Proceedings of the 20th International Students Conference "Modern Analytical Chemistry"

Prague, 19—20 September 2024

Edited by Karel Nesměrák

FACULTY OF SCIENCE
Charles University

Prague 2024

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Preface

Scientific communication, both written and spoken, is one of the basic needs and skills in any scientific field. To help emerging scientists enhance their communication abilities, the Department of Analytical Chemistry, Faculty of Science, Charles University, organizes an annual conference for PhD students in analytical chemistry. You are now holding the proceedings of its 20th anniversary edition, which took place on September 19–20, 2024. During this conference, 45 young scientists from Germany, Poland, and the Czech Republic had the chance to not only meet but also present and discuss their research in analytical chemistry. They exchanged new insights and ideas to advance this crucial field for humanity. Additionally, participating in the conference helped them improve their language skills.

The proceedings feature seven full papers, while the remaining 38 authors chose to publish only abstracts of their lectures. Both sections are organized by the date of receipt and accompanied by indexes to allow navigation through the pages. The topics cover all three areas of instrumental analysis – electroanalytical, separation, and spectral methods – and demonstrate various applications in addressing societal challenges. We hope readers find these papers interesting, rewarding, and enjoyable.

The patronage of the Division of Analytical Chemistry of the European Chemical Society and the Working Group of Analytical Chemistry of the Czech Chemical Society are gratefully welcomed.

Lastly, we extend our heartfelt thanks to all sponsors for their generous support, which made the conference possible, and for their cooperation in many of our other activities.

doc. RNDr. Karel Nesměrák, Ph.D., *editor*

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Electrochemical characterization of scaife polished {110}-oriented boron doped diamond electrodes

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Keywords
dopamine
electrochemical impedance spectroscopy
single-crystal boron doped diamond
scaife polishing
voltammetry

Abstract
This work focuses on the effect of scaife polishing on electrochemical properties of {110}-oriented single-crystal diamond electrodes in context with morphology and boron concentration. Both, as-grown and scaife polished electrode surfaces underwent complex electrochemical and morphological characterization by various techniques. Alterations in heterogenous electron transfer kinetics after polishing of the as-grown surfaces, were evaluated by cyclic voltammetry of outer-sphere and inner-sphere redox systems. No noticeable acceleration of heterogenous electron transfer kinetics was observed for outer-sphere redox markers after scaife polishing of the as-grown surfaces. On the other hand, inner-sphere redox marker $[Fe(CN)_6]^{3-/4-}$ was sensitive to significantly decreased surface roughness, and acceleration of electron transfer kinetics was observed on all electrodes. Electrochemical impedance spectroscopy was utilized to obtain charge transfer resistance. Performance of scaife polished electrodes was tested also in detection of dopamine using square-wave voltammetry. Limits of detection in units of μmol dm^{-3} were achieved.

1. Introduction

Boron doped diamond (BDD) has been widely used as an electrode material due to its exceptional properties, including wide potential window, low background current, chemical stability in harsh environments, ability to generate hydroxyl radicals due to anodic decomposition of water at highly positive potentials, and biocompatibility [1–2]. However, conductivity and heterogenous electron transfer (HET) kinetics, and thus final performance, can be significantly influenced by several factors: i) boron content ([B]), ii) sp^2-bonded carbon content, iii) surface termination (H– *vs.* O–), and iv) crystal orientation.

Heterogenous electron transfer kinetics can be evaluated by cyclic voltammetry (CV) with redox systems, which have inner- or outer-sphere electron transfer mechanisms, making them either sensitive or insensitive to the chemical composition of the electrode surface, respectively. In BDD electrochemistry, the fastest HET kinetics has been demonstrated on H-terminated surfaces, which result from BDD growth in a hydrogen rich environment. However, HET kinetics often deteriorate when BDD is exposed to air due to formation of oxygen-containing functionalities; thus, it is favourable to use a cathodic pre-treatment prior to measurements to renew H-termination and achieve high repeatability [3]. Additionally, HET kinetics can also be enhanced by polishing of the electrode surface. Unlike alumina polishing, which does not change the surface morphology, scaife polishing can, besides removal of oxygen groups, significantly decrease surface roughness [4].

Single-crystal BDD (SC-BDD) offers an opportunity to study individual crystal orientations. Any effect of sp^2-bonded carbon is eliminated due to the absence of grain boundaries. Therefore, conductivity is governed only by boron concentration, which has been found to be related to crystal orientation, and surface termination [5, 6]. SC-BDDs with {100}, {111} or {110} crystallographic orientation have been studied as electrodes in [5–12], where the {110} orientation is the least studied, despite the fact it can be the dominant orientation in polycrystalline diamond [13–14].

In this study, we focused on exploration and comparison of electrochemical properties of i) "as-grown" and, ii) scaife polished, {110}-oriented SC-BDD electrodes in context of their surface morphology and boron doping level. The investigated {110}-oriented SC-BDD electrodes varying in boron concentration [B] ($\{110\}_{250}$, $\{110\}_{500}$, $\{110\}_{1000}$, $\{110\}_{2000}$; the number in subscript relates to B/C ratio during chemical vapor deposition (CVD) of BDD, underwent thorough morphological (scanning electron, atomic and optical microscopies), chemical (Raman spectrometry), electrical (van der Pauw) and electrochemical (CV utilising inner-sphere and outer-sphere redox probes, and electrochemical impedance spectroscopy) characterization. Additionally, the performance of the investigated SC-BDD electrodes in sensing of a neurotransmitter, dopamine, was assessed by square-wave voltammetry.

2. Experimental

2.1 Reagents and chemicals

Analytical grade reagents: potassium hexachloroiridate, hexaammineruthenium chloride, ferrocene methanol, dopamine hydrochloride (all Sigma-Aldrich, Germany), potassium hexacyanoferrate trihydrate, potassium chloride, sodium dihydrogen phosphate dihydrate, isopropanol (all Lach-Ner, Czech Republic), sodium hydroxide, sulphuric acid (all Penta, Czech Republic) were used

as-received. Deionized water (Millipore Mili plus Q system, Billerica, USA) with a resistivity of 18.2 MΩ cm was used to prepare all aqueous solutions.

2.2 Instrumentation

Cyclic voltammetry, square-wave voltammetry and electrochemical impedance spectroscopy measurements were carried out on a μAutolab type III potentiostat equipped with FRA module and controlled by Nova software (Metrohm Autolab, The Netherlands). All electrochemical measurements were performed at laboratory temperature (293±1 K).

