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New Applications of Carbon Paste Electrodes for Determination of Biologically Active Organic Compounds

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Abstract: In this paper, new electrochemical determinations of selected biologically active compounds are described employing various types of carbon paste electrodes (CPE) in batch voltammetric methods (differential pulse voltammetry (DPV), direct current voltammetry (DCV), and cyclic voltammetry (CV)) and in HPLC with electrochemical detection (HPLC-ED) utilizing amperometric CPE sensors. The results for determination of cymoxanil, famoxadone, 2,4-dihydroxybenzophenone, 2-hydroxy-4-methoxybenzophenone, chlortoluron, carboxin, triclosan, diafenthiuron, and propyl gallate are compared and discussed. Glassy carbon spherical microparticles were used as carbonaceous component of the paste. The limits of detection of some analytes were below $1 \cdot 10^{-7}$ mol l^{-1} in both batch and flow methods. The newly developed methods of determination were applied to model samples of drinking and river water, soils, and practical samples of edible oil, toothpaste, soap, and toilet water.

Keywords: Carbon paste electrode; Glassy carbon spherical microparticles; HPLC-ED; Differential pulse voltammetry; Cyclic voltammetry; Biologically active organic compounds.

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Introduction

Carbon paste electrodes (CPE) were introduced into the analytical praxis in 1958 by prof. Ralph. N. Adams [1], and since then, several thousand papers appeared in the literature dealing with the use of carbon pastes in different fields of electroanalytical chemistry, for determinations of numerous inorganic and organic analytes.

The development in this field is documented in many reviews, book chapters and books covering the latest developments, some examples being the reference sources [2-10]. Carbon paste electrodes were introduced into the analytical chemistry with the intention to cover the anodic potential region, as at the time of their introduction, the determinations in cathodic potential region were under thorough and intensive investigation utilizing variations of classic polarography or related methods using liquid mercury as the electrode material. Oxidation reactions were studied using solid electrodes like glassy carbon or noble metal electrodes. The obvious disadvantages of electroactive surface passivation and necessity of cleaning the electrode surface following each measurement compromised these types of electrodes and lead to lower repeatability and reproducibility when comparing with polarographic or voltammetric methods at mercury electrodes.

Carbon pastes as a simple mixture of carbon powder and a suitable pasting liquid are easy to prepare, easy to use for measurements and easy to refresh their surface mechanically by wiping off the surface layer, which was used for measurements and possibly passivated by the products of the electrode reaction. The vast choice of available forms of carbon powders and available pasting liquids when adjusting the properties of carbon pastes electrodes is another advantage, which contributed to quite fast spread in the field of electroanalytical chemistry. Admixture of the third component into the paste composition gave birth to chemically modified carbon paste electrodes (CM CPE) [11]. CM CPE enabled to increase the selectivity or even the specificity of the determinations due to selective reactions of the analytes with the modifier, or to increase the sensitivity of the determinations due to the possibility to adsorb, absorb or extract the analytes onto or into the modified carbon paste.

Another major development in the field was the introduction of biologically modified carbon paste electrodes (BM CPE) [12] with entrapped enzymes. With BM CPE, it was suddenly possible to determine a lot of organic compounds, which were not directly oxidizable at bare CPE, such as carbohydrates, organic acids, amino acids, or peptides. To name at least some other breakthroughs in the field of carbonaceous electrodes it is necessary to mention the introduction of heterogeneous carbon composite electrodes and screen printed electrodes (SPE) [13]. The introduction of film electrode configurations and new carbon materials — e.g. carbon single-wall or multi-wall nanotubes, graphene, etc. — should be briefly mentioned, too [14-17].

Over the last four years several new methods of determination of biologically active organic compound including either environmental pollutants (e.g. pesticides or endocrine disruptors) or food additives have been developed in our UNESCO Laboratory of

Environmental Electrochemistry. The developed methods are summarized here with basic parameters of determination methods. The advantages and disadvantages of the developed methods are discussed, limits of detection are compared and the results of analytes determinations in either model or real samples brought together.

Experimental

Chemicals, Reagents, Stock and Standard Solutions

All chemicals used for the preparation of analyte stock solutions, buffers, mobile phases were of analytical reagent grade or HPLC grade and purchased from Sigma-Aldrich, Lach-Ner (Czech Republic), or Merck. Water used throughout all measurements was deionized by Milli-Q system from Millipore. The stock solutions were prepared in the concentration 1.10^{-3} M in methanol. All measurements were carried out at laboratory temperature.

