VOLTAMMETRIC DETERMINATION OF FLUOREN-9-OL AND 2-ACETAMIDOFLUORENE USING CARBON PASTE ELECTRODES

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Square wave voltammetry and differential pulse voltammetry have been used for the determination of 2-acetamidofluorene and fluoren-9-ol using carbon paste electrodes, following the study of the influence of the carbon paste composition on the voltammetric signals of the analytes. The methods are based on the oxidation of the above compounds and they include adsorptive accumulation of the analyte on the surface of the working electrode. The limit of detection was 1 μmol l^{-1} for fluoren-9-ol in a medium of 0.1 M H_2SO_4, and 40 nmol l^{-1} for 2-acetamidofluorene in Britton–Robinson buffer (pH 7).

Keywords: 2-Acetamidofluorene; Fluoren-9-ol; Carbon paste electrode; Differential pulse voltammetry; Square wave voltammetry; Adsorptive stripping voltammetry; Electroanalysis; Electrochemistry.

Amino derivatives of polycyclic aromatic hydrocarbons (APAH) rank among environmental contaminants which are proved or suspected chemical carcinogens for humans[1,2]. They are mainly anthropogenic and are widespread in the environment as a result of their use as intermediates in the manufacture of various products[3]. Therefore, they could be found in a variety of establishments or in industrial effluents[4]. They are also found in various food products as a result of common cooking procedures such as broiling, frying, barbecuing, heat processing and pyrolysis of protein-rich foods[5]. APAH and hydroxy derivatives of polycyclic aromatic hydrocarbons (OHPAH) are also metabolites of their parent polycyclic aromatic hydrocarbons[6] (PAH) or nitro derivatives of polycyclic aromatic hydrocarbons (NPAH) which are also carcinogenic[7]. Therefore, APAH and OHPAH can be utilized as biomarkers for monitoring the exposure of humans to these parent environmental contaminants and simple and sensitive methods for their determination are of

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great interest. As fluorene and its various derivatives are often abundant in numerous environmental matrices, they are frequently used as model compounds for evaluating the exposition to PAH and their derivatives. The main pathways of fluorene biodegradation by bacteria[8–11] and fungi[12,13] involve the oxygenation of fluorene to fluoren-9-ol (9-HF). On the other hand, fluoren-9-ol can be found also among the metabolites of other PAH, e.g., fluoranthene[14]. Therefore, studies on PAH biodegradation need the methods for the determination of 9-HF. Another fluorene derivative, 2-acetamidofluorene (2-AAF) is widely used as a positive control by toxicologists to study the carcinogenicity and mutagenicity of aromatic amines. The processes of interaction of 2-AAF metabolites and DNA are of major importance for the understanding of tumor formation[15–19]. The National Toxicology Program in the U.S.A. recently included 2-AAF in the list of compounds reasonably anticipated to be human carcinogens based on sufficient evidence of carcinogenicity in experimental animals[20]. The determination of 9-HF and 2-AAF is carried out mostly by chromatographic methods – gas chromatography[8–10,12,14,21] and HPLC [9,13]. However, electroanalytical methods can be successfully used to determine the electroactive derivatives of PAH [22,23] as well. The main advantage of modern electroanalytical methods is their high sensitivity and low cost of analysis. Modern voltammetric methods utilizing carbon paste electrodes have been already successfully applied to the determination of various amino and hydroxy derivatives of PAH [24–26]. The advantages of carbon pastes are associated mainly with their broad potential window, low background current, ease of chemical or biological modification of the carbon paste composition and ease of renewal of the working surface of the carbon paste electrode. Among the disadvantages, it is necessary to mention lower stability of carbon pastes in media containing high amounts of organic solvents but even this drawback could be solved to some extent by using spherical microparticles of glassy carbon[27]. The aim of this work was to develop a voltammetric method for the determination of trace amounts of 9-HF and 2-AAF using their anodic oxidation on carbon paste electrode.

