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Electroanalysis of Benzalkonium Chloride in Ophthalmic Formulation by Boron-doped Diamond Electrode

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Abstract: Benzalkonium chloride (BAK) is a mixture of alkyl benzyl dimethyl ammonium chlorides, which is used primarily as a biocide, surfactant, preservative, and antimicrobial agent in the pharmaceutical industry, in particular in ophthalmologic and nasal solutions. However, BAK may cause harmful consequences on the eye structures of the anterior segment. Control of BAK identity and content is necessary by applying a sensitive detection method. This study unravels the use of a glassy carbon (GC) electrode and a pristine boron-doped diamond electrode (BDD) for the detection of four BAK homologs in a non-aqueous medium using square wave voltammetry (SWV). The BDD provided more reproduc-

bility of the oxidation potential than GC with a correlation coefficient of 0.999. The irreversible oxidation peak was very broad and deconvoluted into 3 peaks corresponding to C₁₂, C₁₄, and combined C₁₆–C₁₈ to reflect their concentration ratio in the mixture. The method was then extended to the detection of the C₁₂ homolog in the ophthalmic formulations with a limit of detection (LOD) of 0.4 µg/mL. The estimated BAK levels in three ophthalmic formulations were in agreement with the specified values by the manufacturers. The results were validated by high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection, confirming the presence of a single homolog (C₁₂) in the eye drops.

Keywords: Benzalkonium chloride · electrochemical method · boron-doped diamond electrode · C₁₂ homologs · high-performance liquid chromatography · eye drops

1 Introduction

Benzalkonium chloride (BAK, C₆H₅CH₂N⁺(CH₃)₂R) Cl⁻) consists of a mixture of alkyl benzyl dimethylammonium chlorides, where *R* represents n-C₈H₁₇ to n-C₁₈H₃₇. The four most important homologs are C₁₂, C₁₄, C₁₆, and C₁₈, cationic surfactants which serve as biocides, and phase transfer agents [1]. BAK is often used as a disinfectant, preservative, and antibacterial agent in the pharmaceutical industry [2]. It also serves as fungicides, spermicides, and virucides [3] with antiviral activities against some enveloped and non-enveloped viruses [3b,4]. Besides bacteria, the C₁₂ homolog is most effective against fungi and yeast, whereas the C₁₄ and C₁₆ homologs display antimicrobial activities against bacteria (Gram-negative and Gram-positive) [2b,5]. The bactericidal activity of BAK (1 mg/mL) can be attributed to the inactivation of bacterial energy-producing enzymes, denaturation of essential proteins, and disruption of the bacterial membrane [6]. The BAK homologs are formulated in pharmaceutical and industrial products below toxicity levels (200 mg/kg), otherwise, it induces toxicity in animals, the environment, and humans [3a,6–7]. Besides the damage of human nose epithelia and exacerbation of rhinitis [8], BAK has been associated with ocular adverse effects, including dry eye, trabecular meshwork degeneration, and ocular inflammation [9].

Analytical separation techniques have been used extensively for BAK determination in pharmaceutical products [1a,10] including high-performance liquid chromatography (HPLC) [2b,5,7a,11], ion chromatography

(IC) [12], capillary electrophoresis (CE) and fluorescence [2b]. Electrochemical approaches provide rapid analysis time and on-site monitoring compared to separation methods. Electrochemical detection of BAK was achieved using a phosphomolybdic acid modified carbon paste electrode (CPE) [13]. Glassy carbon (GC), a mercury-modified GC, and gold (Au) electrodes were used for the trace determination of BAK in cosmetic and oral hygiene products [14]. Of note is an electrochemical strategy using a GC to study BAK's physicochemical properties and their effect on the de-chlorination of allyl chloride with a detection limit of 2.2 mM [15].

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The boron-doped diamond (BDD) electrode offers a wide potential range, and electrochemical stability with a low background current. This study unravels the use of a pristine BDD for the detection of four BAK compounds in a non-aqueous medium. The method was then extended to the detection of the C₁₂ homolog in the ophthalmic formulations. The results are corroborated by HPLC coupled to ultraviolet (UV) detection. The electrochemical oxidation mechanism of BAK on the BDD electrode is proposed. This method is simple and straightforward over the method reported by Gabe et al. [13], which requires the modification of the electrode, as well as provides a lower limit of detection (LOD) than the method of Muthuraman et al. [15].

