DETERMINATION OF 2-NITROPHENOL, 4-NITROPHENOL, 2-METHOXY-5-NITROPHENOL, AND 2,4-DINITROPHENOL BY DIFFERENTIAL PULSE VOLTAMMETRY AND ADSORPTIVE STRIPPING VOLTAMMETRY

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The behaviour of the title substances, which are components of the commercial plant growth stimulator Sviton, in differential pulse voltammetry at a hanging mercury drop electrode was studied and the optimum conditions were established for their quantitation over the concentration region of $1 . 10^{-7}$ to $1 . 10^{-5}$ mol l^{-1}. Additional decrease in the limit of determination was achieved in adsorptive stripping voltammetry, by which 4-nitrophenol and 2-nitrophenol can be determined within the concentration regions of $2 . 10^{-10}$ to $1 . 10^{-7}$ and $2 . 10^{-8}$ to $1. 10^{-7}$ mol l^{-1}, respectively, and 2,4-dinitrophenol and 2-methoxy-5-nitrophenol within the region of $2 . 10^{-9}$ to $1 . 10^{-7}$ mol l^{-1}.

The title nitrophenols are basic constituents of many plant growth stimulators such as the Japanese agent Atonic and the Czech agent Sviton, which are concentrates of the corresponding sodium phenolates, well suited to dilution with water. Toxicologically, the substances are poisons exhibiting appreciable cumulative effects, which block the oxidative phosphorylation in cells[1].

So far, the substances have been quantitated by gas chromatography[2–8], high performance liquid chromatography[2,9–13], paper chromatography[14], thin layer chromatography[2,15,16], UV spectrophotometry[17,18] and Raman spectrometry[19,20]. Extraction with ether[6] and adsorption[8] and ion exchange[21] column chromatography have been employed for purification.

The mechanism of polarographic reduction of 2-nitrophenol, 4-nitrophenol and 2,4-dinitrophenol has been discussed in monographs[22–25] and papers[26–29]. Coulometric reduction of 2,4-dinitrophenol at a constant potential has also been described[30]. More recently, the kinetics and mechanism of the irreversible phenomena involved have been discussed in refs[31,32]. The relationship between the structure and polarographic behaviour of various substituted nitrophenols has been dealt with in ref.[33]. As to the polaro-

graphic behaviour of 2-methoxy-5-nitrophenol, no mention could be found in the literature.

The sensitivity of conventional polarography, which has been applied, for instance, in combination with TLC to the determination of 2,4-dinitrophenol in herbicides[34], fails to meet the present demands placed on the quantitation of residues of the substances in the environment or in various biological materials. Attention has been therefore paid to sensitivity increase by application of pulse polarographic methods[35]. Differential pulse polarography has been employed for the determination of trace amounts of various nitrated pesticides[36–38]. Additional appreciable sensitivity increase for the quantitation of organic nitrocompounds can be achieved by using adsorptive stripping voltammetry[39–41], which also gave good results with nitrated pesticides[42]. Selectivity in the voltammetric determination of nitrophenols can be improved by adding α-cyclodextrin[43,44] and by using a glassy carbon electrode coated with a naphion membrane[43] or a hanging mercury drop electrode[44]. The use of a combined microelectrode for the determination of 2,4-dinitrophenol has also been reported[45].

The present work deals with the determination of 2-nitrophenol, 4-nitrophenol, 2,4-dinitrophenol and 2-methoxy-5-nitrophenol by differential pulse voltammetry (DPV) at a hanging mercury drop electrode (HMDE) and by adsorptive stripping voltammetry (AdSV).

EXPERIMENTAL

Reagents

Stock solutions of the substances: 2-nitrophenol ($C_6H_5NO_3$; CAS name: phenol, 2-nitro; CAS Registry Number: 88-75-5), 4-nitrophenol ($C_6H_5NO_3$; phenol, 4-nitro; 100-02-7), 2,4-dinitrophenol ($C_6H_4N_2O_5$; phenol, 2,4-dinitro; 51-28-5), and 2-methoxy-5-nitrophenol ($C_7H_7NO_4$; phenol, 2-methoxy-5-nitro; 90-05-1) at a concentration of $1 \cdot 10^{-3}$ mol l^{-1} were prepared by dissolving the corresponding quantity of the substance of reagent grade purity (Lachema, Brno, The Czech Republic) in 100 ml of deionized water (Milli-Q plus, Millipore, Bedford, U.S.A.) by means of ultrasound. The purity of the chemicals was checked by HPLC (ref.[46]). More dilute solutions were prepared by diluting the stock solutions with deionized water. All solutions were kept in dark.

