Voltametrické stanovení stopových množství methyloranže pomocí diferenční pulsní voltametrie na stříbrné amalgamové elektrodě

S teoretickými základy moderních polarografických a voltametrických metod jsou posluchači seznámeni během přednášky „Elektrochemické metody" a během přednášky „Organická polarografie a voltametrie". Optimalizace podmínek pro stanovení strukturně příbuzné methylčerveně diferenční pulsní voltametrií je popsána v poslední části tohoto návodu. Z didaktických důvodů je příloha v originále, tj. v anglickém jazyce. Podrobnosti o pevných stříbrných amalgamových elektrodách lze nalézt v článku Novotný L., Yosipchuk B.: Chem. Listy 94, 1118 (2000).

Aparatura: Počítačem řízený EkoTribo Polarograf se softwarem PolarPro verze 2.0 (Polarosensors, Praha). Pracováno bude vždy v tříelektrodovém zapojení s nasycenou argentchloridovou referentní elektrodou a platinovou drátkovou pomocnou elektrodou . Jako pracovní elektroda bude použita stříbrná amalgamová elektroda (Polarosensors, Praha).

Ovládací program

Popis programu: PolarPro je program pro měření a vyhodnocení koncentrace látek ve vzorcích analyzovaných na EkoTribo Polarografu. Program ve verzi Pro umožňuje též návrh a úpravu metod měření. Tento uživatelsky přívětivý program pracuje v operačním prostředí Windows a jeho ovládání je názorné a jednoduché. Zvládnutí tohoto programu usnadňuje snadno dostupná rozsáhlá nápověda.

Požadavky programu: EkoTribo Polarograf; stojánek + převodníková karta Polaro 4 (vše Polarosensors Praha)
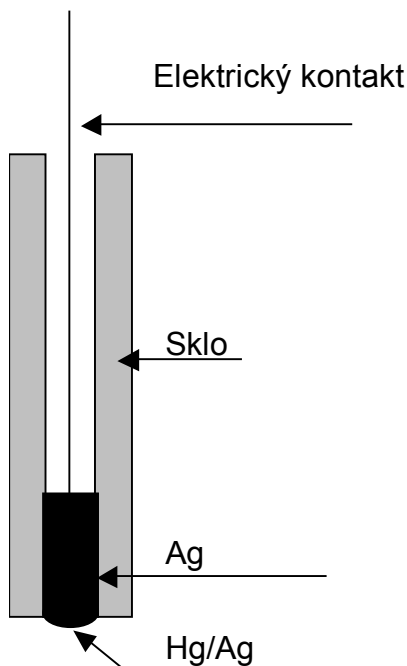Windows 3.1 CE nebo Windows 95, volné 2 MB na disku, myš.
Minimální doporučená sestava: procesor 486, RAM paměť 8 MB.

Omezení programu: maximální počet křivek v jednom souboru – 24; maximální počet vyhodnocovaných látek v jednom souboru – 8

Stříbrná tuhá amalgamová elektroda

Schéma AgSAE

Příprava elektrody k měření

Před zahájením měření se špička tuhé stříbrné amalgamové elektrody ponoří do malého objemu rtuti v semimikrozkumavce na 15 s za intenzivního třepáni. V případě nutnosti je možno rtuťový meniskus obnovit mechanickým přečištěním povrchu elektrody a jejím opětovným kontaktem se rtutí.

Elektrochemická předúprava rtuťové amalgamové elektrody

V prostředí 0,2 M-KCl se na elektrodu vloží potenciál -2,2 V po dobu 300 s za intenzivního míchání roztoku a poté se elektroda opláchne destilovanou vodou.

Pracovní postup:

1. Proměřte vliv pH na diferenční pulsní voltamogramy methyloranže

(c = 1.10^{-5} mol/L) na stříbrné amalgamové elektrodě v prostředí Brittonova-Robinsonova pufru o pH 2-13 a nalezněte pH při kterém látka poskytuje nejlépe vyvinuté a nejsnáze vyhodnotitelné voltamogramy.

2. Při tomto pH změřte kalibrační přímky v koncentračním rozmezí 1.10^{-5} až 1.10^{-7} mol/L s pravidelným krokem (0,2,4,6,8 a 10 v každém koncentračním řádu).