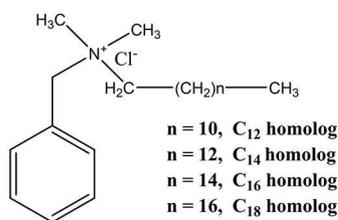
2 Experimental Section

2.1 Chemicals

All chemicals of the highest purity were purchased from Sigma-Aldrich (Dublin, Ireland). Three eye drops (Murine, Optrex clear, and Optrex soothing) were obtained from a local pharmacy in Cork, Ireland. These eye drops mainly contain benzalkonium chloride, boric acid, borax, disodium edetate, naphazoline hydrochloride, and purified water. A certified reference material of BAK 50% (w/v) consists of 50.7% C₁₂, 29.4% C₁₄, 17.0% C₁₆, and 2.8% C₁₈ homologs (Scheme 1). The supporting electrolyte consisting of 10 mM tetrabutylammonium hexafluorophosphate (TBAH)/acetonitrile (ACN) was utilized for the detection of BAK in standard solutions and ophthalmic formulations. A BAK stock solution (20 mg/mL) dissolved in ACN was diluted with the supporting electrolyte to prepare the working solutions with different concentrations.

2.2 Apparatus and Measurements

All electrochemical measurements were performed using a CHI1040A electrochemical workstation (CH Instrument, Austin, TX) equipped with an electrochemical cell consisting of 3 electrodes: BDD (3 mm geometric diameter, 0.1% doped boron, Windsor Scientific, Slough Berkshire, UK), a non-aqueous reference electrode (Ag/Ag⁺, BASi), and a platinum (Pt) wire as a counter electrode (Sigma-Aldrich, Dublin, Ireland). For comparison, a glassy carbon (GC) electrode (3 mm geometric diameter, BASi Analytical Instruments, West Lafayette,



Scheme 1. Structure of benzalkonium chloride homologs.

IN) was obtained. Detailed information on instrumentation, electrode preparation can be found elsewhere [16]. Before measurement, the electrode was cleaned by potential cycling from -1 to $+2$ V vs. Ag/Ag⁺ using 100 mV/s in 10 mM TBAH/ACN until a steady cyclic voltammogram (CV) profile was attained.

2.3 High-performance Liquid Chromatography (HPLC)

The chromatographic analysis was performed on an Agilent HPLC system [16] equipped with the Agilent ChemStation software instrumental control and data acquisition. The separation was performed on an Agilent poroshell 120 EC-C8 column (2.1 × 50 mm, 1.9 μm) using a gradient mobile phase. All the BAK homologs exhibited the maximum absorbance at 260 nm. The mobile phase compositions are A: 0.05% trifluoroacetic acid (TFA) in H₂O, B: 0.05% TFA in (ACN: isopropyl alcohol (IPA)), which was filtered and sonicated before use. The column was equilibrated for 20 min with the mobile phase before injection with an injection volume of 1 μL. The BAK standard solution and ophthalmic formulations were filtered (Econofiltr Nylon, 13 mm, 0.2 μm) before analysis. Different concentrations of BAK were prepared by diluting the BAK stock solution in deionized water. The ophthalmic formulations were used as received without dilution.

2.4 HPLC Method Validation

The proposed method was validated in terms of linear range, linearity, sensitivity, inter-day, and intra-day precision. The detection linearity of BAK was established in the range of 3 to 10 mg/mL in chromatographic analysis. The intra-day (repeatability) was established by calculating the relative standard deviation (R.S.D %) for three measurements at 3 mg/mL within one experiment of the same day. The inter-day (intermediate precision) was assessed by calculating three measurements at 3 mg/mL within three experiments for three different days. Repeatability and intermediate precision were calculated using the retention time and oxidation potential for chromatographic and electroanalysis, respectively.

3 Results and Discussion

3.1 Electrochemical Behavior of BAK

The CV presented only one irreversible oxidation peak at $+1.089$ and at $+1.54$ V for GC and BDD electrodes, respectively (Figure 1). The oxidation peak was also broad as the standard BAK consisted of four different BAK homologs, evincing their similar oxidation potentials.

