

## DETERMINATION OF 5-AMINO-6-NITROQUINOLINE AT A CARBON PASTE ELECTRODE

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*Dedicated to the memory of Professor Jaroslav Heyrovský on the occasion of 50th anniversary of his Nobel Prize for polarography.*

Differential pulse voltammetry, direct current voltammetry, adsorptive stripping voltammetry and HPLC with electrochemical detection were used for the determination of 5-amino-6-nitroquinoline at a carbon paste electrode. The methods are based either on anodic oxidation or cathodic reduction of this substance, whose electrochemical behavior at carbon paste electrode was further studied by cyclic voltammetry. Practical applicability of these methods was demonstrated on the determination of 5-amino-6-nitroquinoline in model samples of drinking and river water. The detection limit was  $2.0 \times 10^{-6}$  mol l<sup>-1</sup> for anodic differential pulse voltammetry in a mixture of Britton–Robinson buffer (pH 11)–methanol 1:1 (v/v) and  $1.6 \times 10^{-7}$  mol l<sup>-1</sup> for HPLC with electrochemical detection ( $E = +1.2$  V) in a mobile phase Britton–Robinson buffer (pH 7)–methanol 1:9 (v/v).

**Keywords:** 5-Amino-6-nitroquinoline; Carbon paste electrode; Differential pulse voltammetry; Cyclic voltammetry; HPLC; Spectrophotometric detection; Electrochemical detection.

Cancer is a leading cause of death in human population all over the world although the tumor frequency in various organs shows marked differences among countries<sup>1,2</sup>. People are continuously exposed to varying amounts of chemicals that have been experimentally shown to have carcinogenic or mutagenic properties<sup>3</sup>.

5-Amino-6-nitroquinoline (5A6NQ) ranks among nitro and amino derivatives of heterocyclic compounds (HC) which, due to chemical composition, properties and occurrence, are often similar to nitro and amino derivatives of polycyclic aromatic hydrocarbons (PAH)<sup>4</sup>. PAH accompanied by HC occur in a variety of complex matrices including petroleum and petroleum distillates, smoked and flame-broiled foods<sup>5</sup>, effluents from a variety of in-

dustrial facilities<sup>6,7</sup>, municipal waste-waters, cigarette smoke, creosote leachate, and airborne emissions from waste incinerators and internal combustion engines<sup>8</sup>. Usual methods for the determination of the above analytes are gas chromatography connected to mass spectrometry or other detection techniques, liquid chromatography with fluorescent, spectrophotometric or electrochemical detection<sup>9-11</sup>.

Heterocyclic amines (HCA) are a group of very harmful compounds, which are often mutagenic and carcinogenic<sup>12</sup>. They are produced when meat is heated above 180 °C for a long period of time<sup>13,14</sup>. We can find them as products of incineration of fossil fuel<sup>15</sup> and also in cigarette smoke<sup>16</sup>. At least 23 HCAs were isolated from food, 10 of them showing mutagenic and carcinogenic properties. In experimental animals most HCAs are metabolized and only 1-2% is excreted in unmetabolized form in urine<sup>17</sup>. Carcinogenity of HCA depends on metabolic activity of detoxication enzymes, mainly liver cytochrom P 450<sup>18</sup>.

Moreover, numerous derivatives of quinoline are used in pharmaceutical industry. Dichloro derivatives of 8-hydroxyquinoline (Endiaron®) for treatment of intestinal infections. 4-Aminoquinoline (chloroquine)<sup>19,20</sup> and 8-aminoquinoline (primaquine)<sup>21-23</sup> are used in treatment of malaria.

The compound under investigation has not been shown to have carcinogenic or mutagenic properties so far (it is not included in the IARC, NTP, OSHA lists). However, its toxicological properties were studied on *Tetrahymena pyroformis*, and were shown to be comparable to 4-amino-2-nitrophenol<sup>24</sup>, which has carcinogenic effects on animals. 5A6NQ was also used for preparation of a new type of radioligands<sup>25</sup> and local anesthetics<sup>26</sup>.

