

Voltammetric determination of Nordiazepam at meniscus modified silver solid amalgam electrode

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(Presented at the XIIIth Conference with International Participation "Present State and Perspectives of Analytical Chemistry in Practice", 1–4 June, 2014, Bratislava)

Abstract: New electrochemical method for determination of Nordiazepam (NDZ) at meniscus modified silver solid amalgam electrode (m-AgSAE) was developed utilizing differential pulse voltammetry (DPV). The pH effect on the current response of NDZ was studied in the mixture of Britton-Robinson buffer (BR) and methanol (9:1) with optimum pH value of 10. The calibration dependences were examined under the optimal conditions and linearity in the range from 2×10^{-6} to 1×10^{-4} mol L⁻¹ with limit of quantification of 1.7×10^{-6} mol L⁻¹ were accomplished.

Keywords: meniscus modified silver solid amalgam electrode, differential pulse voltammetry, Nordiazepam

Introduction

Nordiazepam (NDZ Fig. 1) belonging to a group of 1,4-benzodiazepines is produced as a drug with commercial trade names Nordaz, Stilny, and Calmday (Kintz 2014). This drug as well as other 1,4-benzodiazepines is used for treatment of insomnia, anxiety, panic disorder, and nausea (Roškar and Dolenc 2010).

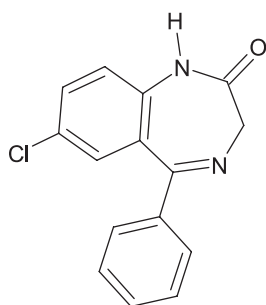


Fig. 1. Chemical structure of Nordiazepam.

Benzodiazepines interact allosterically with neuron GABA_A receptors facilitating the interaction between the receptor and the neurotransmitter, which leads to a decrease in neuronal activity and causes anxiolytic and anticonvulsant effect (Tysczuk 2010, Auta et al. 2010). Overdose causes drowsiness, hypotension, impaired motoric function, and coma (Seger 2004). NDZ is eliminated by metabolism of liver – hydroxylation and conjugation with glucuronic acid. Hydroxylated metabolite (oxazepam) is excreted by bile and urine in the form of glucuronide conjugates (Tysczuk 2010).

From the view of electroanalytical chemistry, benzodiazepines (BDZ) contain azomethine functional group in the position four of the benzodiazepine ring that can be easily reduced at m-AgSAE by a two-electron reduction (Carvalho et al. 2010). Solid amalgam electrodes are easy and cheap to construct and they are stable for several weeks (Mikkelsen and Schröder 2003). These electrodes are non-toxic and they exhibit the same electrochemical properties as mercury electrodes (Tvrdíková et al. 2010). Moreover, they can be used in field analysis (Vaňková et al. 2006). Other methods have also been used for determination of NDZ including high performance liquid chromatography (HPLC), gas chromatography (GC), spectrophotometry, and titrimetric method (Wallace et al. 1979 and Mostafa and Algothani 2010). In comparison with these techniques, voltammetric methods have a lot of advantages as low cost instrumentation, simple process, high selectivity, and speed (Carvalho et al. 2010).

The objective of this work was to find the optimal pH of BR in the mixture with methanol (9:1) as supporting electrolyte for sensitive determination of NDZ and characterize analytical performance of developed method. According to our knowledge, the NDZ determination has not been studied at m-AgSAE till now.

Experimental

Chemicals

All chemicals used for the preparation of solutions were of analytical reagent grade and purchased from

Sigma-Aldrich (Czech Republic), Lachema (Brno, Czech Republic) and Lach-Ner (Neratovice, Czech Republic). Stock solution of NDZ $1 \times 10^{-3} \text{ mol L}^{-1}$ was prepared by dissolving 2.7 mg of NDZ in 10 mL methanol in volumetric flask and kept in refrigerator. 0.2 mol L^{-1} stock solution of KCl was used for electrochemical activation of m-AgSAE. Methanol and BR were used as supporting electrolytes. BR was prepared by mixing of 0.2 mol L^{-1} NaOH with 0.04 mol L^{-1} acids (98 % CH_3COOH , H_3BO_3 , 85 % H_3PO_4). Prior to analysis, particular solution in electrochemical cell was purged with nitrogen gas to remove oxygen. All solutions besides NDZ were prepared using deionized water (Milli-Q plus system, Millipore, USA).