3. Statisticky vyhodnoťte všechny sestrojené kalibrační závislosti a vypočtěte odpovídající meze stanovitelnosti

4. Pomocí sestrojené kalibrační křivky určete koncentraci methyloranže v neznámém vzorku.

Metoda: DP voltametrie

Potenciál		Parametry metody	
počáteční Ein	+150 mV	doba bublání	300 s
konečný Efin	-700 mV	počet měření	3
rychlost	20 mV/s	výška pulsu	- 50 mV
		šířka pulsu	80 ms

Vyhodnocení se provede pomocí podprogramu „HODNOCENÍ", který je součástí používaného programu Polar Pro, verze 2.0.

Upozornění: Do jednoho souboru lze uložit maximálně 24 křivek

Voltammetric determination of N, N-Dimethyl-4 – Amino-2´-
Carboxyazobenzene at a Silver Solid Amalgam Electrode

Key words: N, N- Dimethyl-4 – amino- 2´-carboxyazobenzene,
Voltammetry, Silver Solid Amalgam Electrodes

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Abstract

The voltammetric behaviour of N, N- dimethyl-4-amino-2`- carboxyazobenzene was investigated by Differential Pulse Voltammetry at a meniscus silver solid amalgam electrode (m-AgSAE). Optimum conditions have been found for its determination by DPV at m-AgSAE in the concentration range of 0.4 to 15.2 μmol.l^{-1}.

Introduction

Derivatives of N, N-dimethyl-4- amino-2`-carboxyazobenzene are among the best known genotoxic azocompounds (1) and are often used as model substances in various toxicological studies. It follows from QSAR correlation between the biological activity and the structure of N, N-dimethyl-4- aminoazobenzene derivatives that their carcinogenity generally increases with increasing lipophility (2). Even very small amount of these substances can have a detrimental effect on biological processes (3,4) and thus a great deal of attention has been paid to analytical methods for the determination of trace amounts of these substances in both biological samples and environment (5). The easy polarographic reduction of azocompounds, whose

mechanism is discussed in monographs (6,7) permits the very sensitive determination of a number of genotoxic derivatives of N, N-dimethyl-4-aminoazobenzene (8-10) using modern techniques such as Differential Pulse Polarography (DPP), Differential Pulse Voltammetry (DPV) at a mercury dropping electrodeelectrode or Adsorptive Stripping Voltammetry (AdSV). This work is concerned with the use of differential pulse voltammetry for the determination of trace amounts of a genotoxic azocompound, using a non- toxic meniscus silver solid amalgam electrode (m-AgSAE), which has a good mechanical stability, simple handling and regeneration including an electrochemical pre- treatment of its surface, etc. In absence of specific interactions between the analyte and silver the DPV- peak potentials on m-AgSAE and HMDE are nearly the same. (11)

Experimental

Reagents

The stock solution of N, N-dimethyl-4-amino-2`-carboxyazobenzene (c= 0.1 mmol.l^{-1}) was prepared by dissolving 0.00291 g of the substance (Sigma) in 100 ml of de-ionised water. All the solutions were stored in the dark. It followed from the spectrophotometric study of the stability of these solutions (12) that the solution with concentration of 1 mmol.l^{-1} must be prepared fresh once a month, 0.1 mmol.l^{-1} every week and 0.01 mmol.l^{-1} daily. Britton – Robinson buffers were prepared in a usual way (13). De-ionised water was produced by a Milli-Q $_{plus}$ system.

Apparatus

DPV measurements were carried out using computer driven Eco- Tribo- Polarograph with Polar Pro software, version 4.0 (both POLARO- SENSORS, Ltd., Prague) in combination with a three electrode arrangement with a platinum wire auxiliary electrode and saturated silver/ silver chloride reference electrode, to which all the potential values are referred. The working electrode is a meniscus silver solid amalgam electrode, consisted of a drawn- out glass tube, the bore of which near the tip was filled with a fine silver powder, amalgamated by liquid mercury and connected to an electric contact (14).

Then it was immersed into a small volume of liquid mercury and agitation for 15 seconds. The m- AgSAE could be used for several weeks without major changes,

only its regeneration through mechanical contact with mercury is recommended to be repeated every week.