The aim of this work was to develop a new voltammetric and HPLC-ED method for the determination of trace amounts of 5A6NQ using its anodic oxidation and cathodic reduction at carbon paste electrode (CPE). CPEs are very useful electrochemical sensors for the determination of organic compounds that can be anodically oxidized<sup>26-29</sup>. Their application in cathodic reduction of substances is somewhat complicated by the interference of oxygen dissolved in measured solutions, adsorbed on carbon particles used for the preparation of CPE or dissolved in a pasting liquid. The possibility of using a single electrode for the determination of an organic analyte based on oxidation or reduction of its functional groups can significantly increase the determination reliability. The selectivity of the determination can be greatly increased by a combination of extremely sensitive electrochemical detection with a powerful separation technique, such as HPLC.

## EXPERIMENTAL

### Apparatus

For voltammetric measurements, a computerized voltammetric analyzer Eco-Tribo Polarograph with software PolarPro 4.0 (all Polaro Sensors, Prague, Czech Republic) was used. The following voltammetric parameters were used: scan rate  $20 \text{ mV s}^{-1}$ , for DPV a pulse amplitude of  $50 \text{ mV}$  (for cathodic reactions  $-50 \text{ mV}$ ). The surface of the working carbon paste electrode (2 mm in diameter for voltammetric methods) was renewed mechanically by protruding the piston and smoothing the surface with wet filter paper.

The reference electrode was Ag/AgCl electrode RAE 113 (Monokrystaly, Turnov, Czech Republic) filled with  $3 \text{ M KCl}$ . Platinum wire served as auxiliary electrode. For cathodic voltammetry, oxygen was removed by 5-min bubbling with nitrogen.

Two HPLC-ED systems were used. For anodic detection of 5A6NQ the system consisted of a high-pressure piston pump HPP 5001, an LCI 30 sample injector with  $20\text{-}\mu\text{l}$  sample loop and an LCD 2040 UV/Vis spectrophotometric detector (all Laboratorní přístroje, Praha, Czech Republic). The reverse-phase column Lichrospher<sup>®</sup> C18, 100 ( $5 \mu\text{m}$ ),  $125 \times 4 \text{ mm}$  was used (LichroCART, Merck). The system was operated by CSW 1.7 programme (DataApex, Czech Republic) working in the Windows 95 system (Microsoft). A different HPLC system and column were used for cathodic detection of 5A6NQ to eliminate the interference of oxygen. The system consisted of a Beta 10 serial dual-piston pump, a DG-3014 solvent degasser, valve D with a  $20\text{-}\mu\text{l}$  sample loop and a Sapphire 800 UV/Vis spectrophotometric detector (all Ecom, Prague, Czech Republic). The reverse-phase column Kromasil B C18 ( $7 \mu\text{m}$ ),  $250 \times 4.6 \text{ mm}$  was used (Prochome). The mobile phase flow rate was  $0.5 \text{ ml min}^{-1}$ , the CPE was working in a wall-jet configuration. The system was operated by Clarity 2.3.0 programme (DataApex, Czech Republic) working in the Windows XP system (Microsoft). An ADLC 2 amperometric detector (Laboratorní přístroje, Praha, Czech Republic) was used in both systems.

An ultrasonic bath PS02000A (Powersonic, USA) was used to facilitate dissolution of the analytes. pH of the solutions was measured with a pH meter Jenway 4330 (Jenway, UK) with a combined glass electrode. Spectrophotometer PU 8800 (Philips, UK) was used to study the stability of the stock solution. All experiments were carried out at laboratory temperature.

### Reagents

5-Amino-6-nitroquinoline was obtained from Aldrich. Its stock solution ( $1 \times 10^{-3} \text{ mol l}^{-1}$ ) was prepared by dissolving an accurately weighed amount of the substance in p.a. methanol (Lach-Ner, Neratovice, Czech Republic). Solutions of lower concentrations were prepared by dilution of the stock solution with methanol. A spectrophotometric study of stability of the stock solution demonstrated that it was stable for at least four months.

Britton–Robinson (BR) buffers were prepared in a usual way, by mixing  $0.04 \text{ M}$  phosphoric acid,  $0.04 \text{ M}$  acetic acid and  $0.04 \text{ M}$  boric acid with an appropriate amount of  $0.2 \text{ M}$  sodium hydroxide. Phosphate buffers were prepared from  $0.01 \text{ M}$  sodium dihydrogen phosphate with an appropriate amount of concentrated phosphoric acid. All the chemicals used were of analytical reagent grade (Lachema, Brno, Czech Republic).

For model samples tap drinking water was used; a sample of river water was taken from the Vltava river in the Prague City.