The other chemicals used, viz. acetic acid, phosphoric acid and sodium hydroxide, were also of reagent grade purity (Lachema). Britton–Robinson buffers were prepared conventionally[47].

Apparatus

A PA 3 polarographic analyzer interfaced to an XY 4106 plotter (both Laboratorni pristroje, Prague) was used. The potential sweep rate in the DPV and AdSV measurements was 20 mV s^{-1}, modulation amplitude was –100 mV. The three-electrode connection using a reference calomel electrode and an auxiliary platinum wire electrode was employed. All potentials are reported versus SCE. An SMDE static mercury drop electrode (Laboratorni pristroje, Prague) with a capillary 0.136 mm in diameter, connected as a hanging mercury drop electrode (HMDE), served as the working electrode. The maximum drop size was used, determined by the 160 ms valve opening. The stirrer was operated at 500 r.p.m.

Oxygen was removed from the solutions by nitrogen purging for 10 min; nitrogen was purified for this by passing it through a solution of chromium(II) ions in dilute hydrochloric acid over a zinc amalgam.

Acidity was measured with a PHM 62 pH-meter (Radiometer, Copenhagen, Denmark) equipped with an indicator glass electrode and a reference calomel electrode.

Spectrophotometric measurements were accomplished with a Pye Unicam 8800 instrument (Cambridge, U.K.) in quartz cells 1.0 cm optical pathway.

Procedures

For the voltammetric measurements, the appropriate volume of the solution was added to a 10 ml volumetric flask and diluted to volume with a Britton–Robinson buffer. The solutions so prepared were transferred to the polarographic vessel and freed from oxygen by 10 min nitrogen purging, and the voltammetric curve was recorded. All measurements were carried out in triplicate and statistically processed. Linear regression calculations were based on the least squares method. The limits of determination L_Q were determined as the tenfold standard deviations from 7 analyte determinations at the concentration corresponding to the lowest point in the calibration straight line plot[48].

All measurements were performed at room temperature.

RESULTS AND DISCUSSION

Stability of Stock Solutions

The stability of the stock solutions of the analytes in water was monitored spectrophotometrically in quartz cells 1.0 cm optical pathlength. The absorbances were measured at the absorption peak wavelengths λ_{max}. The absorption spectra of the substances are shown in Fig. 1 (the spectrum of 4-nitrophenol, which is not reproduced, is virtually identical with that of 2-nitrophenol). The wavelengths λ_{max} and the results of measurement are given in Table I, demonstrating that no appreciable decrease in concentration occurs in 3 months. Hence, stored in dark at room temperature, the stock solutions of the substances studied are sufficiently stable.

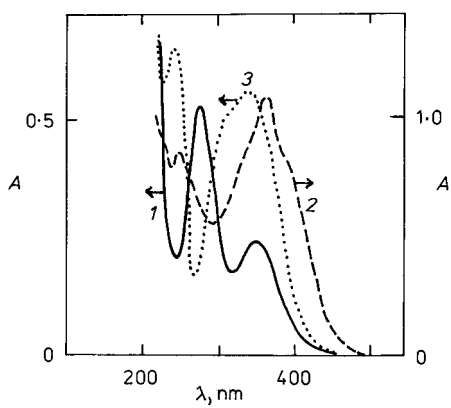

FIG. 1
Absorption spectra of 2-nitrophenol (*1*), 2,4-dinitrophenol (*2*) and 2-methoxy-5-nitrophenol (*3*) at $c = 1 \cdot 10^{-4}$ mol l^{-1}, optical pathlength 1.0 cm