EXPERIMENTAL

Apparatus

The experiments with fluoren-9-ol were performed using a computerized potentiostat Autolab/PGSTAT30 (Eco Chemie, Netherlands) with the GPES 4.9 software in a square wave voltammetry (SWV) mode (frequency 50 Hz, scan rate 300 mV s^{-1} and amplitude 25 mV). For the measurements with 2-AAF, a computerized voltammetric analyzer Eco-Tribo Polaro-

graph connected to a voltammetric stand UMμE and driven by a voltammetric software PolarPro 2.0 (all Polaro Sensors, Prague, Czech Republic) in differential pulse voltammetry (DPV) mode was used. The voltammetric parameters used: scan rate 20 mV s^{-1}, pulse amplitude 50 mV, pulse duration 80 ms, and interval between pulses 40 ms. The working carbon paste electrode (CPE) was a Teflon tube with 2 mm hole filled with a carbon paste with contact through the threaded stainless steel piston. The carbon paste was prepared by thorough mixing 250 mg of carbon powder with 100 μl of paraffin oil Uvasol (Merck). The carbon powders used were graphite powder Acheson 38 (Fisher Scientific, Pittsburgh, U.S.A.) and glassy carbon spherical microparticles 0.4–12 μm in diameter, type 2 (Alfa Aesar, Ward Hill, U.S.A.). Glassy carbon working electrode F 3500 (Radiometer, Copenhagen, Denmark) with active part 3.2 mm in diameter was used for comparative experiments. The reference electrode was silver/silver chloride electrode RAE 113 (Monokrystaly, Turnov, Czech Republic) filled with 1 M KCl, to which all potential values are referred. Platinum wire served as auxiliary electrode. An ultrasonic bath PS02000A (Notus–Powersonic, Vráble, Slovakia) was used to facilitate dissolution of the analytes when preparing their stock solutions. pH of the solutions was measured with a conductivity and pH meter Jenway 4330 (Jenway, U.K.) with a combined glass electrode. All experiments were carried out at laboratory temperature.

Reagents

Fluoren-9-ol and 2-acetamidofluorene were obtained from Aldrich. Stock solutions, 1×10^{-2} M 9-HF and 2-AAF, were prepared by dissolving accurately weighed amounts of the substances in methanol (Lachema, Brno, Czech Republic). Working solutions of lower concentrations were prepared by further dilution of the stock solutions with methanol. 1×10^{-5} M solutions and lower concentrations were also prepared by dissolving the analytes directly in water using sonication. To obtain the analyte concentrations required for voltammetric experiments, the working solutions were added to aqueous solutions of Britton–Robinson buffers or inorganic acids. The methanol content in measured solutions, 1% (v/v), was kept constant. Britton–Robinson buffers were prepared in a usual way (i.e. by mixing a solution which was 0.04 mol l^{-1} in phosphoric acid, 0.04 mol l^{-1} in acetic acid, and 0.04 mol l^{-1} in boric acid with an appropriate amount of 0.2 M sodium hydroxide solution). Other reagents used were of analytical grade (Lachema, Brno, Czech Republic). All solutions were prepared by using deionized water obtained from a MilliQ Plus unit (Millipore, Molsheim, France).

Procedures

The fluoren-9-ol and 2-acetamidofluorene determinations were performed in 0.1 M H_2SO_4 and Britton–Robinson buffer (pH 7), respectively. Before every measurement, the surface of the CPE was renewed by protruding the paste with a piston and by removing thin layer of the paste with a wet filter paper. No other pretreatment of the CPE surface was used. After immersion of the CPE into a voltammetric vessel with 10 ml of a test solution, the voltammogram was recorded after 10 s rest time allowing the test solution to become quiescent. For adsorptive stripping voltammetry, stirring was switched on for a chosen accumulation time. Accumulation at open circuit potential was applied during the accumulation step. Stirring was stopped for a 10 s rest time and the potential scan was started from +0.5 V to obtain the voltammograms. The final potential of the scan was +1.2 and +1.7 V for 2-AAF and 9-HF, respectively. Calibration curves were measured in triplicate (each point; always with surface renewal before new measurement) and their parameters (slope, intercept, corre-

lation coefficient) were calculated. Measured voltammograms were not smoothed. Limit of determination (L_Q) for $\alpha = 0.05$ was calculated using statistic software Adstat, version 2.0 (TriloByte, Czech Republic).