The mobile phase for HPLC contained methanol for HPLC (Chromoservis, Praha, Czech Republic) and aqueous BR or phosphate buffer.

Carbon paste contained 250 mg of spherical microparticles of glassy carbon with diameter 0.4–12  $\mu\text{m}$  (Alpha Aesar, USA) and 90  $\mu\text{l}$  of mineral oil (Fluka Biochemica, Switzerland).

All aqueous solutions were prepared using deionized water obtained from a MilliQ Plus system (Millipore, Molsheim, France).

### Procedures

Solutions for voltammetry were prepared by measuring 5 ml of BR buffer of chosen pH, adding the respective amount of the 5A6NQ stock solution, filling with methanol to 10 ml in a volumetric flask and transferring the solution to the voltammetric vessel. Before measurements the solution in the voltammetric cell was deaerated for 10 min by bubbling with nitrogen.

The solution for measurement of model samples was prepared by mixing 1 ml of BR buffer (pH 11), 4 ml of drinking or river water, the respective amount of the 5A6NQ stock solution and filling with methanol to 10 ml.

Calibration curves were measured in triplicate and evaluated by a linear regression method. The limit of detection ( $L_D$ ) for the flow method was calculated as an amount of 5A6NQ, which gave the signal three times higher than the background noise ( $S/N$ ). For batch methods the limit of detection was calculated using the whole calibration line<sup>30</sup>.

## RESULTS AND DISCUSSION

### *Voltammetric Determination of 5-Amino-6-nitroquinoline*

First the influence of pH of the supporting electrolyte on the voltammetric behavior of the test substance was investigated. Because of the low solubility of tested 5A6NQ in water, a mixture of BR buffer with methanol 1:1 (v/v) was used. Then the oxidation of the amino group was studied by the anodic direct current (DC) or differential pulse (DP) voltammetry. The effect of pH on the peak current is depicted in Fig. 1. The figure shows a shift of peak potential to less positive values with increasing pH, which can be explained by preliminary protonation of the test substance. The optimum medium for the measurement of anodic 5A6NQ calibration dependences was BR (pH 11) and methanol (1:1).

Cathodic DC and DP voltammetries utilize the reduction of the nitro group. The effect of pH on the peak current is depicted in Fig. 2. The figure shows a shift of the peak potential to less positive values with decreasing pH due to easier reduction of nitro group on protonation in more acidic medium. Cathodic voltammetry on CPE is complicated by the presence of oxygen, not only in the measured solution but also in the carbon paste. The oxygen from the solution could be easily removed by nitrogen purging

while that in the paste can sometimes severely interfere with the reaction under study making the measurement impossible. The optimum medium for measurement of cathodic 5A6NQ calibration dependences was of BR buffer (pH 11)–methanol (1:1).

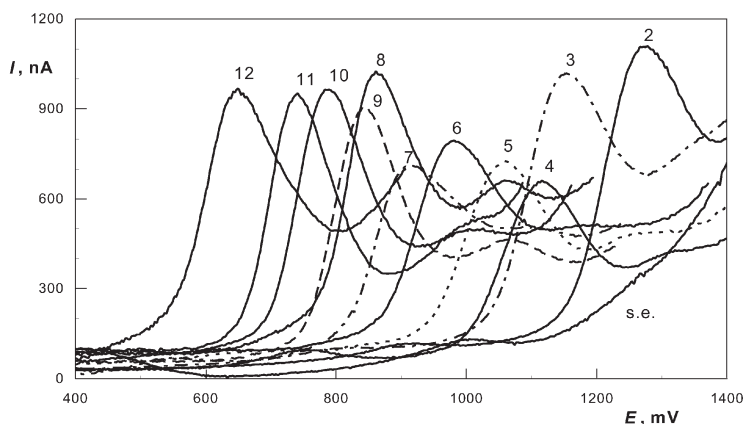


FIG. 1  
Anodic DP voltammograms of  $1 \times 10^{-4}$  M 5A6NQ in BR buffer–methanol (1:1) medium. The pH values of BR buffer are equal to the curve numbers 2–12, s.e. supporting electrolyte BR buffer (pH 2)–methanol (1:1)

