

The First Study of the Electrochemical Behavior of Mephenoxalone and its Determination Using Screen-Printed Sensors With Chemically Deposited Boron Doped Diamond Electrode

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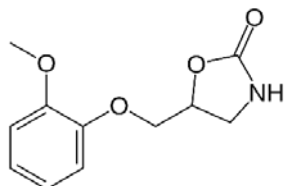
Abstract

Electrochemical oxidation of a mephenoxalone (MNL) was firstly studied using boron doped diamond electrode (BDDE). At different pH, an irreversible anodic peak was observed at potential of +1.375 V (vs. Ag/AgCl). The voltammetric method for MNL determination was developed using square wave voltammetry. This method provides low limit of detection (5.5×10^{-8} mol L⁻¹), wide linear dynamic range (1.0×10^{-7} - 3.0×10^{-5} mol L⁻¹), and good recovery in the analysis of model samples as well as pharmaceutical preparation Dimexol 200 mg. The developed method was successfully transferred to the commercially available and lab-made screen-printed sensors with chemically deposited BDDE.

Key words: Mephenoxalone, Dimexol, Electrochemistry, Voltammetry, Screen-printed sensors, Boron doped diamond electrode.

Introduction

Mephenoxalone (MNL, IUPAC name: 5-[(2-methoxyphenoxy)methyl]-1,3-oxazolidin-2-one) is the active substance in pharmaceuticals which has a nephroprotective effect and helps with anxiety in humans¹⁻³, so it acts as a tranquilizer⁴⁻⁷. MNL has relaxing properties for muscles and provides a pronounced sedation without causing serious side effects in humans^{6,8}. MNL in combination with paracetamol has an analgesic character⁹. Studies in dogs and rats have shown that long-term exposure to MNL leads to anemia, weight loss, hemolytic anemia and death in animals^{1,2,10,11}. Therefore, such animal side effects require clear and precise control of the concentration of MNL in the environment.



Scheme 1: Structural formula of mephenoxalone.

To date, different instrumental detection methods are used to determine MNL, such as high-performance liquid chromatography with fluorescence detection^{4,12,13}, spectrophotometry³, and nuclear magnetic resonance¹⁴ (NMR). All of these methods are exact, sensitive and selective. On the other hand, they are also expensive, instrumentally demanding, time consuming, and often require complicated sample preparation before analysis. An acceptable alternative may be electrochemical methods, which are characterized by no less accuracy, speed, simplicity, low cost and relatively simple sample preparation.

Until now, there is no information on the electrochemical behavior of MNL. Also, there is no information about the possibility of voltammetric determination of MNL in the literature. Therefore, this work will be devoted to the study of the electrochemical behavior of MNL at BDDE and to the development of a method for its determination. BDDE was chosen as working electrode due to its excellent electrochemical properties including a wide available potential window (3 V), low background current, good chemical resistance, low current noise, resistance to passivation, and high hardness¹⁵⁻¹⁸. BDDEs have been used frequently to determine a variety of drugs and health-hazardous substances¹⁹. In recent years, more and more scientific progress is focused to the miniaturization of equipment, speeding up the process and reducing the cost of analysis of various substances. Screen-printed sensors (SPE) are a partial solution to these problems, as they reduce the amount of aliquot to be analyzed and allow field analysis to be performed using mini-potentials. Therefore, in this work, the possibility of using printed sensors with chemically deposited BDDE (SP/BDDE) to determine the MNL was also be tested.

Experimental

Britton-Robinson buffer (BRB, pH 2–12) was prepared by mixing the acidic and alkaline components under a pH meter. The acidic component was a 0.04 M solution of H₃PO₄, H₃BO₃ and CH₃COOH. The alkaline component was created by 0.2 M NaOH. 0.1 M HNO₃, 0.05 M H₂SO₄, 0.1 M H₂SO₄, 0.1 M HCl, 0.1 M HClO₄ were diluted from concentrated 65 % HNO₃, 96 % H₂SO₄, 70 % HCl, 64.5 % HClO₄ (all from Ing. Petr Švec-PENTA s.r.o., Czech Republic). 1×10⁻³ M solution of MNL (Sigma-Aldrich) was prepared by dissolving the appropriate batch in acetonitril (Ing. Petr Švec-PENTA s.r.o., Czech Republic) and stored in a refrigerator. Solutions with lower concentrations were prepared fresh by diluting them with BRB daily. The pharmaceutical preparation Dimexol 200 mg (Glenmark Pharmaceuticals Distribution s.r.o., Czech Republic) was analyzed as a practical sample.

