ELECTROANALYSIS OF SOME CATECHOLAMINES AT A SINGLE-WALL NANOTUBES MODIFIED CARBON PASTE ELECTRODE

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Catecholamines (CAs) are important neurotransmitters and hormones in mammalian species. They are highly reactive and are readily oxidized to aminochromes. That enables the use of electrochemical method for their determination. In this paper, differential pulse voltammetric (DPV) determination and HPLC with amperometric detection of dopamine (D), epinephrine (E), norepinephrine (NE) and L-Dopa (DOPA) is developed and optimized. The carbon paste electrode (CPE) prepared of carbon powder CR2 and single-wall carbon nanotubes (SWCNT) (50:50 m/m) was used. The miniaturized body for CPE was used to reduce consumption of all chemicals. In Britton–Robinson buffer solution pH 6, the limits of detection 1×10^{-6}, 2×10^{-6}, 2×10^{-6} and 8×10^{-6} mol dm^{-3} have been found for DPV determination of D, E, NE and DOPA, respectively. HPLC was carried out using 0.1 M phosphate buffer pH 3:methanol (95:5) mobile phase containing 10 mM EDTA. The above described CPE in a wall-jet arrangement was used for amperometric detection. The limit of detection of each analyte in a mixture was 2×10^{-6} mol dm^{-3}. Practical application of this method on spiked urine samples was verified.

Keywords: Carbon paste electrode; Carbon nanotubes; Catecholamines; Differential pulse voltammetry; HPLC-ED; Electrochemistry; Voltammetry; HPLC.

Catecholamines (CAs) are compounds with a dihydroxyphenyl group and an amino group. The main endogenous CAs are dopamine (D), epinephrine (E), norepinephrine (NE) and L-Dopa (DOPA) (Fig. 1). They can act as hormones or neurotransmitters in several physiological and pathological situations related to both the autonomic and central nervous systems[1]. Thus, the determination of CA levels in biological fluids is a useful tool for the correct diagnosis of related diseases. High plasma and/or urine levels of this compound[2,3] are associated with states of anxiety in humans, are a marker for neuroblastoma[4] and pheochromocytoma[5,6] and are used for the diagnosis

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of cardiovascular diseases[7]. Catecholamines drugs are used in the treatment of bronchial asthma, hypertension, heart failure associated with organic heart diseases and cardiac surgery[8].

High-performance liquid chromatography with electrochemical detection (HPLC-ED) is most often used technique for analysis of catecholamines and their metabolites because of its high sensitivity and selectivity[9–12]. The coulometric detection[13,14] is the most sensitive method of ED, but the detector is rather expensive. Catecholamines are easily converted into quinone species by electrochemical oxidation. ED thus enables to obtain high sensitivity without derivatization. Fluorimetric detection after derivatization of CAs [15], fluorescence detection[16], chemiluminescence detection[17,18] and mass spectrometry[19] have been also reported. Other methods for CAs assays and studies involve capillary electrophoresis[20,21] and flow injection analysis[22,23].

Carbon nanotubes (CNT), discovered in 1991 [24] have unique structural, geometric, mechanical and chemical properties. There are two types of CNT, single-wall carbon nanotubes (SWCNT) and multi-wall carbon nanotubes (MWCNT). SWCNT, consisting of hollow cylindrically wound graphite sheet, are real single large molecules. MWCNT have a structure of rolling graphene sheets to make several layers spaced by van der Waals distance. CNT can behave as metals or semiconductors depending on the structure and their unique properties make them very attractive for the design of electrochemical biosensors[25]. MWCNT are more often used for the preparation of carbon paste electrode[26–29,43]. CPE have been widely used in determination of catecholamines[30–34], drugs, biomolecules and other organic species because of easy preparation and wider potential window depending on experimental conditions. Recently, extensive reviews covering the field of the use of carbon paste electrodes in electroanalytical chemistry

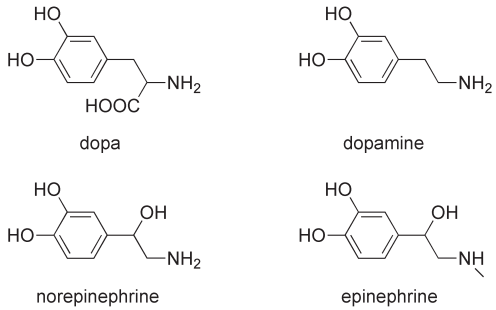

FIG. 1
Structural formulas of tested catecholamines

have been published[35–39]. CNT as a unique electrode material[44], but mainly as an electrode modifier[45–47] are also used for determination of CAs.