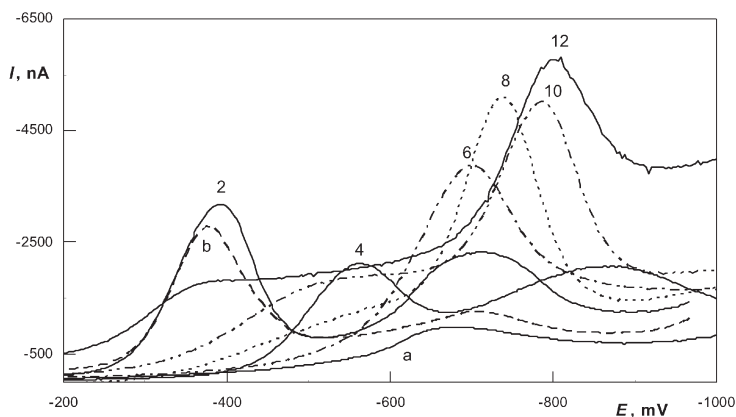


FIG. 2  
Cathodic DP voltammograms of  $1 \times 10^{-4}$  M 5A6NQ in BR buffer–methanol (1:1) medium. The pH values of BR buffer are equal to the curve numbers 2–12, a supporting electrolyte 0.1 M  $\text{H}_3\text{PO}_4$  (pH 1.6)–methanol (1:1), b  $1 \times 10^{-4}$  M 5A6NQ in 0.1 M  $\text{H}_3\text{PO}_4$  (pH 1.6)–methanol (1:1)

Optimum conditions for voltammetric determination of 5A6NQ are summarized in Table I. The calibration curves were measured in concentration range  $2 \times 10^{-6}$ – $1 \times 10^{-4}$  mol l<sup>-1</sup>. The possibility of increasing the sensitivity of the determination by adsorptive accumulation of the analyte on the surface of CPE was investigated. The influence of accumulation potential on the peak current of  $8 \times 10^{-6}$  M 5A6NQ was measured in the range from 0 to -0.8 V, with the accumulation time ( $t_{acc}$ ) from 30 s to 10 min in BR buffer (pH 2, 7 and 11), always in a mixture with methanol (1:1). As the effect

TABLE I  
Parameters of the calibration lines for voltammetric determination of 5A6NQ on CPE

| Method       | Supporting electrolyte                                    | Slope<br>mA l mol <sup>-1</sup> | Intercept<br>nA | $R^2$ <sup>a</sup> | $L_D$ <sup>b</sup> × 10 <sup>6</sup><br>mol l <sup>-1</sup> |
|--------------|---|---------------------------------|-----------------|--------------------|---|
| Anodic DCV   | MeOH-BR (pH 11) 1:1                                       | 8.73                            | 21.7            | 0.9963             | 3.1   |
| Anodic DPV   | MeOH-BR (pH 11) 1:1                                       | 6.89                            | 8.1             | 0.9959             | 2.0   |
| Cathodic DCV | MeOH-0.1 M H <sub>3</sub> PO <sub>4</sub><br>(pH 1.6) 1:1 | -24.7                           | -81.0           | 0.9963             | 1.5   |
| Cathodic DPV | MeOH-0.1 M H <sub>3</sub> PO <sub>4</sub><br>(pH 1.6) 1:1 | -18.3                           | -33.1           | 0.9959             | 1.3   |
| Cathodic DPV | MeOH-BR (pH 11) 1:1                                       | -16.2                           | -95.7           | 0.9933             | 1.9   |

<sup>a</sup>  $R^2$ , coefficient of determination; <sup>b</sup>  $L_D$ , limit of detection.

TABLE II  
Parameters of the calibration lines for voltammetric determination of 5A6NQ in model samples of drinking and river water, measured in BR buffer (pH 11)-methanol (1:1)

| Method         | Concentration range<br>mol l <sup>-1</sup> | Slope<br>mA l mol <sup>-1</sup> | Intercept<br>nA | $R^2$ <sup>a</sup> | $L_D$ <sup>b</sup> × 10 <sup>6</sup><br>mol l <sup>-1</sup> |
|----------------|--|---------------------------------|-----------------|--------------------|---|
| Drinking water |  |                                 |                 |                    |   |
| Anodic DCV     | 8 × 10 <sup>-6</sup> –1 × 10 <sup>-4</sup> | 7.96                            | -4.7            | 0.9983             | 7.4   |
| Anodic DPV     |  | 6.78                            | -7.2            | 0.9981             | 7.2   |
| River water    |  |                                 |                 |                    |   |
| Anodic DCV     | 6 × 10 <sup>-6</sup> –1 × 10 <sup>-4</sup> | 8.43                            | 18.5            | 0.9975             | 5.6   |
| Anodic DPV     |  | 6.75                            | -7.5            | 0.9986             | 5.5   |

<sup>a</sup>  $R^2$ , coefficient of determination; <sup>b</sup>  $L_D$ , limit of detection.

of the analyte accumulation in 50% methanol was not significant, the amount of methanol was decreased to 5%, but without any effect. Neither adsorptive accumulation of the analyte on CPE nor its extraction into the electrode body occurred so that no increase in sensitivity was reached.