Electrochemical Apparatus and Other Instrumentation

An electrochemical system Eco-Tribo Polarograph equipped with PolarPro software (PolaroSensors, Prague, Czech Republic) and PalmSens system equipped with PSTrace 2.4 software (PalmSens, Netherlands) were used for voltammetric batch measurements. HPLC flow system consisted of gradient pump Beta, injection valve with 20 μl loop, column Kromasil 100-7 μm, C18, 150 x 4,6 mm, degasser DG-4014 (all from Ecom, Prague, Czech Republic), and amperometric detector ADLC 2 (Laboratorní přístroje, Prague, Czech Rep.).

Amperometric detector worked in three electrode system and consisted of working carbon paste electrode in wall-jet arrangement, Ag | AgCl | 3M KCl reference, and Pt wire counter electrode (both Monokrystaly; Turnov, Czech Republic), similarly as during batch voltammetric measurements. The parameters of differential pulse voltammetric measurements were scan rate, v = 20 $mV \cdot s^{-1}$, the pulse height (amplitude), ΔE = 50 mV, and the pulse width of 100 ms. pH values were measured by a digital pH-meter with combined glass electrode (Jenway, United Kingdom).

The stability of stock solutions was checked in 1 mm quartz cuvettes using a spectrophotometer Agilent 8453 (Agilent Technologies, USA). Ultrasound system PSO2000A (Powersonic, USA), shaker (model “Vortex Genie 2”; Scientific Industries, Inc., USA), magnetic stirrer MS 3000 (Jenway, UK) were further used. River water samples from Vltava

river, Prague, were filtered through ProFill Plus PVDF (0.45 μm) and MS Nylon Membrane Filter (0.22 μm, Membrane Solutions, USA) filters. Soil samples from Prague, Modrany, were sieved to the fraction below 120 mesh and, after drying, were used for the preparation of spiked model samples.

All experiments were performed at least in triplicate. The statistical differences were calculated using the probability factor $p = 0.05$. The quantification limits were calculated as the concentration of the analyte which gave a signal three times the standard deviation of the signal of the lowest evaluable concentration.

Carbon Paste and Other Electrodes

Carbon pastes were prepared by thorough hand-mixing of 0.25 g of glassy carbon spherical microparticles (Alfa Aesar, USA) with 50 to 120 μl of mineral oil (Sigma). Both components had been homogenized manually in a mortar, the paste was packed into the piston driven electrode teflon body with the inner diameter of 2 mm, and thus prepared CPE was used for the measurements next day. The electrode surface was renewed mechanically before each batch measurement by wiping off the used carbon paste layer. The miniaturized glassy carbon paste electrode (minGCPE) consisted of *Teflon*®-based capillary with 0.5 mm inner diameter filled with carbon paste. In case of HPLC-ED measurements, the electrode surface was not renewed between the measurements as the mobile phase flow was cleaning the surface sufficiently and CPE was used in a configuration of wall-jet in an overflow vessel. Composite fiber rod electrode (CFRE) was made from a carbon fiber rod of 2 mm outer diameter (Midwest Products Co., Indiana, USA), glassy carbon electrode was a product of Metrohm, Herisau, Switzerland, with 2 mm outer diameter.

Results and Discussion

Development and Optimization of the Method

The voltammetric behavior of all the analytes studied made 1.10^{-4} mol l^{-1} in concentration was investigated using differential pulse voltammetry (DPV), direct current voltammetry (DCV) or cyclic voltammetry (CV) in Britton-Robinson (BR) buffers of pH 2-12 in mixtures with methanol (MeOH), in appropriate ratios reflecting the solubility of each analyte.

BR buffers were used purposely as they cover wide range of pH values with comfortable adjusting the ratios of its main components. From these studies, the optimum conditions for respective determinations were found and used for measurement of calibration dependences. Then, the parameters of the calibration lines were calculated together with analytes limits of detection or limits of determination. The attempts to decrease the limits of determination by adsorptive accumulation of the analytes on the surface of working electrodes were not too successful as the studied analytes were relatively well soluble in aqueous or mixed methanol-aqueous media and exhibited low tendency to adsorb at hydrophobic electrode surface.