3. Results and discussion

Redox systems with outer-sphere (surface chemistry insensitive, $FcMeOH^{+/0}$, $[Ru(NH_3)_6]^{3+/2+}$, $[IrCl_6]^{2-/3-}$) and inner-sphere (surface chemistry sensitive, $[Fe(CN)_6]^{3-/4-}$) nature of electron transfer were utilized in CV experiments to evaluate alterations in HET kinetics. In the case of $[Ru(NH_3)_6]^{3+/2+}$, no noticeable acceleration of HET kinetics (Table 1), except electrode $\{110\}_{500}$ (26 mV lower peak to peak separation ΔE_p when comparing as-grown and polished surface) was observed. Concerning $FcMeOH^{+/0}$, almost no differences in ΔE_p values due to polishing were observed (Fig. 1). In the case of surface sensitive redox probe ($[Fe(CN)_6]^{3-/4-}$) with inner-sphere electron transfer mechanism, high effectivity of scaife polishing was demonstrated, as ΔE_p values noticeably decreased (to 81 mV – 95 mV for all studied SC-BDD electrodes; Table 1 and Fig. 2) improved, due to significantly reduced surface roughness.

Furthermore, electrochemical impedance spectroscopy measurements support the results obtained from CV experiments with $[Fe(CN)_6]^{3-/4-}$. Charge transfer resistance RCT decreased with increasing B/C in the gas phase during CVD on both, as-grown and scaife polished surfaces, due to increasing conductivity for all studied SC-BDD (Table 2), as well as it decreased as result of scaife polishing of the as-grown surfaces, demonstrating the polishing effectivity.

Table 1
Comparison of ΔE_p values of $[Fe(CN)_6]^{3-/4-}$ and $[Ru(NH_3)_6]^{3+/2+}$ for as-grown and scaife polished surfaces.

SC-BDD	$\Delta E_p([Fe(CN)_6]^{3-/4-})$ / mV		$\Delta E_p(Ru(NH_3)_6]^{3+/2+})$ / mV	
	as-grown	scaife polished	as-grown	scaife polished
$\{110\}_{250}$	137	81	70	68
$\{110\}_{500}$	232	95	95	69
$\{110\}_{1000}$	134	90	73	71
$\{110\}_{2000}$	122	83	63	63

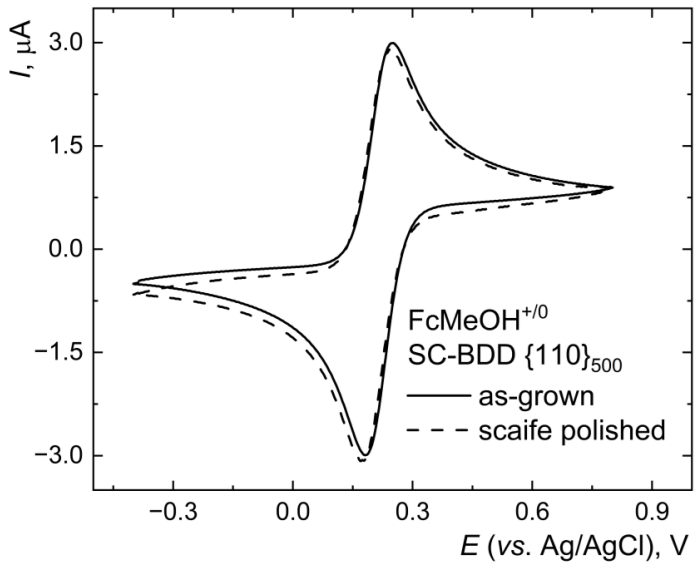

Fig. 1 Cyclic voltammograms of 1 mmol dm^{-3} $FcMeOH^{+/0}$ in 1 mol dm^{-3} KCl, recorded on as-grown and scaife polished $\{110\}_{500}$ SC-BDD electrode

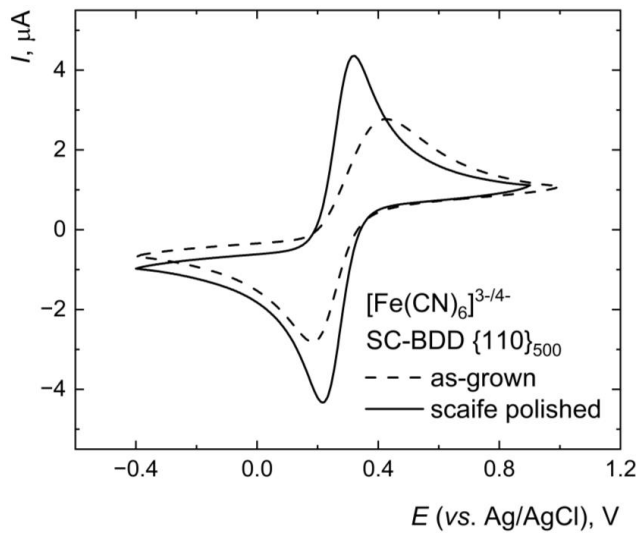

Fig. 2 Cyclic voltammograms of 1 mmol dm^{-3} $[Fe(CN)_6]^{3-/4-}$ in 1 mol dm^{-3} KCl, recorded on as-grown and scaife polished $\{110\}_{500}$ SC-BDD electrode

Table 2
Charge transfer resistance (R_{CT}) of as-grown and scaife polished surfaces calculated from electrochemical impedance spectroscopy.

SC-BDD	R_{CT} / kΩ	
	as-grown	scaife polished
$\{110\}_{250}$	127	17.0
$\{110\}_{500}$	126	8.16
$\{110\}_{1000}$	89.4	4.88
$\{110\}_{2000}$	66.3	4.55

Square-wave voltammetry measurements were performed on scaife polished {110}-oriented SC-BDD ($\{110\}_{250}$ and $\{110\}_{2000}$), with both, H- and O-terminated surface. Linear dynamic range was very wide for both electrodes and surface terminations (from units of μmol dm^{-3} up to 100 μmol dm^{-3}). The widest linear dynamic range was observed on H-BDD $\{110\}_{2000}$. Limit of detection values (0.22 to 3.17 μmol dm^{-3}) achieved are comparable with values obtained on porous, polycrystalline [15] and chem-mechanically polished BDD electrodes [14]. Lower background, and therefore lower limit od detection and limit od quantification, and higher sensitivity were achieved on electrodes with high B/C ratio (2000 ppm), probably due to sufficiently high conductivity.