The practical applicability of the developed voltammetric methods was demonstrated by the determination of 5A6NQ in model samples of drinking and river water (Table II, Fig. 3). The slopes of the calibration lines in drinking and river water correspond to the values in deionized water. The obtained limits of detection are slightly higher due to higher noise in model samples.

Cyclic voltammetry was used to investigate reversibility of the 5A6NQ reaction on CPE. The reversibility of electrochemical oxidation was studied in BR buffer (pH 2, 7 and 11)–methanol (1:1) medium, the reversibility of electrochemical reduction in 0.1 M  $\text{H}_3\text{PO}_4$  (pH 1.6)–methanol (1:1). Cyclic voltammograms were measured with scan rates 5–100  $\text{mV s}^{-1}$  always starting from potential 0 V (Fig. 4). The behavior of 5A6NQ during five consecutive cycles was studied for the scan rate 20  $\text{mV s}^{-1}$  (Fig. 5). It follows from the results that both oxidation and reduction are irreversible under the given conditions. They are controlled by both diffusion and adsorption (direct proportionality was not observed for the dependence of the peak cur-

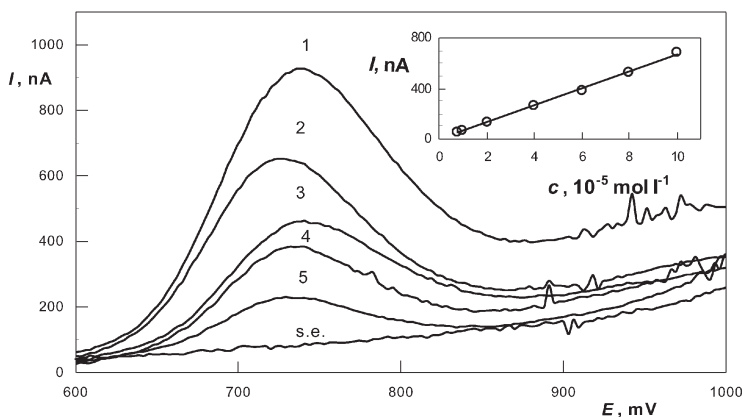


FIG. 3

Anodic DP voltammograms of 5A6NQ in model samples of drinking water measured in BR buffer (pH 11)–methanol (1:1). Concentration of 5A6NQ: 1  $10 \times 10^{-6}$ , 2  $8 \times 10^{-6}$ , 3  $6 \times 10^{-6}$ , 4  $4 \times 10^{-6}$ , 5  $2 \times 10^{-6}$   $\text{mol l}^{-1}$ ; s.e. supporting electrolyte BR buffer (pH 11)–methanol (1:1)

rent on scan rate or on the square root of the scan rate). It is also clear that the electrode surface is passivated during consecutive runs so that renewal of the electrode surface between measurements is essential.

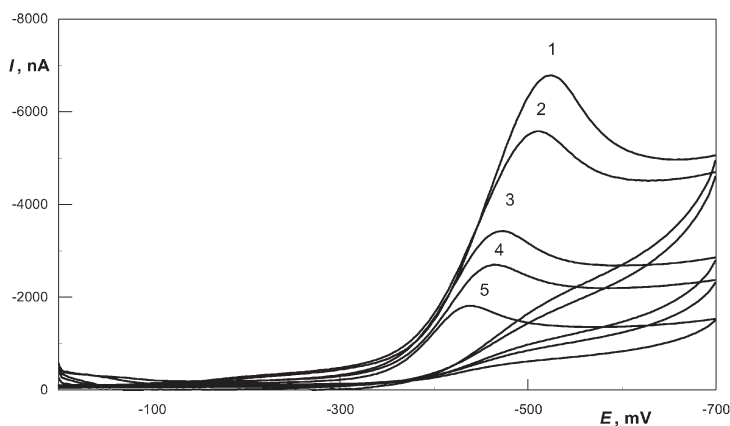


FIG. 4

Cyclic voltammograms of  $1 \times 10^{-4}$  M 5A6NQ in 0.1 M  $\text{H}_3\text{PO}_4$  (pH 1.6)–methanol (1:1),  $E_{\text{in}} = 0$  V; scan rate: 1 100, 2 50, 3 20, 4 10, 5 5  $\text{mV s}^{-1}$

