

Voltammetric Determination of Diazepam at Meniscus-Modified Silver Solid Amalgam Electrode

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Abstract: Voltammetric methods for determination of Diazepam (DZ) were developed. Techniques differential pulse voltammetry and DC voltammetry were used for determination of DZ at meniscus-modified silver solid amalgam electrode (m-AgSAE). The effect of the pH on the intensity of signal of DZ was studied in a mixture of Britton-Robinson buffer + methanol (9:1) and 0.1 M NaOH + methanol (9:1); the optimal medium for analysis was a mixture of 0.1 M NaOH + methanol (9:1). The developed method has been tested on the determination of DZ in a sample of commercial drug (Diazepam).

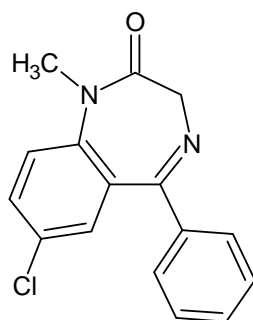
Keywords: Meniscus modified silver solid amalgam electrode; DC voltammetry; Differential pulse voltammetry; Diazepam; Determination.

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Introduction

Diazepam (DZ) is produced as drug with commercial trade names Apaurin, Seduxen, Stesolid, Diazepam, and Desitin. In medicine it is used for anxiolytic and anticonvulsant effect [2,3]. DZ is entering human body by oral and injection way. DZ is bound to proteins in plasma and is easily dissolved in lipids.

This drug is easily divided to all tissues and it is eliminated by metabolism of liver [4,5]. The so-called demethylation is a process within which DZ is converted to nordiazepam (NDZ). NDZ is hydroxylated into oxazepam leaving body in urine [5,6]. Excessive use of DZ can lead to psychological dependence and increases also desire for alcohol. Patient with overdose has one or more of the following symptoms: drowsiness, hypotension, impaired motoric function, and a coma. The oral LD50 (lethal dose, 50 %) of DZ is 720 mg/kg for mice and 1240 mg/kg for rats. Patients taking 2000 mg DZ falls into a deep coma. Electroanalytical methods, such as DC voltammetry and DPV, can be used for determination of this drug because DZ contains double bond in its structure that is easily reduced [6]. Modified silver solid amalgam electrode has several advantages. The advantages of m-AgSAE are its non-toxicity, robustness, and easy manipulation [7].



Scheme 1:
The chemical structure of Diazepam (DZ).

Experimental

Chemicals and Solutions

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 DZ 1×10^{-3} M was prepared by dissolving 2.8 mg of DZ in 10 mL methanol (MeOH) in volumetric flask and kept in refrigerator. 0.2 M stock solution of KCl was used for electrochemical activation of m-AgSAE. Methanol, 0.1 M stock solution of NaOH and Britton-Robinson buffer(s) (BR-B) were used as the supporting electrolytes. BR-B was prepared by mixing of 0.2 M NaOH with 0,04 M acid ingredient (98% CH_3COOH , H_3BO_3 , 85% H_3PO_4). Analyzed samples were purged with nitrogen gas to remove oxygen. All solutions besides DZ were prepared using deionized water from laboratory purification system (Millipore-Q plus, Millipore, USA).

Electrochemical Apparatus

A three-electrode system consisting of (the working) meniscus-modified silver solid amalgam electrode (ca. 0.5 cm², Polaro-Sensors, Czech Republic), Ag|AgCl|3M KCl reference, and Pt-plate (Monokrystal, Turnov, Czech Republic) serving as the counter electrode were connected to electrochemical analyzer Eco-Tribo polarograf and controlled via the software PolarPro 5.1 Pro (Polaro-Sensors). Three different pretreatments; namely, amalgamation, electrochemical activation, and regeneration were performed with the working electrode, m-AgSAE, or its surface, respectively.

Voltammetric Measurements

Differential pulse voltammetry (DPV). Differential pulse voltammograms were recorded using the following parameters: scan rate, $v=20$ mV s⁻¹; pulse height, -50 mV; pulse width, 80 ms. The potential scan was carried out between -0.3 and -1.5 V for measurements of the pH effect, -1 and -1.4 V for the concentration dependence (calibrations).

DC voltammetry (DCV). DC voltammograms were recorded at scan rate of 20 mV s⁻¹. The potential scan was carried out in the same way as above.