4. Conclusions

Scaife polished {110}-oriented SC-BDD electrodes showed no noticeable acceleration of HET kinetics for outer sphere redox markers, compared to the as-grown SC-BDD electrodes. On the other hand, a significant acceleration of HET kinetics was demonstrated for surface chemistry sensitive $[Fe(CN)_6]^{3-/4-}$. Detection and quantification limits for dopamine, obtained by square-wave voltammetry were comparable to those achieved on porous, polycrystalline, and chem-mechanically polished BDD electrodes. The performed experiments show that smooth SC-BDD surfaces are promising candidates for other applications, such as surface modification and functionalization.

Acknowledgments

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Analysis of degradation reactions in lithium-ion cell electrolytes during thermal and electrochemical aging

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Keywords
chromatography
electrolyte analysis
ionic conductivity
lithium-ion cells
mass spectrometry

Abstract
The electrification of the transportation sector results in an increasing demand of lithium-ion cells over the last couple years. The lifetime of electric vehicles and therefore it's lithium-ion cells is a topic which has been focused on. The cell lifetime is influenced by the electrolyte composition and stability regarding degradation reactions. Thermal and electrochemical aging reactions in the electrolyte lead towards electrolyte degradation. In this research electrolyte degradation reactions and their influence on the cell performance are analyzed by means of HPLC-MS and IC-MS to investigate the electrolyte degradation reactions. The effect of the degradation on the cell performance is examined by ionic conductivity.

1. Introduction

The Green Deal represents the measures with which Europe wants to become the first climate-neutral continent [1]. Electric vehicles play an essential part in the transportation sector to reach these CO_2 emission goals. Their energy storage systems is composed of lithium-ion cells and as a result, topics such as cell lifetime are becoming more important [2]. The lifetime of a lithium-ion cell is closely linked to the electrolyte stability at elevated temperatures and during cycling (charging and discharging) [3]. The electrolyte is composed of a conductive salt and a variation of carbonates operating as the electrolyte solvent (Fig. 1). Understanding the ongoing aging processes in lithium-ion cells is a key step towards the elimination of the degradation and longer cell lifetime [4].

In this research the degradation of lithium-ion cell electrolytes due to thermal and electrochemical aging is investigated by means of IC-MS and HPLC-MS and its effect on the electrolyte conductivity is analyzed. Furthermore, a variation of conductive salt concentrations and its impact on the electrolyte conductivity is examined.

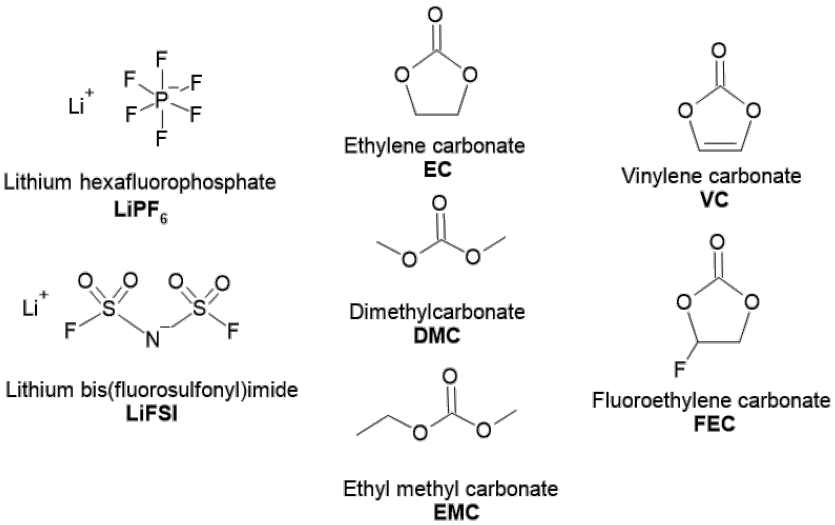

Fig. 1 The chemical formulas of the single components in the electrolyte. On the left the conductive salt: lithium hexafluorophosphate ($LiPF_6$) and lithium bis(fluorosulfonyl)imide (LiFSI). In the middle a variation of electrolyte solvents: ethylene carbonate (EC), ethyl methyl carbonate (EMC), dimethyl carbonate (DMC). On the right side the electrolyte additives: vinylene carbonate (VC) and fluoroethylene carbonate (FEC).

2. Experimental

2.1 Reagents and chemicals

Conductive salts lithium hexafluorophosphate ($LiPF_6$), lithium bis(fluoromethyl)-sulfonyl)amide (LiFSI) and the electrolyte solvents ethyl methyl carbonate (EMC), ethylene carbonate (EC) and the electrolyte additives vinylene carbonate (VC), fluoro ethylene carbonate (FEC) were mixed in an argon filled glovebox with O_2 and H_2O values below 1 ppm.

Single layer lithium-ion cells with 100 mAh were assembled in the dry room with a dew point lower than –60 °C. The cells were built out of $LiNi_8Mn_1Co_1O_2$ (NMC) for the cathode material and for the anode material Si-Graphite was used.

2.2 Instrumentation

Ionic conductivity measurements were performed in a TSC 1600 closed GC cell from RHD instruments consisting of a platinum working electrode and a glassy carbon counter electrode. The cell was connected to an Autolab (Metrohm) controlled by Nova (2.1.6) The conductivity for each electrolyte was measured at the following temperatures (0 °C, 10 °C, 20 °C, 25 °C, 30 °C and 40 °C) in a frequency range of 1–10.000 Hz.

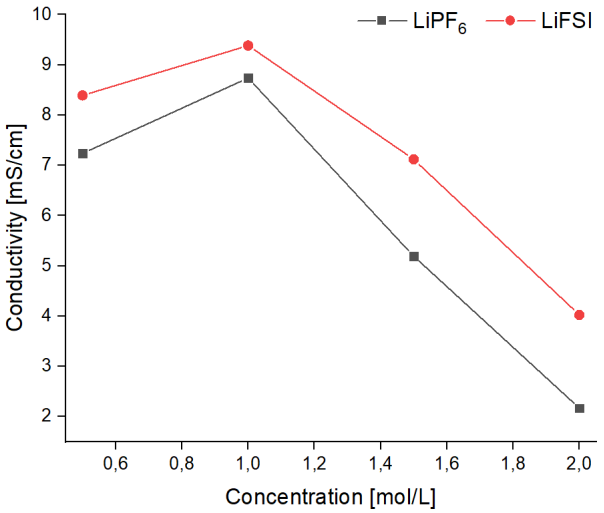

Fig. 2 Ionic conductivity measurements of electrolytes containing 0.5–2.0 mol/L LiFSI (red circles) and 0.5–2.0 M $LiPF_6$ (black squares) at 25 °C.