Differential Pulse Voltammetry

Britton–Robinson buffers were chosen as the base electrolytes for the substances studied, based on their known DC-polarographic behaviour[32,35,37]. Figure 2 demonstrates that 2-nitrophenol, 4-nitrophenol and 2-methoxy-5-nitrophenol exhibit a single peak within the pH 2 – 12 region (4-nitrophenol gives 2 peaks at pH 12), whereas 2,4-dinitrophenol displays 2 peaks across the entire pH region. The pH-dependences of the peak potentials (E_p) and peak currents (I_p) are given in Table II. The following parameters were obtained for the linear relation

$$E_p \text{ (mV)} = a \text{ pH} + b :$$

For 2-nitrophenol:
$a = -53.0$, $b = +11.0$ at pH 2 – 7 ($r = 0.9966$),
$a = -95.5$, $b = +303.0$ at pH 7 – 10 ($r = 0.9998$),
$a = -30.0$, $b = -350.0$ at pH 10 – 12 ($r = 0.9999$).
For 4-nitrophenol:
$a = -58.1$, $b = -47.5$ at pH 2 – 7 ($r = 0.9979$),
$a = -126.0$, $b = +418.5$ at pH 7 – 10 ($r = 0.9941$),
$a = -27.5$, $b = -559.1$ at pH 10 – 12 ($r = 0.9672$).
For 2-methoxy-5-nitrophenol:
$a = -59.9$, $b = -6.4$ at pH 2 – 8 ($r = 0.9975$),
$a = -97.5$, $b = +297.5$ at pH 8 – 11 ($r = 0.9964$)
(r is the correlation coefficient).

These dependences are consistent with the following concept. Within the pH 2 – 6 range, non-dissociated molecules predominate in the solution and preliminary protonation takes place, giving rise to the observed E_p vs pH dependence. The hydroxy group

TABLE I
Stability of the substances in water[a] ($c = 1 \cdot 10^{-3}$ mol l^{-1})

Compound	λ_{max}, nm	*t*, days			
		1	8	30	90
2-Nitrophenol	280	100.0	98.5	96.8	95.0
4-Nitrophenol	280	100.0	99.6	99.0	98.5
2-Methoxy-5-nitrophenol	340	100.0	99.1	98.3	98.2
2,4-Dinitrophenol	357	100.0	99.0	98.5	98.4

[a] In relative concentrations (%) with respect to the initial concentration.

dissociates gradually at pH 6 – 10 and is responsible for the increase in the slope of the plot. Dissociated molecules of the analyte prevail in the solution at pH > 10 and are electrochemically reduced directly, without prior protonation, so that the E_p vs pH dependence is less pronounced in this pH range. The observed behaviour of 2-nitrophenol and 4-nitrophenol in DPV is consistent with that in DC polarography[26–28]. For 2,4-dinitrophenol, based on analogy with its behaviour in DC polarography[30], it is suggested that the first peak corresponds to the reduction of the nitro group in position 2 whereas the second peak corresponds to the reduction of the nitro group in position 4. The observed dependences of E_p on the concentrations of the substances studied are apparently due to the irreversible nature of the phenomena involved, although passivation of the electrode, whose surface is not renewed during the measurement, may play a role as well.