A series of experiments with square wave voltammetry (SWV) was then conducted at the following optimal parameters: amplitude (E_{sw}) of 50 mV, a potential increment (ΔE) of 4 mV, and frequency (f) of 5 Hz ($t = 1/f$, s). SWV was conducted to obtain the lowest detection limit

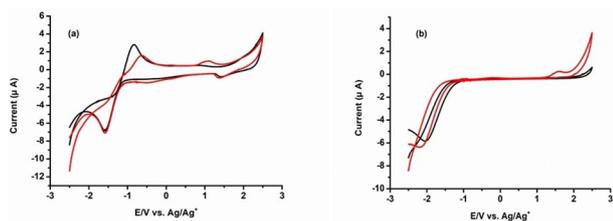


Fig. 1. CV response in the absence (black) and presence (red) of 80 µg/mL for BAK on the (a) GC and (b) BDD electrodes vs. Ag/Ag⁺. Electrolyte: 10 mM TBAH/ACN with a scan rate 100 mV/s.

for BAK. Compared to differential pulse voltammetry, SWV provides higher sensitivity and thus it was chosen for quantitation. The use of SWV indicated BAK

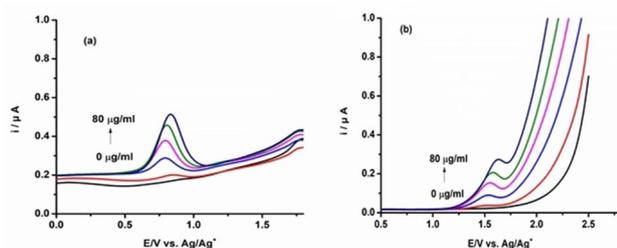


Fig. 2. SWVs of different BAK concentrations (4–80 µg/mL) at (a) GC and (b) BDD electrodes vs. Ag/Ag⁺. Electrolyte: 10 mM TBAH/ACN and a scan rate 100 mV/s.

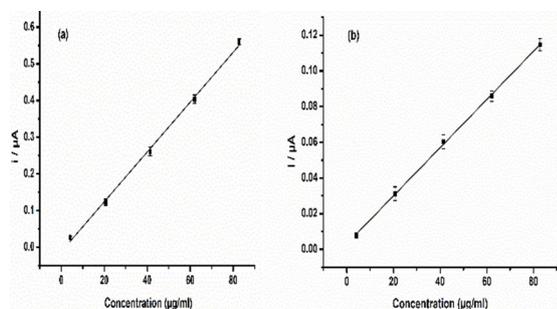


Fig. 3. Calibration plot of different BAK concentrations (4–80 µg/mL) at (a) GC and (b) BDD electrodes vs. Ag/Ag⁺ in 10 mM TBAH/ACN. The error bar was estimated from three different values for each analyte concentration.

oxidation on the GC and BDD electrodes in the linear range 4–80 µg/mL (Figure 2, 3) with an excellent correlation coefficient (R^2) of greater than 0.996 (Table 1). The estimated detection limit ($S/N=3$) was 0.68 µg/mL and 0.4 µg/mL for the GC and BDD electrodes, respectively. The reproducibility of the electrodes for BAK detection was estimated from three repeated measurements with 40 µg/mL of BAK. The R.S.D % value of the potential was 3.43 % for GC and 0.4510 % for BDD. Table 1 displays a comparison of analytical performance between the BDD and GC electrode toward the BAK oxidation. Compared to GC electrode, the BDD electrode exhibits excellent sensitivity and repeatability, and high resistance to fouling [17]. Consequently, BDD was selected for all subsequent experiments to develop a chemosensor for BAK homologs in the ophthalmic formulation.

The BDD electrode was also subject to cathodic treatment to form an adsorption layer with hydrogen formation (H-BDD), as opposed to the anodic treatment to form reactive hydroxyl radicals ($\cdot\text{OH}$) on the BDD surface (BDD- $\cdot\text{OH}$) [18]. The BDD electrode was treated with 0.5 M sulfuric acid at +3 V or –3 V for 30 min and detailed information of such treatment is available elsewhere [19]. The anodic peak of BAK was observed at +1.794 V after anodic treatment and at +1.652 V after cathodic treatment and the response signal was slightly lower than that of bare BDD (Figure S1). The effective surface area was calculated using the Randles-Sevcik equation