Conductive salt concentrations and ionic electrolyte degradation products were analyzed by means of a professional IC AnCat MSM-HC MCS by Metrohm A Metrosep C4 100/4.0 column was used for the separation of the cationic components and the anionic components were separated on a Metrosep A Supp 5-250/4.0 column. The IC was connected to a 6130 single quadrupole by Agilent Technologies. The anionic flow was analyzed using an API-ES ionization (3 kV) in a range from 50–300 *m/z*.

Electrolyte solvents and nonionic aging products were identified on a Thermo Scientific Vanquish HPLC. The samples were separated on a Raptor F5 column (150×2.1 mm) with a pore diameter of 2.7 µm using a 0.4 mL/min flow rate. The HPLC was connected to a Thermo Scientific Orbitrap Exploris 120. A heated ESI (2 kV) was used for the ionization and analyzed in a mass range of 20–400 *m/z* and 150–1500 *m/z*.

3. Results and discussion

3.1 Conductive salt concentration in electrolytes and it's effect on conductivity

The conductivity is an important factor for the electrolyte. It displays the ability to transport lithium-ions between the electrodes. A variety of conductive salts and conductive salt concentrations are known in literature each of them showing advantages and disadvantages [5]. Figure 2 compares the ionic conductivity of electrolytes with two conductive salts over a concentration range (0.5–2.0 mol/L) indicating that the highest ionic conductivity can be achieved with a 1 mol/L concentration. The conductive salt LiFSI shows a higher ionic conductivity compared to LiPF6. Finding the optimum conductive salt concentration and therefore a high conductivity is essential for mixing electrolytes in high performance lithium-ion cells.

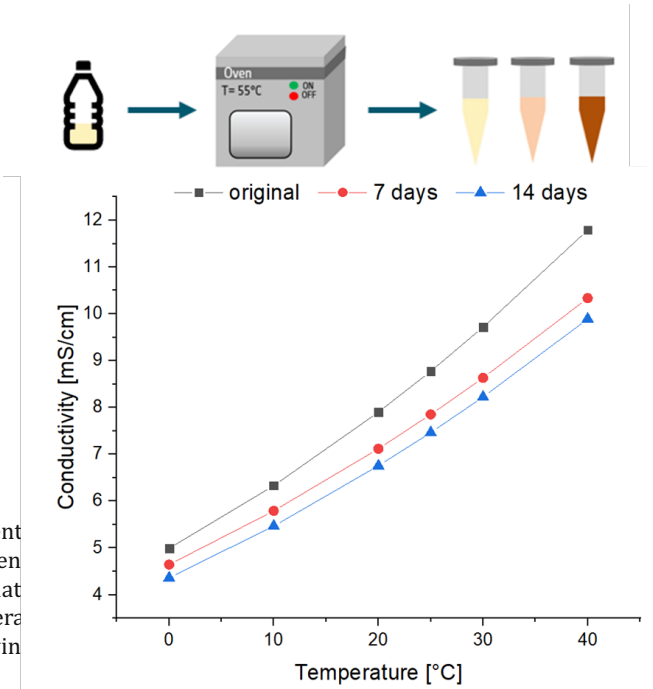

Fig. 3 Conductivity measusrement [mS/cm] of 1 M $LiPF_6$ in ethylen carbonate and ethyl methyl carbonat (1:1) electrolyte at different tempera tures after the aging in the dryin chamber at 55 °C for 7 and 14 days.

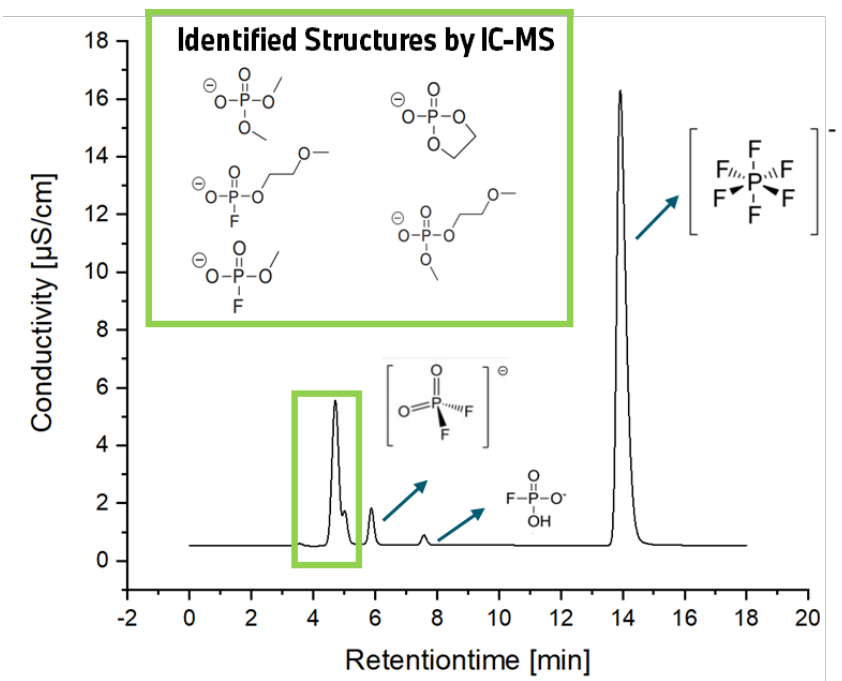

Fig. 4 IC-MS chromatogram from the anionic column for 1 M $LiPF_6$ in ethylene carbonate and ethyl methyl carbonate (1:1) after the aging in the drying chamber at 55 °C for 14 days. By MS a variation of different structures could be identified such as: dimehtyl phosphate, methoxyethyl fluoro-phosphate, methyl fluorophosphate, ethylene phosphate, methoxymethyl methyl phosphate.