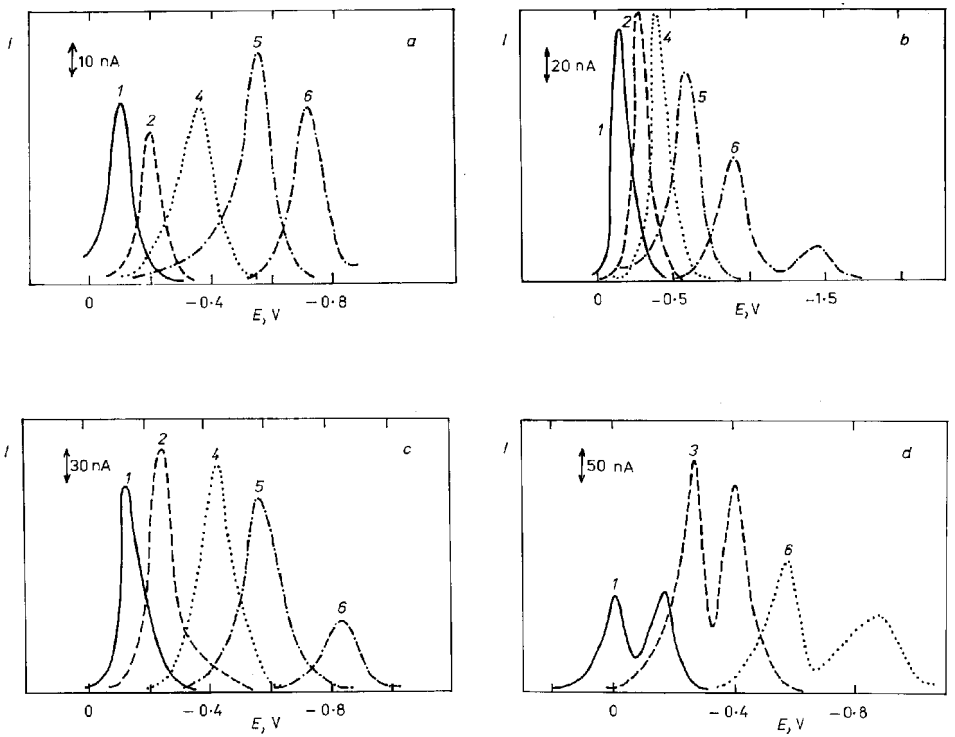

FIG. 2

Differential pulse voltammograms of 2-nitrophenol (*a*), 4-nitrophenol (*b*), 2-methoxy-5-nitrophenol (*c*) and 2,4-dinitrophenol (*d*) at $c = 1 \cdot 10^{-5}$ mol l^{-1} in Britton–Robinson buffer solutions at pH: *1* 2.0, *2* 4.0, *3* 6.0, *4* 7.0, *5* 9.0, *6* 12.0

TABLE II
Effect of pH on DP voltammograms of the substances (c = 1 . 10^{-5} mol l^{-1}) in Britton–Robinson buffer solutions

Compound	Peak parameters	pH									
		2.01	3.00	4.00	5.02	6.01	7.00	8.01	9.00	10.00	11.00	12.01
2-Nitrophenol	E_p, mV	–105	–135	–205	–250	–305	–365	–460	–560	–650	–680	–710
	I_p, nA	110	115	100	106	111	116	125	153	142	134	121
4-Nitrophenol	E_p, mV	–170	–220	–280	–330	–390	–475	–575	–740	–830	–870	–885[a] –1 460[a]
	I_p, nA	169	182	180	175	176	168	131	98	127	82	86[a] 22[a]
2-Methoxy-5-nitrophenol	E_p, mV	–136	–190	–235	–295	–360	–435	–490	–575	–665	–785	–810
	I_p, nA	205	217	242	232	200	225	225	192	90	85	70
2,4-Dinitrophenol	E_p^1, mV	5	–57	–120	–200	–260	–315	–355	–410	–445	–490	–565
	I_p^1, nA	157	247	277	362	385	382	370	375	375	337	215
	E_p^2, mV	–160	–205	–265	–335	–395	–465	–550	–720	–820	–870	–850
	I_p^2, nA	167	245	315	345	297	302	212	122	130	127	92

[a] The 2 values correspond to 2 peaks emerging (peaks 1 and 2, respectively).

From the analytical point of view, i.e. with respect to the peak height, peak shape, repeatability and facile evaluation, pH 5 emerged as optimum. This pH was used in the subsequent examination of other dependences. Table III demonstrates that DPV measurements should be made in a constant – and short – time from the solution preparation.

The dependence of the DPV peak height (measured from the straight line connecting the side minima) on the analyte concentration is linear over the region of $2 \cdot 10^{-7}$ to $1 \cdot 10^{-4}$ mol l^{-1} for all of the substances studied. The linear regression parameters and limits of determination are given in Table IV.

Adsorptive Stripping Voltammetry

For all substances at $c = 1 \cdot 10^{-7}$ mol l^{-1}, the dependence of the DPV peak height on the time of accumulation was measured in Britton–Robinson buffer solutions at pH 5. The height increased with time (Fig. 3), which bear out the occurrence of adsorption accumulation of the analytes on the surface of the hanging mercury drop electrode. The asymptotic character of this dependence is apparently related to the maximum possible coverage of the working electrode with the substance adsorbed.