$$I_p = (2.69 \times 10^5) n^{3/2} A D_0^{1/2} v^{1/2} c_0^*$$

where n is the number of electron transfer ($n=1$), A is the effective surface area, D_0 is the diffusion coefficient ($7.6 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$), v is the scan rate and c_0^* is the concentration of $\text{K}_3[\text{Fe}(\text{CN})_6]$. Cyclic voltammetry at different scan rates (10–1000 mV/s) was performed using the electrode immersed in a solution of 1 mM $\text{K}_3[\text{Fe}(\text{CN})_6]$ in 0.1 M KCl. From the slope of the plot, $I_p = f(v^{1/2})$ (figure not shown) the effective surface areas of the bare, anodic treated, and cathodic treated BDD were found to be 2.36×10^{-3} , 0.83×10^{-3} and $2.25 \times 10^{-3} \text{ cm}^2$ respectively. The surface area of the bare BDD was higher than that after the treatments, which was attributed to the higher response signal. Thus, the bare BDD electrode was used for further experiments. The deconvolution of this broad unsymmetrical peak resulted in three separated peaks at 1.54 V, 1.63 V, and 1.66 V, which could be assigned for

Table 1. Calibration plots and LOD using SWV at the GC and BDD electrodes.

	Linear range (µg/mL)	Linear regression equation (y in µA, C in µg/mL)	Correlation coefficient (R^2)	R.S.D % (n=3) ^a	LOD ^b
Glassy Carbon	4–80	$y = 0.0068 C - 0.0118$	0.9973	3.43	0.68 µg/mL (1.7 µM)
Bare BDD	4–80	$y = 0.00135 C + 0.00299$	0.9993	0.451	0.4 µg/mL (1 µM)

^a R.S.D (%) calculated from three SWV measurements for the potential at 40 µg/mL of BAK (n=3). ^b LOD ($S/N=3$).

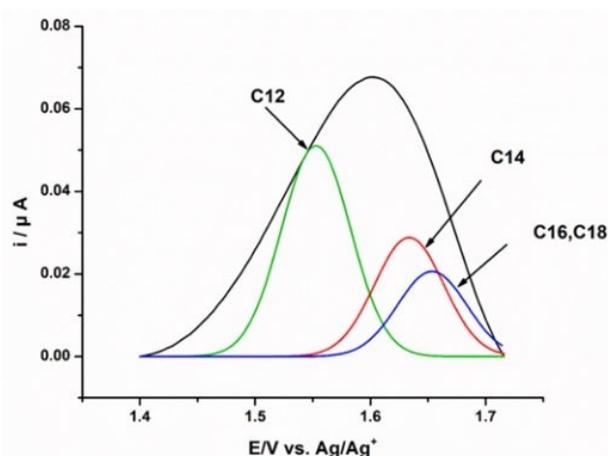


Fig. 4. Resolved SWV of the standard mixture at 80 µg/mL of BAK after the application of peak deconvolution using Origin Pro 8.5.1. on the BDD electrode vs. Ag/Ag⁺ using 10 mM TBAH/ACN.

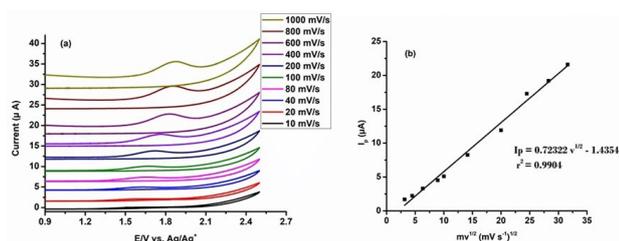
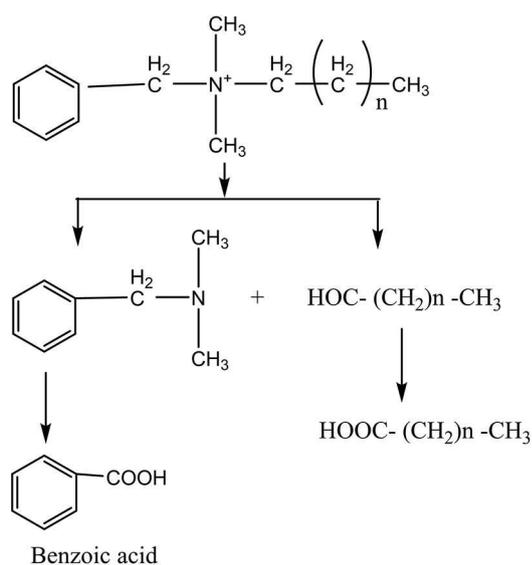


Fig. 5. (a) CV responses with the varying scan rate from 10 to 1000 mV/s using 0.15 mg/mL of BAK in 10 mM TBAH/ACN. (b) A linear relationship between I_p and $v^{1/2}$.