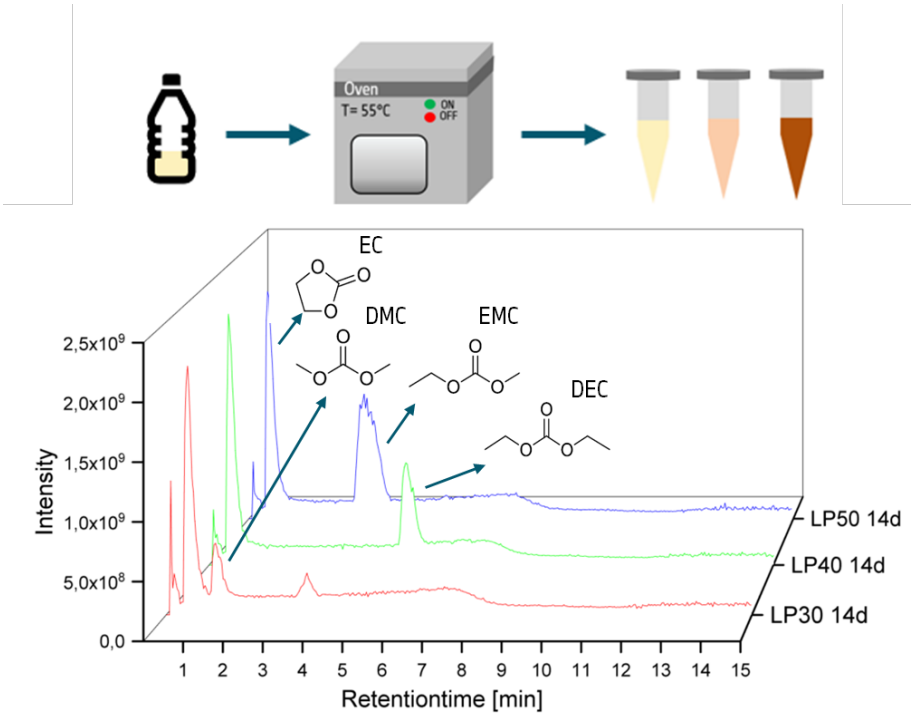

Fig. 5 HPLC-MS chromatogram for three different electrolytes (a) 1 M $LiPF_6$ in ethylene carbonate and dimethyl carbonate (1:1), (b) 1 M $LiPF_6$ in ethylene carbonate and diethyl carbonate (1:1), (c) 1 M $LiPF_6$ in ethylene carbonate and ethyl methyl carbonate (1:1), after the aging in the drying chamber at 55 °C for 14 days.

3.2 Electrolyte degradation due to thermal and electrochemical aging

The electrolyte can undergo various aging processes in a lithium-ion cell one of them being thermal aging at elevated temperatures. A variation of degradation reactions can occur in the electrolyte, reducing the conductivity and therefore the performance of the cell. Figure 3 shows a conductivity measurement of a 1 mol/L $LiPF_6$ in EC and EMC (1:1) electrolyte at a variation of temperatures after 7 and 14 days of aging at 55 °C. A significant conductivity loss can be seen after 7 and 14 days indicating a variation of degradation reactions occurring in the electrolyte.

By means of IC-MS the ionic degradation products can be investigated as shown in Fig. 4. At elevated temperatures the hexafluorophosphate anion shows an accelerated reaction with H_2O leading to electrolyte degradation products such as fluorophosphate and difluorophosphate. Furthermore, a reaction of the hexafluorophosphate anion with the electrolyte solvents such as dimethyl carbonate (DMC), ethyl methyl carbonate (EMC) or ethylene carbonate (EC) can be observed. Herein, the methyl and ethyl end groups get transferred to the conductive salt leading to degradation products like dimethyl phosphate or ethylene phosphate [6–9].

Nonionic degradation products can be analyzed by means of HPLC-MS as shown in Fig. 5. Storing electrolyte at elevated temperatures initiates the transesterification reaction. Herein the carbonates used as electrolyte solvents can undergo a degradation reaction in which the end groups are exchanged [10, 11].

During the first charging of the cell (formation process) the electrolyte is subjected to many electrochemical degradation reactions. The investigation and understanding of the electrolyte degradation during this process is essential for performing further lifetime studies [12, 13].

4. Conclusions

The electrolyte in lithium-ion cells plays a significant role for the performance and lifetime of the cell. It could be shown that an electrolyte containing 1 mol/L LiFSI leads to the highest ionic conductivity within the analyzed electrolytes. Furthermore, degradation reactions and their influence on the cell performance were investigated. The ionic degradation products resulting from a reaction of the anionic hexafluorophosphate with the electrolyte solvents could be identified by means of IC-MS. Transesterification and oligomerization products could be analyzed by means of HPLC-MS leading towards the understanding of those reaction mechanisms. The effect of thermal aging of electrolyte on the performance of the was shown by conductivity measurements and for a better understanding of the degradation reaction some degradation products could be identified by HPLC-MS and IC-MS.

Acknowledgments

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Characterization of electrochemically visualized latent fingerprints

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Keywords
electrochemical deposition
FTIR
latent fingerprint
polyphenazine
profilometry

Abstract
This work presents an electrochemical approach to the visualization of fingerprints on brass cartridges with the aim of characterizing how visualization using polyphenazine dyes affects latent fingerprints. Cyclic voltammetry and chronoamperometry have been used to deposit thin polymer films that visualize fingerprints. After visualization the individual layers (substrate/fingerprint/polymer film) and their interfaces were characterized profilometrically, spectroscopically (FTIR) and via SEM. With the help of SEM images, it was possible to assess the quality and morphology of the applied polymer layer. Profilometry showed the homogeneity and surface roughness of the deposited layers. Based on the FTIR spectra, it can be concluded that polymer films of dyes are deposited on the surface of the substrates due to the observed changes and band intensities belonging to the mono- and polymer form of neutral red and toluidine blue, respectively. The electrochemical visualization method proved to be usable, gentle and relatively fast.

1. Introduction

Several techniques are used in the contemporary forensic science to visualize latent fingerprint; which are divided into physical, physico-chemical, chemical and optical methods [1, 2]. The nature of the surface on which the fingerprint is to be visualized affects the choice of the appropriate technique [1–3]. Non-destructive optical visualization techniques exploit the optical properties of fingerprints [2] and also provide important information about the molecular structure of the fingerprint. These techniques are based on infrared absorption, Raman scattering, etc. [2, 3].

Latent fingerprints are composed of a mixture of hydrophilic and hydrophobic substances from the eccrine and sweat glands of the skin [4]. A large number of inorganic and organic substances can therefore be found in a fingerprint. In addition to the chemical structure, spectroscopic data can provide information on the adhesion of the individually formed layers to the metal substrate surface [3].

A chemical agent bound or dissolved in the area of the applied fingerprint is used in physico-chemical methods of visualization [2]. These methods include: cyanoacrylate vapors, multiple metal deposition, electrochromic film deposition [5], and others [2]. From the point of view of criminology, the visualization of latent fingerprint on cartridge cases is of great importance. There are only a few techniques that can successfully make such fingerprints visible [2, 3, 5–7]. One of these is visualization using polyphenazine dyes such as poly(neutral red) and poly(toluidine blue), which are prepared from their respective monomers, neutral red and toluidine blue.