RESULTS AND DISCUSSION

Determination of Fluoren-9-ol

Preliminary experiments using the glassy carbon electrode have shown that the 9-HF oxidation peak is observed at ca. +1.5 V (Fig. 1). However, the anodic potential windows of the carbon pastes prepared from various kinds of graphite powders were too narrow to obtain well-developed analytical peaks of 9-HF oxidation. When glassy carbon spherical microparticles were admixed to graphite powder in various ratios (from 0.2 to 1.0), the resulting carbon pastes showed better electrochemical properties with increasing content of glassy carbon spherical microparticles, while maintaining favourable mechanical properties with good plasticity and stability. Figure 1 illustrates the dependence of the shape of the recorded SWVs on the composition of carbon paste. It can be seen that the carbon paste containing glassy carbon spherical microparticles allows to obtain well defined 9-HF

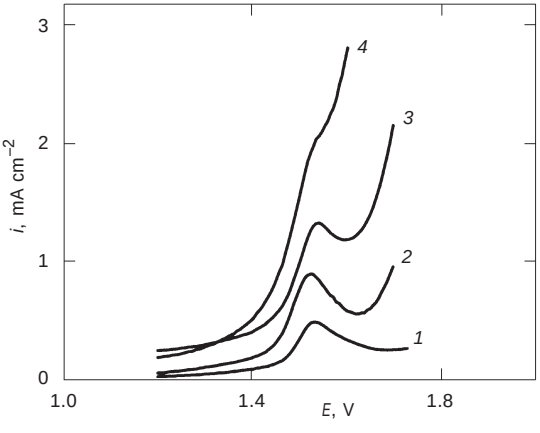

FIG. 1
Square wave voltammograms of 9-HF (c = 0.01 mol l^{-1}) in 0.1 M H_2SO_4 using glassy carbon (*1*) and carbon paste (*2–4*) electrodes based on glassy carbon spherical microparticles (*2*), mixture of glassy carbon microparticles and graphite powder 1:1 (*3*), and pure graphite powder (*4*). Current density values on *y*-axis are used for comparison of voltammograms obtained using the electrodes with different surface areas

oxidation peaks. Although carbon pastes containing a mixture of glassy carbon spherical microparticles and graphite powder (in the ratio 0.2–0.8 w/w) had better plasticity and the pastes could be more easily extruded from the electrode body, the voltammetric peaks of 9-HF were somewhat lower and broader. Therefore, carbon paste based on glassy carbon spherical microparticles was used for further measurements with 9-HF. Britton–Robinson buffers and various inorganic acids were used to find the optimum medium for 9-HF determination. The effect of pH on peak potentials and peak currents is depicted in Fig. 2. The dissociation of hydroxy group results in higher electron density at the redox centre which leads to easier electrochemical oxidation. This fact explains the observed shift of peak potentials to less positive values with increasing pH. However, the anodic border of the potential window moves to the negative side as well. In alkaline medium, the peak currents are lower than in acid medium and voltammetric peaks are broader. The highest and best developed analytical signals were obtained at low pH values, the optimum medium being 0.1 M H_2SO_4 where the calibration dependences were measured. The parameters of the calibration curves are summarized in Table I.

Among the advantages of CPEs is the possibility to utilize adsorptive or extractive accumulation of the analytes and thus to decrease their limits of determination. Figure 3 illustrates the effect of adsorptive accumulation in stirred solution on peak currents of square wave voltammetric peaks of

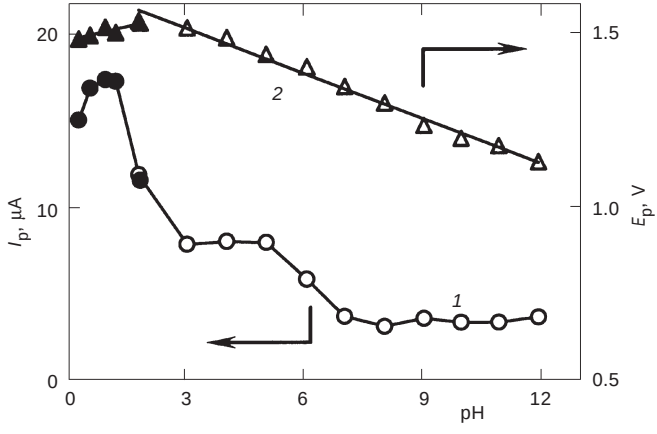

FIG. 2

The effect of pH on 9-HF (c = 0.1 mmol l^{-1}) peak currents (*1*) and peak potentials (*2*). The filled and empty symbols – H_2SO_4 and Britton–Robinson buffers, respectively. SWV on CPE based on glassy carbon spherical microparticles

9-HF for 50 and 100 μM concentrations. To achieve higher sensitivity, 1 min accumulation time was selected as optimum for measuring the calibration dependences. A further increase in the accumulation time did not markedly enhance the signal. The influence of accumulation potential on the peak