Scheme 2. A postulated electrochemical oxidation of BAK is deciphered from the biodegradation of this compound by microorganisms or chemical oxidation [22].

C₁₂, C₁₄, and combined C₁₆–C₁₈. The area of each peak over the total broad peak area agreed well with their concentration percentage in the standard mixture. Peak deconvolution of the BAK standard mixture is shown in Figure 4.

The peak potential for irreversible systems normally shifted toward positive potentials with increasing scan rate (v) [20], as shown in Figure 5a. A linear relationship between the oxidation peak current and $v^{1/2}$ indicated electrode kinetics were diffusion-controlled (Figure 5b). Such behavior is normally anticipated for the BDD electrode, due to the low adsorption of analyte on the electrode surface [20b, 21].

Despite that the exact mechanism of electrochemical oxidation of BAK is not known, *Aeromonas hydrophila* sp. K is capable of utilizing BAK as a sole source of carbon and energy [22]. The initial attack on BAK is the central fission of Calkyl-N bond by an oxygen-dependent dehydrogenase to form an alkanal and benzyldimethylamine (BDMA) as shown in Scheme 2. Perhaps, this is a general strategy of several microorganisms to gain access to the alkyl chains of BAK [23]. The alkanal could be oxidized to a corresponding acid via a beta-oxidation pathway [23b]. A similar degradation pathway has been proposed from the oxidation of BAK by S²O₈²⁻/Fe²⁺ [24]. In this context, this pathway for BAK is postulated for electrochemical oxidation, considering the OH[•] radical, a powerful reactive oxygen species (ROS) generated by the BDD electrode. Of course, the exact mechanism of the electrochemical oxidation of BAK by BDD is a subject of future endeavors.

3.2 Electroanalysis of BAK in Ophthalmic Formulations

The optimal BDD method was applied to the determination of the BAK in three ophthalmic samples. A representative SWV of a Murine ophthalmic sample analyzed by the BDD is shown in Figure 6a whereas the SWVs of Optrex Clear and Optrex are displayed in Figure S2. The SWVs show only one peak around +1.5 V

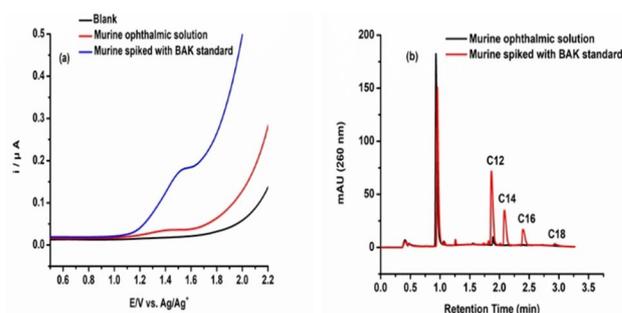


Fig. 6. (a) SWVs of Murine ophthalmic solution and spiked with 40 µg/mL of BAK on the bare BDD electrode vs. Ag/Ag⁺ in 10 mM TBAH/ACN. (b) HPLC-UV gradient analysis of Murine ophthalmic solution and spiked with 3 mg/mL of BAK analyzed by gradient HPLC-UV. Mobile phase: A: 0.05 % TFA in H₂O, B: 0.05 % TFA in (ACN: IPA; 50:50), flow rate: 0.3 mL/min, temperature 30 °C.

because only C_{12} is present in the eye drops. The estimated BAK concentration in the ophthalmic formulations was 0.1 (0.01 %), 0.051 (0.0051 %), and 0.039 (0.0039 %) mg/mL for Murine, Optrex Clear, and Optrex Soothing, respectively (Table S1). The determined amounts of BAK in the three samples were in agreement with the labeled values of BAK by the manufacturers.

