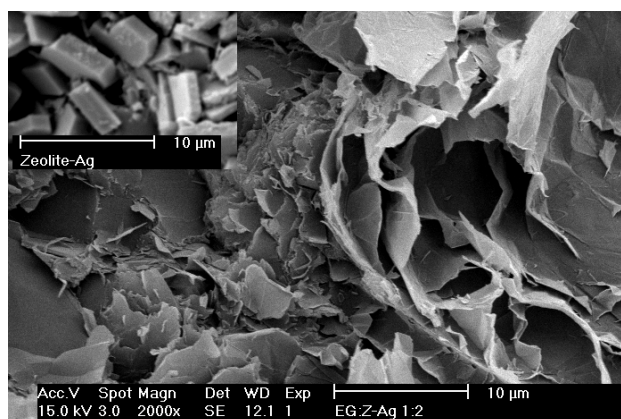
2. Results and Discussion

The prepared EG-Ag-Z-Epoxy composite electrode showed good mechanical strength and electrical conductivity ($18.28 \text{ S} \cdot \text{cm}^{-1}$).

2.1. Surface characterization

SEM imaging has been used to provide qualitative information about distribution of expanded graphite and Ag-doped zeolite zones and some surface features of this composite electrode. Figure 1 illustrates comparatively the images of Ag-zeolite particles and EG-Ag-Z-Epoxy composite electrode and exhibited a closely spaced expanded graphite zones with random distribution and orientation due to the irregular shapes of both the expanded graphite particles and Ag-zeolite particles in epoxy matrix. The Ag-zeolite particles are invisible on the composite surface because they are completely embedded into the epoxy resin, and are rarely exposed at the electrode surface. In addition, a SEM image of Romanian Ag-doped zeolite is shown in the inset of Figure 1 for comparison.

Figure 1. SEM image of EG-Ag-Z-Epoxy composite electrode. Inset: SEM image of Ag modified zeolite.



2.2. Voltammetric measurements

Figure 2 depicts a detail of CVs of EG-Ag-Z-Epoxy composite electrode in 0.1 M NaOH supporting electrolyte involving repetitive first scans. It can be seen that the EG composite electrode containing Romanian zeolite with small amount of Ag^+ cations distributed randomly in the zeolite structure displays complex behaviour with several electrochemically distinct species. The anodic part of the voltammogram is characterized by the occurrence of the anodic peaks corresponding to the electroformation of soluble $[\text{Ag}(\text{OH})_2]^-$ complex species (the first peak, shoulder), to the electroformation of Ag_2O (the second peak), and to the formation of AgO (the third peak) [16,17]. The cathodic part of the cyclic voltammograms is characterized by the occurrence of cathodic peaks corresponding to the electroreduction of AgO to Ag_2O , the reduction of Ag^+ located in different sites of zeolite [12]. However, it must be noticed that the anodic current is very stable, while the cathodic current is stable up to the reduction of Ag^+ species. The stability of CVs could be owned to time-consuming process of the soaking of embedded zeolite particles.

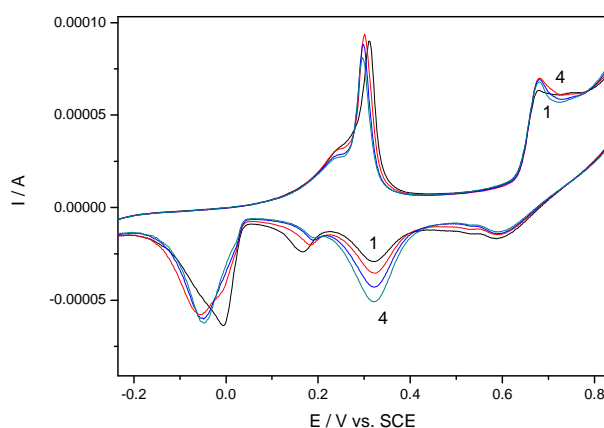
An investigation of the effect of the scan rate on the peaks currents and potentials corresponding to the redox $\text{AgO}/\text{Ag}_2\text{O}$ couple was carried out (Figure 3). The linear proportionality of the current peak

vs. square root of the scan rate (Inset a of Figure 3) indicated that the process was generally diffusion step controlled and the practically zero intercepts suggested that adsorption steps and surface interactions were negligible. Also, from the variation of peak potentials with scan rate (Inset b of Figure 3), using the treatment proposed by Laviron [15], kinetics parameters for the heterogeneous electron transfer at EG-Ag-Z-Epoxy composite electrode were determined in relation with redox couple of Ag(II) and Ag(I) oxides. Taking into account that for scan rates higher than 300 mVs^{-1} , the difference between the anodic and cathodic peak was higher than 200 mV, and the values of $\Delta E = E_p - E_p'$ were proportional to the logarithm of scan rate, the transfer coefficient (α) and the apparent charge transfer rate constant (k_s) for electron transfer between the electrode and surface deposited layer were determined. A plot of $E_p = f(\log v)$ yields two straight lines with slope equal to $2.3RT/\alpha nF$ and $2.3RT/(1-\alpha)nF$ for the cathodic and anodic peak. Using such a plot and the equation:

$$\log k_s = \alpha \log(1 - \alpha) + (1 - \alpha) \log \alpha - \log(RT / nFv) - \alpha(1 - \alpha)nF\Delta E_p / 2.3RT \quad (1)$$

where the values of α and k_s were 0.42 and 0.36 s^{-1} , respectively, for EG-Z-Ag-Epoxy in the presence of 0.1 M NaOH subjected to AgO /Ag₂O couple.

Figure 2. Detail of repetitive cyclic voltammograms (CVs) of EG-Ag-Z-composite electrode in 0.1 M NaOH supporting electrolyte; potential range of -0.5 to 1.25 V/SCE; scan rate 0.05 Vs^{-1} ; 1-4: initial CV-the fourth repetition of CV



A series of resultant CVs obtained directly over the concentration range 0.1 mM – 2.6 mM for urea standard solutions, corresponding to curves 1-13, is illustrated in Figure 4. The anodic current peaks corresponding to AgO formation increased progressively linearly with urea concentration. Also, the two cathodic current peaks recorded at about 0 and 0.2 V vs. SCE increased with urea concentrations but the current within this potential range was not considered due to the fact it was not stable in the absence of urea (see Figure 2). A very unexpected and unusual oxidation peak occurred immediately after the reduction of AgO to Ag₂O on backward scanning in cathodic sense, which increased also linearly with urea concentration.

Figure 3. Cyclic voltammograms (CVs) of EG-Ag-Z-composite electrode in 0.1 M NaOH supporting electrolyte at different scan rates: 1-0.01, 2-0.02, 3-0.03, 4-0.04, 5-0.06, 6-0.08, 7-0.1 $\text{V}\cdot\text{s}^{-1}$; Insets: a)-The anodic peak current versus the square root of scan rate, b)-Experimental variation of ΔE versus the logarithm of the scan rate.

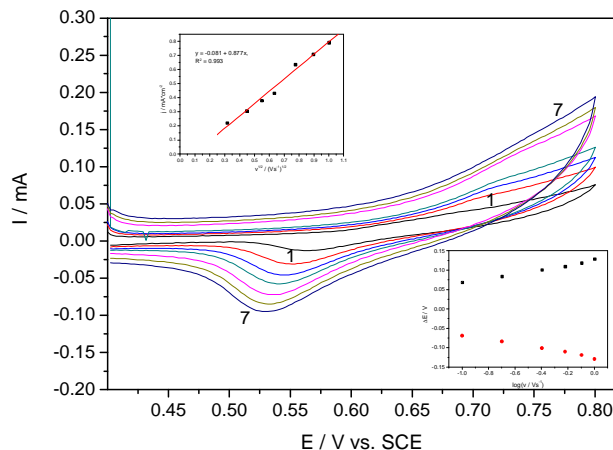
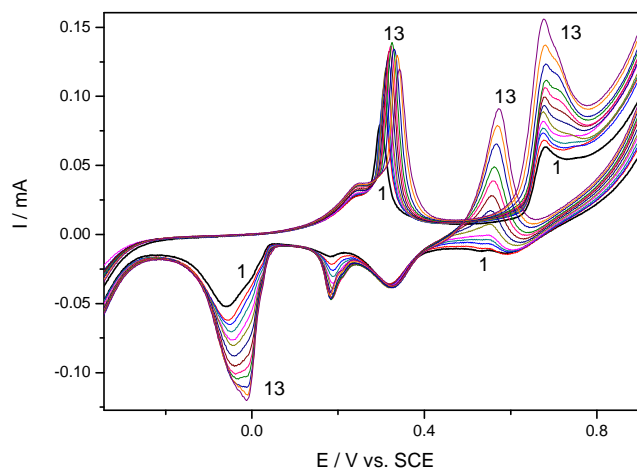


Figure 4. Cyclic voltammograms of EG-Ag-Z-Epoxy composite electrode in 0.1 M NaOH supporting electrolyte (1) and in the presence of different urea concentrations: 2-0.1 mM; 3-0.2 mM; 4-0.3 mM; 5-0.4 mM; 6-0.6 mM; 7-0.8 mM; 8-1 mM; 9-1.2 mM; 10-1.4 mM; 11-1.8 mM; 12-2.2 mM; 13-2.6 mM; potential scan rate $0.05 \text{ V}\cdot\text{s}^{-1}$; potential range: -0.5 to 1.25 V