The effect of the working electrode potential on the DPV peak height was examined at a constant time of accumulation of 90 s in stirred solutions. No appreciable effect was observed for 2-nitrophenol; its concentration dependences were therefore investigated at a potential of accumulation of 0 V. In such conditions the peak height dependence on concentration, applying accumulation for 60 s in stirred solutions, is linear across the concentration region of $(2 - 10) \cdot 10^{-8}$ mol l^{-1} (Table V). The standard deviation of experimental points from the regression straight line was 0.19 nA. Distorted voltammograms, difficult to evaluate, were obtained if the time of accumulation was

TABLE III
Stability of the substances ($c = 1 \cdot 10^{-6}$ mol l^{-1}) in Britton–Robinson buffer at pH 5.0[a]

Compound	*t*, min			
	10	20	30	60
2-Nitrophenol	100	97.6	89.9	75.0
4-Nitrophenol	100	98.7	95.4	93.2
2-Methoxy-5-nitrophenol	100	99.1	96.6	93.1
2,4-Dinitrophenol	100	99.0	98.7	97.4

[a] In relative concentrations (%) with respect to the initial concentration.

extended; this may be associated with the limited stability of 2-nitrophenol in the medium used.

TABLE IV
Parameters of calibration curves for the determination by DPV at a HMDE in Britton–Robinson buffer solutions at pH 5.0

Compound	c, mol l^{-1}	Slope mA mol^{-1} l	Intercept nA	r^a	$L_Q{}^b$ mol l^{-1}
2-Nitrophenol	$(1 - 10) . 10^{-5}$	6.77	–42.0	0.9864	–
	$(1 - 10) . 10^{-6}$	6.80	–0.13	0.9939	–
	$(2 - 10) . 10^{-7}$	6.95	–0.09	0.9991	$1.3 . 10^{-7}$
4-Nitrophenol	$(1 - 10) . 10^{-5}$	15.9	58.0	0.9994	–
	$(1 - 10) . 10^{-6}$	15.7	6.8	0.9994	–
	$(2 - 10) . 10^{-7}$	14.3	0.6	0.9988	$1.6 . 10^{-7}$
2-Methoxy-5-nitrophenol	$(1 - 10) . 10^{-5}$	13.8	89.0	0.9916	–
	$(1 - 10) . 10^{-6}$	13.2	6.1	0.9976	–
	$(2 - 10) . 10^{-7}$	12.8	1.2	0.9988	$1.2 . 10^{-7}$
2,4-Dinitrophenol	$(1 - 10) . 10^{-5,c}$	14.5	221.0	0.9916	–
	$(1 - 10) . 10^{-5,d}$	15.3	161.0	0.9938	–
	$(1 - 10) . 10^{-6,c}$	16.8	–2.9	0.9973	–
	$(1 - 10) . 10^{-6,d}$	17.2	10.6	0.9966	–
	$(2 - 10) . 10^{-7,c}$	14.8	1.5	0.9905	$1.3 . 10^{-7}$
	$(2 - 10) . 10^{-7,d}$	15.2	3.3	0.9932	$1.4 . 10^{-7}$

[a] Correlation coefficient; [b] limit of determination; [c] for peak 1; [d] for peak 2.

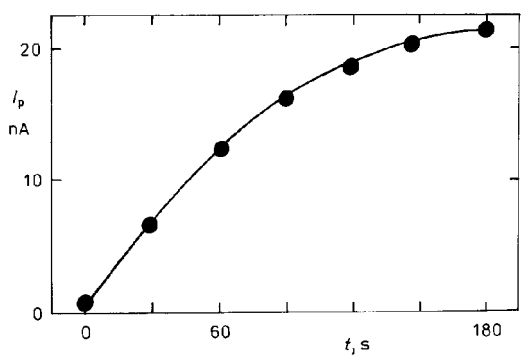

FIG. 3
Dependence of peak current on time of accumulation for 2-methoxy-5-nitrophenol at $c = 1 . 10^{-7}$ mol l^{-1} in stirred Britton–Robinson buffer solution, pH 5.0

Some dependence of the peak height on the potential of accumulation, though not very pronounced, was observed for 4-nitrophenol. The concentration dependences were therefore measured at a potential of accumulation of –0.2 V. The dependences were linear across the region of $2 . 10^{-10}$ to $1 . 10^{-7}$ mol l^{-1} (Table V). The standard deviation

TABLE V
Parameters of calibration curves for the determination by AdSV at a HMDE in Britton–Robinson buffer solutions at pH 5.0