Electrochemical Apparatus

A three-electrode system consisting of (working) meniscus modified silver solid amalgam electrode (approx. 0.5 cm^2 , Polaro-Sensors, Czech Republic), $\text{Ag}|\text{AgCl}|3 \text{ mol L}^{-1} \text{ KCl}$ reference electrode, and Pt-plate (Monokrystal, Turnov, Czech Republic) serving as the counter were connected to electrochemical analyzer Eco-Tribo Polarograph with software PolarPro v. 5.1 (Polaro-Sensors, Praha, Czech republic). Three pretreatment steps as amalgamation, electrochemical activation, and regeneration were performed with working electrode m-AgSAE.

Pretreatment of m-AgSAE

The amalgamation was carried out once a week. The tip of m-AgSAE was immersed into the liquid mercury for 15 seconds.

The electrochemical activation was carried out under the potential -2.2 V in $0.2 \text{ mol L}^{-1} \text{ KCl}$ stirred solution for 300 seconds. The procedure was realized either after the amalgamation step or pause longer than one hour.

The regeneration was performed in the measured solution. The regeneration lasting 30 seconds before each measurement was carried out by periodical switching every 0.1 seconds between the potentials -1400 mV and -800 mV .

Voltammetric Measurements

Differential pulse voltammograms were recorded using the following parameters: scan rate: $v = 10 \text{ mV s}^{-1}$; pulse height: -50 mV and pulse width: 80 ms . The potential scan was carried out between -0.4 and -1.4 V for measurement of the pH effect, the range -0.9 and -1.3 V was used for measurement of concentration dependence.

Spectrophotometric apparatus and measurements

Spectrophotometric measurements were performed on Pye-Unicam PU 8800 UV/VIS Spectrophotometer (Cambridge, Great Britain). Spectrum of

NDZ solution in methanol ($1 \times 10^{-4} \text{ mol L}^{-1}$) was recorded from 200 to 400 nm against methanol as blank in 1 cm quartz cell. NDZ stock solution was measured every 7 days for a period of the month.

Results and discussion

Stability of NDZ stock solution

The stability of NDZ stock solution was studied by UV spectrophotometry. The absorption spectrum of NDZ contains one maximum at 239 nm . The values of absorbance measured at this wavelength (Table 1) proved the sufficient stability of NDZ solution for a month.

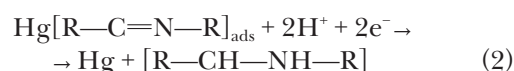
Tab. 1. Stability of NDZ stock solution.

Days	1	7	14	21	28
A_{239}	1.502	1.494	1.482	1.49	1.493
(%)	100	99.5	98.6	99.2	99.4

Electrochemical behaviour of NDZ

Based on our former results (Samiec et al. 2014) obtained for determination of Diazepam at m-AgSAE a mixture of BR and methanol was chosen as a possible medium for study of the current response of NDZ. DPV was applied in the mixtures of BR and methanol (9:1) of varying pH value containing $1 \times 10^{-3} \text{ mol L}^{-1}$ NDZ exhibited voltammograms showed in Figs. 2a and 2b. The highest current signal was obtained in mixture of BR and methanol (9:1) of the pH value 10; the current response decreased significantly at pH 11 and 12 in comparison to Diazepam exhibiting the increasing current responses in the pH range 11–13.