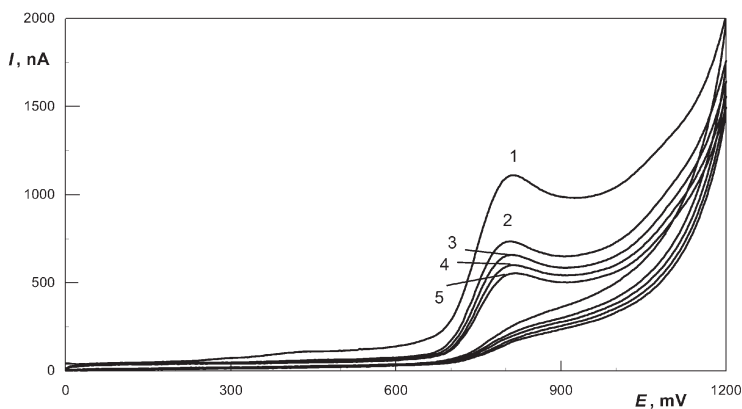


FIG. 5

Cyclic voltammograms of  $1 \times 10^{-4}$  M 5A6NQ in BR buffer (pH 11)–methanol (1:1),  $E_{\text{in}} = 0$  V, scan rate 20  $\text{mV s}^{-1}$ ; cycles: 1 first, 2 second, 3 third, 4 fourth, 5 fifth



### *HPLC Determination of 5-Amino-6-nitroquinoline with Spectrophotometric and Electrochemical Detection*

5A6NQ can occur in more complex matrices, where it is not possible to use the batch voltammetric methods without previous separation for its determination. One of the possibilities is the application of HPLC with amperometric detection. Therefore, optimum conditions for electrochemical detection of 5A6NQ were further investigated. The mobile phase containing 90% methanol was used to obtain a low retention time. The amperometric detector of the wall-jet type was placed behind the UV/Vis detector operating at 295 nm. First, optimum pH value of the mobile phase was found from the region compatible with the used column. Hydrodynamic voltammograms were measured in BR buffers of pH 3, 5 and 7, always in a mixture with methanol (1:9) for potentials from 0 to +1.4 V (Fig. 6), when employing electrochemical oxidation of the amino group, or in 0.01 M phosphate buffer pH 2, 4 and 6, always in a mixture with methanol (1:9) for potentials from 0 to -1.4 V (Fig. 7), when employing electrochemical reduction of the nitro group. HPLC chromatograms in optimal conditions are shown in Fig. 8. Table III summarizes the parameters of the measured calibration straight lines and optimum media. The calibration curves were measured in the concentration range  $2 \times 10^{-7}$ – $1 \times 10^{-4}$  mol l<sup>-1</sup>.

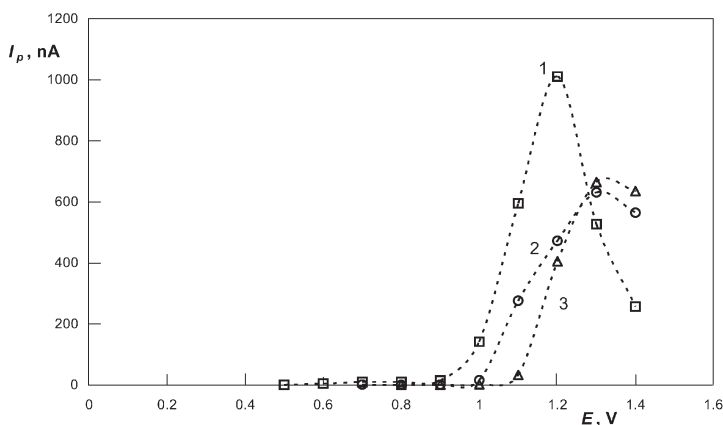


FIG. 6

Hydrodynamic voltammograms of 20  $\mu$ l of  $1 \times 10^{-4}$  M 5A6NQ solution on CPE in the mobile phase BR buffer (pH: 1, 2, 5, 3, 3)–methanol (1:9)

It can be observed that the electrochemical detection based on reduction of 5A6NQ does not give such good results as the detection based on oxidation of 5A6NQ. This is caused by a higher noise in amperometric detection at negative potentials.

