Department of Analytical Chemistry[1], Faculty of Chemical and Food Technology, Slovak University of Technology, Department of Pharmaceutical Chemistry[2], Faculty of Pharmacy, Comenius University, Bratislava, Slovakia

# Interaction of some esters of 2-, 3-, 4-alkoxyphenylcarbamic acids with surface-bound DNA at a dsDNA modified electrode

**Study of local anaesthetics: part 164**

M. Vaníčková[1], J. Labuda[1], J. Lehotay[1], J. Čižmárik[2]

*Received February 12, 2003, accepted February 19, 2003*

*Prof. RNDr. J. Čižmárik, PhD, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Comenius University, Odbojárov 10, 832 32 Bratislava 2, Slovak Republic*
*cizmarik@fpharm.uniba.sk*

*Pharmazie 58: 570–572 (2003)*

An interaction of some derivatives of 2-, 3-, 4-alkoxyphenylcarbamic acids with dsDNA was investigated using the anodic voltammetric signal at a DNA-based electrochemical biosensor. Significant, however, reversible association was found, particularly in weak acidic and neutral solution where the protonated forms of the drug molecules occur which are electrostatically bound to the negatively charged DNA backbone. Total drug liberation from the DNA layer within 1 min. was observed in blank buffer solution without drug molecules.

## 1. Introduction

DNA-based biosensors with a surface DNA layer represent powerful tools for the investigation of host-guest interactions between DNA and small molecules such as drugs and other chemicals [1–5]. At these sensors, the surface-bound DNA can mimic, to some degree, the behavior of native DNA bound in cells. In principle, the structural interaction of small aromatic molecules with DNA can occur through three modes: electrostatic binding to the negatively charged sugar-phosphate backbone, groove binding and intercalation between the base pairs of double helix. In recent years, conventional voltammetric electrodes modified by a thin DNA layer have been used as simple devices for electrochemical investigation of interactions of anticancer drugs based on antibiotics [6–10] and platinum compounds [11] as well as antidepresive [12, 13] and other drugs [14, 15]. In this paper, we report for the first time an association interaction of three local anaesthetics such as pyrrolidinocyclohexylester of 3-pentyloxyphenylcarbamic acid (pentacaine, PC) [16], 2-(diethylamino)-1-methylester of 2-heptyloxyphenylcarbamic acid (carbiocaine, CC) [17], and piperidinoethylester of 2-heptyloxyphenylcarbamic acid (heptacaine, HC) [18] as well as three potential local anaesthetics based on 1-methyl-2-piperidinoethylester derivatives of 2-, 3-, 4-hexyloxyphenylcarbamic acid (denoted as 16 V, 17 V, and 18 V, resp.) [19] with dsDNA attached to the surface of a carbon paste screen-printed voltammetric electrode as the signal transducer. The aim of this study was the characterization of a drug-DNA association using the voltammetric signal of individual drugs at the DNA-based biosensor.

**Table: $pK_a$ values DPV peak potentials at DNA/SPE ($E_p$, V vs. Ag/AgCl/SPE) and rate constants of the desorption from DNA/SPE in blank buffer solution**

| | $pK_a$ | $E_p$ (V) | $k_{desorp}$ ($s^{-1}$) |
|---|---|---|---|
| PC | 8.6 | 0.843 | – |
| CC | 8.8 | 0.694 | 0.0072 |
| HC | 8.9 | 0.658 | – |
| 16 V | 7.32 | 0.585 | 0.218 |
| 17 V | 7.31 | 0.813 | 0.137 |
| 18 V | 7.63 | 0.548 | 0.007 |

Conditions: 0.01 mol/l phosphate buffer pH 7.0 for PC, CC and HC and 0.01 mol/l acetate buffer pH 4.8 for 16 V, 17 V and 18 V. Symbols: see text

## 2. Investigations, results and discussion

All compounds under study undergo electrochemical oxidation at both bare electrode without DNA (denoted as SPE) and DNA modified electrode (DNA/SPE). In differential pulse voltammetry (DPV) and cyclic voltammetry (CV), individual derivatives exhibit one anodic peak in the region of 0.49 to 0.84 V vs the reference Ag/AgCl/SPE corresponding to the redox change localized at nitrogen atom of the ester group (Table). In solution, the drug molecules adsorb on the surface of bare working electrode and associate with DNA at DNA/SPE as it follows from a significant increase in the peak current at DNA/SPE comparing to bare SPE (Fig. 1). This accumulation of the drug is time dependent. Using the detection scheme of DNA/SPE treatment in the drug solution for its accumulation and then transfer of the sensor into blank buffer solution without drug for the DPV measurement, the drug signal reaches a saturation level after 30 s for 16 V, 60 s for 17 V and 18 V in 0.01 mol/l acetate buffer pH 4.8 with −0.2 V as optimum accumulation potential as well as after 60 s for HC and 180 s for PC and CC in 0.01 mol/l phosphate buffer pH 7.0 under open circuit (no effect of accumulation potential was found within the range from −0.20 V to +0.40 V).