In this work, the voltammetric behavior of CAs was studied at a single-wall nanotubes modified carbon paste electrode (SWCNT-CPE) and then the SWCNT-CPE was tested as amperometric detector in a wall-jet arrangement in flow system. In both cases the miniaturized body of CPE [38] was used. This body allowed the voltammetric measurements in a volume of 1 ml which led to decrease of consumption of both the analytes and the electrode paste material.

EXPERIMENTAL

Reagents

Dopamine, epinephrine, norepinephrine and L-Dopa were purchased from Sigma–Aldrich (Germany). Britton–Robinson (B–R) buffers were prepared by mixing a solution of 0.04 M phosphoric acid, 0.04 M acetic acid and 0.04 M boric acid with the appropriate amount of 0.2 M sodium hydroxide solution. Chemicals for the preparation of the B–R buffers (all p.a. purity) were obtained from Sigma–Aldrich. Deionized water from Millipore Q-plus System (Millipore, USA) was used for all experiments. The 1×10^{-4} M CA stock solutions were prepared by dissolving test substances in deionized water. The solution of appropriate concentration was prepared for each measurement by proper diluting each CA stock solution with B–R buffer of appropriate pH. Carbon paste was prepared from carbon powder "CR2" (crystal graphite 2 μm, Maziva Tyn, Czech Republic), single-wall carbon nanotubes (Alfa Aesar, Germany) (50:50 m/m) and mineral oil Nujol (Fluka, Germany) (30% w/w). Mobile phase for HPLC contained methanol (LiChrosolv® Gradient grade, Merck, Germany), 0.1 M phosphate buffer solution pH 3 and EDTA (final concentration 10 mmol dm^{-3}). For model urine samples analysis, the addition of sodium dodecyl sulfate (final concentration 1 mmol dm^{-3}) to the mobile phase was used ensuring the formation of miceles for masking proteins[11–13].

Apparatus

Differential pulse voltammograms were obtained with PalmSens potentiostat (Palm Instruments BV) controlled by PalmSensPC software (Ivium Technologies, The Netherlands). Measurements were carried out in a three-electrode system consisting of a working carbon paste electrode in a miniaturized electrode body (University of Pardubice, Czech Republic)[38], a silver|silver chloride, 3 M KCl reference electrode RE-5B (BASi, USA) and a platinum wire as the auxiliary electrode (Monokrystaly, Czech Republic). DPV parameters were: applied potential range 0 to +1000 mV, scan rate 20 mV s^{-1}, pulse amplitude +50 mV, pulse time 0.070 s. An HPLC system consisted of a high-pressure pump HPP 5001 (Laboratorní přístroje, Prague, Czech Republic), injector valve CI-30 (Laboratorní přístroje, Prague, Czech Republic) with 10 μl loop, a column Kromasil 100-5 μm, 250 × 4.6 mm (VDS Optilab, GmbH, Germany) and UV/VIS detector LCD 2040 (Laboratorní přístroje, Prague, Czech Republic), with software CSW 32 (DataApex Ltd., Czech Republic). Amperometric detection using the miniaturized CPE in a wall-jet arrangement[41] was carried out with PalmSens (Palm Instruments BV) controlled by PalmSensPC software (Ivium Technologies, The Netherlands).

The same reference and auxiliary electrodes as in voltammetric measurements were used and the electrodes were immersed in a mobile phase in an overflow vessel. A 4330 Conductivity & pH Meter (Jenway Ltd., UK) fitted with the combined glass electrode was employed to measure the pH of the solutions. The pH meter was calibrated with aqueous buffers at laboratory temperature.