TABLE I
Calibration data for voltammetric determination of fluoren-9-ol (GCPE) and 2-acetamidofluorene (CPE)

Method	c, μmol l^{-1}	Slope mA mol^{-1} l	Intercept, μA	R	L_Q[a], mol l^{-1}
		Fluoren-9-ol, 0.1 M H_2SO_4			
SWV	100–300	188	–1.6	0.9996	1×10^{-4}
AdS SWV (t_{acc} = 1 min)	10–100	313	–1.6	0.9980	1×10^{-5} (1×10^{-6})[b]
		2-Acetamidofluorene, Britton–Robinson buffer (pH 7)			
DPV	1–10	47	–0.002	0.9977	2×10^{-7}
AdS DPV (t_{acc} = 1 min)	0.05–0.1	223	–0.006	0.9984	4×10^{-8}

[a] L_Q, limit of determination; [b] with background subtraction (see the text).

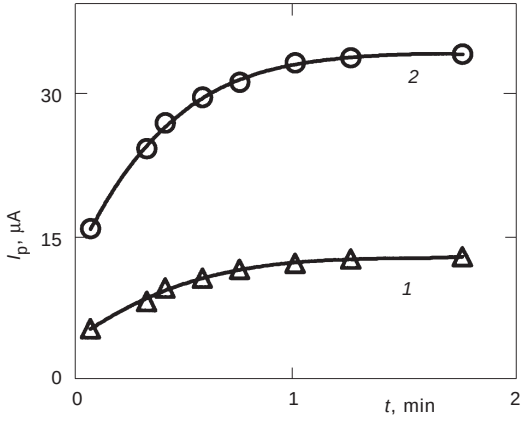

FIG. 3
The effect of the adsorptive accumulation time on peak heights of 9-HF: 50 μM 9-HF (*1*) and 100 μM 9-HF (*2*) in 0.1 M H_2SO_4. AdS SWV on CPE based on glassy carbon spherical microparticles

current of 0.1 M solution was studied for potentials from 0 to +0.7 V in 0.1 M H_2SO_4. As there was no pronounced effect of accumulation potential on the 9-HF peak currents, the accumulation at open circuit potential was chosen for measurement of calibration dependences. They were measured in the concentration range from 10 to 600 μmol l^{-1}. Although some deviation from linearity was observed for higher concentrations, the dependences were close to linear for low concentrations or for narrower intervals of 9-HF concentrations (Fig. 4). The oxidation peaks are very reproducible – relative standard deviation is 2–3% (n = 5) for concentration range 50–300 μmol l^{-1}. For lower concentrations, the relative standard deviation increases to 7–8%. The detection limit calculated from measured calibration dependences (see Table I) was checked by performing repeated measurements with 10 μM 9-HF and 1 min accumulation time. Figure 5a illustrates the shape of analytical peaks of 9-HF at 10 μM concentration. It can be seen that the shape of the peaks is not much convenient for graphic evaluation; however, using computerized peak search and evaluation, the relative standard deviation still does not exceed 10%. Thus, the 10 μM concentration was evaluated as a detection limit when no baseline correction was used. However, when using software subtraction of the background current, the shape of the voltammetric signal improves dramatically and, consequently, the detection limit for adsorptive stripping square wave voltammetric (AdS SWV) determination (at open circuit potential, t_{acc} = 1 min) of 9-HF on CPE

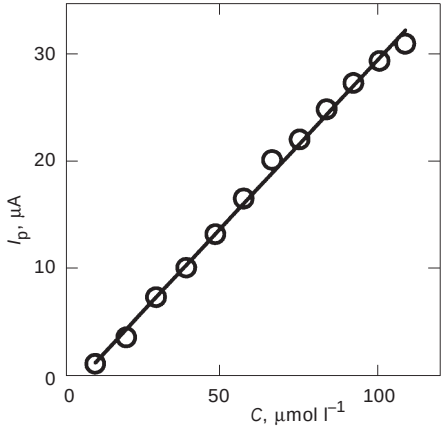

FIG. 4
Concentration dependence of 9-HF. AdS SWV on CPE based on glassy carbon spherical microparticles. 1 min accumulation time at open circuit in 0.1 M H_2SO_4

based on glassy carbon spherical microparticles decreases about 10 times – to 1 μmol l^{-1} (Fig. 5b).