Voltammetric measurements were performed using Autolab PGSTAT204 (Metrohm Autolab, Netherlands) equipped with software Nova 2.1. Measurements were performed in a three-electrode set up with bulk BDDE (BioLogic, active surface area of 7.07 mm², inner diameter of 3 mm, B/C ration during deposition 1000 ppm) as a working electrode (WE), saturated argentchloride electrode (Ag/AgCl (KCl, sat.)) as a reference (RE) and platinum wire as a counter (CE) electrode (both Monokrystaly, CZ). Commercially available SP/BDDE (DropSens, active surface area of 10.17 mm², inner diameter of 3,6 mm, B/C – producer does not provide this information) whih WE from BDD, CE from carbon, and RE from silver. The lab-made (LM) SP/BDDE was the second one consisted of BDD as WE as well as CE and silver RE (active surface area of 0.785, 3.14, 7.07 mm², inner diameter of 1, 2, 3 mm, B/C 312,500 ppm). In all cases, BDDEs were activated at the beginning of the working day by performing 20 cyclic voltammograms in the potential range from initial potential (E_{in}) of -1000 mV to switching potential (E_{switch}) of +2200 mV directly in the supporting electrolyte used. Activation procedure was terminated at the positive potential value, *i.e.*, at the final potential (E_{fin}) +2200 mV. It was found that there was no need to reactivate or regenerate the electrode surfaces in any way between particular measurements. The measurement took place on the O-terminated electrode surface. Prior to measurements with SP/BDDEs, only as many cycles were performed until the recorded signals were stable.

Parameters of calibration curves and confidence intervals were calculated on the level of significance 0.05. Limit of detection (*LOD*) and limit of quantification (*LOQ*) were calculated from the calibration dependences as three times standard deviation and ten times standard deviation of an intercept divided by a slope.

Results and discussion

Voltammetric behavior of MNL ($2.0 \times 10^{-5} \text{ mol L}^{-1}$) was firstly studied using the bulk BDDE in slightly acidic medium of BRB of pH 5 at the scan rate of 100 mV s^{-1} . The obtained cyclic voltammograms are shown in **Figure 1A**. On the obtained voltammograms there is one clearly expressed peak at the potential +1375 mV, and a little pronounced second peak +1580 mV. On the cathode part of the voltammograms there are no corresponding reduction peaks, which indicates that this oxidation reaction is irreversible. As it was found from the following studies of the effect of pH of the electrolyte on the anode signals, the optimal pH value is 9, because only one peak of MNL oxidation was observed. When using a solution of 0.1 M HNO_3 , two distinct, separated peaks of anodic oxidation of MNL are observed, but the results are not reproducible. When using different types of acids (H_2SO_4 , HCl , HClO_4) as a supporting electrolyte, the same situation is observed as in the BRB buffer, the peaks are not separated. Based on the dependences of the peak height on the scan rate, respectively on the square root of the scan rate it follows that these reactions are controlled by diffusion. When studying the electrochemical behavior of MNL at different electrodes (bulk BDDE, SP/BDDE and LM-SP/BDDE) there is a shift of the oxidation peak potential to a more negative region, which is most associated with the use of different reference electrodes. The height of the oxidation peaks is slightly lower on the SPS compared to the bulk BDDE (**Figure 1B**).