This work is focused on the characterization of fingerprints electrochemically visualized with polyphenazine dyes using FTIR spectroscopy, SEM and profilometry. Together, these methods provide a range of important information about the nature and structure of (i) the fingerprint, (ii) the deposited polymer film, and also iii) the metal/fingerprint/polymer interface.

2. Experimental

2.1 Reagents and chemicals

For cleaning the brass substrates, redistilled water, acetone and ethanol were used. Visualization was carried out using a solution of neutral red or toluidine blue, phosphate buffer (KH_2PO_4, $NaHPO_4.12H_2O$), KNO_3 and redistilled water. All commercial chemicals were obtained from Lachema, Lach-ner or Penta (Czechia) and were consumed without further purification.

2.2 Instrumentation

Deposition of polymer layers was performed an Autolab PGSTAT12 (Metrohm, Netherlands) with a three-electrode system. As a reference electrode, Ag|AgCl (3 mol L^{-1} KCl) was used. Platinum plate (4 cm^2) as a counter electrode and brass substrates as a working electrode were used. For the working electrode in the form of a plate, alligator clip was applied, whereas the working electrode in the form a cartridge was connected using a special electrode contact.

Measurements were conducted using a scanning electron microscope Mira 3 LMH (Tescan, Czechia); an infrared microscope Nicolet iN10 MX (Thermo Fisher Scientific, USA) with a nitrogen-cooled MCT-A detector, spectral range of 4000–650 cm^{-1}, spectral resolution of 4 cm^{-1}, 64 scans, spatial resolution of the reflection technique of 25 μm and a 3D optical surface profiler NewView™ 9000 (Zygo, USA) with 10× magnification objective and numeric aperture of 0.28.

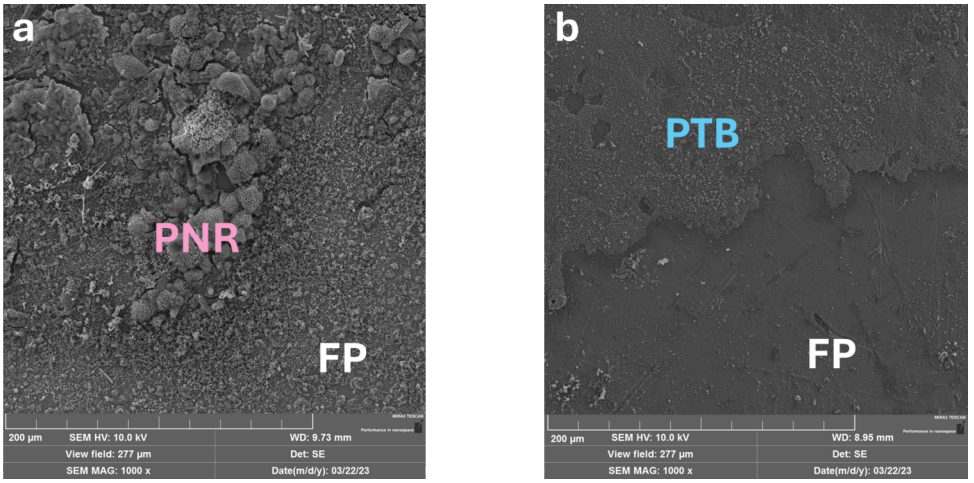

Fig. 1 Image of SEM texture of the interface in secondary electrons mode: (a) poly(neutral red), PNR, and fingerprint, FP, (b) poly(toluidine blue), PTB, and fingerprint, FP.

2.3 Electrochemical visualization

The visualization of sebaceous fingerprints on brass substrates was carried out under these conditions: (i) neutral red was deposited by cyclic voltammetry: the potential range from –200 to +500 mV (*vs.* Ag|AgCl) with a scan rate of 50 mV s^{-1} and 8 (for unfired cartridges) or 4–6 (for fired cartridges) cycles, solution: 2 mmol L^{-1} neutral red, 0.1 mol L^{-1} KNO_3 in phosphate buffer (pH = 7); (ii) toluidine blue was deposited by chronoamperometry: applied potential +500 mV (*vs.* Ag|AgCl) for 120 s (for unfired cartridges) or 40–60 s (for fired cartridges), solution: 5 mmol L^{-1} toluidine blue in 0.1 mol L^{-1} solution of KNO_3.

3. Results and discussion

Figure 1 shows the interface of the polymer layers and sebaceous fingerprints. It is clear that poly(neutral red) forms larger particles compared to poly(toluidine blue), as described by Hermochová et al. [7]. Figure 1a demonstrates an indistinct transition between poly(neutral red) and fingerprint, which was likely due to the sorption of the monomer/dimer form of neutral red. Figure 1b, on the other hand, displays a sharp transition between poly(toluidine blue) and fingerprint.

For another characterization of the brass substrate with polymer layers, optical profilometry was used. The quality of the poly(neutral red) polymer layer that was applied to visualize the remaining fingerprint left on the brass cartridge was investigated. Using this technique, surface irregularities and roughness were detected. A pure poly(neutral red) polymer without fingerprint with a surface roughness of 29.333 µm is captured in Fig. 2a. This finding supports the theory

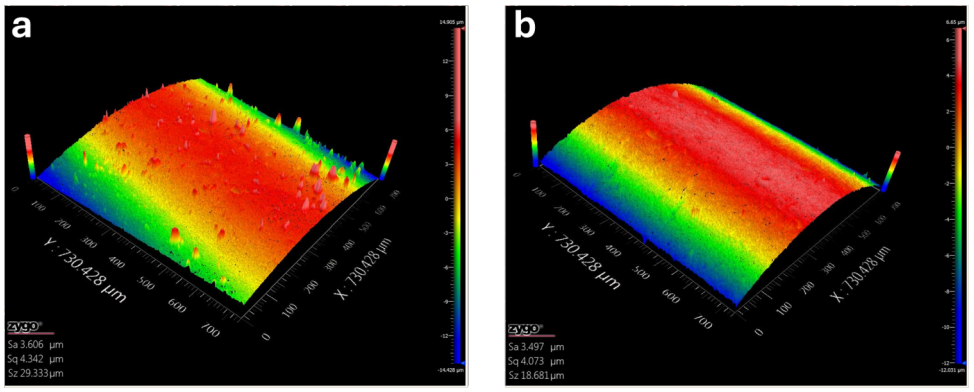

Fig.2 Profilometry image of polymer layers on brass cartridges: (a) poly(neutral red), (b) poly(toluidine blue).

that a monomeric neutral red starts to aggregate at the fingerprint/poly(neutral red) interface, and therefore the resulting layer exhibits much greater roughness. The poly(toluidine blue) polymer layer itself is captured in Fig. 2b, the roughness of which is 18.681 µm. The results show that the roughness difference between the poly(neutral red) and poly(toluidine blue) layers is nearly 10.652 µm. Thus, the roughness of the poly(neutral red) layer itself is much greater than of the poly-(toluidine blue), which again confirms the greater homogeneity of the poly(toluidine blue).