In the case of HPLC-ED, the hydrodynamic voltammograms of 1.10^{-4} mol l^{-1} analyte in a potential range from +0.7 to +1.2 V were measured in suitable mobile phases compatible with the used column; gradient elution was utilized in some measurements. The results of all newly developed methods of analytes determination together with their basic analytical parameters are summarized in Table I.

Cymoxanil and famoxadone are pesticides which could appear in the environment in mixtures. Therefore, methods of determination were studied for both compounds: for cymoxanil by DPV in cathodic potential region using other types of electrodes than CPE and for famoxadone by DPV using minGCPE (see Fig. 1), and GCE [18]. For cymoxanil, comparable limits of detection were obtained using DPV on both types of studied electrodes, for famoxadone, much better results were obtained using DPV at minGCPE.

The electrode surface of minCPE was renewed by cutting off the outer part of capillary filled with the paste; the repeatability of the renewal was checked by measuring ten successive curves with and without renewal of the electrode surface. The calculated repeatability of peak heights was around 8 % which was about twice as much in the comparison with the same experiment performed using GCE. Nevertheless, minGCPE enabled determination of even submicromolar concentrations of famoxadone in model samples of river water and in soil samples. The presence of either cymoxanil or famoxadone in the mixed sample does not negatively influence the voltammograms of the other analyte, so that both substances could be measured simultaneously in one sample, in either cathodic or anodic potential region.

Herbicide chlortoluron was determined by two batch voltammetric methods using two types of GCPE and by HPLC-ED at GCPE. Best results were obtained with HPLC-ED, where the limit of chlortoluron detection was below $4 \cdot 10^{-8}$ mol l^{-1}. Even DPV at minGCPE gave the limits of chlortoluron detection below $1 \cdot 10^{-7}$ mol l^{-1}.

Table I: *Parameters of newly developed methods of determination of selected biologically acive organic compounds.*

Compound	Electrode	Method	Optimum medium	Concentration range (M)	LOD (M)	Real or model matrix	Ref.
cymoxanil	CFRE	DPV	BRB pH 4 : MeOH (9:1)	$1\cdot10^{-5} – 6\cdot10^{-7}$	$5.9\cdot10^{-7}$	soil 0.3 mg.kg^{-1}, river water $1.3\cdot10^{-6}$ M	18
	GCE	DPV	BRB pH 7 : MeOH (9:1)	$1\cdot10^{-5} – 4\cdot10^{-7}$	$5.6\cdot10^{-7}$		18
famoxadone	GCE	DPV	BRB pH 4 : MeOH (9:1)	$1\cdot10^{-5} – 6\cdot10^{-7}$	$6.3\cdot10^{-7}$		18
	minGCPE	DPV	BRB pH 2 : MeOH (9:1)	$1\cdot10^{-5} – 2\cdot10^{-7}$	$1.4\cdot10^{-7}$	soil 0.03 mg.kg^{-1}, river water $4.9\cdot10^{-7}$ M	18
2,4-dihydroxy-benzophenone (BP-1)	GCPE	HPLC-ED, +1.1 V	PhAcB pH 6 : MeOH grad	$1\cdot10^{-4} – 4\cdot10^{-7}$	$1.4\cdot10^{-7}$	in urine following SPE $9.9\cdot10^{-9}$ M	19
	GCE	HPLC-ED, +1.1 V	PhAcB pH 6 : MeOH grad	$1\cdot10^{-4} – 4\cdot10^{-8}$	$1.3\cdot10^{-8}$		19
2-hydroxy-4-methoxy-benzophenone (BP-2)	GCPE	HPLC-ED, +1.1 V	PhAcB pH 6 : MeOH grad	1·10-4 – $4\cdot10^{-7}$	$2.2\cdot10^{-7}$	in urine following SPE $2.1\cdot10^{-8}$ M	19
	GCE	HPLC-ED, +1.1 V	PhAcB pH 6 : MeOH grad	$1\cdot10^{-4} – 4\cdot10^{-8}$	$3.2\cdot10^{-8}$		19
	GCPE	DPV	BRB pH 12 : MeOH (99:1)	$1\cdot10^{-4} – 1\cdot10^{-6}$	$6.0\cdot10^{-7}$	tap water $5.5\cdot10^{-7}$ M	20
chlortoluron	GCPE	DPV	BRB pH 3 : MeOH (9:1)	$1\cdot10^{-4} – 8\cdot10^{-7}$	$3.7\cdot10^{-7}$	river water $8.3\cdot10^{-7}$ M, soil 0.93 mg.kg^{-1}	21
	GCPE	HPLC-ED, +1.3 V	BRB pH 4 : MeOH (4:6)	$1\cdot10^{-4} – 2\cdot10^{-7}$	$3.3\cdot10^{-8}$	river water $5.7\cdot10^{-8}$ M, soil 0.086 mg.kg-^{1}	21
	minGCPE	DPV	BRB pH 3 : MeOH (9:1)	$1\cdot10^{-4} – 4\cdot10^{-7}$	$8.7\cdot10^{-8}$	river water, $1.0\cdot10^{-7}$ M, soil 1.3 mg.kg^{-1}	21
carboxin	GCPE	DPV	BRB pH 2 : MeOH (99:1)	$1\cdot10^{-4} – 1\cdot10^{-7}$	$1.1\cdot10^{-7}$	tap water $3.2\cdot10^{-7}$ M, river water $6.1\cdot10^{-7}$ M	22
	minGCPE	DPV	BRB pH 2 : MeOH (99:1)	$1\cdot10^{-4} – 1\cdot10^{-7}$	$3.3\cdot10^{-7}$	tap water $4.9\cdot10^{-7}$ M, river water $6.4\cdot10^{-7}$ M	22
	GCPE	HPLC-ED, +1.2 V	BRB pH 5.5 : MeOH (4:6)	$1\cdot10^{-4} – 1\cdot10^{-7}$	$1.1\cdot10^{-7}$	tap water $1.6\cdot10^{-7}$ M, river water $3.1\cdot10^{-7}$ M	22
triclosan	GCPE	DPV	BRB pH 11 : MeOH (9:1)	$1\cdot10^{-4} – 1\cdot10^{-7}$	$1.2\cdot10^{-7}$	tap water $1.2\cdot10^{-7}$, river water $2.0\cdot10^{-7}$, tooth paste, soap	23