Determination of 2-Acetamidofluorene

It has been found that 2-AAF oxidation peaks can be obtained with CPEs in a wide range of pH values of Britton–Robinson buffers and the peak potentials of DP voltammograms vary from ca. +1.0 V at pH 2 to ca. +0.6 V at pH 11 (Fig. 6). As the 2-AAF oxidation proceeds more easily in comparison with 9-HF, common and less expensive carbon powder was utilized for the preparation of carbon paste instead of glassy carbon spherical microparticles. Although the highest peaks were observed at pH 11, the optimum medium of pH 7 has been chosen for measuring the calibration dependences because 2-AAF peaks were better developed and more reproducible in this medium. As amides are susceptible to hydrolysis, the stability of 2-AAF solutions was tested measuring the SWVs of 2-AAF with carbon paste and glassy carbon electrode. When working at laboratory temperature, no decrease in peak heights was observed during 30 min time periods in all tested media of Britton–Robinson buffers, pH 2 to 12.

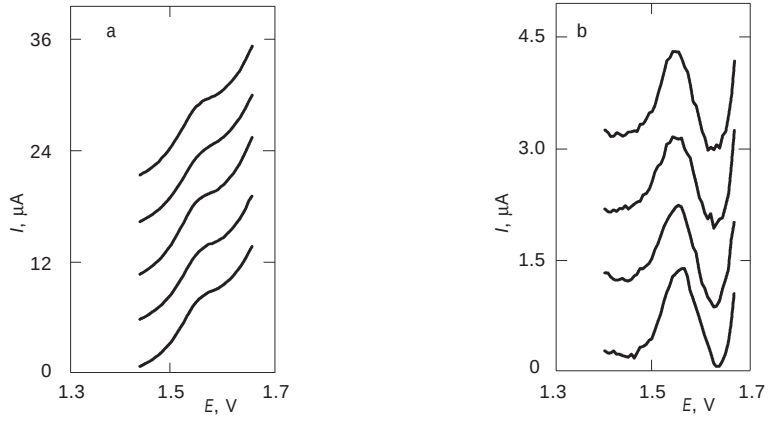

FIG. 5

The repetitive analytical signals at low concentrations of 9-HF without (a) and with (b) background line subtraction. AdS SW voltammograms on CPE based on glassy carbon spherical microparticles: 10 μM 9-HF (a), 5 μM 9-HF (b) in 0.1 M H_2SO_4. 1 min adsorptive accumulation time

The adsorptive accumulation at CPE under open circuit conditions can be used to enhance the sensitivity of the 2-AAF and 9-HF determination. A typical dependence of the peak height of 2-AAF on the adsorptive accumulation time is shown in Fig. 7. The effect of the accumulation can

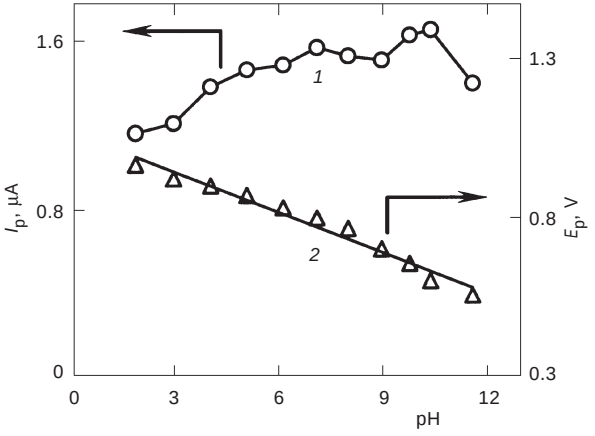

FIG. 6
The effect of pH on 2-AAF peak currents I_p (*1*) and peak potentials E_p (*2*). Conditions: 0.1 mM 2-AAF in Britton–Robinson buffers, DPV on CPE based on graphite powder

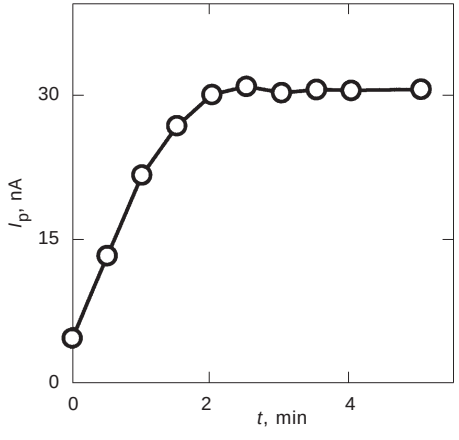

FIG. 7
The effect of the adsorptive accumulation time on peak heights of 0.2 μM 2-AAF. AdS DPV on CPE based on graphite powder, Britton–Robinson buffer (pH 7)

be seen also from a comparison of the dependences of peak heights on the 2-AAF concentration in Fig. 8 where the sensitivity after 1.5 min accumulation is about three times higher. It should be noted that the accumula-

