# NANOPOROUS GRAPHITE IN ELECTROCHEMICAL SENSORS AND ITS USAGE FOR DETERMINATION OF METANEPHRINES

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**Abstract**

Electrochemical sensors are used for detection of antioxidants, vitamins, drugs, pesticides, catecholamines and other electroactive compounds. Cells of the detectors usually contain three electrodes (the working, the reference and the auxiliary) and a potential difference is applied between the working electrode and the reference electrode. The auxiliary electrode provides this potential difference to be constant. Carbon-based working electrodes are made from glassy carbon, pyrolytic carbon and porous graphite. The pore size of the graphite is approximately 0.2 μm so it is a nanoporous material with large internal surface area.

Electrochemical detection (ECD) is very selective and sensitive method. Ultra-high performance liquid chromatography (UHPLC) with ECD is very useful tool for food analysis, clinical diagnostic, toxicology, prediction of xenobiotics metabolism, protein research etc. – mainly in coupling with mass spectrometry.

Our project aims to develop a new kit for the determination of plasma free metanephrines, diagnostic markers of tumor pheochromocytoma. The determination was performed by ion-pair UHPLC with electrochemical Coulochem III detector.

**Keywords:**

Nanoporous Graphite, Electrochemistry, Coulochem, Metanephrines

## 1. INTRODUCTION

Electrochemical detectors are used for detection of electroactive compounds (antioxidants, vitamins, drugs, pesticides, carbohydrates, biogenic amines, catecholamines etc.). Cells of the detectors usually contain three electrodes – the working, the reference and the auxiliary. A potential difference is applied between the working electrode and the reference electrode. This potential is the driving force for oxidation or reduction of analytes at the surface of the working electrode. The counter (auxiliary) electrode provides all of the current flow needed to maintain a constant potential difference between the working and reference electrodes. The current changes from the electrochemical reaction are amplified and are presented as peaks on the recording device. When the electrolysis is 100%, the peak area is related to the quantity of sample injected by Faraday's law. [1,2,3,4]

### 1.1 Electrochemical cells

Electrochemical cells can be in several different arrangements. The eluent can flow through (coulometric "flow-through"), flow at (amperometric "wall jet"), or passed over (amperometic "thin layer") the working

electrode. The “flow-through” sensor is the most efficient. Flow of a mobile phase passes through the porous electrode and causes extremely small diffusion distances. [2,4]

Carbon-based working electrodes of coulometric cells are made from glassy carbon, pyrolytic carbon and porous graphite. The pore size of the graphite is approximately 0.2 μm. Graphite is a layered material having very thin atomic layer. Nanoporous materials have large internal surface areas. Porous nanocomposites of hydrophobic carbon layers and active metal oxides are required for high efficiency catalytic performance and specific adsorption under moisture atmosphere. [1,4]

### 1.2 Coulometric detection

Coulometric detection allows complete oxidation / reduction in flow-through nanoporous graphite cells and low detection limits without special sample pre-treatment. Identification and quantification at the fg level are feasible. Single or two-cell Coulochem detection or CoulArray multi-channel detection (which provides chromatograms of electroactive compounds at different potentials simultaneously) is possible to use coupled to HPLC. [1,2,3]

Electrochemical detection (ECD) is very selective and sensitive method. Ultra-high performance liquid chromatography (UHPLC) with ECD is very useful tool for food analysis, clinical diagnostic, toxicology, prediction of xenobiotics metabolism, protein research etc. – mainly in coupling with mass spectrometry (MS). [1,3,5,6] Our Institute of Nutrition and Diagnostics (RADANAL Ltd.) uses HPLC/CoulArray, UHPLC/Coulochem and UHPLC/EC/MS coupling as well. We are developing a new kit for the determination of plasma free metanephrines, diagnostic markers of tumor pheochromocytoma (PHEO), by UHPLC/Coulochem III.

### 1.3 Electrochemistry coupled to mass spectrometry

Electrochemistry coupled to mass spectrometry is a method which is able to mimic xenobiotics metabolism in a human body (the oxidative phase I metabolism catalyzed by cytochromes P450 and the conjugative phase II metabolism). This purely instrumental method can also predict stability and environmental persistence of compounds, simulate peptide and protein cleavage, disulfide bond reduction, oxidative stress etc. It generates intermediates and unstable metabolites and almost real-time monitors these unstable products. EC/MS gives us also information about sensitivity of a substrate towards oxidation and regions on the molecule where oxidations are likely to take place. It is versatile, reagent-free, simple but efficient low-cost instrumentation. Moreover EC/MS is useful for synthesis of standards. [5,6,7,8]

## 2. ELECTROCHEMICAL DETERMINATION OF PLASMA FREE METANEPHRINES

Liquid chromatography with Coulochem detector is used for determination of catecholamines and their O-methylated metabolites, metanephrines, in most of clinical laboratories. We are developing a new kit for the determination of plasma free metanephrines. These compounds play an important role in the diagnosis of pheochromocytoma. This adrenal medulla tumor synthesizes, stocks, metabolizes and mostly secretes catecholamines. [9] For this reason it is possible to use significantly elevated concentrations of catecholamines and their metabolic products as diagnostic markers of this tumor. The determination of metanephrines, mainly normetanephrine (NMN), metanephrine (MN) and 3-methoxytyramine (3-MT), is preferred against the determination of catecholamines because tumor cells produce free metanephrines continuously and irrespective of the release of catecholamines. Moreover 3-MT is a biomarker for metastatic PHEO. [10,11]

### 2.1 Experimental

Solid phase extraction (SPE) with ion exchange columns was used for a pre-treatment of plasma samples. The determination was performed by ion-pair UHPLC with electrochemical Coulochem III detector. A core-shell column and a mobile phase consisted of mixed buffer and acetonitrile (total pH 2.9) were used.