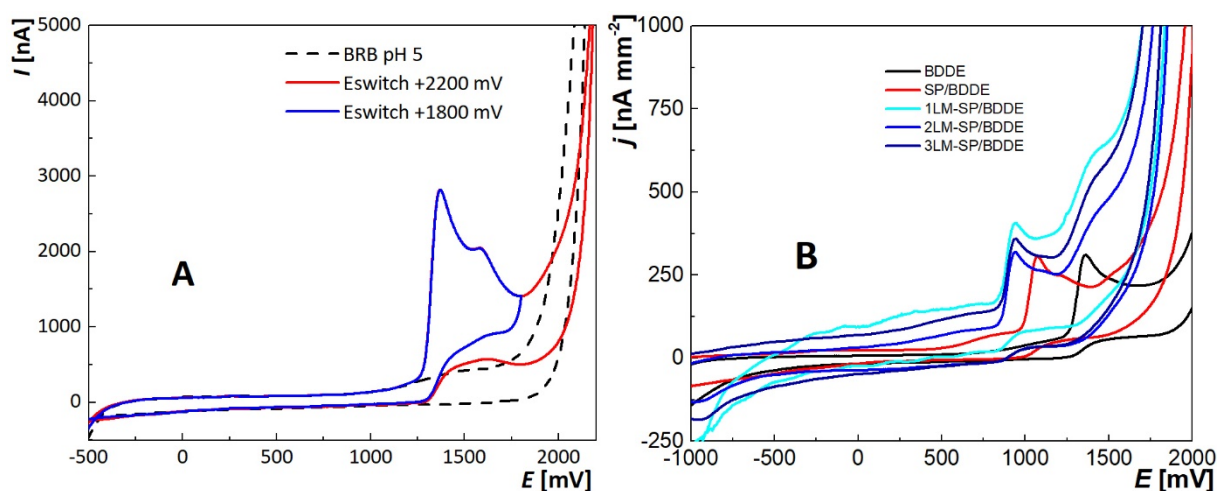


Fig. 1. Cyclic voltammograms of $2.0 \times 10^{-5} \text{ mol L}^{-1}$ MNL recorded on the BDDE (A) and on bulk BDDE, SP/BDDE, and LM-SP/BDDEs recalculated to current densities: Method – CV, $E_{\text{in}} = E_{\text{fin}} = -1500 \text{ mV}$, $E_{\text{switch}} = +2200 \text{ mV}$, $v = 100 \text{ mV s}^{-1}$; supporting electrolyte – BRB (pH 5 (A) and 9 (B)).

The square wave voltammetry (SWV) provides higher current peaks of MNL compared to the differential pulse voltammetry (DPV). Therefore, this method was applied for its determination and the BRB (pH 9) was chosen as the supporting electrolyte. The next stage was optimizing of SWV parameters – scan rate (v) = 50 mV s^{-1} , modulation amplitude (A) = $+40 \text{ mV}$, and frequency (f) = 25 Hz . Moreover, the insertion of pre-treatment procedures such as CV, cathodic and anodic polarization and polishing were tested. Highest and well repeatable peak was achieved after cycling. Therefore, this procedure was chosen as the most suitable. Finally, the concentration dependences were measured and the basic statistical parameters as linear dynamic range (LDR), LOD, and LOQ were calculated (**Table 1**). The ranges of linear dependence of peak height on MNL concentration for all electrodes are shown in **Figure 2A**.

Table 1

Statistical parameters for MNL determination

Electrode	LRD [mol L ⁻¹]	LOD ^a [mol L ⁻¹]	LOQ ^a [mol L ⁻¹]	RSD ₁₁ ^b [%]
BDDE	1.0×10 ⁻⁷ –3.0×10 ⁻⁵	5.5×10 ⁻⁸	1.8×10 ⁻⁷	0.89
SP/BDDE	3.0×10 ⁻⁷ –2.1×10 ⁻⁵	9.4×10 ⁻⁸	3.1×10 ⁻⁷	0.65
1LM-SP/BDDE	4.0×10 ⁻⁷ –3.0×10 ⁻⁵	9.9×10 ⁻⁸	3.3×10 ⁻⁷	0.66
2LM-SP/BDDE	3.0×10 ⁻⁷ –3.0×10 ⁻⁵	7.4×10 ⁻⁸	2.5×10 ⁻⁷	0.60
3LM-SP/BDDE	5.0×10 ⁻⁷ –3.0×10 ⁻⁵	5.8×10 ⁻⁸	1.9×10 ⁻⁷	0.95

^aCalculated from the recorded concentration dependence in the range of 5×10⁻⁷–1×10⁻⁵ mol L⁻¹.^bCalculated from the 11 repeated measurements at the concentration of MNL of 5×10⁻⁶ mol L⁻¹.