Results and Discussion

Optimization of Experimental Parameters

Effect of pH. Electrochemical behavior of drug was studied with DC voltammetry and DPV at different pH of BR-B and MeOH (9:1) (pH 12) and in 0.1 M NaOH and MeOH (9:1) (pH 13.2) containing 1.10⁻³ M DZ. Fig. 1 and Fig. 2 indicate voltammograms of DZ in solution of BR-B and MeOH (9:1), whereas Fig. 3 shows voltammogram of DZ in 0.1 M NaOH and MeOH (9:1). The highest signal was provided in 0.1 M NaOH and MeOH (9:1). This medium was chosen as optimal for the determination of DZ.

Calibration Measurements. The concentration dependence was measured under the optimum conditions in the concentration range from 1 × 10⁻⁴ to 6 × 10⁻⁶ M with DC voltammetry and from 1 × 10⁻⁴ to 2 × 10⁻⁶ M with DPV (see Figs. 4-7). Limit of determination was found to be 6.6 × 10⁻⁶ M with DCV and 1 × 10⁻⁶ M with DPV.

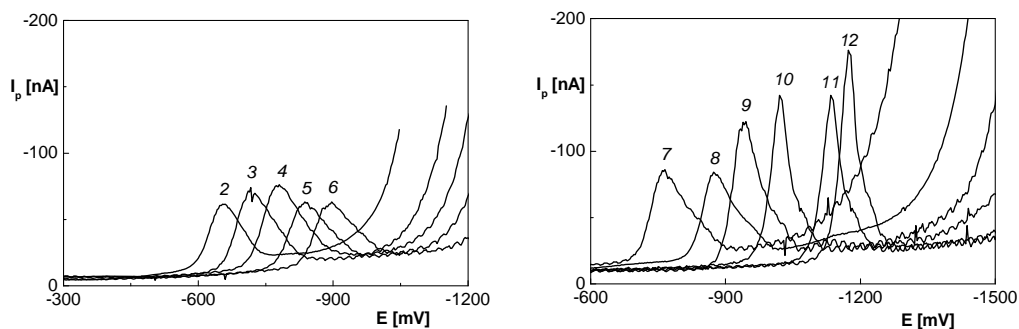


Fig. 1: The current responses of DZ with differential pulse voltammetry at different pH of BR-B and MeOH (9:1). Number describing the curves corresponds to the actual pH of solution of BR-B and MeOH (9:1).

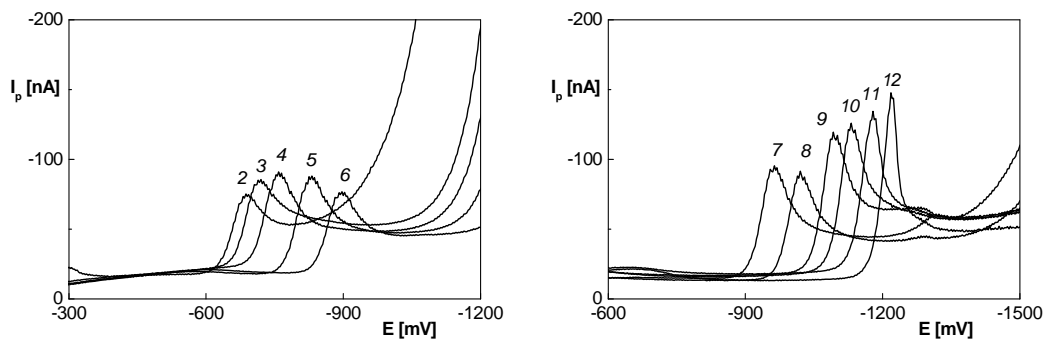


Fig. 2: The current responses of DZ with DC voltammetry at different pH of BR-B and MeOH (9:1). Number above the curves corresponds to the actual pH of BR-B and MeOH (9:1).

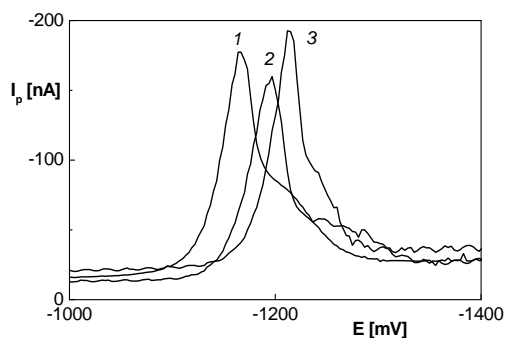


Fig. 3: The current responses of DZ with DPV in BR and MeOH (9:1) and 0.1 M NaOH and MeOH (9:1). BR and MeOH (9:1) pH 12 (1), 0.01 M NaOH and MeOH (9:1) pH 13 (2), 0.1 M NaOH and MeOH (9:1) pH 13.2(3).