The anodic current from forward branches of CVs recorded at +0.68 V/SCE depends linearly on the urea concentration in the range 0.1-2.6 mM, with the sensitivity of $0.033 \text{ mA}\cdot\text{mM}^{-1}$. Also, under the same conditions, the current recorded on backward scan at the potential about +0.55 V vs. SCE showed a better sensitivity, as $0.042 \text{ mA}\cdot\text{mM}^{-1}$. For both potential values, the linear calibration plots of the current peaks vs. concentration with very good correlation parameter higher than 0.99 were obtained. The linear voltammetric data recorded on both direct and reverse scanning were obtained in the presence of different urea concentrations. As example, in Figure 5 the linear scan voltammograms obtained on reverse scanning (from 1.25 V to -0.5 V vs. SCE) are presented, and a non-linear

dependence of the current peak height vs. urea concentration was reached (inset of Figure 5). This unexpected behaviour could be the result of continuously remove of the surface layers of Ag_2O in the presence of ammonium as a product of catalyzed hydrolysis of urea with formation of soluble complex of diammine silver ($\text{Ag}(\text{NH}_3)_2^+$), thus allowing the oxidation to proceed as the electrode is not oxidized in depth [18]. The CVs shown in Figure 6 present the comparative results in the presence of urea and ammonium ions and the behaviour similarity, as increase of the secondary oxidation peak, support this affirmation.

Figure 5. Linear scan voltammograms of EG-Ag-Z-Epoxy composite electrode in 0.1 M NaOH supporting electrolyte (1) and in the presence of different urea concentrations: 2-0.5 mM; 3-1 mM; 4-2 mM; 5-3 mM; 6-4 mM; potential scan rate 0.05 Vs^{-1} ; potential range: +1.25 to -0.5 V (arrow indicates the scanning direction). Inset: The dependence of linear scan voltammetric signal recorded at +0.55 V/SCE vs. urea concentrations.

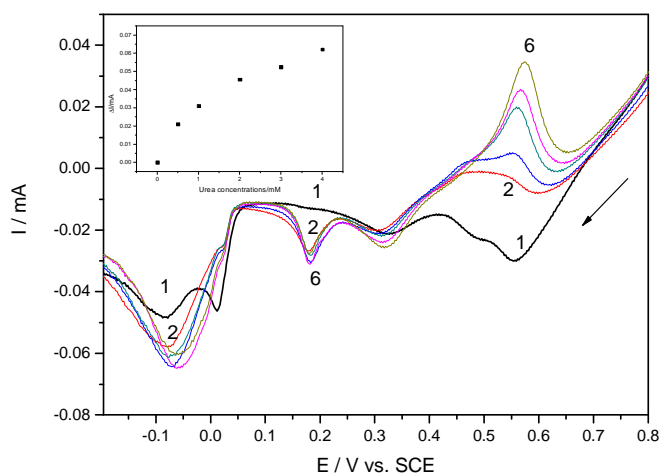
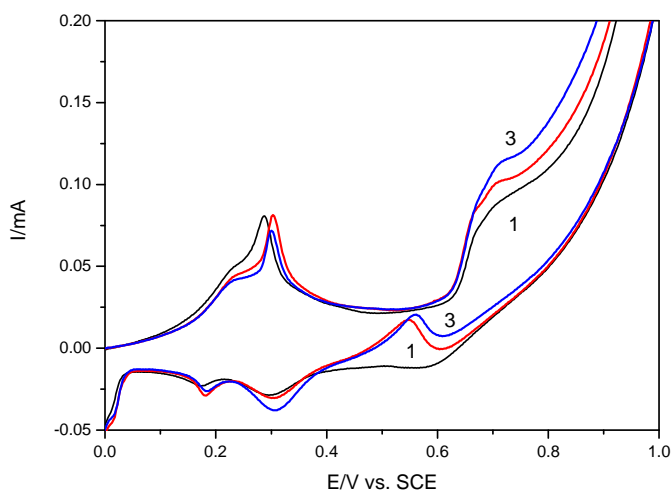


Figure 6. Cyclic voltammograms of EG-Ag-Z-Epoxy composite electrode in 0.1 M NaOH supporting electrolyte (1) in the presence of 0.8 mM urea (2) and in the presence of 0.2 mM ammonium (3).