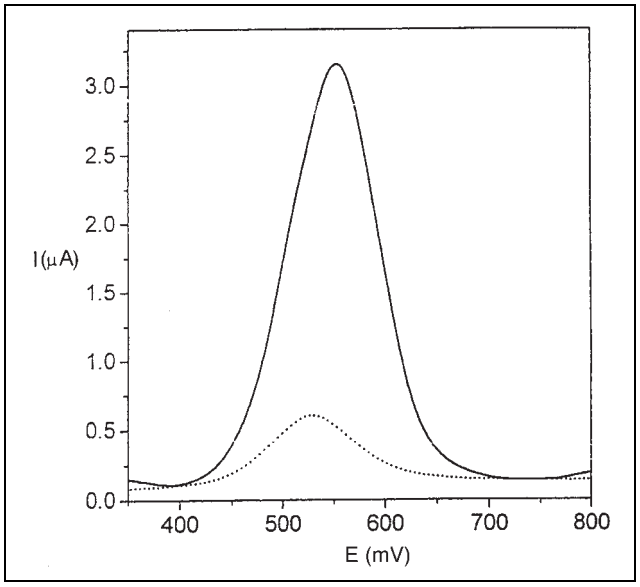


Fig. 1: Differential pulse voltammograms of 1-methyl-2-piperidinoethylester of 4-hexyloxyphenylcarbamic acid (18 V) obtained at DNA/SPE (full line) and SPE (dotted line) after 60 s accumulation from $9 \times 10^{-6}$ mol/l drug solution in 0.02 mol/l acetate buffer pH 4.8

The ratio of DPV anodic peak currents of individual drugs measured at DNA/SPE and bare SPE is the same (1.3 : 1.4 : 1.0 for PC, CC, and HC and 1.0 : 5.0 : 2.5 for 16 V, 18 V and 17 V). Moreover, parameters of the DPV anodic peaks depend on the pH value of buffers used for drug accumulation and voltammetric measurement in blank solution (all the buffers used were of the same ionic strength of 0.01 mol/l). Fig. 2 shows typical changes of the peak current and the peak potential which can be explained by the presence of protonated and deprotonated forms of the drug molecules (for $pK_a$ values see Table). All these observations confirm the same mechanism of drug oxidation at both DNA/SPE and unmodified SPE as

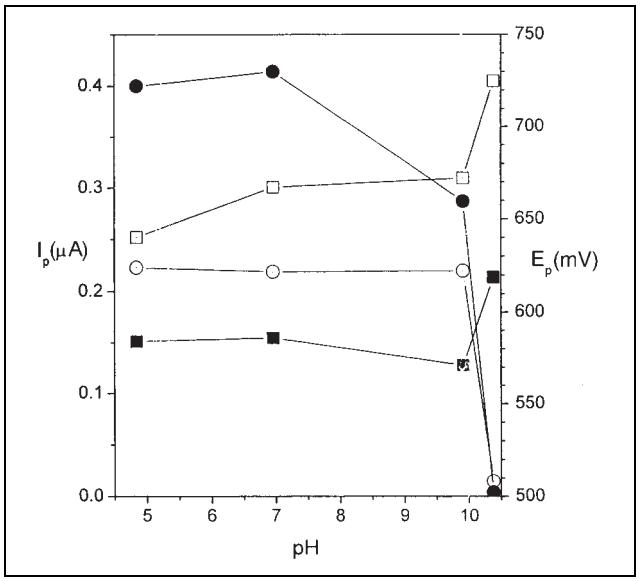


Fig. 2: Dependence of DPV peak current (○, ●) and peak potential (□, ■) of carbisocaine (CC) on pH of solution (0.02 mol/l acetate buffer pH 4.8, 0.005 mol/l phosphate buffer pH 6.9, and 0.10 mol/l Britton-Robinson buffer pH 9.91 and 10.38). Conditions: 120 s accumulation from $5 \times 10^{-7}$ mol/l drug solution at open circuit, DPV record at DNA/SPE (●, ■) and SPE (○, □) after the electrode transfer into blank supporting electrolyte