Compound	Electrode	Method	Optimum medium	Concentration range (M)	LOD (M)	Real or model matrix	Ref.
diafenthiuron	GCPE	DPV	BRB pH 5 : MeOH (1:1)	$1 \cdot 10^{-4} – 1 \cdot 10^{-5}$	$1.5 \cdot 10^{-5}$		24
	GCPE	DCV, CV	BRB pH 3 : MeOH (1:1)	$1 \cdot 10^{-4} – 1 \cdot 10^{-5}$	$1.7 \cdot 10^{-5}$		24
propyl gallate	GCPE	DPV	BRB pH 5 : MeOH (4:1)	$1 \cdot 10^{-4} – 1 \cdot 10^{-7}$	$6 \cdot 10^{-8}$	edible oil	25
	GCPE	HPLC-ED, +0.8 V	PB pH 4 : MeOH (1:1)	$1 \cdot 10^{-4} – 1 \cdot 10^{-7}$	$3.9 \cdot 10^{-8}$	edible oil	25

Abbreviations & symbols used: BRB Britton-Robinson buffer, minGCPE capillary glassy carbon paste electrode, CFRE composite fiber rod electrode, CV cyclic voltammetry, DCV direct current voltammetry, DPV differential pulse voltammetry, GCE glassy carbon electrode, GCPE glassy carbon paste electrode, HPLC-ED high performance liquid chromatography with electrochemical detection, MeOH methanol, PhAcB phosphate-acetate buffer.