Procedures

DPV measurements were performed in an unstirred and not deaerated B–R buffer at a laboratory temperature. Miniaturized body of CPE was used in order to reduce the consumption of all chemicals. Small voltammetric cell enabling the measurements in a volume of 1 ml was used. Accumulations were performed in a stirred, not deaerated B–R buffer solution. The calibration curves were measured in triplicate and their statistical parameters (e.g. slope, intercept, limit of determination) were calculated using the least squares linear regression method. The detection limits were calculated as the concentration of an analyte which gave a signal three times the background noise (S/N = 3). Composition of carbon paste (carbon powder CR2:SWCNT (50:50 m/m)) was chosen on the basis of previous measurements, when SWCNT were tested as a modifier of CPE in order to prepare more sensitive electrode[40]. The paste was carefully hand-mixed for about half an hour in a mortar, the electrode body was filled by a paste and the carbon paste was allowed to homogenize till the next day when the measurements were started. The electrode surface was mechanically renewed before each measurement. Carbon nanotubes were purified by a treatment with a concentrated nitric acid as written in the literature[42]. The volume of 1 ml of the measured solution was used employing the carbon paste in a miniaturized body of electrode. The mobile phase for HPLC of CAs was chosen on the basis of the literature search[11–13]. It consisted of methanol and 0.1 M phosphate buffer pH 3 (5:95 v/v) and 10 mM EDTA, for urine model samples the mobile phase further contained 1 mM sodium dodecyl sulfate to mask the presence of proteins or other urine constituents[11–13]. Therefore, the urine samples were injected without previous extraction or pretreatment as the addition of sodium dodecyl sulfate to the mobile phase can mask the possibly present long chained constituents. All measurements were performed at laboratory temperature. The results of the determination of CAs using HPLC-ED were compared with the results obtained using HPLC with UV detection of CAs.

RESULTS AND DISCUSSION

DPV Determination of Dopamine, Epinephrine, Norepinephrine and L-Dopa at SWCNT-CPE

In our previous study[40] the influence of different types of carbon powders and different amounts of added modifier SWCNT into the carbon paste on the voltammetric behavior of E was studied. From this study followed the optimum carbon powder and optimum ratio between the carbon powder and SWCNT which was kept constant in this study.

The influence of pH on DPV of all CAs at a SWCNT-CPE was investigated in the range of pH 2 to 12. D provided one well-defined peak in the whole

pH range. The highest peak was obtained at pH 5 and 6. Both E and NE gave one peak in the range of pH 2–10. In this pH range, the peak broadened and decreased its height with increasing pH. In B–R buffer pH 11 and

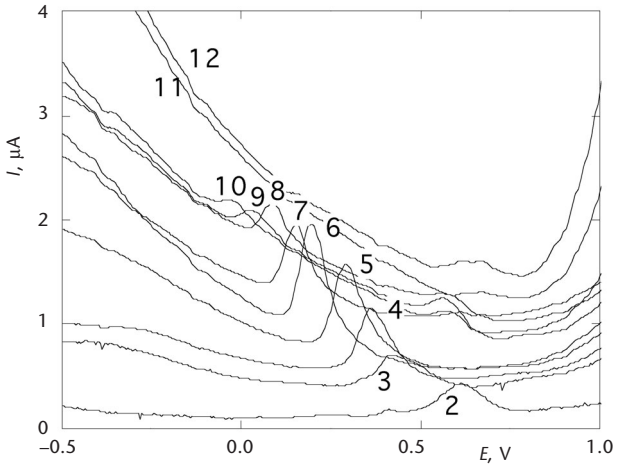

FIG. 2
DP voltammograms of 1×10^{-4} M epinephrine, measured at SWCNT-CPE in B–R buffer at pH 2–12. The numbers indicate pH of supporting electrolyte

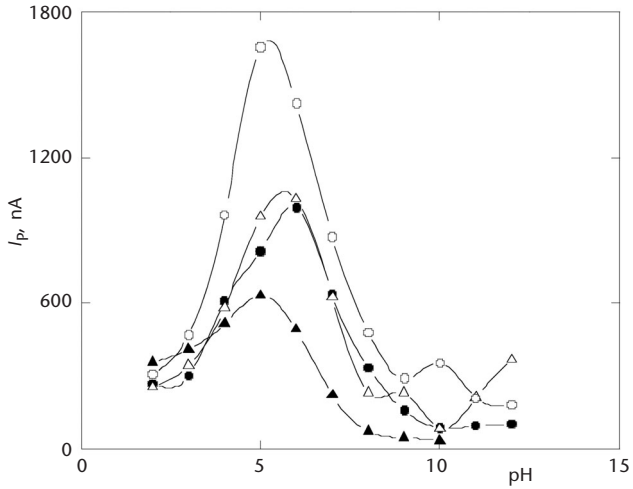

FIG. 3
Dependence of peaks heights (I_p) on pH: 1×10^{-4} M D (○), E (●), NE (△) and DOPA (▲) in B–R buffer pH 2–12. Measured by DPV at SWCNT-CPE