3.3 HPLC Analysis of BAK

HPLC equipped with a C8 column and UV-detection was then conducted for the analysis of the BAK homologs in the ophthalmic formulations. Initially, the gradient condition was optimized with varying organic solvent composition and analysis time (Figure S3–5). The calibration curves of four homologs are presented in Figure S6 whereas Table S2 demonstrates the proportions of homologs in the BAK standard solutions. The optimized HPLC-UV method was then applied for the determination of BAK in three different ophthalmic formulations. The HPLC data confirmed the presence of only the C_{12} homolog in the ophthalmic formulations with the following corresponding concentration (mg/mL): 0.097 (Murine) in Figure 6b, 0.047 (Optrex Clear), and 0.035 (Optrex Soothing) (Figure S7). The content of BAK homologs in three ophthalmic solutions is presented in Table S3. Such a result was not completely unexpected as the C_8 and C_{10} homologs have very weak bactericidal activity. The C_{12} and C_{14} homologs exhibit antibacterial effects at concentrations spanning 8–400 $\mu\text{g/mL}$, and C_{12} -benzalkonium chloride is the most effective component of the BAK homologs [25]. The Pharmacopoeia of Japan prescribes that BAK is composed mainly of $n\text{-}C_{12}H_{25}$ and $n\text{-}C_{14}H_{29}$ homologs but no specific amount of these two homologs are stipulated [25]. In the US National Formulary XVI, the proportion of the C_{12} and C_{14} homologs must be over 40 % and 20 %, respectively [25]. The solubility and the critical micelle concentration of the BAK homologs with carbon chain length above C_{14} is extremely low [26]. Of importance is the binding to serum albumin, as C_{14} -BAK binds to serum albumin 2.5–3.7 times more compared with C_{12} -BAK. For a 10 % solution of human serum with 0.65 % protein as albumin, about 70–90 % of C_{12} -BAK with concentrations of 500–1000 $\mu\text{g/mL}$ remains unbound [25]. Despite all BAK homologs causing

cytotoxicity and corneal barrier dysfunction, the degree of corneal toxicity has different concentration dependency among the BAK homologs. C_{14} -BAK (0.005 %) damages the epithelium, whereas C_{12} -BAK has no effect at this concentration [27]. Stronger concentrations are caustic and damage the corneal endothelium irreversibly. In this context, ophthalmic solutions are formulated only with the C_{12} homolog with the highest bactericidal activity and minimal cytotoxicity. As a result, BAK is mostly used as a preservative in eye drops with concentrations ranging from 0.004 to 0.01 %, detectable at the BDD and by HPLC-UV as reported earlier.

The analytical performance of the HPLC system for baseline separation and detection of the BAK homologs is summarized in Table S4. The regression coefficient (R^2) of four homologs was greater than 0.94. The repeatability was in the range of 0.13–0.35 % and intermediate precision in the range of 0.30–0.75 %, confirming the suitability of this method for BAK determination. Electroanalysis of BAK by BDD or even with GC offers better limits of detection, compared with HPLC-UV detection (Tables 2, S5). However, the latter offers a baseline separation of 4 common BAK homologs and identifies the presence of C_{12} -BAK in ophthalmic formulations. However, C_{12} -BAK is commonly formulated in ophthalmic solutions due to its low toxicity and high bactericidal activity. Electroanalysis is sufficient for the rapid and sensitive detection of this homolog in ophthalmic formulations.

4 Conclusions

An efficient and simple method was described for detecting BAK in ophthalmic formulations at the BDD electrode. The rapid HPLC method confirmed the presence of only the C_{12} homolog in three different eye drops considering this homolog exhibits minimal cytotoxicity with the highest bactericidal activity, compared to other BAK homologs. The BDD electrode offers a lower detection limit compared with HPLC-UV detection. Ideally, an analytical method for BAK must differentiate and quantitate the homologs in the BAK mixture with C_{12} , C_{14} , and C_{16} as proportions of the alkyl groups. Thus, HPLC equipped with a downstream BDD electrode may be used for the separation and sensitive detection of BAK

Table 2. Comparison of the proposed method for the detection of BAK homologs on the BDD electrode and GC electrode with the literature methods.

Method	Analyte	Linear range	Correlation coefficient (R^2)	Detection limit	Reference
BDD	C_{12} – C_{14} – C_{16} – C_{18}	4–80 $\mu\text{g/mL}$ (0.01–0.2 mM)	0.9993	0.4 $\mu\text{g/mL}$ (1 μM)	Present work
GC	C_{12} – C_{14} – C_{16} – C_{18}	4–80 $\mu\text{g/mL}$ (0.01–0.2 mM)	0.9973	0.68 $\mu\text{g/mL}$ (1.7 μM)	Present work
Carbon paste electrodes	BAK	0.13×10^{-3} –0.17 mM	0.9824	0.1 μM	[13]
GC	C_{16}	0.01–0.48 mM	N.R. ^a	2.2 mM	[15]

^a N.R., not reported

homologs, formulated in pharmaceutical preparations [16]. If necessary, the electrode can be coated with Nafion [28], a negatively charged polymer to enhance the selective adsorption of BAK homologs. In this context, the electrode can be deposited with the negatively charged sulfobutylether-beta-cyclodextrin-doped poly(N-acetyltiramine) [29].