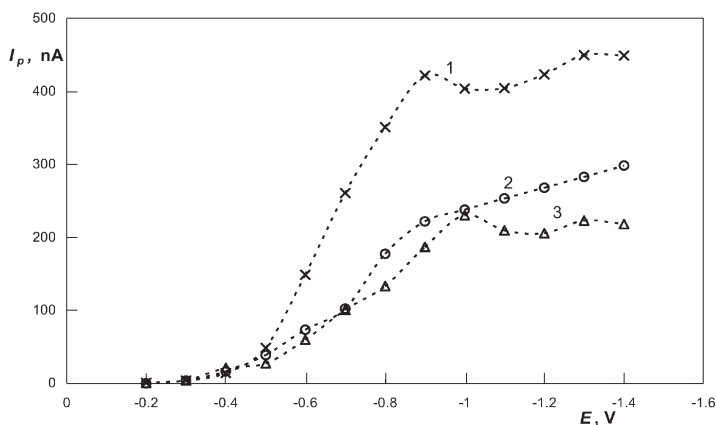


FIG. 7

Hydrodynamic voltammograms of 20  $\mu\text{l}$  of  $1 \times 10^{-4}$  M 5A6NQ solution on CPE in the mobile phase 0.01 M phosphate buffer (pH: 1 2, 2 4, 3 6)–methanol (1:9)

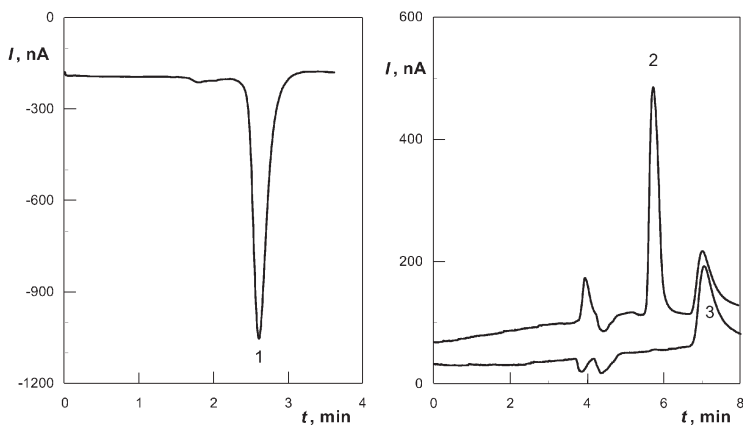


FIG. 8

HPLC-ED chromatograms of 20  $\mu\text{l}$  of  $1 \times 10^{-4}$  M 5A6NQ solution on CPE in the mobile phases: BR buffer (pH 7)–methanol (1:9),  $E = +1.2$  V (1); 0.01 M phosphate buffer (pH 2)–methanol (1:9),  $E = -0.9$  V (2). Injection of 20  $\mu\text{l}$  of methanol gives oxygen peak (3)

TABLE III  
Calculated limits of detection for HPLC determination of 5A6NQ

| Method              | Mobile phase                     | $L_D^a \times 10^7$<br>mol l <sup>-1</sup> |
|---------------------|----------------------------------|--|
| HPLC UV/Vis, 295 nm | MeOH-BR (pH 7) 9:1               | 1.1  |
| HPLC ED, +1.2 V     | MeOH-BR (pH 7) 9:1               | 1.6  |
| HPLC UV/Vis, 295 nm | MeOH-phosphate buffer (pH 2) 9:1 | 1.3  |
| HPLC ED, -0.9 V     | MeOH-phosphate buffer (pH 2) 9:1 | 4.9  |

<sup>a</sup>  $L_D$ , limit of detection.

## CONCLUSIONS

It has been proved that both batch voltammetry and amperometry at carbon paste electrode in flowing systems is applicable in the determination of micro- and submicromolar concentrations of genotoxic 5A6NQ. Both electrochemical oxidation of amino group and electrochemical reduction of nitro group can be used. In HPLC, the anodic detection methods utilizing the oxidation of amino group of 5A6NQ are preferred over the cathodic reduction of nitro group. The cathodic detection gives lower detection limits which are comparable with HPLC with spectrophotometric detection of 5A6NQ. In the case of batch voltammetric methods the limits of detection of 5A6NQ for both anodic and cathodic methods are comparable.