12, the compounds gave no oxidation peak (Fig. 2). The peak of DOPA was increasing till pH 6 then there was a small peak at pH 7 which disappeared at higher pH values. The peak potentials of all CAs shifted negatively with increasing pH. The dependence of the peak heights of CAs on pH is shown in Fig. 3. It was found that the oxidation peaks of CAs are best-defined and highest in the Britton–Robinson buffer pH 6, which was used for the following calibration measurements (Table I). Figures 4a–4d show DP voltammograms and calibration curves of D, E, NE and DOPA. D provided the highest peak and thus the lowest limit of detection 1×10^{-6} mol dm^{-3}. DOPA gave the highest limit of detection 8×10^{-6} mol dm^{-3}. E and NE gave the similar results, which is caused by the similar side chain in the structure of these compounds.

HPLC-ED Determination of Dopamine, Epinephrine, Norepinephrine and L-Dopa at SWCNT-CPE

The tested SWCNT-CPE was further used as amperometric wall-jet detector in a flow system. On the basis of the literature[11–13] the mobile phase containing methanol–0.1 M phosphate buffer pH 3 (5:95 v/v) and 10 mM EDTA was used. The optimal potential of working electrode was found out by measuring the hydrodynamic voltammograms (the dependence of peak height on the potential of the working electrode), see Fig. 5.

The hydrodynamic voltammograms were measured in the potential range from +0.3 to +1.4 V and the potential +0.7 V was chosen as optimal for the detection of mixture of all four CAs. Under optimized instrument conditions, the individual peaks were identified using standard addition method.

TABLE I
Parameters of calibration straight lines for DPV determination of D, E, NE and DOPA at SWCNT-CPE

Analyte	Concentration range, mol dm^{-3}	Slope nA mmol^{-1} dm^3	Intercept nA	Correlation coefficient	RSD %	L_D mol dm^{-3}
Dopamine	1×10^{-6}–1×10^{-4}	14.3	+40.4	0.998	4.0	1×10^{-6}
Epinephrine	2×10^{-6}–1×10^{-4}	9.9	+28.9	0.988	9.1	2×10^{-6}
Norepinephrine	2×10^{-6}–1×10^{-4}	[a]	[a]	0.996	4.1	2×10^{-6}
L-Dopa	8×10^{-6}–1×10^{-4}	3.7	+25.7	0.970	9.7	8×10^{-6}

[a] A nonlinear dependence.

Test substances were eluted in order NE – E – DOPA – D (Fig. 6). All peaks were eluted in less than 10 min. The calibration curve of the mixture of CAs was measured using HLPC-ED within the concentration range from 1 × 10^{-4} to 2 × 10^{-6} mol dm^{-3}. The achieved limit of detection was 2 × 10^{-6} mol