Acknowledgments

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Data Availability Statement

The data that supports the findings of this study are available in the supplementary material of this article

References

- [1] a) J. Dudkiewicz-Wilczyńska, J. Tautt, I. Roman, *J. Pharm. Biomed. Anal.* **2004**, *34*, 909–920; b) S. H. K. P., M. S. Thayyil, B. M. S. K. Deshpande, V. K. Rajan, *J. Mol. Liq.* **2018**, *251*, 487–491.
- [2] a) R. Mallik, S. Raman, X. Liang, A. W. Grobin, D. Choudhury, *J. Chromatogr. A* **2015**, *1413*, 22–32; b) S. J. Prince, H.-J. McLaury, L. V. Allen, P. McLaury, *J. Pharm. Biomed. Anal.* **1999**, *19*, 877–882; c) T. Mitani, A. I. E. Elmarhomy, L. Dulamjav, E. Anu, S. Saitoh, S. Ishida, Y. Oyama, *Chem.-Biol. Interact.* **2017**, *268*, 31–36; d) H.-Y. Choi, Y.-H. Lee, C.-H. Lim, Y.-S. Kim, I.-S. Lee, J.-M. Jo, H.-Y. Lee, H.-G. Cha, H. J. Woo, D.-S. Seo, *Part. Fibre Toxicol.* **2020**, *17*, 5.
- [3] a) I. Ferrer, E. T. Furlong, *Environ. Sci. Technol.* **2001**, *35*, 2583–2588; b) E. G. Romanowski, K. A. Yates, R. M. Q. Shanks, R. P. Kowalski, *J. Ocul. Pharmacol. Ther.* **2019**, *35*, 311–314.
- [4] D. R. Lazzaro, K. Abulawi, M. E. Hajee, *Eye Contact Lens* **2009**, *35*, 329–332.
- [5] H. K. Trivedi, M. C. Patel, *J. Chem.* **2010**, *7*, 1514–1522.
- [6] F. Ferk, M. Mišfik, C. Hoelzl, M. Uhl, M. Fuerhacker, B. Grillitsch, W. Parzefall, A. Nersesyan, K. Mičičeta, T. Grummt, V. Ehrlich, S. Knasmüller, *Mutagenesis* **2007**, *22*, 363–370.
- [7] a) P. Pérez, E. Fernández, R. Beiras, *Water Air Soil Pollut.* **2009**, *201*, 319–330; b) L.-P. Labranche, S. N. Dumont, S. Levesque, A. Carrier, *J. Pharm. Biomed. Anal.* **2007**, *43*, 989–993; c) M. van Berkel, F. A. de Wolff, *Hum. Toxicol.* **1988**, *7*, 191–193.
- [8] N. F. Johnson, *J. Aerosol Med. Pulm. Drug Deliv.* **2018**, *31*, 1–17.
- [9] a) C. Baudouin, A. Labbé, H. Liang, A. Pauly, F. Brignole-Baudouin, *Prog. Retinal Eye Res.* **2010**, *29*, 312–334; b) S. Datta, C. Baudouin, F. Brignole-Baudouin, A. Denoyer, G. A. Cortopassi, *Invest. Ophthalmol. Visual Sci.* **2017**, *58*, 2406–2412.
- [10] a) J. Zakrajšek, V. Stojić, S. Bohanec, U. Urleb, *Acta Chim. Slov.* **2015**, *62*, 72–82; b) M. Jovović, N. Kostić, B. Jančić-Stojanović, A. Malenović, *J. Liq. Chromatogr. Relat. Technol.* **2012**, *35*, 231–239.
- [11] a) K. A. Shaikh, A. T. Patil, *Food & Drugs* **2013**, *1*, 14–21; b) K. L. N. Rao, K. P. Reddy, K. S. Babu, K. S. Raju, K. V. Rao, J. V. Shaik, *Int. J. Res. Pharm. Sci.* **2010**, *1*, 473–480; c) M. S. Fortunato, S. Baroni, A. J. Gonzalez, J. D. A. Roncancio, A. Storino, C. Parise, E. Planes, A. Gallego, S. E. Korol, *Water Air Soil Pollut.* **2019**, *230*, 14; d) A. Agarwal, S. Tiwari, K. Nagariya, *J. Drug Delivery Ther.* **2013**, *3*, 26–30; e) X. M. Wang, K. Kasperski, *Anal. Methods* **2018**, *10*, 2512–2525; f) J. Mehta, K. Patidar, N. Vyas, *E-J. Chem.* **2010**, *7*, 11–20; g) H. AlAani, Y. AlNukkary, *J. Appl. Pharmacol.* **2016**, *6*, 080–089.