Before starting the work, as well as after every pause longer than one hour the electrochemical pre- treatment of AgSAE was applied in 0.2 M KCl at –2.2 V under stirring of the solution for 300 seconds and then rinsed with distilled water. The diameter of m-AgSAE disc is 0.54 mm (15).

Where not stated otherwise, work with m-AgSAE was carried out at a scan rate of 20 mV/s and the pulse amplitude of –50 mV. Air oxygen was removed from the measured solution by bubbling with nitrogen for five minutes. Before every measurement the solution was stirred with mechanical stirrer for ten seconds.

The solution pH was measured with PHM 62 digital pH meter (Radiometer, Copenhagen, Denmark) using combined electrode.

All the measurements were carried out at laboratory temperature.

Procedures

The calibration curves were measured in triplicate and evaluated by the least squares linear regression method. The limits of determination and detection were calculated as the tenfold standard deviation from 7 analyte determinations at the concentration corresponding to the lowest point on the appropriate calibration straight line (16).

The electrochemical cell was filled with 10 ml of Britton- Robinson buffer from pH 2 to 11, containing 2 ?mol.l-1 of the test substance in order the influence of pH to be defined. Oxygen was removed from the measured solutions by purging with nitrogen for five minutes and stirred, using mechanical stirrer.

Results and discussions

The influence of pH on the differential pulse voltammograms of N, N-dimethyl-4-amino-2`-carboxyazobenzene is documented by Fig.1 The dashed line corresponds to the baseline from which the peak height was measured.
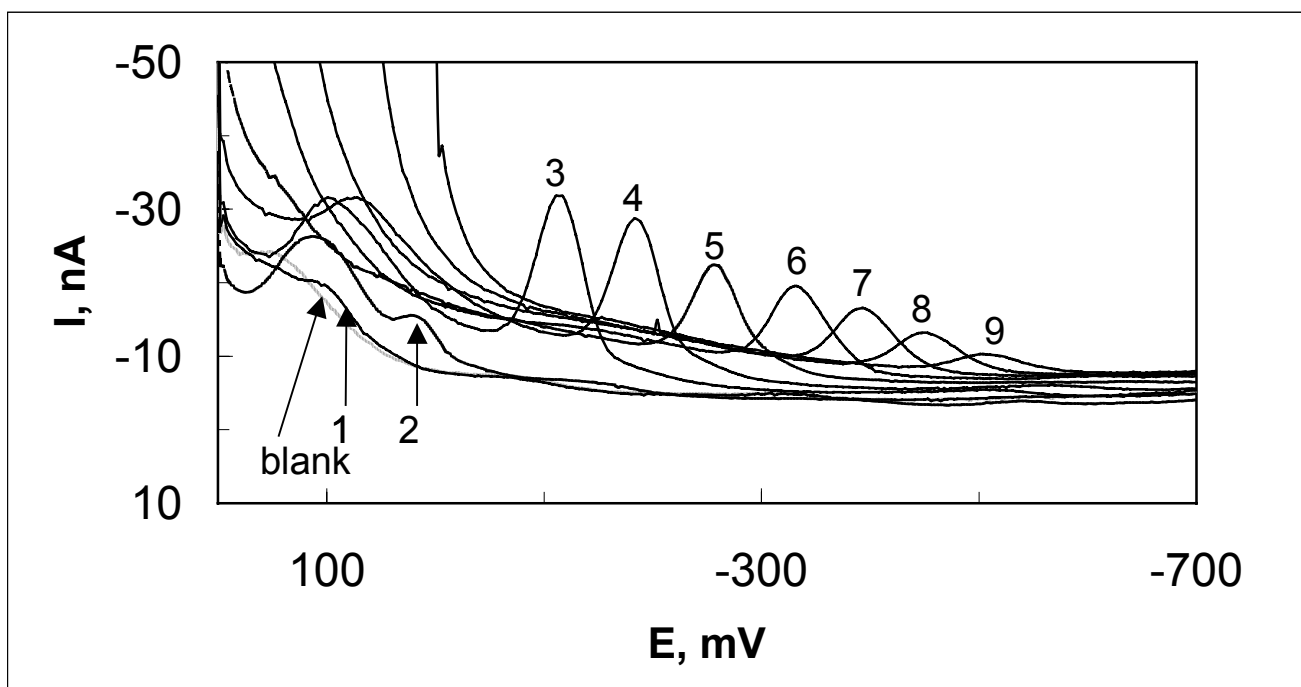
Fig.1

Differential pulse voltammograms of N, N- dimethyl-4-amino-2`-carboxyazobenzene, c= 2 μmol.l^{-1} at m-AgSAE in a Britton- Robinson buffer, pH: (1) 2.0, (2) 3.0, (3) 5.0, (4) 6.0, (5) 7.0, (6) 8.0, (7) 9.0, (8) 10.0, (9) 11.0.