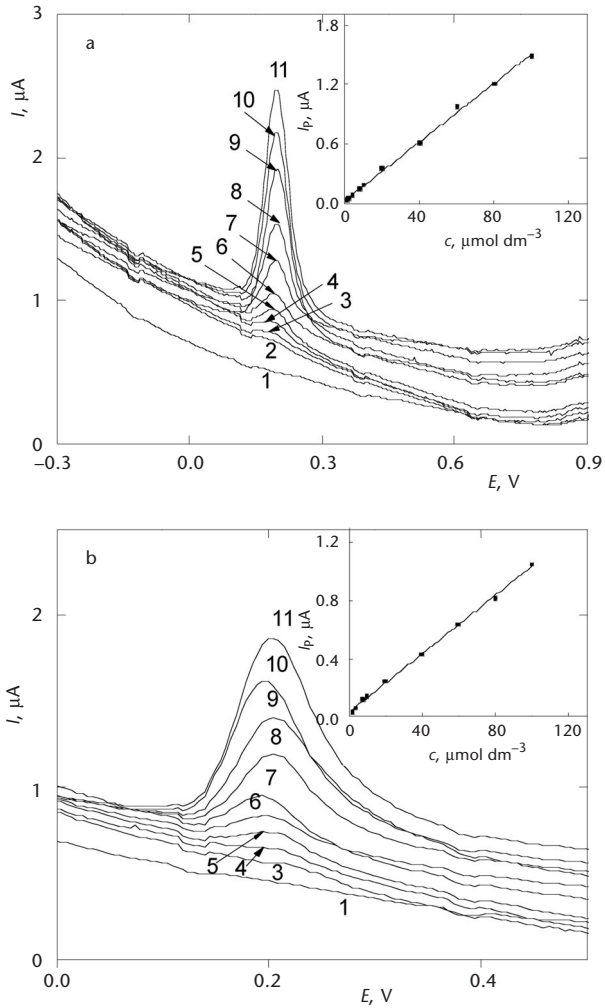

FIG. 4

DP voltammograms of dopamine (a), epinephrine (b), norepinephrine (c) and L-Dopa (d), measured at SWCNT-CPE in B–R buffer at pH 6; c(D) = 0 (1), 1 × 10^{-6} (2), 2 × 10^{-6} (3), 4 × 10^{-6} (4), 8 × 10^{-6} (5), 10 × 10^{-6} (6), 20 × 10^{-6} (7), 40 × 10^{-6} (8), 60 × 10^{-6} (9), 80 × 10^{-6} (10), 100 × 10^{-6} (11) mol dm^{-3}. Calibration dependence is in the inset

dm^{-3}. Practical applicability of the method was tested on model samples of human urine. In that case, sodium dodecyl sulfate (final concentration 1 mmol dm^{-3}) was added to the mobile phase. The presence of SDS in mobile phase caused the rise of pressure in the flow system and therefore, the flow rate was decreased to 0.8 ml min^{-1} to protect the column. The change of flow rate led to shift of analytes retention times, which is well visible in

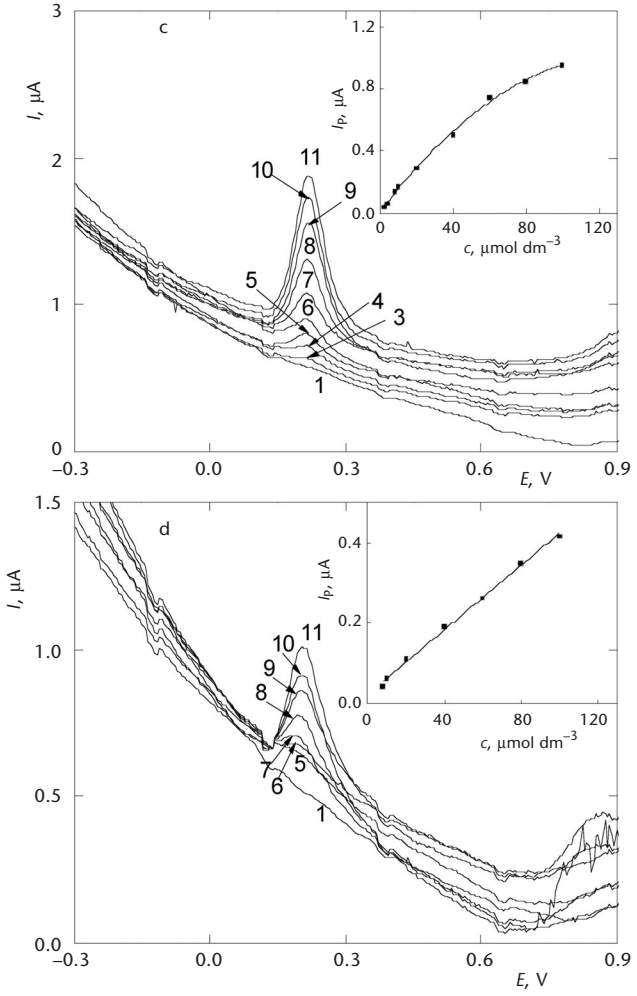

FIG. 4
(*Continued*)