2.3. Electrocatalytic voltammetric/amperometric determination of urea

The results obtained by using the differential pulse voltammetry on EG-Ag-Z-Epoxy composite electrode in 0.1 M NaOH and in the presence of different urea concentrations, and the variation of pulsed voltammetric peaks with urea concentrations (Inset of Figure 7) are shown in Figure 7. The electroanalytical parameters for the concentration ranges where linear proportionality was reached are also presented in Table 1. It has to be mentioned that analytical signals were recorded after preliminary conditioning at the potential value of +1.25V/SCE for 30 seconds in the presence of urea. No analytical signals occurred without preconditioning, an aspect that strengthens the idea that the signal corresponds to a soluble complex of diammine silver.

Figure 7. Differential pulse voltammograms recorded on EG-Ag-Z-Epoxy composite electrode with a potential scan rate 0.05 Vs^{-1} between 0 and 1V vs. SCE in a 0.1 M NaOH supporting electrolyte (1) and in the presence of different urea concentrations: 2-13: 0.2 mM -2.4 mM, urea concentration adding step of 0.2 mM. Inset: The dependence of differential pulsed voltammetric signal recorded at +0.38 V/SCE vs. urea concentrations

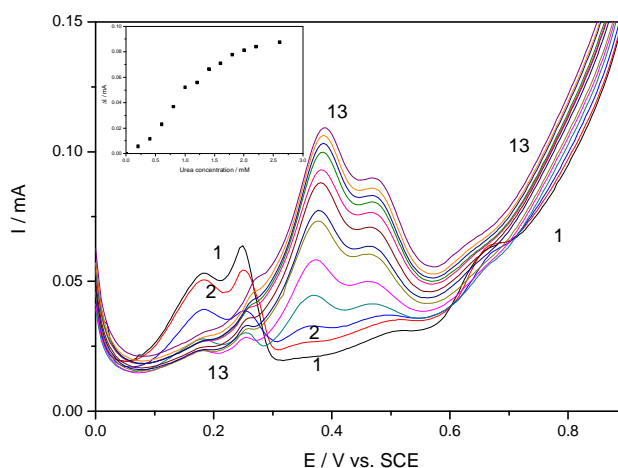


Table 1. Electroanalytical performance of EG-Z-Ag-Epoxy electrode for the detection of urea for the concentration range of 0.2 to 1.4 mM.

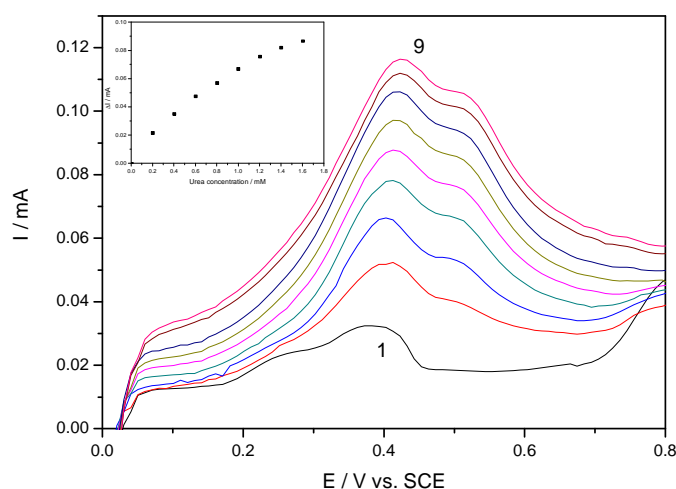
| Peak potential | Technique used | Sensitivity ($\text{mA}\cdot\text{mM}^{-1}$) | Correlation coefficient (R^2) |
|----------------|----------------------|--|-----------------------------------|
| +0.68 | CV | 0.033 | 0.994 |
| +0.55* | CV | 0.044 | 0.993 |
| +0.41 | SWV | 0.058 | 0.990 |
| +0.38 | DPV | 0.044 | 0.991 |
| +0.55 | CA | 0.030 | 0.999 |
| +0.38 | Preconcentration/DPV | 0.065 | 0.990 |

* recorded on the reverse scanning of CV

Differential pulsed voltammetric experiments show that the peaks corresponding to the electroformation of soluble $[\text{Ag}(\text{OH})_2]^-$ complex species and the electroformation of Ag_2O are influenced by increasing urea concentration. This may be the result of the competitive adsorption between ammonium and hydroxyl ion or a change in mechanism. The both peaks results from the formation of a soluble complex between ammonium and silver species.

The similar results were obtained also by using and square-wave voltammetry, and the voltammograms in relation with urea concentrations are presented in Figure 8. For the whole concentration range studied no linear increase of the amperometric signal vs. concentration was reached (inset of Figure 8). However, it has to be mentioned that shape of square-wave voltammograms is different versus differential-pulsed voltammograms, and the presumptive electroformation of soluble $[\text{Ag}(\text{OH})_2]^-$ complex species was not evidenced.