Compound	E_{acc} [a] V	c mol l^{-1}	Slope mA mol^{-1} l	Intercept nA	r [b]	L_Q [c] mol l^{-1}
2-Nitrophenol	0	$(2–10) . 10^{-8}$ [d]	65.0	0.9	0.9952	$2.4 . 10^{-8}$
4-Nitrophenol	–0.2	$(1 – 10) . 10^{-8}$ [e]	103.0	0.3	0.9955	–
		$(1 – 10) . 10^{-9}$ [f]	211.0	2.3	0.9831	–
		$(2 – 10) . 10^{-10}$ [g]	510.0	1.4	0.9910	$2.8 . 10^{-10}$
2-Methoxy-5-nitrophenol	0	$(1 – 10) . 10^{-8}$ [h]	122.0	1.1	0.9966	–
		$(2 – 10) . 10^{-9}$ [d]	1 009.0	0.8	0.9947	$1.7 . 10^{-9}$
2,4-Dinitrophenol	0	$(1 – 10) . 10^{-8}$ [h]	167 [i]	–2.5 [i]	0.9933 [i]	–
			168 [j]	–1.7 [j]	0.9972 [j]	–
		$(2 – 10) . 10^{-9}$ [d]	910 [i]	–2.1 [i]	0.9986 [i]	$1.5 . 10^{-9}$
			950 [j]	–1.3 [j]	0.9984 [j]	$1.5 . 10^{-9}$

[a] Potential of accumulation; [b] correlation coefficient; [c] limit of determination; [d – h] time of accumulation in stirred solution (s): [d] 60, [e] 180, [f] 240, [g] 360, [h] 15; [i] for peak 1; [j] for peak 2.

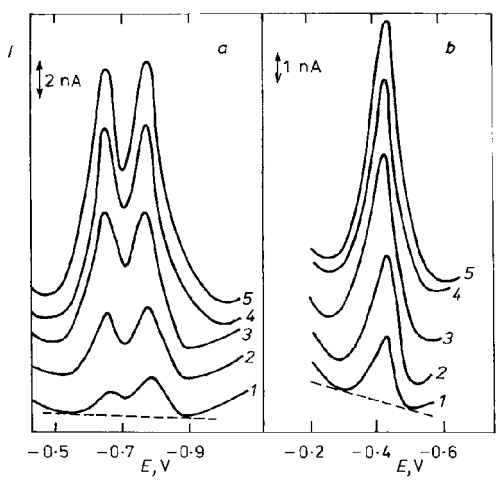

FIG. 4
Adsorptive stripping voltammograms of 2,4-dinitrophenol (*a*) and 2-methoxy-5-nitrophenol (*b*) in stirred Britton–Robinson buffer solutions at pH 5.0, with accumulation for 60 s. Analyte concentration (10^{-9} mol l^{-1}): *1* 2, *2* 4, *3* 6, *4* 8, *5* 10. Dashed lines are baselines from which the peak heights were measured

of experimental points from the regression straight line was 0.017 nA within the range of $(2 - 10) \cdot 10^{-10}$ mol l^{-1}. Extension of the time of accumulation to more than 360 s resulted in distorted voltammograms which could not be evaluated.

For 2-methoxy-5-nitrophenol and 2,4-dinitrophenol, the peak height was virtually independent of the potential of accumulation, and a potential of 0 V was therefore applied in the concentration dependence measurement. The concentration dependences were linear over the region of $2 \cdot 10^{-9}$ to $1 \cdot 10^{-7}$ mol l^{-1} for both substances (Table V). The standard deviation of the experimental points from the regression straight line within the range of $(2 - 10) \cdot 10^{-9}$ mol l^{-1} was 0.21 and 0.20 nA for the two substances, respectively. The voltammograms were strongly distorted and could not be evaluated if the time of accumulation was longer than 60 s. Voltammograms of the two substances at concentrations of $(2 - 10) \cdot 10^{-9}$ mol l^{-1} are shown in Fig. 4.

In conclusion, the new analytical methods are sufficiently sensitive to quantitate trace amounts of the substances studied. Since the differences between the peak potentials of the various nitrophenols are small, their mixtures cannot be analyzed directly, the components must be first separated by thin layer chromatography[49]. Residues of the substances in plants can be determined following separation by the method[49] based on sample homogenization, extraction with ether, preconcentration on a column containing a C18 reverse phase immobilized on a suitable sorbent such as Separon SGX, and chromatographic treatment on a Silufol UV 254 thin layer (Kavalier, Sázava, The Czech Republic). This preliminary separation has already been applied to the determination by differential pulse polarography; in comparison with the latter, however, the new methods are 1 to 2 orders of magnitude more sensitive.

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