The potential of conditioning cell was set at +400 mV. The working potentials were +100 mV (1st electrode) and -350 mV (2nd electrode).

### 2.2 Results and Discussion

During the development of the kit we have progressively optimized the whole method - pre-treatment of plasma samples, isolation of metanephrines, mobile phase composition, analytical conditions etc. We tested different centrifugation procedures for the pre-treatment of plasma samples and various SPE columns, SPE sorbents and SPE reagents for the isolation of free metanephrines from samples. We tried several buffers as mobile phases for UHPLC and different settings of UHPLC/Coulochem III (various columns, speeds of flow rate, column temperatures and potentials at the cells of the detector were tested).

Plasma samples with increased levels of metanephrines (established in the laboratory of 1st Faculty of Medicine at Charles University in Prague) were analyzed using of our method. Comparison of analysis results in our laboratory and in the laboratory in Prague is in Table 1. The results from both laboratories are very similar.

Metanephrine occurs in a healthy human body in the maximum concentration of 100 pg/mL and normetanephrine in the max. concentration of 160 pg/mL. At least one of the analytes (NMN, MN) in the tested plasma samples (Table 1) exceeds the limit value. Patients (who have provided their blood for these determinations) have probably the tumor pheochromocytoma in their body.

**Table 1** Comparison of analysis results of plasma samples with increased levels of metanephrines detected in our laboratory and in the laboratory of 1st Faculty of Medicine (Charles University in Prague)

| | Measured at the Charles University | | Our measurements | |
|---|---|---|---|---|
| **Sample No.** | **NMN (pg/mL)** | **MN (pg/mL)** | **NMN (pg/mL)** | **MN (pg/mL)** |
| **328** | 46 | 317 | 54 | 364 |
| **368** | 977 | 710 | 1011 | 623 |
| **410** | 2901 | 72 | 2855 | 92 |
| **432** | 187 | 41 | 187 | 45 |
| **449** | 168 | 53 | 179 | 58 |
| **464** | 1139 | 284 | 1153 | 263 |

## 3. CONCLUSIONS

Electrochemical detection is very selective and sensitive method. Coulometric nanoporous graphite cells (pore size 0.2 μm) allow complete oxidation / reduction of electroactive compounds and very low detection limits (at the fg level). Liquid chromatography with ECD is used in food analysis, clinical diagnostic and toxicology.

We developed a method (using of UHPLC/Coulochem III) which can reliably reveal the tumor pheochromocytoma in human blood plasma samples. Our method can be successfully used for the determination of plasma free metanephrines (NMN, MN, 3-MT) in tens of pg/mL range. Institute of Nutrition and Diagnostics (RADANAL Ltd.) will offer the new kit for the determination of plasma free metanephrines at the beginning of year 2015.

Electrochemical cells coupled to MS can be very useful tool for prediction of xenobiotics metabolism and stability, for simulation of peptide and protein cleavage, disulfide bond reduction, oxidative stress etc. and also for synthesis of standards. EC/MS provides detailed information on possible metabolites and elucidates

harmfulness of compounds which affect us. Institute of Nutrition and Diagnostics comes under a small amount of workplaces which have and use UHPLC/EC/MS.

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**LITERATURA**

[1] HSUEH, C. J., JANYASUPAB, M., HUI LEE, Y., LIU, C. C. Coulometric Analysis. In: KREYSA, G., OTA, K. I., SAVINELL, R. F., editors. *Encyclopedia of Applied Electrochemistry.* New York: Springer New York, 2014, p. 275-283. ISBN: 978-1-4419-6995-8.

[2] http://www.esainc.com/resources/detector_tech/how_ec_works, downloaded August 26th, 2014.

[3] NOVÁKOVÁ, L., DOUŠA, M. *Moderní HPLC separace v teorii a praxi I.* Praha: Europrint, 2013. ISBN: 978-80-260-4244-0.

[4] FLANAGAN, R. J., PERETT, D., WHELPTON, R. *Electrochemical Detection in HPLC. Analysis of Drugs and Poisons.* Cambridge: The Royal Society of Chemistry, 2005, p. 6-11, 23-29. ISBN: 978-0-85404-532-7.

[5] JURVA, U. *Electrochemistry on-line with mass spectrometry: Instrumental methods for in vitro generation and detection of drug metabolites.* Göteborg: Mediagruppen Intercopy, 2004.

[6] JAHN, S., KARST, U. *J. Chromatogr. A*, 2012, 1259, p. 16-49.

[7] ZANGER, U. M. Introduction to Drug Metabolism. In: ANZENBACHER, P., ZANGER, U. M., editors. *Metabolism of Drugs and Other Xenobiotics.* Weinheim: Wiley-VCH, 2012, p. 287-289.

[8] ROESER, J. *Dissertation.* Groningen: University of Groningen, 2013.

[9] KRŠEK, M. *Endokrinologie.* Praha: Galén, 2011.

[10] PACÁK, K. *Feochromocytom.* Praha: Galén, 2008.

[11] EISENHOFER, G., LENDERS, J. W. M., SIEGERT, G., BORNSTEIN, S. R., FRIBERG, P., MILOSEVIC, D., MANNELLI, M., LINEHAN, W. M., ADAMS, K., TIMMERS, H. J., PACÁK, K. *Eur. J. Cancer*, 2012, 48, p. 1739-1749.