The infrared spectra (Fig. 3) of fingerprints visualized on unfired cartridges using polymerization of neutral red (a, b) exhibit a broad band with a maximum at 3387 cm^{-1} and a side maximum at 3542 cm^{-1}. The bands at 2925, 2853, 1742, 1419 and 1352 cm^{-1} correspond to the sebaceous fingerprints. The broad band with a maximum at 1037 cm^{-1} is probably a vibration of phosphate ions, which originate from the supporting electrolyte of the phosphate buffer [8]. These spectra are practically identical to the fingerprint spectra visualized by poly(neutral red) on the cartridges even after firing (c) and (d). Only the C=O peak at 1742 cm^{-1} and the maxima of the $-CH_3$ and $-CH_2-$ stretching vibrations are weaker. The spectra are very similar to those of a thin film of electrochemically polymerized neutral red on a brass substrate, in which the last peaks of the fingerprints are missing (e). The interpretation of this spectrum and its comparison with the spectrum of pure neutral red are described in Broncová et al. [3]. The spectrum (f) of the monomer/dimer anchored on the polymer coated plate was compared with that of the powdered monomeric form of neutral red (g). There were adsorbed neutral red molecules at the fingerprint/poly(neutral red) interface, which was also confirmed by SEM.

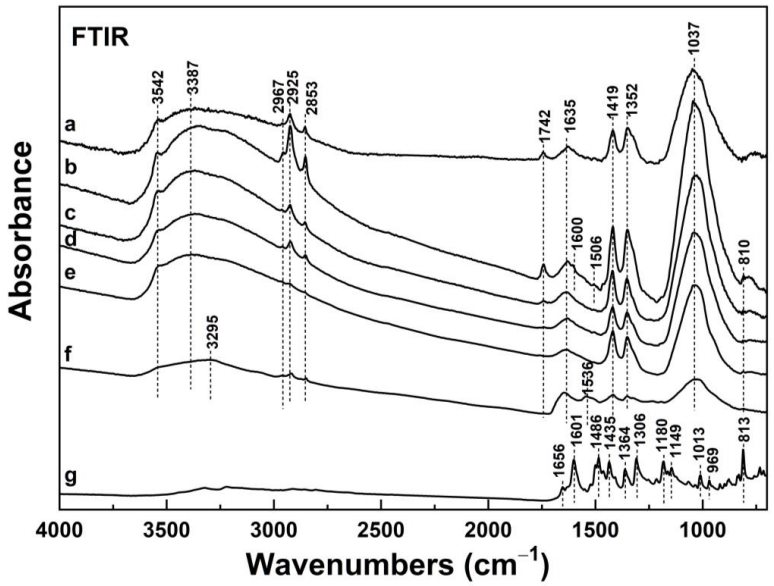

Fig. 3 FTIR spectrum of visualized fingerprints with a deposited layer of poly(neutral red), polymer and powder monomer: (a), (b) visualized fresh fingerprint before firing; (c), (d) visualized fingerprint residue after firing; (e) structure of the poly(neutral red) polymer layer without fingerprint on the plate; (f) adsorbed form of neutral red on the plate; (g) neutral red monomer in powder form.

4. Conclusions

Fingerprints visualized under optimal conditions for the electrochemical deposition of polyphenazine dyes on brass cartridges were characterized spectroscopically, using SEM and profilometrically. Profilometry showed that the roughness of the two polymer films was fundamentally different, with the poly(neutral red) film showing greater roughness compared to poly(toluidine blue), which was consistent with the SEM results. The surface morphology of the fingerprint/poly(neutral red) *vs.* fingerprint/poly(toluidine blue) interface confirms that the adsorbed monomer/dimer form of neutral red extends more into the fingerprint at the fingerprint/poly(toluidine blue) interface than in the case of poly(toluidine blue). The poly(toluidine blue) film is more homogenous. FTIR spectra allowed us to assess how the molecular structure of fingerprints changes simultaneously due to the deposition conditions of both dyes and the structure of the polymer films in the presence of fingerprints. The combination of all the described methods helped to determine the nature of both polymer films in fingerprint visualization as well as the fingerprint properties.

Acknowledgments

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Development of a novel hydride atomizer based on atmospheric pressure glow discharge for atomic absorption spectrometry

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Keywords
atmospheric pressure glow discharge
atomic absorption spectrometry
hydride generation

Abstract
A new type of hydride atomizer for atomic absorption spectrometry (AAS) based on atmospheric pressure glow discharge (APGD) was constructed. Subsequently, APGD atomization conditions were optimized individually for As, Se and Sb as model analytes being introduced into the atomizer by means of hydride generation (HG). Under optimum conditions the performance of the novel APGD hydride atomizer in terms of sensitivity and limit of detection (LOD) was compared to other two hydride atomizers working on different principles. An externally heated quartz tube atomizer (QTA) as the most common hydride atomizer in HG-AAS was used as a reference. A recently described dielectric barrier discharge (DBD) plasma atomizer was used for comparison. The sensitivity and LODs reached in the novel APGD atomizer are comparable to those observed in QTA and DBD, respectively. The LODs reached for individual analytes in APGD were 0.14 ng ml^{-1} As, 0.11 ng ml Se^{-1}, and 0.25 ng ml Sb^{-1}.

1. Introduction

Some analytically important elements such as As, Sb, Bi, Se, Pb, Sn, Te or Ge can be efficiently converted from a liquid sample into their volatile hydrides [1], yielding a 100% analyte introduction efficiency into the spectrometric detector, and surpassing thus, by an order of magnitude, the performance of common pneumatic nebulizers (5–10%). Moreover, the analyte hydride is separated from sample matrix using this approach [1, 2]. Atomic absorption spectrometry (AAS) offers affordable instrumentation, method robustness and operator friendliness

for routine use. Externally heated quartz tube atomizers (QTA) are the most common hydride atomizers in hydride generation (HG) AAS [1]. Recently, the dielectric barrier discharges (DBD), i.e., low power and low temperature plasmas sustained by alternating voltage at atmospheric pressure, have been proven as promising hydride atomizers at least for some of the elements listed above [2]. To overcome difficulties with atomization of some hydride forming elements in DBD, especially Pb, Sn or Ge, also other types of atmospheric plasma discharges are searched for as alternative hydride atomizers. Among them, atmospheric pressure glow discharges (APGD) seem to be promising. Their applicability to hydride atomization and free atom excitation with detection by optical emission spectrometry (OES) has been already proven recently [3].