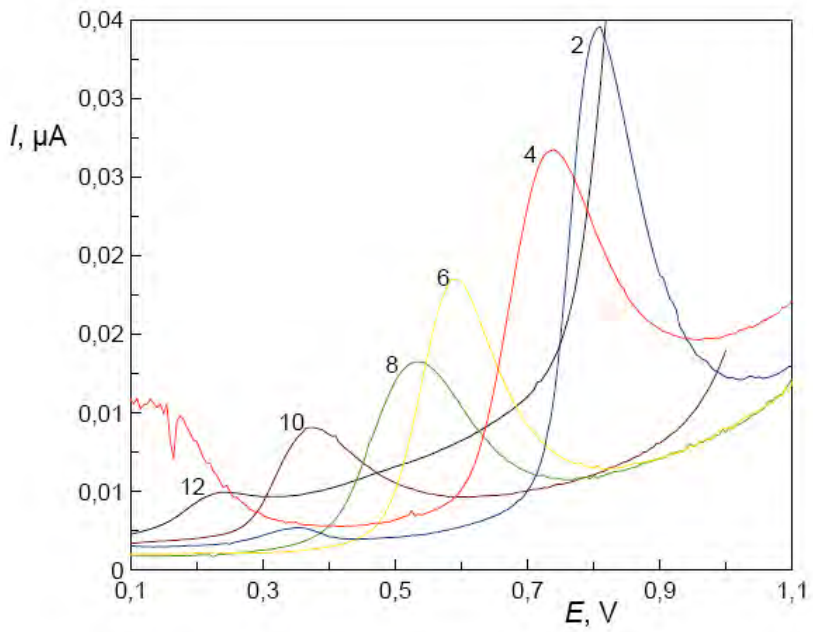

Fig. 1: *Anodic DP voltammograms of famoxadone (c = 1.10^{-5} mol l^{-1}) at a miniaturized carbon paste electrode in BR buffer –methanol (9:1) medium with pH values of the BR buffer indicated above the voltammograms (over 2-12 range).*

Limits of detection in model matrices were similar to those in pure supporting electrolytes. The relative standard deviation of HPLC-ED peak of chlortoluron was 3.3 % (Fig. 2). Such a good repeatability together with low limit of electrochemical detection on GCPE and the separation power of HPLC clearly demonstrated that this is a superior method of determination of this herbicide in model samples with real environmental matrices.

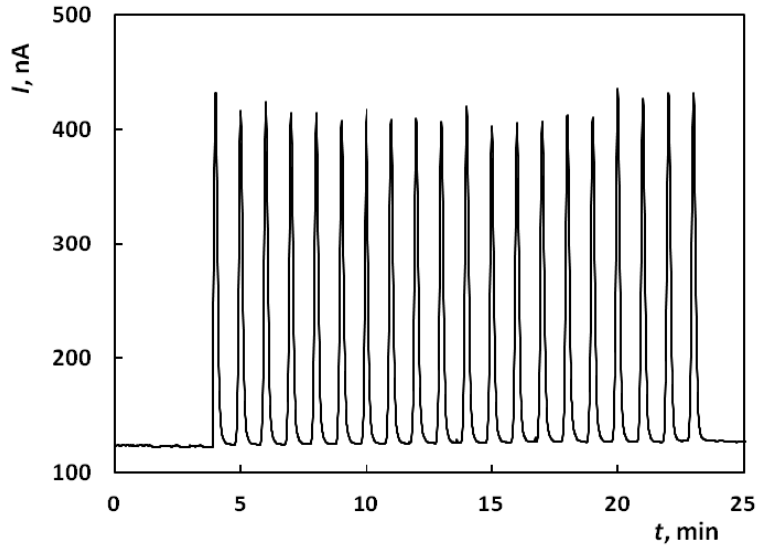

Fig. 2: *Chromatograms of twenty successive injections of 1.10^{-4} mol l^{-1} chlortoluron in a mixed deionized water-methanol (4:6, v/v) without the renewal of electrode surface.* Measured by HPLC-ED at GCPE in wall-jet arrangement (E =+1.3 V), injected 20 μl of the sample, mobile phase BR buffer pH 4 – methanol (4:6, v/v).

In the case of carboxin, quite comparable results were obtained when developing new methods of determination using both voltammetric batch methods and HPLC with amperometric detection using carbon paste electrodes [22]. The limits of detection were in all cases below micromolar level. With the minGCPE, it was possible to perform the measurements in volumes of 1 ml. During the method development for carboxin determination it was found out that the analyte does not passivate the electrochemically active surface of carbon paste. This was not surprising in case of HPLC with amperometric detection but rather astonishing in voltammetric batch methods. Even when using the miniaturized carbon paste electrode, the repeatability of twenty consecutive measurements without the surface renewal gave the relative standard deviation for peak height of 1.10^{-4} mol l^{-1} carboxin only 3.6 %, see Fig. 3.