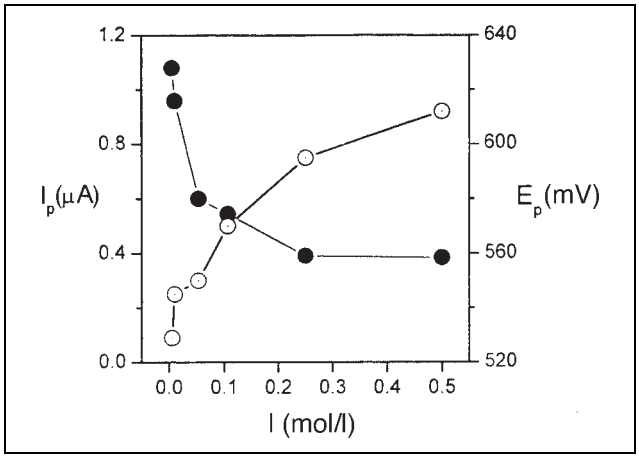


Fig. 3: Dependence of DPV peak current (●) and peak potential (○) of 1-methyl-2-piperidinoethylester of 4-hexyloxyphenylcarbamic acid (18 V) on ionic strength of acetate buffer solution of the concentration 0.01, 0.02, 0.1, 0.2, 0.5 and 1.0 mol/l. Conditions: DNA/SPE, 60 s accumulation from $1 \times 10^{-6}$ mol/l drug solution in 0.02 mol/l acetate buffer pH 4.8 at −0.2 V, DPV record in the same solution

well as the accumulation of the protonated molecules (in weak acidic and neutral solutions) within DNA.

Binding properties of the drugs to DNA were studied using buffer solutions of different concentration (i.e. different ionic strength). An increase in the drug DPV current at low buffer concentration (Fig. 3) again indicates a predominantly electrostatic character of the drug-DNA bond as a consequence of low competition of buffer cations with the protonated drug particles at an association with the negatively charged DNA backbone. Cyclic voltammetry performed at various polarization scan rates is a typical technique for the evaluation of transport properties of an analyte. The effect of scan rate on the drug signal was examined after transfer of the DNA/SPE sensor with the accumulated drug molecules into blank supporting electrolyte. Linear dependence of the CV peak current vs. scan rate within 0.010 to 0.250 V/s was obtained at both SPE and DNA/SPE which indicates the oxidation of surface attached drug. Thus the drug molecules at DNA/SPE are without any significant movement within microenvironment of the DNA layer.

The drug association with DNA is reversible and the drug molecules can be removed at a treatment of the DNA/SPE in blank buffer solution of high ionic strength (0.1 mol/l

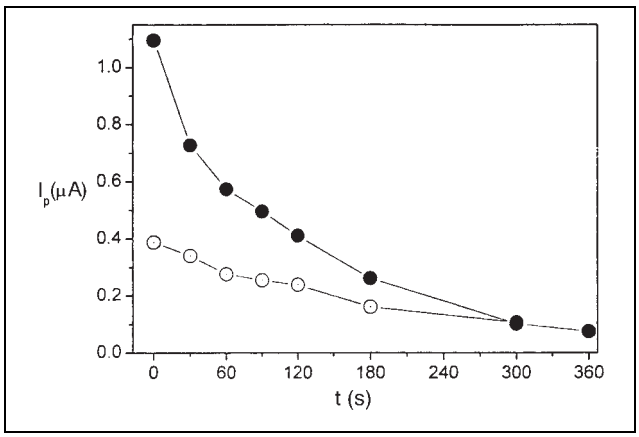


Fig. 4: Desorption of carbisocaine (CC) from DNA/SPE (●) and SPE (○) in blank 0.01 mol/l phosphate buffer pH 7.0. Conditions: 180 s accumulation from $5 \times 10^{-7}$ mol/l drug solution at open circuit, DPV record after the electrode transfer