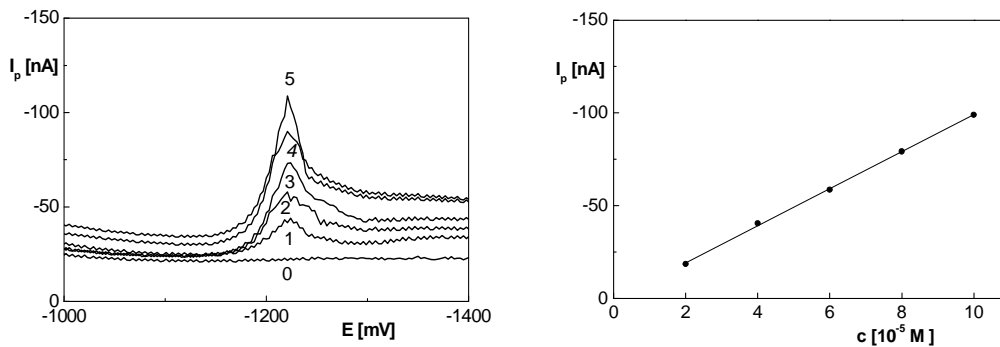


Fig. 4: DC voltammograms of DZ in 0.1 M NaOH and MeOH (9:1). DZ concentration: 0 (0), 2 (1), 4 (2), 6 (3), 8 (4), 10 (5) $\times 10^{-5}$ M.

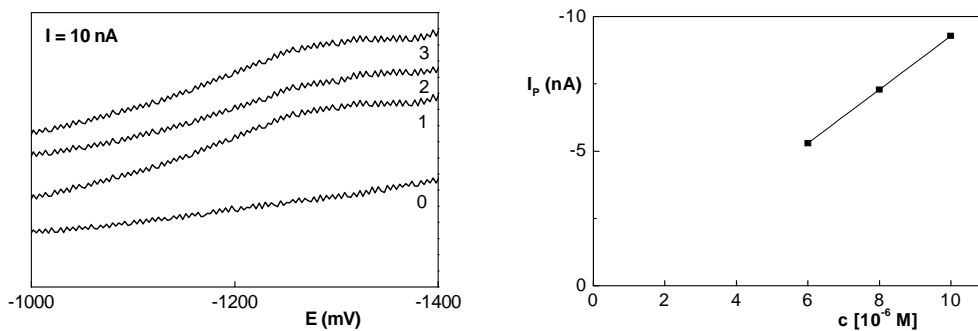


Fig. 5: DC voltammograms of DZ in 0.1 M NaOH and MeOH (9:1). DZ concentration: 0 (0), 2 (1), 4 (2), 6 (3) $\times 10^{-6}$ M.

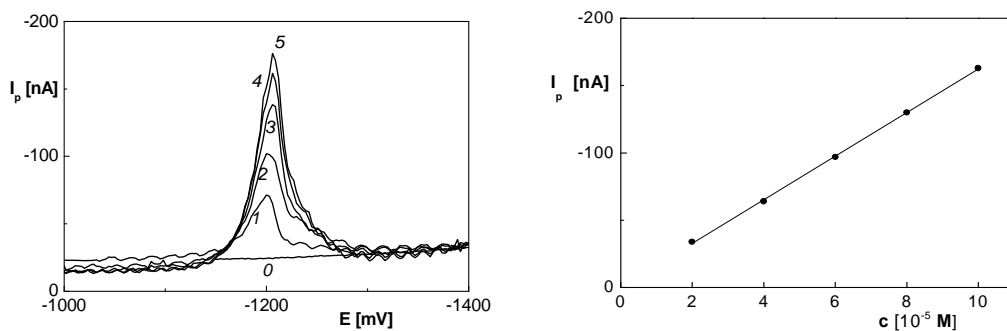


Fig. 6: DPV voltammograms of DZ in 0.1 M NaOH and MeOH (9:1). DZ concentration 0 (0), 2 (1), 4 (2), 6 (3), 8 (4), 10 (5) $\times 10^{-5}$ M.