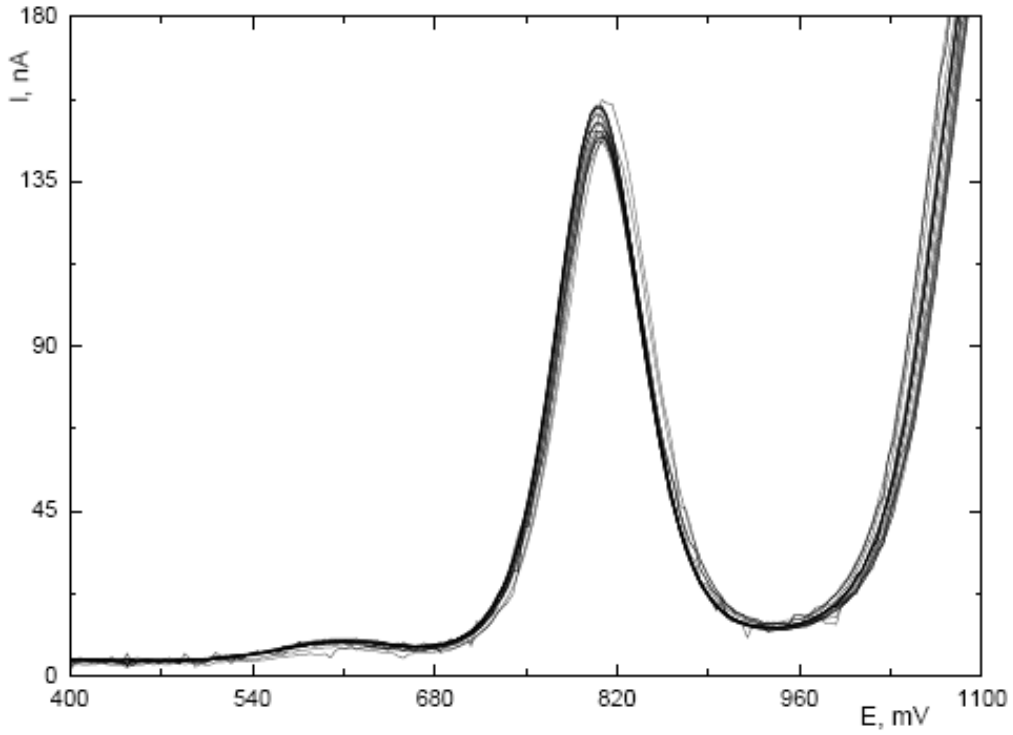

Fig. 3: *DP voltammograms of twenty consecutive measurements of 1.10^{-4} mol l^{-1} carboxin at minGCPE in BR buffer pH 2 mixture with methanol (99:1, v/v).*

The miniaturized CPE is well suited for large scale monitoring of environmental samples requiring just small sample volumes of matrices while HPLC-ED proved to be superior when complicated matrices are to be analyzed requiring separation power of HPLC.

Another class of compounds studied were benzophenone derivatives [19, 20] which are suspected endocrine disruptors. The new methods for determination of 2,4-dihydroxybenzophenone and 2-hydroxy-4-methoxybenzophenone using HPLC-ED at GCPE were developed. Following the measurement of hydrodynamic voltammograms in conceivable mobile phases, the optimum conditions of determination were found for both analytes, Fig. 4. Under these conditions, the limits of detection of both analytes were deeply in submicromolar region. When working with urine samples, solid phase extraction was used, which resulted in further decrease of LOD down to the nanomolar concentration region. Also, the addition of sodium dodecyl sulphate ($2 \cdot 10^{-4}$ M SDS) to mobile phase was tested, as SDS masks urine proteins and in some cases enables to work directly with urine samples. Although the limits of detection of both benzophenone derivatives somewhat increased, it was still possible to work with untreated urine in submicromolar concentration region.