The dependence of peak potential Ep, mV of N, N-dimethyl-4-amino-2`-carboxyazobenzene, c= 2 μmol.l^{-1} on the pH 2-12 measured by DPV at m-AgSAE is shown on Fig.2.
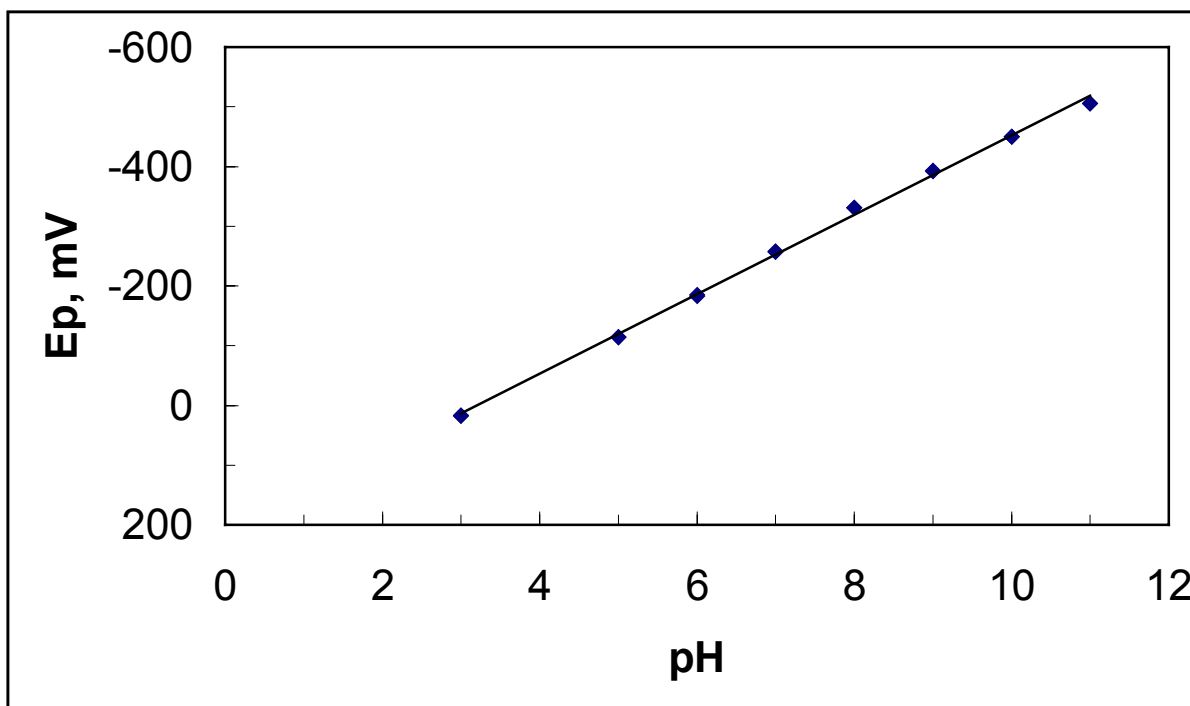
Fig. 2

The dependence of peak potential Ep, mV of N, N-dimethyl-4-amino-2`-carboxyazobenzene, c= 2 μmol.l^{-1} vs. pH, measured by DPV at m-AgSAE.