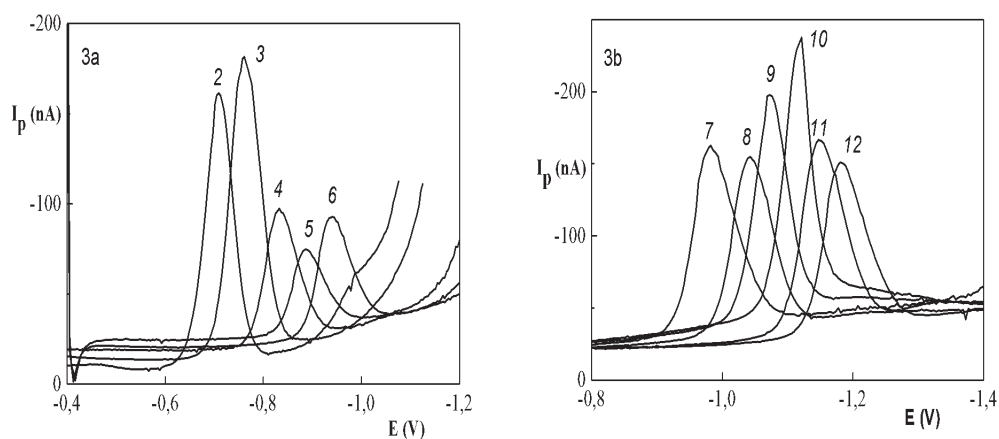
The pH effect of supporting electrolyte on the peak potential of NDZ can be described by equation $E \text{ (V)} = -0.047 \text{ pH} - 0.640$ ($R = 0.9988$). The slope value indicates that reduction mechanism of azomethine involves transferring of two electrons and two protons at m-AgSAE, according to the previously stated reactions (Carvalho et al. 2010) evidenced below:



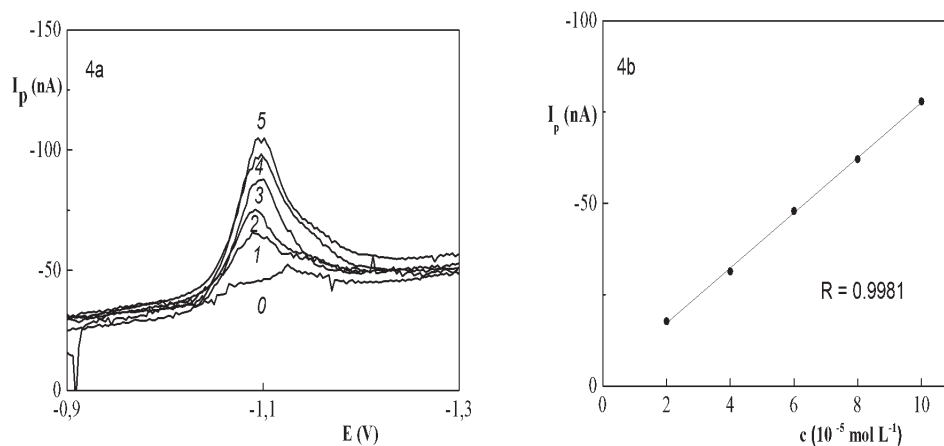
The mixture of BR and methanol (9:1) of the pH value 10 was selected as an optimal supporting electrolyte for further measurements.