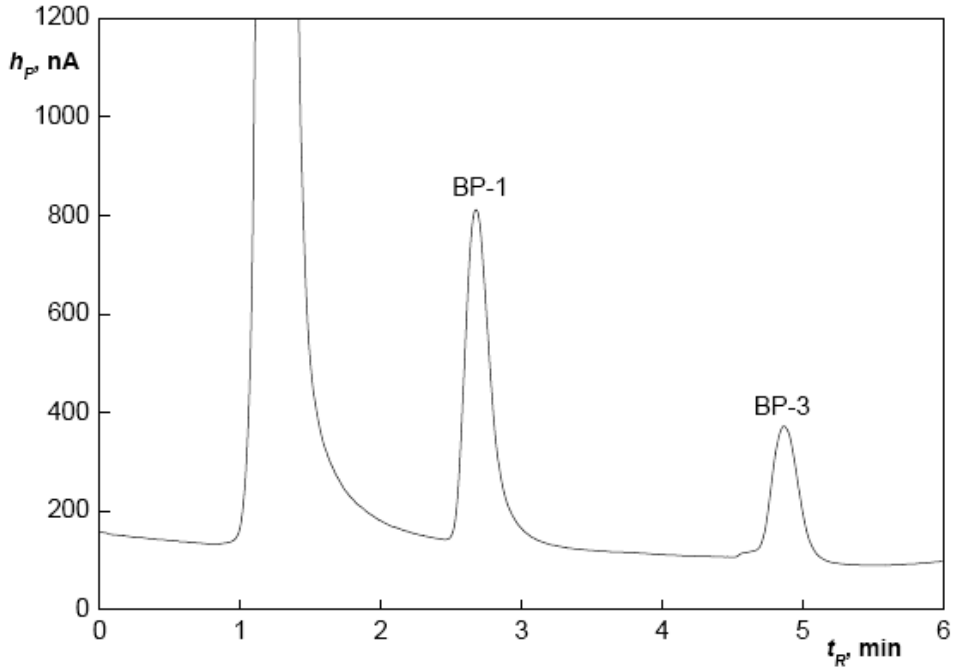

Fig. 4: *Chromatogram of a mixture of BP-1 and BP-3 ($1 \cdot 10^{-4}$ mol·l^{-1} each). Measured by HPLC-ED at GCPE (250 mg : 120 μl) at +1,1 V. Column Kromasil 100-7 μm, C18, 150 x 4,6 mm, injected 20 μl, linear gradient - 0 min: 70 % methanol 5 min: 91 % methanol, BRB pH 6, flow rate 1 ml·min^{-1}.*

Propyl gallate as an important and widely used synthetic antioxidant and preservative agent, frequently used as additive in oils and greases, was determined using differential pulse voltammetry and HPLC-ED using carbon paste electrodes [25].

Propyl gallate is quite easily oxidized due to the presence of three hydroxyl groups on the aromatic benzene ring and depending on pH gives oxidation signals at relatively low potentials. Following the optimization of conditions for DP voltammetric and HPLC-ED determinations, and the evaluation of method parameters, both two newly developed methods were successfully used for the determination of propyl gallate in samples of edible oils.

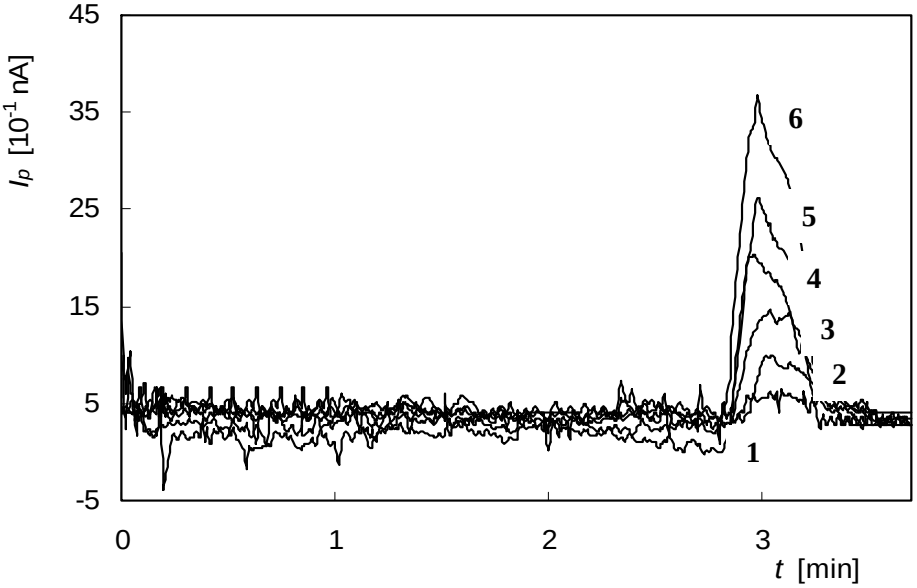

Fig. 5: *Propyl gallate chromatograms obtained by HPLC-ED at a GCPE detector in 0.01 mol·l^{-1} phosphate buffer pH 4 – methanol (50 %, v/v) mobile phase, column LiChroCART® 125-4, Superspher 100, RP-18, F_m = 1,0 ml·min^{-1}, injected 10 µl, E = +0.8 V, c (PG) = 1·10^{-7} (1); 2·10^{-7} (2); 4·10^{-7} (3); 6·10^{-7} (4); 8·10^{-7} (5); 1·10^{-6} (6) mol·l^{-1}.*

Carbon paste electrodes even in their simple bare form proved to be an effective tool for electrochemical methods of determination of a number of biologically active organic compounds, which are easily applicable in both batch and flow arrangements. Especially highly sensitive electrochemical detection in tandem with HPLC is a powerful tool for environmental, food or medicinal analysis.