Figure 8. Square-wave voltammograms recorded on EG-Z-Ag-Epoxy electrode with a potential scan rate 0.05 Vs^{-1} between 0 and 1V vs. SCE in a 0.1 M NaOH supporting electrolyte (1) and in the presence of different urea concentrations: 2-9: 0.2 mM -1.6 mM, urea concentration adding step of 0.2 mM. Inset: The dependence of square-wave voltammetric signal recorded at +0.41 V/SCE vs. urea concentrations.



Batch amperometric measurements at constant applied potential (+0.55 V vs. SCE) prove that EG-Ag-Z-Epoxy composite electrode works well as amperometric sensor for urea determination. A typical example of amperometric response to successive adding of 0.4 mM urea is presented in Figure 9.

When the concentration of urea was higher than 1.4 mM, a response platform as a "catalytic saturation" was observed, showing a characteristic in a formal accordance with the Michaelis-Menten catalytic kinetic mechanism. The kinetic parameters could be obtained from the electrochemical version of the Lineweaver-Burk equation [19]. The apparent Michaelis-Menten type constant, K'_M , and I_{max} were determined by analysis of the slope and intercept of the plot of the reciprocals of the steady-state current recorded at 50 seconds versus urea concentration (Figure 10). The K'_M value of the EG-Ag-Z-Epoxy electrode was 1.6 mM and $I_{max} = 0.05 \text{ mA}$. Also, the sensitivity calculated as I_{max}/K'_M ratio was $0.03 \text{ mA} \cdot \text{mM}^{-1}$.

Figure 9. Chronoamperometric response of EG-Ag-Z-Epoxy composite electrode recorded at +0.55 V/SCE to successive adding of 0.4 mM urea (1-0.1 M NaOH supporting electrolyte; 2-8: 0.4 mM-2.8 mM urea).

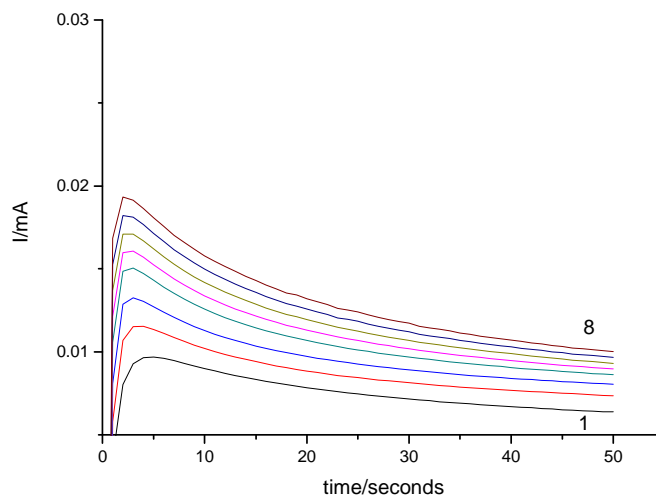
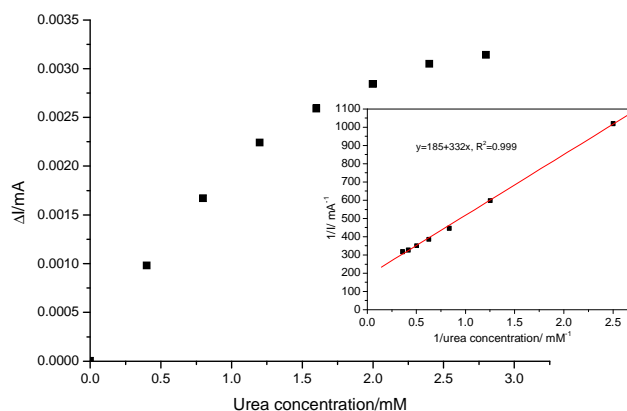


Figure 10. Calibration plot of EG-Z-Ag-Epoxy electrode for successive adding of 0.4 mM urea in 0.1 M NaOH; applied potential +0.55 V vs. SCE, conditioning at potential value of +1.25V vs. SCE for 30 seconds; Inset: Lineweaver Burke plot.



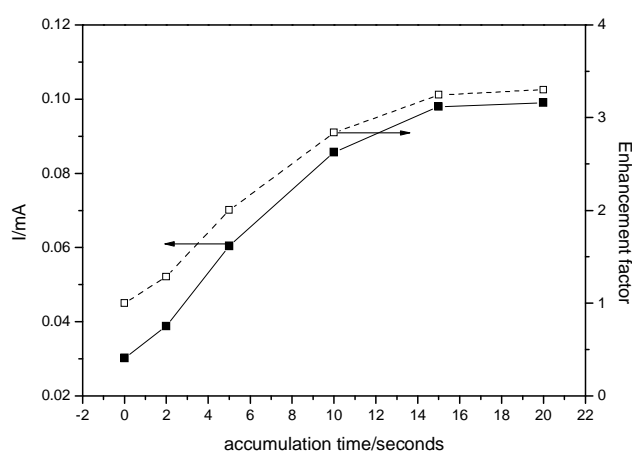
Under these working conditions, electroanalytical performances of EG-Z-Ag-Epoxy for urea determination was reached. Based on sorption properties of clinoptilolite for urea [20], the further experiments were carried out to apply a preconcentration-voltammetric detection scheme to get the better electroanalytical performances for urea detection.

