Electrochemical Techniques in Monitoring of Nervous System Drugs
Elektrochemické techniky při monitoringu léčiv nervového systému)

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Abstract
Drugs of the nervous system (ATC group N) belong generally to the most commonly (mis)used substances worldwide. Their determination and monitoring of these drugs and their metabolites in various body fluids or environmental matrices represent a challenge for analytical chemists. Our research is focused on the application of electrochemical methods in the monitoring of the most frequently prescribed and the newly introduced nervous system drugs of precisely specified structures and/or of defined properties. For toxicological and pharmacokinetic reasons, emphasis has been placed on investigating the reaction mechanisms of their metabolite formation. New or alternative to commonly used electroanalytical methods applicable for monitoring and characterization of target compounds in their pure state, in body fluids, wastewaters, and other environmental matrices, has been developed. To improve the chemometric parameters and sensing characteristics, attention was be paid to the construction of new electrochemical sensors, detectors (e.g. screen-printed, 3D printed), or cells, based on (modified/unmodified) traditional or nontraditional materials, preferably usable at the point-of-care.

Keywords: Nervous system drugs, Active pharmaceutical ingredient, Metabolites, Electrochemistry, Analytical chemistry, Electrode materials, Modifications, Body fluids, Biologically active compounds, Blood.

Introduction
The human nervous system can be affected by a wide range of diseases and medical conditions. For the treatment of psychiatric and neurologic patients, more than 200 drugs are available which have been discovered and developed during the last 60 years [1]. Due to different definitions of terms used in literature by various authors and in different laws, it is very complicated to differentiate between the terms „drug“ (defined in the US) and "medicinal product" (defined by EU laws). Nevertheless, in this text, the term "drug" can be used for the term “medicinal product” as well as “active pharmaceutical ingredient”.

In psychiatry and neurology, patient populations that may particularly benefit from precise drug determination are children, adolescents, pregnant women, elderly patients, and individuals with intellectual disabilities, among others [1]. Therefore, monitoring and determination of drugs and their metabolites are relevant in many areas of medicinal, analytical, and environmental chemistry, biochemistry, pharmacy, etc., e.g.:

a) In the field of drug development and production, where it is necessary to determine the precise content of active pharmaceutical ingredients (APIs) that a drug should contain.
b) Another important area of drug analysis is “therapeutic drug monitoring” (TDM), i.e., the quantification and interpretation of drug and their metabolite concentrations in body

fluids (urine, blood, etc.) to optimize pharmacotherapy. Information on doses of drugs in the human body is of high importance because it can be used to reduce side effects, in the case of antidepressants it is possible to mention: e.g., loss of libido, inability to orgasm, nausea, headache, apathy, loss of motivation, vivid dreams, nightmares, hypotension, dizziness or weight gain. TDM considers the interindividual variability of pharmacokinetics and thus enables personalized pharmacotherapy [1, 2]. Non-response at therapeutic doses, uncertain drug adherence, suboptimal tolerability, or pharmacokinetic drug-drug interactions are typical indications for TDM. However, the potential benefits of TDM to optimize pharmacotherapy can only be obtained if the method is adequately integrated into the clinical treatment process [1].

Moreover, many drug metabolites actively contribute to the overall clinical effect of the parent compound. Therefore, the knowledge of formed active and inactive metabolites and quantification of the parent drug, play important roles in TDM. In the case of many newly introduced drugs, the information about their metabolism is missing [3].

c) Determination of the selected APIs and/or of their metabolites in body fluids can provide an answer, to whether the desired drug has been administered or not (e.g. in the case of psychiatric patients who refuse drug administration).
d) The last step in the fate of any drug consists in its release into the environment (via urine, feces, etc.) in unchanged form (e.g., in the USA, there were about 250 million antidepressant prescriptions in the year 2010, which represents a great environmental burden). Moreover, some of the investigated APIs or their combinations are toxic, mutagenic, or carcinogenic [4]. Therefore, it is necessary to monitor their levels in waste, rivers, and drinking waters.
e) Drug and metabolite analyses play important role in toxicology too. According to the papers and annual reports of the Czech Toxicological Information Center (TIC) [5, 6], the nervous system drugs (N group according to the Anatomical Therapeutic Chemical (ATC) classification system [7]) have been the most frequent cause of calls to the Czech TIC, i.e., more than 12.2 % (ca. 29 200) of all calls (ca. 240 000) and 31.2 % of all calls dealing with drugs (ca. 93 000) since 2005. The drugs from the N group were (mis)used in suicide attempts in more than 55 % of cases, accidentally in 19.3 %, and in ca. 9 % of cases, it was a medical error.