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## REFERENCES

1. Sugimura T.: *Toxicology* **2002**, 17, 181.
2. Stratil P., Kubáň V.: *Chem. Listy* **2004**, 98, 379.
3. Wogan N. G., Hecht S. S., Felton J. S., Conney A. H., Loeb L. A.: *Semin. Cancer Biol.* **2004**, 14, 473.
4. Zima J., Stoica A. I., Zítová A., Barek J.: *Electroanalysis* **2005**, 18, 158.
5. Tomaniová M., Kocourek V., Hajšlová J.: *Chem. Listy* **1997**, 91, 357.
6. Barek J., Bencko V., Cvačka J., Šuta M.: *Chem. Listy* **1998**, 92, 794.
7. Leotz-Gartziandia E., Tatry V., Carlier P.: *Environ. Monit. Assess.* **2000**, 65, 155.
8. White P. A.: *Mutat. Res.* **2002**, 85, 515.
9. Barek J., Cvačka J., Moreira C., Zima J.: *Chem. Listy* **1996**, 90, 805.
10. Barek J., Mejstřík V., Švagrová I., Zima J.: *Chem. Listy* **1994**, 88, 341.

11. Barek J., Cvačka J., Muck A., Quaiserová V., Zima J.: *Electroanalysis* **2001**, 13, 799.
12. Stratil P., Kubáň V.: *Chem. Listy* **2005**, 99, 3.
13. Felton J. S., Knize M. G., Dolbeare F. A., Wu R.: *Environ. Health Perspect.* **1994**, 102, 201.
14. Jakzyn P., Agudo A., Ibáñez R., García-Closas R., Pera G., Amiano P., González C. A.: *J. Nutr.* **2004**, 134, 2011.
15. Okumura L. L., Stradiotto R. N.: *Electroanalysis* **2007**, 19, 709.
16. Vineis P., Pirastu R.: *Cancer Causes Control* **1997**, 8, 346.
17. Naqao M., Wakabayashi K., Ushijima T., Toyota M., Totsuka Y., Sugimura T.: *Environ. Health Perspect.* **1996**, 104, 497.
18. Weisburger J. H.: *Mutat. Res.* **2002**, 9, 506.
19. Fitch C. D.: *Life Sci.* **2004**, 74, 1957.
20. Lo W. L., Tsang C. W.: *J. Hong Kong Med. Assoc.* **1986**, 38, 43.
21. Basco L. K., Ringwald P.: *Antimicrob. Agents Chemother.* **2003**, 47, 1391.
22. Haskelberg L.: *J. Org. Chem.* **1947**, 12, 434.
23. Queener S. F., Barlett M. S., Nasr M., Smith J. W.: *Antimicrob. Agents Chemother.* **1993**, 37, 2166.
24. González M. P., Díaz H. G., Cabrera M. A., Ruiz R. M.: *Bioorg. Med. Chem.* **2004**, 12, 735.
25. Karramkam M., Dollé F., Valette H., Basret L., Bromoullé Y., Hinnen F., Vaufrey F., Franclin C., Bauray S., Coulon C., Ottaviani M., Delaforge M., Loc'h C., Bottlaender M., Crouzel C.: *Bioorg. Med. Chem.* **2002**, 10, 2611.
26. Goda F. E., Aziz A., Ghoneim H. A.: *Bioorg. Med. Chem.* **2005**, 13, 3175.
27. Švancara I., Vytřas K., Barek J., Zima J.: *Crit. Rev. Anal. Chem.* **2001**, 31, 311.
28. Zima J., Švancara I., Pecková K., Barek J. in: *Progress on Drinking Water Research* (M. H. Lefebvre and M. M. Roux, Eds), Chap. 5. Nova Science Publishers, New York 2008.
29. Zima J., Švancara I., Barek J., Vytřas K.: *Crit. Rev. Anal. Chem.* **2009**, 39, 204.
30. Miller J. N., Miller J. C.: *Statistics and Chemometrics for Analytical Chemistry*, 4th ed., p. 120. Pearson, Harlow 2000.