2.4. Influence of accumulation time

The accumulation time is very important for using EG-Z-Ag-Epoxy electrode in a preconcentration-voltammetric detection scheme, because it could influence the degree of adsorption on the electrode surface. An important role that may be played by zeolite is its availability to concentrate species within

its porous structure [21]. This would also increase the amount of species available for reaction. To determine the enhancement factor as the ratio of peak current after and before sorption process, the effect of accumulation time on anodic peak current densities were investigated by DPV. Figure 13 shows the dependence of the useful signal corresponding to the anodic peak current corresponding to urea determination and enhancement factor on the accumulation time for 0.4 mM urea. By increasing accumulation time up to 15 minutes the amount of urea at electrode surface increased leading to the enhancement of useful signal. The enhancement factor of about 3 revealed an effective concentration effect of EG-Z-Ag-Epoxy electrode on urea. Also, it is well-known that the kinetics of the accumulation process depends on the nature of zeolite-modified electrode. The accumulation time of 15 minutes needed to reach the equilibrium suggested a diffusion effect of the solution in the bulk of the electrode, may be due to the electrode surface porosity [10]. The better electroanalytical performance obtained by using preconcentration- differential pulsed voltammetric detection scheme is gathered also in Table 1.

Figure 11. Peak current density responses and enhancement factor for the oxidation of 0.4 mM urea for EG-Z-Ag-Epoxy electrode, as a function of the accumulation time, with background current density subtraction. Detection was performed in 0.1 M NaOH supporting electrolyte by DPVs recorded at 0.38 V/SCE.



The equations of the linear dependencies of the current vs. urea concentration, with corresponding characteristic analytical parameters, working conditions and techniques applied in various situations are gathered in Table 1. The limit of detection (LD) determined as 0.05 mM urea, was evaluated based on $3S_B/b$ [22], where S_B is the standard deviation of the mean value of 5 voltammograms of the blank and b is the slope of the straight line in the analytical curve by using square-wave voltammetry. The reproducibility of the electrode using the above-mentioned technique was evaluated for three replicates measurements of urea detection. The relative standard deviation (RSD) of 3.2 % showed the good reproducibility of the electrode. A recovery test was also performed by analyzing three parallel tap water samples, which contain $60.07 \text{ mg}\cdot\text{dm}^{-3}$ urea. This test was run in 0.1 M NaOH as supporting electrolyte and a recovery of 98 % was found.

3. Experimental Section

The expanded graphite-Ag-zeolite-epoxy (EG-Z-Ag-Epoxy) composite electrode was obtained from two-component epoxy resin (LY5052, Araldite) mixed with conductive expanded graphite (EG) fillers powder (Conductograph, SGL Carbon) and silver modified zeolite (clinoptilolite). The ratio between the components was chosen to reach 20 weight percent (w/w) content of expanded graphite, 40 weight percent (w/w) content of silver-modified zeolite. Ag-modified zeolite with a content of 0.008 mg Ag /g zeolite was prepared using natural zeolite from Mirsid, Romania, with 68% wt. clinoptilolite as we previously described [23]. It was not possible to add the full amount of EG and Z-Ag to the matrix resin directly, due to the high surface area of the graphite flakes, therefore, the mixing was performed in a roll-mill at room temperature. The two parts of the epoxy were mixed together and the full amount of the EG was added in steps forming a thick paste. Then the epoxy was treated in a hot press at 50 °C for 60 minutes. Simultaneously the material was shaped in a plate of 1 mm thickness. The plate was slowly cooled down (for about 12 h) to the room temperature. The electrical conductivity of the electrode by four points resistance measurement [24] was found to be $18.28 \text{ S}\cdot\text{cm}^{-1}$. Discs with a surface area of 19.63 mm^2 were embedded in polyethylene and electrical contacts were made using a copper wire. Prior to use, the working electrode was gradually cleaned, first polished with abrasive paper, then polished with (0.3-0.1 μm) alumina powder in aqueous suspension on a pad and rinsing with distilled water. A Scanning Electron Microscope (Philips CM30T) was used to observe the working electrode surface.