The electrochemical methods (ECM) or electrochemical detectors have not been so widely used for the determination of N-group drugs in practice (excluding such as benzodiazepines (with reducible azomethine group), drugs containing other electrochemically active groups (e.g., nitro), N-oxide and carbonyl groups [8] and which were previously studied.

Experimental part

Working electrodes

The material of the working electrode plays a crucial role across various electroanalytical techniques. Among others, we used the following types of electrodes:

a) **3D printed electrodes.** 3D printing belongs to the most promising approaches toward simple, rapid, and inexpensive production of various sensors, electrodes, and cells [9-13], with prospective use for the determination of various nervous drugs and their metabolites [14, 15]. 3D printable sensors are highly customizable [16] and can be (mass)produced in many unique geometries. For electroanalytical applications, 3D printing has been used for the production of various electrodes, flow cells, channel-flow, and wall-jet electrodes, e.g., for trace analysis. 3D printed cells with low internal volume and large working electrode surface are worth as a tool for sensitive detection of electroactive drugs, for the study of electrochemically generated products of their redox reactions in EC-MS system [17].

b) **Boron-doped diamond electrodes.** Mercury-containing (liquid dropping, hanging, or solid amalgam) electrodes are limited in their application in the positive potential region. This disadvantage can be solved by their substitutions e.g. by boron-doped diamond electrodes (BDDE) with a wide potential window [18-24]. These electrodes can be successfully used for nervous drug determinations [24, 25]. Some properties (electron transfer, redox potential, complexation abilities) can be changed by their modifications (e.g., using nanoparticles of Au, Ag, Pd). The introduction of screen-printed boron-doped diamond electrodes (SP-BDDE) is a promising tool for medicinal purposes as well [26].

c) **Mercury-based electrodes.** The application of electrodes based on liquid mercury in medicinal practice seems to be very complicated due to its toxicity. On the other hand, it has been undoubtedly scientifically proved that amalgam is not a toxic material. However, waste material from disposable amalgam SPEs could represent a significant environmental burden. Nevertheless, bare [27], or modified [28] solid amalgam electrodes are suitable to replace (in most cases ideal) liquid mercury electrodes in research laboratories [29] in the elucidation of reaction mechanisms, metabolite formations, etc. Cheap amalgams could replace expensive gold in enzymatic reactors [30, 31] used for TDM too.

Analysis of real samples, separation techniques - Hollow-fiber microextraction

The added excipients can present a huge complication disturbing API determination. They must be filtrated or separated by similar processes. Either drugs or their metabolites can be bound to various proteins (enzymes, transporters, carrier proteins, etc.). We devoted our attention to the application of hollow-fiber microextraction (HF-LPME) [32] to pre-concentrate the analyte from a biological sample and remove interferences. Three-phase HF-LPME is used for extraction of ionizable analytes from donor solution in its neutral form across a supported liquid membrane to acceptor solution, where the analyte is present in its ionized form, entrapping and pre-concentrating the analyte [33, 34]. Acceptor solutions can be analyzed by voltammetric techniques without any additional treatment.

Additional data on analytes - ion transfer voltammetry

Protolytic and partitioning equilibria are essential for the evaluation of the bioavailability of drugs. There is a lack of information on experimental physicochemical parameters of some N-group drugs, especially of the atypical and newly introduced ones. The methodology based on ion transfer voltammetry (ITV) at supported room-temperature ionic liquids (RTILs) membrane, to the development of which the applicant's laboratory substantially has contributed, can provide a broad range of both theoretical and practical results of the electrochemical or analytical significance (estimation of the standard Gibbs energies of ion transfer from water to RTIL, to establish a scale of the absolute potential differences, etc. [35, 36]. The obtained results on N-drugs can be supported by available DFT calculations [35].

Investigated drugs

We focus our attention on the most frequently prescribed nervous system drugs in the Czech Republic [5], modern, newly introduced ones, and atypical antipsychotics [37]. We focused on the following APIs: a) Electrochemically active; b) containing nitrogen heterocycles, c) containing protonatable nitrogen; d) quaternary (nitrogen) bases; extractable using HF-LPME; complexable (ionizable) using some heavy metal cations (e.g., Cu^{2+}, Cu^{+}, Ag^{+}, Au^{+}, Au^{3+}).

