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Review

Electrochemistry/mass spectrometry as a tool in metabolism studies—A review

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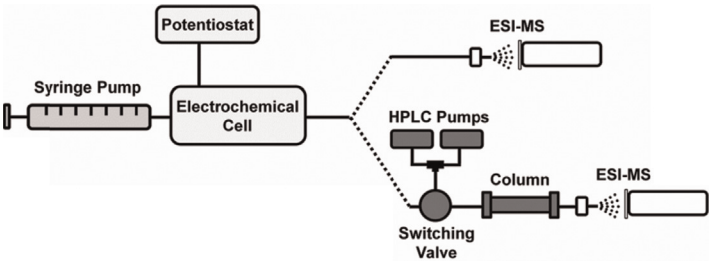
HIGHLIGHTS

- EC/MS provides information on the oxidative metabolism of drugs.
- LC may be added to provide polarity information of the metabolites.
- Metabolism information may be obtained without animal experiments.

GRAPHICAL ABSTRACT



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ABSTRACT

The combination of electrochemistry (EC) and mass spectrometry (MS) has become a more and more frequently used approach in metabolism studies in the last decade. This review provides insight into the importance of metabolism studies during the drug development process and gives a short overview about the conventionally used methods since electrochemistry is often intended to substitute or minimize animal-based studies. The optimization of the electrochemical conditions is of great importance for a successful comparison with *in vitro* approaches. The type of metabolism reactions, which can be simulated by EC, has been extended with new cell types and working electrodes. Although the mechanism differs from the enzyme-catalyzed turnover, electrochemistry can be used to simulate a significant number of the respective reactions.

An expanded set-up consisting of EC, a chromatographic separation and MS allows to distinguish between an electrospray ionization (ESI) in-source and an electrochemical oxidation and provides information on the polarity of the electrogenerated compounds. A main advantage of EC for metabolite generation is the possibility to isolate reactive species because of the purely instrumental approach. Especially when a preparative electrochemical cell with a larger working electrode surface is used, metabolites can be generated in sufficient quantities for their subsequent structure elucidation. Besides, the compounds can also be used for selective trapping experiments with different cell components such

Abbreviations: ACN, acetonitrile; ADME, adsorption, distribution, metabolism, excretion; Ag/AgCl/Cl, silver/silver chloride, reference electrode; APAP, acetaminophen, paracetamol; APPI, atmospheric pressure photoionization; AQ, amodiaquine; AQQI, amodiaquinequinone imine; AUX, auxiliary electrode, counter electrode; BDD, boron-doped diamond; CA, carbonic anhydrase; CYP, cytochrome P450 enzymes; CYS, cysteine; DHP, dihydropyridinium ion; DNA, deoxyribonucleic acid; EC, electrochemistry; EC/MS, electrochemistry coupled to mass spectrometry; ESI, electrospray ionization; EU, European Union; FA, formic acid; FT-MS, Fourier transform-mass spectrometer; GC, glassy carbon; GSH, glutathione; GST, glutathione-S-transferase; HLM, human liver microsomes; HSA, human serum albumin; HV, high voltage; LGA, β-lactoglobulin A; LV, low voltage; NAPQI, *N*-acetyl-*p*-benzoquinone imine; NAT, *N*-acetyltransferase; Q, quadrupole; REF, reference electrode; RLM, rat liver microsomes; ROS, reactive oxygen species; S9, a liver tissue homogenate fraction; SHE, standard hydrogen electrode; SRM, selected reaction monitoring; TCL, ticlopidine; TSP, thermospray; TP, thienopyridinium ion; UDP, uridine diphosphate; UGT, uridine diphosphate glucuronosyltransferase; WE, working electrode.

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as small peptides, proteins or DNA bases. Current and possible future developments and applications of EC are presented and discussed as well.

Contents

Helene Faber studied Food Chemistry at the University of Münster, Germany and finished her final thesis in the group of U. Karst on HPLC with biochemical detection. After obtaining her 2nd state exam for certified food chemists in 2010, she rejoined the group of U. Karst for her Ph.D. thesis in the field of electrochemistry coupled to mass spectrometry, which she finished in 2013. Her research interests are in the field of LC/MS and EC/LC/MS with particular focus on pharmaceutical, food chemistry and environmental chemistry applications.

Uwe Karst obtained his Ph.D. in Analytical Chemistry at the University of Münster, Germany in 1993. He moved to the University of Colorado in Boulder, CO, USA as a postdoctoral research associate. After his return to the University of Münster, he finished his Habilitation in 1998. In 2001, he took over a position as Full Professor of Chemical Analysis at the University of Twente, The Netherlands. He accepted his current position as Chair of Analytical Chemistry at the University of Münster in 2005. His research interests focus on hyphenated analytical techniques and their (bio)medical and pharmaceutical applications, including elemental speciation analysis, metallomics, mass spectrometric imaging and electrochemistry/MS.

Martin Vogel studied Chemistry at the University of Münster, Germany and earned his Ph.D. in 2001 in the field of derivatizing techniques for LC and LC/MS. He took over a position as Lecturer at the University of Twente, The Netherlands in 2001. In 2006, he accepted a position as Lecturer at the University of Münster and was promoted to Senior Lecturer in 2012. He is currently Chairman of the Working Party of Analytical Chemistry of the German Chemical Society GDCh and has organized and co-organized several international meetings in the fields of separation sciences and hyphenated techniques. His main research interests focus on hyphenated techniques based on chromatography, including LC/MS and EC/LC/MS.

1. Introduction

Although the combination of electrochemistry and mass spectrometry often is considered to be a very recent combination of methods, it was first described in the scientific literature more than forty years ago. Already in 1971, Bruckenstein and Gadde introduced electrochemistry coupled to mass spectrometry (EC/MS) for the *in situ* detection of electrochemical oxidation products. They used a porous platinum electrode, which was in contact with a solution to be electrolyzed and with the vacuum inlet of the mass spectrometer [1]. With this approach, they studied gaseous products, e.g., oxygen, generated upon electrochemical oxidation of 0.1 M perchloric acid. With the development of thermospray ionization (TSP), it was possible to detect non-volatile and polar compounds in solution. In 1986, Hambitzer and Heitbaum were the first to study oxidation processes (in this case of *N*,*N*-dimethylaniline) with online-EC/TSP–MS. They observed the formation of dimers and trimers depending on the applied oxidation potential [2]. The high flow rates used in TSP–MS (1–2 mL min^{-1}) adversely affected the conversion efficiency of the electrochemical oxidation. Therefore, the interest in the online coupling of EC and MS declined in the following years. However, after the introduction of the electrospray ionization interface (ESI), a revival of EC/MS was observed, as it was now possible to analyze thermally labile and non-volatile compounds in solution. The direct coupling of EC to ESI–MS was first investigated in detail by Van Berkel et al. with respect to cell design and coupling mode (floated or decoupled from the ESI high voltage) [3–5]. They showed that the direct coupling of EC and MS allowed (1) the electrochemical ionization of neutral compounds, (2) the study of electrode reactions, and (3) the preconcentration of silver ions and the related signal enhancement in ESI–MS using anodic stripping