The electrochemical behaviour/performance of the electrode was studied by cyclic voltammetry (CV), linear-scan voltammetry (LSV), square-wave voltammetry (SWV), differential pulse voltammetry (DPV) and chronoamperometry (CA). Subsequently, an electrochemical pre-treatment by three repetitive cyclings between -0.5 V to 1.25 V vs. SCE in 0.1 M NaOH supporting electrolyte was performed. All measurements were carried out using an Autolab potentiostat/galvanostat PGSTAT 302 (Eco Chemie, The Netherlands) controlled with GPES 4.9 software and a three-electrode cell, with a saturated calomel electrode as reference electrode, a platinum counter electrode and the composite working electrode. DPV is a pulse technique, dependent on applied parameters, i.e., a scan rate of $25 \text{ mV}\cdot\text{s}^{-1}$, a pulse modification of 50 mV in amplitude and 50 ms in duration at intervals of 200 ms. Also, SWV is a pulse technique and the parameters applied was the frequency of 10 Hz, amplitude of 0.45 mV, and the scan rate of $50 \text{ mV}\cdot\text{s}^{-1}$.

Accumulation was performed at open circuit potential (OCP) in the analyte solution, the electrode was then removed and rinsed with distilled water, and immersed in the detection cell for recording the voltammetric curve. Regeneration was achieved by mechanical polishing and above-mentioned electrochemical pretreatment.

4. Conclusions

An EG-Ag-Z-Epoxy composite electrode prepared from Romanian natural zeolitic volcanic tuff showed good mechanical strength and electrical conductivity. A random distribution and orientation of expanded graphite particles and Ag-zeolite particles in epoxy matrix was shown by SEM.

This composite electrode displayed a complex behaviour in 0.1 M NaOH supporting electrolyte with several electrochemically distinct species, involving the redox couple of AgO and Ag₂O. The moderate apparent charge transfer rate across the modified electrode interface within the potential range of two types of silver oxides should be correlated with particular structural characteristics of Ag-modified zeolite and suggests a charge transfer mechanism partially based on surface-mediated electron transfer [13].

This modified electrode exhibited an electrocatalytic effect via redox couple of AgO and Ag₂O towards urea oxidation allowing its determination in aqueous solution. The results obtained by all techniques used, *i.e.*, CV, LSV, DPV, SWV, CA supposed that the mechanism of urea determination by EG-Ag-Z-Epoxy composite electrode in 0.1 M NaOH was the result of continuously remove of the surface layers of Ag₂O in the presence of ammonium as a product of catalyzed hydrolysis of urea with formation of soluble complex of diammine silver (Ag(NH₃)₂⁺).

The electroanalytical sensitivity for the determination of urea at EG-Ag-Z-Epoxy composite electrode ranged from 0.030 to 0.065 mA·mM⁻¹ function of the technique used.

A moderate enhancement of electroanalytical sensitivity for the determination of urea at EG-Ag-Z-Epoxy composite electrode was reached by applying a chemical preconcentration step prior to voltammetric/amperometric quantification.

Taking into account that the application of this sensor in the electroanalysis of real water/wastewater samples or clinical diagnosis, our future research will be focused on to evaluate the influence of urea-associated chemicals as potential interfering agents.

Acknowledgements

This research was supported by the Romanian National Research of Excellence Programs- CEEX, RIWATECH-Grant 62/03.10.2005, PROAQUA-Grant 631/03.10.2005.

References

1. Iida, Y.; Suganuma, Y.; Matsumoto, K.; Satoh, I. Novel determination system for urea in alcoholic beverages by using an FIA system with an acid urease column. *Anal. Sci.* **2006**, *22*, 173-176.
2. Ferrer, S.J.; Granados, S.G.; Bedioui, F.; Ordaz, A.A. Amperometric detection of urea in aqueous solution by poly(Ni-cyclam) film-modified glassy carbon electrode. *Electroanalysis.* **2003**, *15*, 70-73.
3. Jin, J.-H.; Paek, S.-H.; Lee, C.-W.; Min, N.K.; Hong, S.-I. Fabrication of amperometric sensor based on nano-porous silicon technology. *J. Korean Phys. Soc.* **2003**, *42*, 735-738.
4. Yang, J.-K.; Ha, K.S.; Baek, H.S.; Lee, S.S.; Seo, M.L. Amperometric determination of urea using enzyme-modified carbon paste electrode. *Bull. Korean Chem. Soc.* **2004**, *25*, 1499-1502.
5. Veeneman, J.M.; Kingma, H.A.; Stellard, F.; de Jong, P.E.; Reijngoud, D.-J.; Huisman, R.M. Comparison of amino acid oxidation and urea metabolism in haemodialysis patients during fasting and meal intake. *Nephrol. Dial Transplant.* **2004**, *19*, 1533-1541.