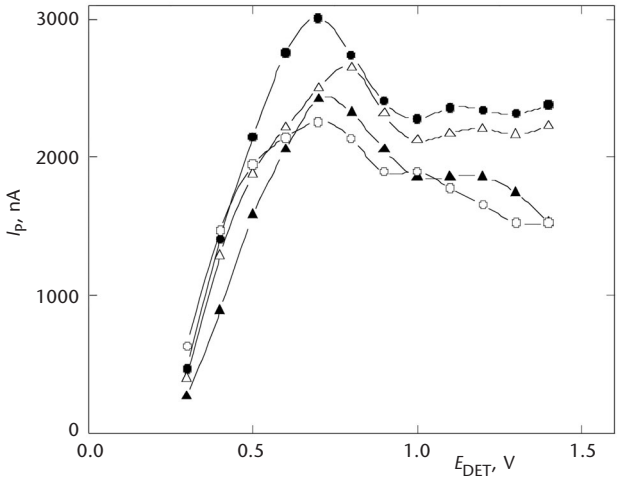

FIG. 5
Dependence of peaks heights (I_p) on E_{DET}: injected 10 µl of 1 × 10^{-4} M D (○), E (●), NE (△) and DOPA (▲). Measured by HPLC-ED at SWCN-CPE with mobile phase methanol:0.1 M phosphate buffer pH 3 (5:95 v/v) containing 10 mM EDTA at flow rate 1 ml min^{-1}

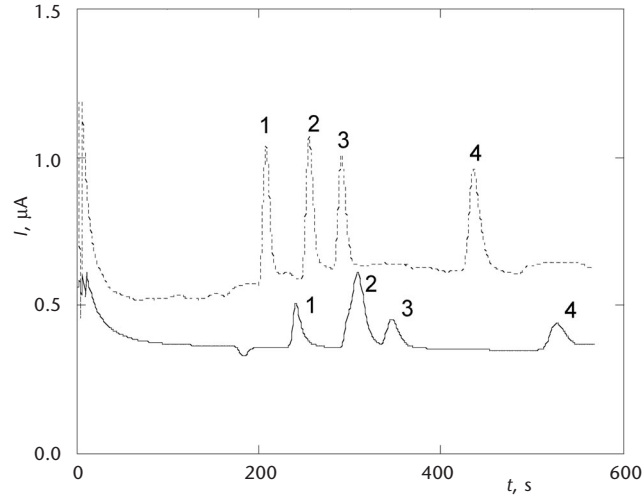

FIG. 6
HPLC-ED chromatograms of a mixture of 1 × 10^{-4} M NE (1), E (2), DOPA (3) and D (4) in mobile phase (dashed line) and a urine sample (solid line), 10 µl injected, E_p = 0.7 V, mobile phase methanol:0.1 M phosphate buffer pH 3 (5:95 v/v) with 10 mM EDTA (+1 mM sodium dodecyl sulfate for urine samples), flow rate 1 ml min^{-1}

Fig. 6. Figure 6 shows the comparison of HPLC-ED chromatograms of a mixture of D, E, NE and DOPA dissolved in a mobile phase or in human urine. The peaks were well separated in both cases. UV spectrometric detection was tested for these analytes and results show that amperometric detection is more suitable than UV detection. The other electrochemically active urine constituents were less pronounced when using selective electrochemical detection than universal UV detection (not shown).

CONCLUSION

A new method using a miniaturized carbon paste electrode body was developed for the determination of D, E, NE and DOPA by DPV at SWCNT-CPE. In Britton–Robinson buffer solution pH 6, the limits of detection have been found 1×10^{-6}, 2×10^{-6}, 2×10^{-6} and 8×10^{-6} mol dm^{-3} for the determination D, E, NE and DOPA, respectively. SWCNT were tested as a material for modification of carbon paste in order to prepare more sensitive electrode and to reach low limits of detection. Although the analytes gave higher peaks on SWCNT modified carbon paste electrode than on bare carbon paste, limits of detection of all the analytes were slightly higher than on bare electrodes, probably due to higher noise when working with SWCNT modified carbon pastes. On the other hand, the use of a miniaturized body of CPE allowed the measurements in low volumes of the analyzed solutions and decreased the consumption of all the chemicals. Further, it was shown that SWCNT-CPE could be used for electrochemical detection of catecholamines in RP-HPLC. The limit of detection of each CAs in a mixture was 2×10^{-6} mol dm^{-3}. The electrode was also usable for HPLC-ED determination of CAs in model urine samples. The amperometric detection proved to be more suitable than UV detection because in UV detection the urine constituents coincided with the peaks of studied analytes.

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