The method of linear regression yielded the relationship

Ep (mV)= -66.495pH + 213.21 (correlation coefficient 0.9990) over the region of pH 2.0- 11.0. The highest, best developed and most easily evaluated peak was obtained at pH 5.0 (see Fig.1). Fig.3 presents the differential pulse voltammograms of the tested substance at m-AgSAE in a Britton-Robinson buffer, pH 5.0, concentration range from 1.9 to 15.2 μmol.l^{-1}. The dashed line corresponds to the base line from which the peak height was measured.
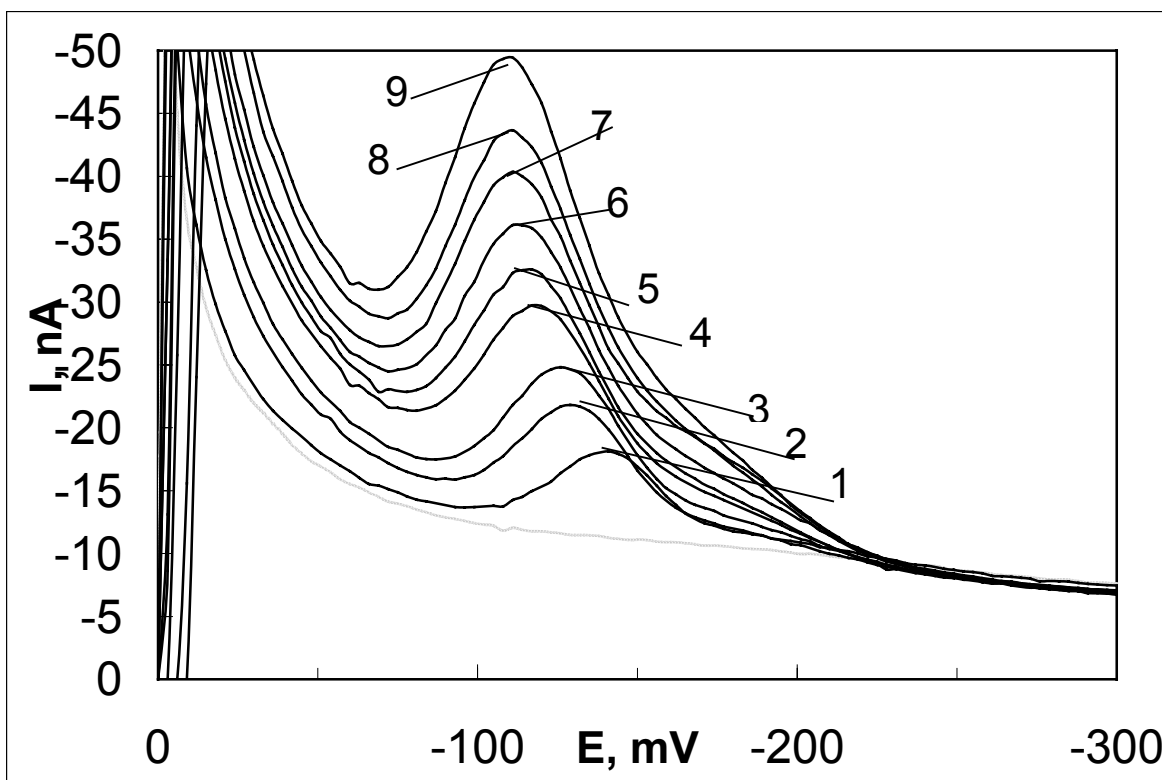
Fig.3

Differential pulse voltammograms of N, N-dimethyl-4-amino-2`-carboxyazobenzene at m-AgSAE in a Britton – Robinson buffer, pH 5.0, c, $\mu mol.l^{-1}$: (0) base electrolyte, (1) 1.9, (2) 3.8, (3) 5.6, (4) 7.4, (5) 9.0, (6) 10.7, (7) 12.2, (8) 13.7, (9) 15.2

The calibration curves are linear within the concentration range of 1.9 to 15.2 $\mu mol.^{l-1}$ and their parameters are given in Table I.