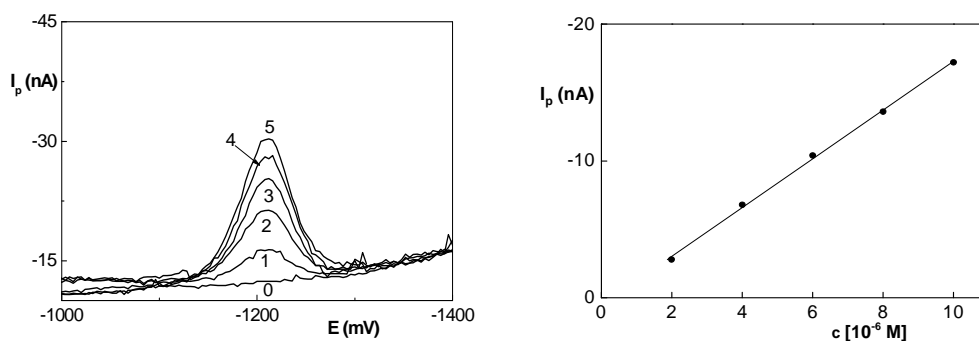


Fig. 7: DC voltammograms of DZ in 0.1 M NaOH and MeOH (9:1). DZ concentration 0 (0), 2 (1), 4 (2), 6 (3), 8 (4), 10 (5) $\times 10^{-6}$ M.

Analysis of Real Sample. Applicability of the developed method was tested on the determination of the drug sample Diazepam (Zentiva, 2 mg). Determination was performed by means of the standard addition method, with two consecutive additions of 100 μ l of the analyte stock solution – DZ.

The drug sample was analysed with the aid of the DPV technique, which is illustrated by the corresponding DP voltammograms in Fig. 8; the results of this test determination being summarized in Table I (see overleaf).

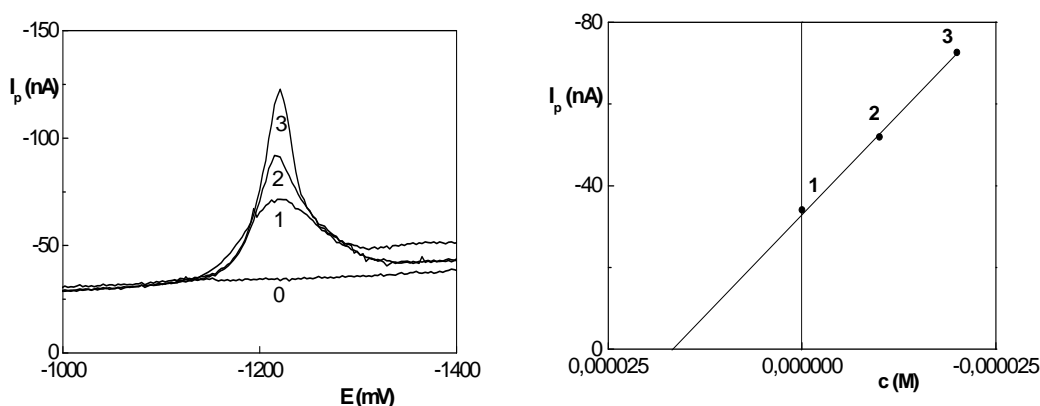


Fig. 8: Determination of DZ with the standard addition method: 0.1 M NaOH and MeOH (9:1) 0 (0), sample of DZ (1), 100 μ l (2), 200 μ l (3) (1×10^{-3} M DZ).

Table I: Summary of the declared and found content of DZ in the drug sample Diazepam.

Medium	Declared content (mol·l ⁻¹)	Found (mol·l ⁻¹)	Yield (%)
0.1 M NaOH + MeOH (9:1)	2×10^{-5}	1.65×10^{-5}	82.5

Conclusions

The simple voltammetric method for determination of diazepam at m-AgSAE was developed, when a mixture of 0.1 M NaOH and MeOH (9:1) was selected as the optimal background electrolyte. The linearity of calibration curves was examined and proved in the range from 1×10^{-4} to 6×10^{-6} M using DC voltammetry and from 1×10^{-4} to 2×10^{-6} M with the DPV. Limit of quantification was estimated to be 6.6×10^{-6} M with DC voltammetry and 1.0×10^{-6} M when using DPV. The method was successfully used for the determination of diazepam in a commercially marketed formulation *Diazepam* (Slovakofarma, Hlohovec; SVK); the content found being 1.65×10^{-5} mol·l⁻¹.

Acknowledgements

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