- [12] L.-H. Wang, *J. Chromatogr. Sci.* **2002**, *40*, 326–330.
- [13] M. Gaber, H. M. Abu Shawish, A. M. Khedr, K. I. Abed-Almonem, *Mater. Sci. Eng. C* **2012**, *32*, 2299–2305.
- [14] L.-H. Wang, S.-J. Tsai, *Anal. Chim. Acta* **2001**, *441*, 107–116.
- [15] G. Muthuraman, K. Chandrasekara Pillai, I.-S. Moon, *Catalysts* **2019**, *9*, 99.
- [16] P. E. Hayes, J. D. Glennon, J. H. T. Luong, *Electroanalysis* **2020**, *32*, 2027–2035.
- [17] a) J. H. Luong, K. B. Male, J. D. Glennon, *Analyst* **2009**, *134*, 1965–1979; b) F. Shang, Y. Liu, S. Hrapovic, J. D. Glennon, J. H. Luong, *Analyst* **2009**, *134*, 519–527.
- [18] S. C. B. Oliveira, A. M. Oliveira-Brett, *J. Electroanal. Chem.* **2010**, *648*, 60–66.
- [19] a) T. Kondo, K. Honda, D. A. Tryk, A. Fujishima, *Electrochim. Acta* **2003**, *48*, 2739–2748; b) B. Marselli, J. Garcia-Gomez, P. A. Michaud, M. A. Rodrigo, C. Cominellis, *J. Electrochem. Soc.* **2003**, *150*, D79–D83; c) H. Girard, N. Simon, D. Ballutaud, M. Herlem, A. Etcheberry, *Diamond Relat. Mater.* **2007**, *16*, 316–325.
- [20] a) L. Janíková-Bandžuchová, R. Šelešovská, K. Schwarzová-Pecková, J. Chýlková, *Electrochim. Acta* **2015**, *154*, 421–429; b) A. J. Bard, L. R. Faulkner, J. Leddy, C. G. Zoski, *Electrochemical methods: fundamentals and applications*, Vol. 2, Wiley New York, **1980**.
- [21] K. Peckova, J. Barek, *Curr. Org. Chem.* **2011**, *15*, 3014–3028.
- [22] M. A. Patrauchan, P. J. Oriol, *J. Appl. Microbiol.* **2003**, *94*, 266–272.
- [23] a) C. Van Ginkel, J. Van Dijk, A. Kroon, *Appl. Environ. Microbiol.* **1992**, *58*, 3083–3087; b) C. G. van Ginkel, *Biodegradation* **1996**, *7*, 151–164.
- [24] J.-M. Hong, Y.-F. Xia, Q. Zhang, B.-Y. Chen, *Journal of the Taiwan Institute of Chemical Engineers* **2017**, *78*, 230–239.
- [25] K. Jono, T. Takayama, M. Kuno, E. Higashide, *Chem. Pharm. Bull.* **1986**, *34*, 4215–4224.
- [26] Y. N. E. Takahashi, M. Koike, M. Kobayashi, *Kaimen Kasseizi Handbook*, Kohgaku Tosho, Tokyo, **1968**.
- [27] M. Uematsu, T. Kumagami, K. Shimoda, M. Kusano, M. Teshima, H. Sasaki, T. Kitaoka, *Cornea* **2010**, *29*, 1296–1301.
- [28] A. Buzid, G. P. McGlacken, J. D. Glennon, J. H. T. Luong, *ACS Omega* **2018**, *3*, 7776–7782.
- [29] F. Shang, L. Zhou, K. A. Mahmoud, S. Hrapovic, Y. Liu, H. A. Moynihan, J. D. Glennon, J. H. T. Luong, *Anal. Chem.* **2009**, *81*, 4089–4098.

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