Conclusions

We have reported on new methods for the determination of biologically active compounds, including pesticides, herbicides, food additives and suspected endocrine disruptors, at carbon paste electrodes.

The developed batch voltammetric methods were used for determination of famoxadone and chlortoluron in soil and river water samples, carboxin and triclosan in tap and river water samples, propyl gallate in edible oils and 2-hydroxy-2-methoxybenzophenone in tap water, and developed HPLC-ED methods for determination of benzophenones in urine, carboxin in tap and river water and propyl gallate in edible oils.

All the developed methods had limits of analyte detection at submicromolar detection. Using miniaturized carbon paste electrode, it was also possible to measure in volumes of 1 ml. Carbon paste electrode still present quite sensitive alternative for electroanalytical method development and in combination with HPLC they offer complex and highly sensitive method able to be used even for complicated environmental matrices

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References

1. R.N. Adams, *Anal. Chem.* ***30*** (1958) 1576.
2. K. Kalcher, *Electroanalysis* ***2*** (1990) 419.
3. K. Kalcher, J.-M. Kauffmann, J. Wang, I. Švancara, K. Vytřas, C. Neuhold, Z. Yang, *Electroanalysis* ***7*** (1995) 5.
4. I. Švancara, K. Schachl, *Chem. Listy* ***93*** (1999) 490.
5. I. Švancara, K. Vytřas, J. Barek, J. Zima, *Crit. Rev. Anal. Chem.* ***31*** (2001) 311.
6. K. Kalcher, I. Švancara, R. Metelka, K. Vytřas, A. Walcarius, in: The Encyclopedia of Sensors, Vol. 4, (C. A. Grimes, E.C. Dickey, M.V. Pishko, Eds.), 283. ASP: American Scientific Publishers, Stevenson Ranch 2006.
7. I. Švancara, K. Vytřas, K. Kalcher, A. Walcarius, J. Wang, *Electroanalysis* ***21*** (2009) 7.
8. K. Vytřas, I. Švancara, R. Metelka, *J. Serb. Chem. Soc.* ***74*** (2009) 1021.
9. J. Zima, I. Švancara, J. Barek, K. Vytras, *Crit. Rev. Anal. Chem.* ***39*** (2009) 204.
10. I. Švancara, K. Kalcher, A. Walcarius, K. Vytřas, Electroanalysis with carbon paste electrodes. CRC Press, Boca Raton 2012.
11. T. Kuwana, W.G. French, *Anal. Chem.* ***36*** (1964) 241.

12. W. Matuszewski, M. Trojanowicz, *Analyst* **113** (1988) 735.
13. C.L. Wang, K.E. Creasy, B.R. Shaw, *J. Electroanal. Chem.* **300** (1991) 365.
14. C.A. Frysz, D.D.L. Chung, *Carbon* **35** (1997) 858.
15. Y. Kureishi, H. Shiraishi, H. Tamiaki, *J. Electroanal. Chem.* **13** (2001) 496.
16. M.D. Rubianes, G.A. Rivas, *Electrochem. Commun.* **5** (2003) 689.
17. S.B. Hocevar, I. Svancara I., K. Vytras, B. Ogorevc, *Electrochimica Acta* **51** (2005) 706.
18. D. Bavol, Master Thesis, Charles University in Prague, 2013.
19. B. Fähnrichová, Master Thesis, Charles University in Prague, 2013.
20. V. Molitor, Bachelor Thesis, Charles University in Prague, 2013.
21. L. Houšková, Master Thesis, Charles University in Prague, 2012.
22. R. Jarošová, Master Thesis, Charles University in Prague, 2013.
23. P. Malá, Bachelor Thesis, Charles University in Prague, 2012.
24. J. Markvart, Bachelor Thesis, Charles University in Prague, 2013.
25. M. Vysoká, Master Thesis, Charles University in Prague, 2010.