Table I

C, $\mu mol.l^{-1}$	Slope, $mA.mol^{-1}l$	Intercept, nA	Correlation coefficient	Limit of determination, $\mu mol.l^{-1}$	Limit of detection, $\mu mol.l^{-1}$
0.4- 15.2	-1.15	-1.32	0.9872	9.65	1.17

Parameters of the calibration straight lines for the determination of N, N-dimethyl-4-amino-2`-carboxyazobenzene using DPV at m-AgSAE in a Britton- Robinson buffer, pH 5.0

Fig.4 represents the calibration curve for the determination of the tested substance in a Britton- Robinson buffer, pH 5.0 using DPV at m-AgSAE, concentration range from 1.9 to 15.2 μmol.l^{-1}.
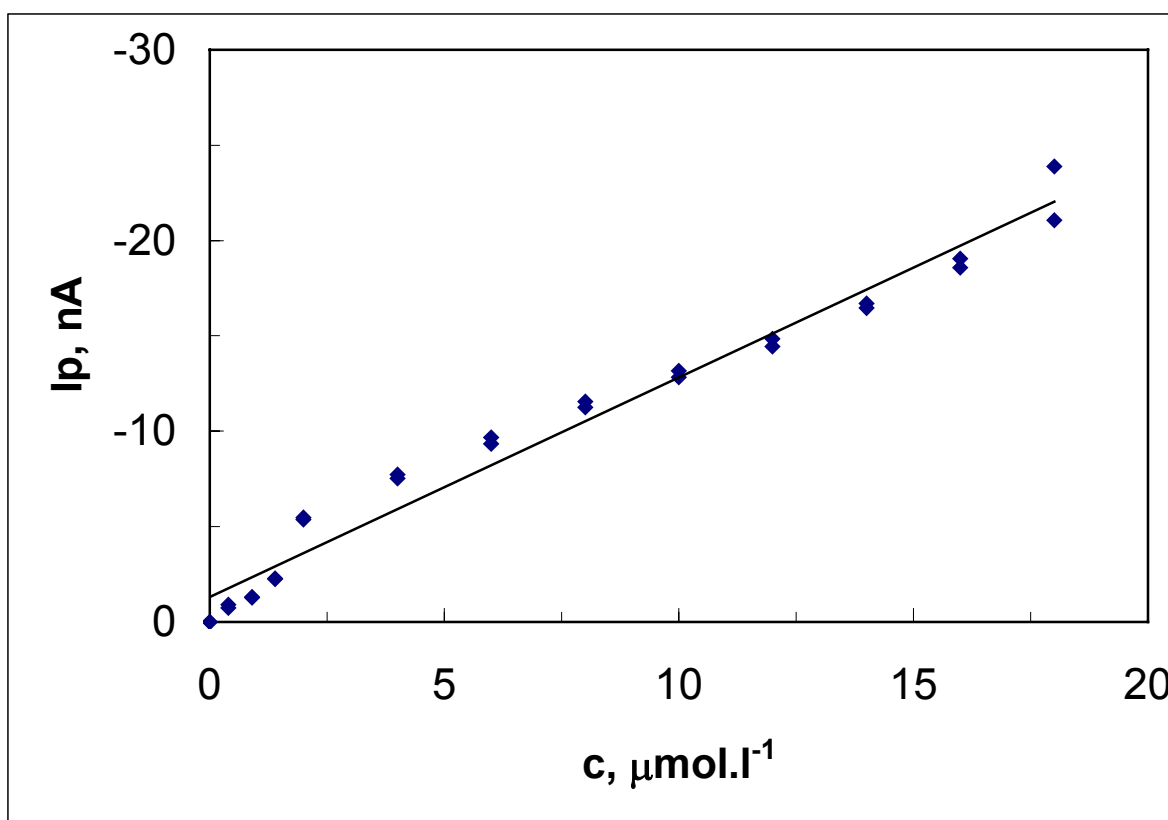

Fig.4

Calibration curve for the determination of N, N-dimethyl-4-amino-2`-carboxyazobenzene in a Britton- Robinson buffer, pH 5.0 using DPV at m- AgSAE, concentration range 1.9- 15.2 ?mol.l^{-1}

Conclusions

The limits of determination using m-AgSAE is around 1.17 ?mol.l^{-1}, i.e. only slightly higher than for differential pulse polarography on a dropping mercury electrode or differential pulse voltammetry on a hanging mercury dropping electrode. Therefore, AgSAE is suitable sensor for the determination of submicromolar concentrations of azocompounds. It has a good reproducibility and sensitivity and in many cases AgSAE represents an effective and simpler alternative to the HMDE.

Among other aspects, the solid amalgam electrodes provide good mechanical stability, simple handling and new fields of application in electrochemical techniques.

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