Results and Discussions

As an example of realized experiments, we can demonstrate voltammograms of the drug venlafaxine, recorded using a glassy carbon electrode, the carbon filament electrode, and the TDMA-TFPB membrane cell.

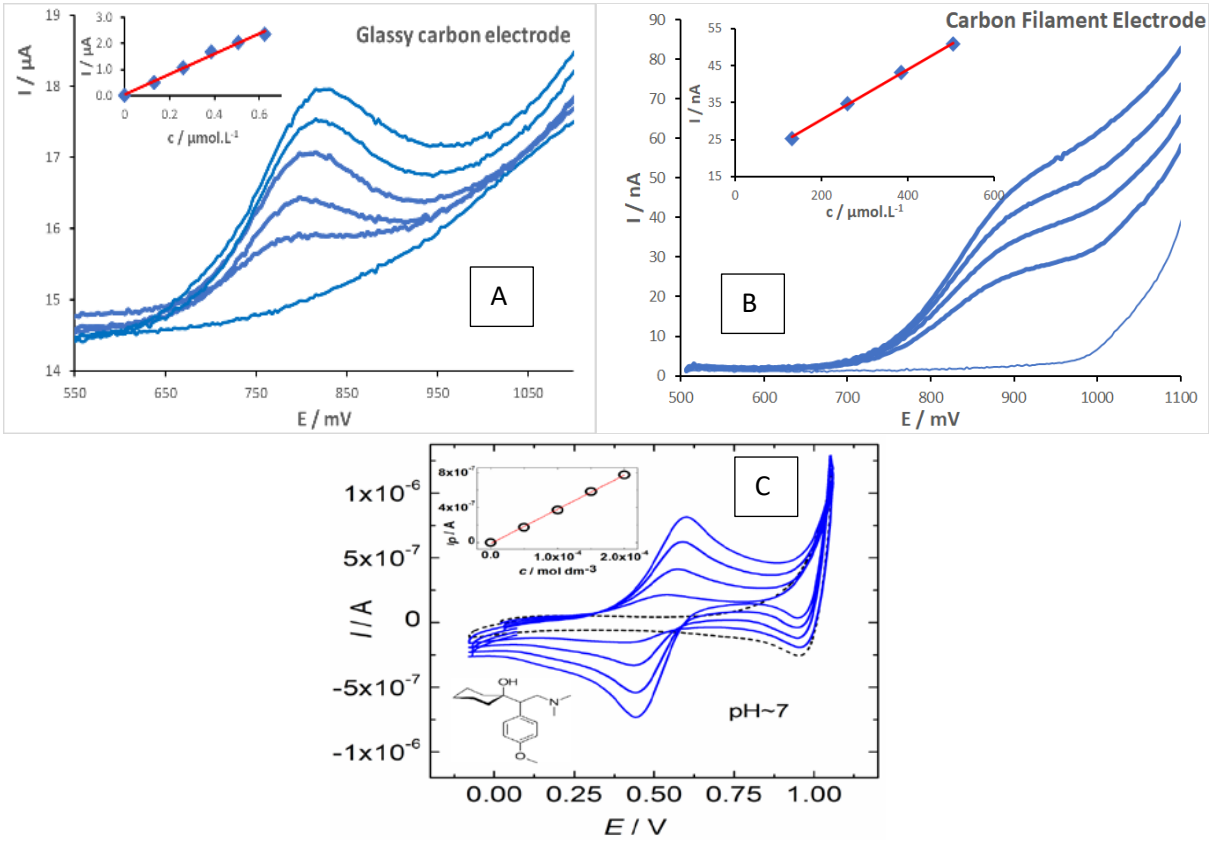

Fig. 1: A) DP voltammograms of venlafaxine recorded using the glassy carbon electrode; B) DP voltammograms of venlafaxine recorded using the carbon filament electrode; C) Cyclic voltammograms of Venlafaxine recorded using the TDMA-TFPB membrane cell.

Conclusions

The development of new sensors/electrodes/cells/systems and hyphenated systems for TDM and analysis of drugs and their metabolites is one of the most important trends in modern analytical chemistry. ECMs can be extremely useful not only as the alternative analytical methods, but e.g. in the elucidation of metabolites formation, and reaction mechanisms in human bodies. The main advantages of ECMs are low investment and running costs, simplicity, portability, and easy miniaturization and applicability at point-of-care. Therefore, great attention in this project will be focused on new electrode materials as well as on surface pretreatment or modification of traditional bare electrodes to improve their sensing properties.

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