Analytical performance of method

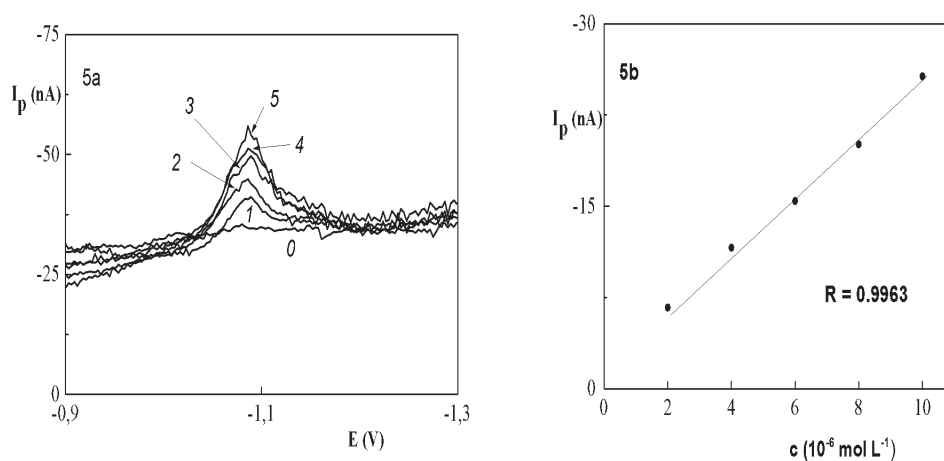
The concentration dependences were measured under the optimum conditions in the concentration range from 2×10^{-5} to $1 \times 10^{-4} \text{ mol L}^{-1}$ (Figs. 3a,



Figs. 2a, 2b. The DPV current responses of NDZ ($1 \times 10^{-4} \text{ mol L}^{-1}$) at different pH of BR and methanol (9:1) mixture. Number of the curves denotes the actual pH of the mixture.



Figs. 3a, 3b. DP voltammograms of NDZ in the mixture of BR and methanol (9:1). NDZ concentration: 0 (0), 2 (1), 4 (2), 6 (3), 8 (4), 10 (5) $\times 10^{-5} \text{ mol L}^{-1}$.



Figs. 4a, 4b. DP voltammograms of NDZ in the mixture of BR and methanol (9:1). NDZ concentration: 0 (0), 2 (1), 4 (2), 6 (3), 8 (4), 10 (5) $\times 10^{-6} \text{ mol L}^{-1}$.

3b) and in the concentration range from 2×10^{-6} to $1 \times 10^{-5} \text{ mol L}^{-1}$ (Figs. 4a, 4b). Limit of quantification (LOQ) was calculated as $10\sigma/a$ where σ is standard deviation from the lowest measured concentration and a is slope of the calibration line. Repeat-

ability of the method was calculated from three successive measurements of $2 \times 10^{-6} \text{ mol L}^{-1}$ NDZ (RSD = 8.37 %).

The LOQ value of $1.7 \times 10^{-6} \text{ mol L}^{-1}$ is higher in comparison with determination of other benzodi-

azepines by voltammetric methods. For example, LOQ for Diazepam was found to be 7 nmol L^{-1} in the case of adsorptive cathodic stripping voltammetry at the lead film electrode (Tysczuk 2010). The LOQ of 73 nmol L^{-1} for Diazepam can be also achieved on modified carbon paste electrode (Chaves et al. 2006). However, these methods are based on adsorption of NDZ enabling the higher sensitivity.

Conclusion

The voltammetric method for determination of Nordiazepam at meniscus modified silver solid amalgam electrode was developed. Stability of stock solution NDZ was proved for one month. The mixture of BR and methanol (9:1) of pH 10 was found as the optimal supporting electrolyte. The linearity of the concentration dependences was proved in the range from 2×10^{-6} to $1 \times 10^{-4} \text{ mol L}^{-1}$. Limit of quantification was calculated to be $1.7 \times 10^{-6} \text{ mol L}^{-1}$. Repeatability of the method was computed to be 8.37 (RSD %). The obtained results represent the first study on the electrochemical behaviour of Nordiazepam and on a possibility to determine it on meniscus modified silver solid amalgam electrode. The study on improvement of the method as regards to sensitivity, interference study and practical feasibility is in progress.

Acknowledgement

This research was financially supported by Ministry of Education Youth and Sports, Czech Republic (MSM 0021620857), Grant Agency of Czech Republic (project P206/10/P087) and OU SGS -SGS05/PřF/2014.

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