In the following step, this method was verified in analysis of model solutions with MNL concentrations of 1.0×10⁻⁶ and 5.0×10⁻⁶ mol L⁻¹ using the standard addition method (SAM). The analysis was 5 times repeated and the corresponding statistical parameters were calculated. In addition, the developed method was also applied in the analysis of the medicinal product Dimexol 200 mg. In general, the obtained results were correct and very well repeatable ($RSD_5 < 2.6\%$), which confirms that the method is suitable for the determination of MNL.

The final step was to check the reproducibility of the results between the individual pieces of electrodes of the same type. This feature is especially important with regard to the possibility of using SPE as disposable sensors. And it is known that the reproducibility of printed sensors can be a problem. For this purpose, 10 repeated measurements of 1.0×10⁻⁵ mol L⁻¹ MNL were performed on a series of all tested sensors and the obtained peak heights are summarized in **Figure 2B** after recalculation to the current densities. It is clear that all the types of sensors investigated show very good reproducibility ($RSD_5 < 5.0\%$).

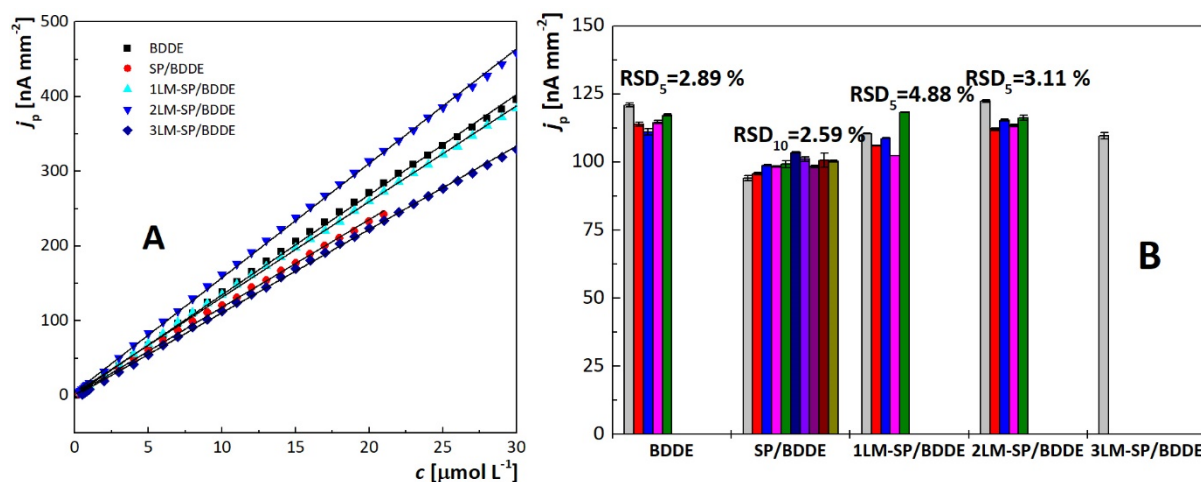


Fig. 2. Dependences of current densities on MNL concentration obtained on all tested electrodes (A) and peak height diagram of MNL (1.0 × 10⁻⁵ mol L⁻¹) obtained on all tested electrodes (B): Method – SWV, supporting electrolyte – BRB (pH 9), $E_{in} = 0$ mV, $E_{fin} = +2000$ mV, $v = 50$ mV s⁻¹, $A = +40$ mV, $f = 25$ Hz.

Conclusion

In this work, the electrochemical behavior of mephenoaloxone on a BDDE was studied for the first time. The oxidation reaction of MNL is irreversible and is controlled by diffusion. The method for determination of MNL was developed, which was successfully applied in the analysis of model solutions and commercially available pharmaceutical preparation Dimexol 200 mg. This new method was tested also using SPE with chemically deposited BDDE. The repeatability of the voltammetric measurement of MNL on one sensor as well as the

reproducibility on a series of SP/BDDEs was confirmed very good. The results indicate the suitability of the developed method for MNL determination applying different types of BDD sensors, *i.e.*, different arrangement of an electrochemical cell with BDDE as working electrode.

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