6. Behrendt, J.; Arevalo, E.; Gulyas, H.; Nlederste-Hollenberg, J.; Niemiec, A.; Zhou, J.; Otterpohl, R. Production of value added products from separately collected urine. *Wat. Sci. Techn.* **2002**, *46*, 341-346.
7. Eggenstein, C.; Borchardt, M.; Diekmann, C.; Gründig, B.; Dumscaht, C.; Cammann, K.; Knoll, M.; Spener, F. A disposable biosensor for urea determination in blood based on a ammonium-sensitive transducer. *Bios. Bioelectron.* **1999**, *14*, 33-41.
8. Walcerz, I.; Stanislaw, G.; Koncki, R. Potentiometric enzyme electrode in a flow injection system for the determination of urea in human serum samples. *Anal. Chim. Acta.* **1998**, *369*, 129-137.
9. Walcarius, A. Zeolite-modified paraffin-impregnated graphite electrode. *J. Solid State Electrochem.* **2006**, *10*, 469-478.
10. Walcarius, A.; Rozanska, S.; Bessiere, J.; Wang, J. Screen-printed zeolite-modified carbon electrodes. *Analyst.* **1999**, *124*, 1185-1190.
11. Valdés, M.G.; Cordoves-Pérez, A.I.; Díaz-García, M.E. Zeolites and zeolite-based materials in analytical chemistry. *Trends Anal. Chem.* **2006**, *25*, 24-30.
12. Walcarius, A. Implication of zeolite chemistry in electrochemical science and applications of zeolite-modified electrodes. In *Handbook of zeolite science and technology*; Auerbach, S.M., Carrado, K.A., Dutta P.K. Eds.; Marcel Dekker: New York, USA, 2003; pp. 721-783.
13. Walcarius, A. Factors affecting the analytical applications of zeolite modified electrodes: indirect detection of nonelectroactive cations. *Anal. Chim. Acta.* **1999**, *388*, 79-91.
14. Hamlaoui, M.L.; Reybier, K.; Marrakchi, M.; Jaffrezic-Renault, N.; Martelet, C.; Kherrat, R.; Walcarius, A. Development of a urea biosensor based on a polymeric membrane including zeolite. *Anal. Chim. Acta.* **2002**, *466*, 39-45.
15. Laviron, E. General expression of the linear potential sweep voltammogram in the case of diffusionless electrochemical systems. *J. Electroanal. Chem.* **1979**, *101*, 19-28.
16. Mentus, S.V.; Bošković, I.; Pješčić, J.M.; Grudić, V.; Bogdanov, Ž. Tailoring the morphology and electrocatalytic properties of electrochemically formed Ag/TiO₂ composite deposits on titanium surfaces. *J. Serb. Chem. Soc.* **2007**, *72*, 1403-1418.
17. Jovič, B.M.; Jovič, V.D. Electrochemical formation and characterization of Ag₂O. *J. Serb. Chem. Soc.* **2004**, *69*, 153-166.
18. Hampson, N.A.; Lee, J.B.; Morley, J.R.; Scanlon, B. Oxidations including silver.V. The oxidation of primary aliphatic amines. *Can. J. Chem.* **1969**, *47*, 3729-3736.
19. Kamin, R.A.; Wilson, G.S. Rotating ring-disk enzyme electrode for biocatalysis kinetic studies and characterization of the immobilized enzyme layer. *Anal. Chem.* **1980**, *52*, 1198-1205.
20. Byler, D.M.; Gerasimowicz, W.V.; Stockette, V.M.; Eberl D.D. Infrared spectroscopic examination of the interaction of urea with the naturally occurring zeolite clinoptilolite. *Microchem. J.* **1991**, *44*, 130-139.
21. Li, Y-J.; Liu, C-Y. Silver-exchanged zeolite Y-modified electrodes: size selectivity for anions. *J. Electroanal. Chem.* **2001**, *527*, 117-120.
22. Codognoto, L.; Machado, S.A.S.; Avaca, L.A. Square wave voltammetry on boron-doped diamond electrodes for analytical determinations. *Diamond Relat. Mater.* **2002**, *11*, 1670-1675.

23. Orha, C.; Manea, F.; Pop, A.; Burtica, G.; Fazekas Todea, I. Obtaining and characterization of zeolitic materials with antibacterial properties. *Rev. Chim.* **2008**, *59*, 173-177.
24. Mironov, V.S.; Kim, J.K.; Lim, S.; Cho, W.K. Comparison of electrical conductivity data obtained by four-electrode and four-point probe methods for graphite-based polymer composites. *Polymer Testing.* **2007**, *26*, 547-555.

© 2008 by the authors; licensee Molecular Diversity Preservation International, Basel, Switzerland. This article is an open-access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/3.0/>).