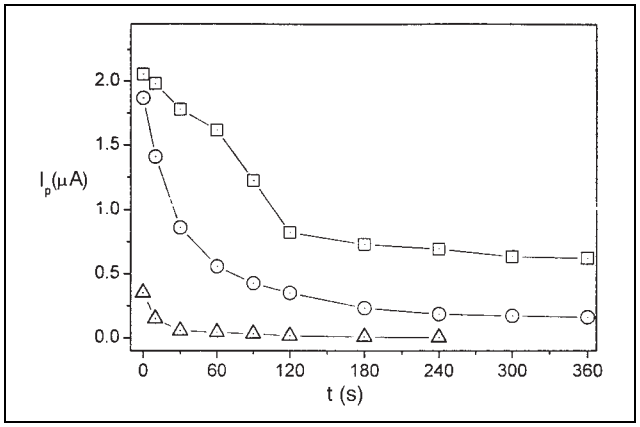


Fig. 5: Desorption of the drugs 16 V (△), 17 V (□) and 18 V (○) from DNA/SPE in blank 0.01 mol/l acetate buffer pH 4.8. Conditions: accumulation from $2x10^{-6}$ mol/l drug solution for 30 s (16 V) and 60 s (17 V and 18 V) at −0.2 V, DPV record after the electrode transfer

phosphate buffer or 0.2 mol/l acetate buffer) for 1 min under stirring. This was used for the regeneration of the biosensor which can be used repeatedly. The desorption from DNA/SPE into blank buffer was investigated using the same DNA modified electrode which was regenerated in blank buffer solution of high concentration after each measurement of the accumulated drug. Typical drug signals vs. time relationships are depicted in Figs. 4 and 5. A relatively fast liberation of individual drugs from the DNA is seen comparing to the desorption from bare SPE. The rate constants obtained as slopes of the ln(DPV peak current) vs. time dependence (Table) demonstrate the difference in the drug adsorption at DNA/SPE and SPE and they confirm a labile character of the drug binding to DNA.

In conclusion, a reversible association of the compounds under study with surface-bound DNA was found. This interaction is significant particularly in weak acidic and neutral solution where the protonated forms of the molecules are associated electrostatically with the negatively charged dsDNA backbone. The association does not lead to DNA damage and the molecules can be removed by dissociation of the drug-DNA adducts in blank buffer solution.

## 3. Experimental

### *3.1. Apparatus and reagents*

A computerized voltammetric analyzer ECA pol, model 110 (Istran, Bratislava, Slovakia) fitted with a screen-printed three-electrode assembly (FACH, Prešov, Slovakia) including a carbon working electrode (SPE, 25 $mm^2$ surface area) together with a silver/silver chloride reference electrode (Ag/AgCl/SPE with the potential of 0.284 V vs conventional Ag/AgCl/sat. KCl electrode) and a carbon counter electrode was used for voltammetric measurements. The bare working electrode was preconditioned electrochemically by polarization at 1.7 V for 60 s and then it was chemically modified by covering with 5 µl of DNA stock solution and leaving the electrode to dry overnight. The measurements were carried out in a 5 ml glass one-compartment voltammetric cell at room temperature (22 °C).

Calf thymus dsDNA was obtained from Merck (1.24013.0100) and used as received. Its stock solution (5 mg/ml) was prepared in 0.010 mol/l Tris-HCl buffer of pH 8.0 with $1 \times 10^{-3}$ mol/l EDTA solution and stored at −4 °C. The drugs were synthesized as described [16–19]. Their $1 \times 10^{-2}$ mol/l aqueous stock solutions were stored at −4 °C. All other chemicals were of analytical reagent grade purity and they were used as received. Deionized, double distilled water was used throughout.

### *3.2. Procedures*

A newly prepared biosensor was immersed into blank buffer solution for 10 min. Prior to measurement, the drug was accumulated at the electrode from its solution in 0.01 mol/l phosphate buffer pH 7.0 under open circuit conditions or in 0.01 mol/l acetate buffer pH 4.8 at −0.20 V polarization potential and for given time under stirring. The DP voltammogram was recorded immediately in the same solution or after a transfer of the sensor into blank buffer solution without drug within the potential range of 0.2 to 0.9 V at the pulse amplitude 100 mV, pulse duration 40 ms and scan rate of 25 mV/s. The DPV peak current was evaluated after correction to the baseline. Then the sensor was regenerated in 0.1 mol/l phosphate buffer pH 7.0 or 0.2 mol/l acetate buffer pH 4.8) for 2 min under stirring. A negligible drug signal was checked by the DPV record in blank buffer solution.

Acknowledgements: This work was supported by the Grant Agency VEGA of the Slovak Republic (Grant No. 1/9253/02, 1/8213/01) and by the project No. 035/2001. The authors thank L. Hrašková and E. Jakubková for their technical assistance.

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