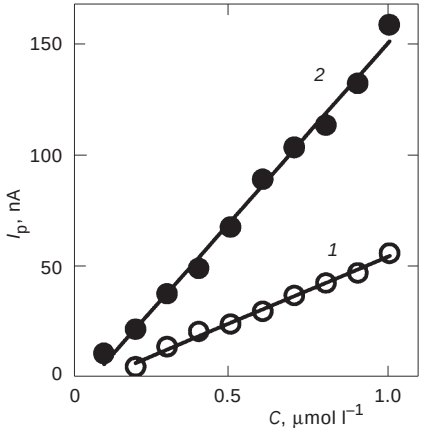

FIG. 8
Concentration dependences of 2-AAF over the concentration range 0.1–1 μmol l^{-1}. AdS DPV using CPE based on graphite powder. Measured without accumulation (*1*) and with 1.5 min accumulation time under open circuit conditions (*2*), Britton–Robinson buffer, pH 7

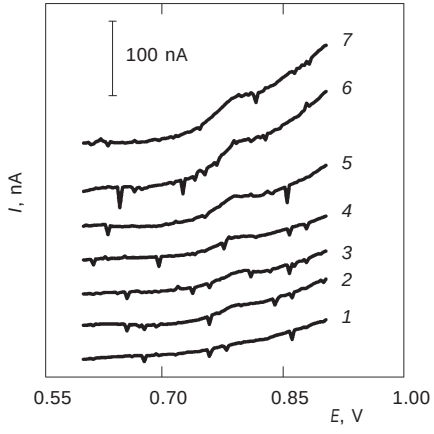

FIG. 9
AdS DP voltammograms of 2-AAF at the lowest concentration range measured. Britton–Robinson buffer (pH 7) (*1*), 50 (*2*), 60 (*3*), 70 (*4*), 80 (*5*), 90 (*6*), 100 (*7*) nM 2-AAF in Britton–Robinson buffer (pH 7). CPE based on graphite powder, 3 min accumulation time at open circuit potential

tion is not necessary for the determination of 2-AAF concentrations higher than 1 μmol l^{-1}. The 2-AAF calibration dependences are strictly linear up to 10 μmol l^{-1} (linear dynamic range from 40 nmol l^{-1} to 10 μmol l^{-1}; see Table I). For higher concentrations, complete coverage of the electrode surface by adsorbed 2-AAF results in nonlinear calibration dependences.

The relative standard deviations (RSD) of 2-AAF peaks depend on the concentration range – they are ca. 5 and 6–8% for 10–100 and 1–10 μM concentration ranges, respectively. However, RSDs do not exceed 10% for the lowest concentration range tested – 0.01–0.1 μmol l^{-1}. Figure 9 shows the AdS DP voltammograms of 2-AAF in the lowest measured concentration range. It follows from the voltammograms that the oxidation peaks are very flat and the evaluation of their heights is subject to errors, even when using good software. This results in a relatively high intercept. In principle, the evaluation of the limit of detection as three standard deviations gives a value of ca. 20 nmol l^{-1}; however, the electronic noise and shape of the analytical signals leads to the conclusion that realistic detection limit for 2-AAF is ca. 40 nmol l^{-1} which is comparable with limits of detection when using HPLC or GC.

CONCLUSIONS

The optimum conditions for the determination of fluoren-9-ol using square wave voltammetry and adsorptive stripping square wave voltammetry on the carbon paste electrode based on spherical microparticles of glassy carbon and of 2-acetamidofluorene using differential pulse voltammetry and adsorptive stripping differential pulse voltammetry on carbon paste electrode based on graphite powder have been found. The limit of determination was 1 μmol l^{-1} for 9-HF in 0.1 M H_2SO_4 for AdS SWV with 1 min analyte accumulation, and 40 nmol l^{-1} for the determination of 2-AAF in Britton–Robinson buffer (pH 7) for AdS DPV with 3 min analyte accumulation. Relative standard deviations did not exceed 10% for both AdS SWV determination of 9-HF and AdS DPV determination of 2-AAF in the lowest concentration ranges.

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