Atmospheric pressure glow discharges (APGDs) are non-equilibrium gas discharges formed between two electrodes powered by high voltage (~ 1–10 kV). The discharge current is most often in the range of 10–100 mA, and the discharge gap varies from 1 to 5 mm [4]. The discharge is usually powered by direct current or in a pulsed mode. A promising and extensively explored is APGD arrangement in which one of the electrodes is a liquid, e.g., a solution of the analyzed sample. As a result of thermal evaporation, cathode sputtering, and plasma-induced generation of volatile species, analyte species are transported to the discharge where they are atomized, excited, and ionized. The detector in such systems is most often OES, and less often AAS or MS.

The aim of this work was to construct an APGD atomizer design and its power supply source compatible with AAS detection and assess its potential to atomize hydrides of As, Se and Sb.

2. Experimental

2.1 Reagents and chemicals

All reagents were of analytical grade or higher purity. The solutions and dilutions were made with deionized water (< 0.1 mS cm^{-1}, Ultrapur, Watrex, USA). Working standards were prepared from 1000 mg l^{-1} stock solutions of As(III) purchased from Merck (Germany), Sb(III) provided by Fluka (Germany) and Se(IV) purchased from Sigma-Aldrich (Germany). A diluted HCl (1 mol l^{-1}) served as the matrix for working standard solutions as well as the blank being prepared from 37% HCl (p.a., Merck, Germany). The reductant was a solution of $NaBH_4$ (Sigma Aldrich, Germany) in 0.4% (m/v) KOH (Lach-Ner, Czech Republic). Solid NaOH pellets (p.a., Penta, Czech Republic) were used as a filling of the gas phase dryer. Also, another type of dryer based on a NafionTM membrane (tube model MD-110-12P, 12″ long, 0.11″ i.d., Perma Pure, USA) was tested. The gases used Ar (99.996%) and He (99.998 %) were purchased from SIAD Czech Ltd. (Czech Republic).

Table 1
Optimum hydride generation conditions and AAS parameters for selected analytes.

Analyte	Hydride generation conditions			AAS parameters	
	HCl / mol l^{-1}	$NaBH_4$/KOH / %	Dryer	λ /nm	Slit / nm
As	1.0	1.0/0.4	NaOH	193.7	0.5
Se	1.0	0.5/0.4	without dryer	196.0	1.0
Sb	1.0	0.5/0.4	Nafion	217.6	0.2

2.2 Instrumentation

2.2.1 Atomic absorption spectrometry

A Varian SpectrAA 300/400 (GBC, Australia) atomic absorption spectrometer without backround correction was employed as the detector. Hollow cathode lamps (HCL) or boosted HCL (superlamps) were employed as radiation sources. The operation conditions for individual elements are listed in Table 1.

2.2.2 Chemical vapor generator

An in-house made, flow injection hydride generation system based on a peristaltic pump (Ismatec, Switzerland), analogous to that described in ref. [5] was employed. Analyte standards were injected manually by a six port injection valve (V-451, IDEX Health-Science, USA) with a loop of 0.50 ml volume into the flow of the carrier liquid (4.0 mlmin^{-1}) before mixing with the reductant (1.2 ml min^{-1}). The optimum concentration levels of carrier liquid (HCl) and reductant ($NaBH_4$) were analyte dependent, and their values are summarized Table 1. The reaction mixture was merged downstream with a flow of carrier gas (Ar if not stated otherwise) controlled by a mass flow controller (Omega Engineering, USA) and directed to the quartz gas-liquid separator (GLS) with a forced outlet. If not stated otherwise, analyte hydrides generated were introduced through a dryer into the APGD atomizer, while liquid waste was drained from the bottom of the GLS. The dryer was realized either by a polypropylene cartridge (100 mm long, 15 mm i.d.) filled with solid NaOH pellets, or a NafionTM membrane tube model MD-110-12P (12″ long, 0.11″ i.d., Perma Pure, USA). The latter one used 1.0 l min^{-1} Ar as a drying gas. The dryers were always installed downstream the GLS.

2.2.3 APGD atomizer

The design for APGD atomizer was derived from QTA atomizer using the quartz body of exactly the same dimensions. As a consequence, the optical arm adjusted in the optical axis of the spectrometer as well as the inlet arm serving to introduce the analyte hydride from GLS are virtually the same allowing direct comparison of

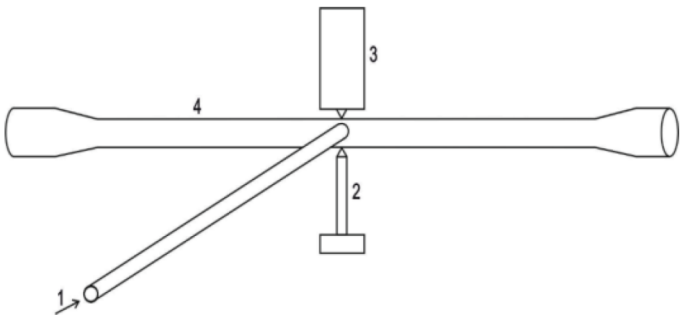

Fig. 1 Scheme of atmospheric pressure glow discharge atomizer: (1) inlet arm, (2) tungsten anode, (3) brass cathode with tungsten tip, (4) optical arm.

analytical performance of both atomizers. In contrast to the QTA, the APGD atomizer has two holes drilled against each other (2mm i.d. and 4mm o.d.) in the center of its optical arm through into which the electrodes are inserted. Figure 1 depicts the scheme of APGD atomizer. Both electrodes are tip-shaped. An in-house made pulsed DC power supply source was employed. The input voltage of this source could be varied from 0 to 12 V DC being converted to a high voltage in a pulsed mode in the power supply source. The input voltage of the source was optimized in this work since the corresponding high voltage in the pulsed mode remains to be characterized in a near future in a collaborating laboratory focused on plasma diagnostics.

3. Results and discussion

3.1 Effect of discharge gas nature

Argon is the most commonly used carrier gas in HG-AAS with QTA atomizers due to its inertness and relatively low cost being substituted by He only in special applications. In plasma discharges such as DBD or APGD the carrier gas serves also as a discharge gas affecting thus the electron number density and formation of energetic species (metastables, ions, excited species) being involved in analyte hydride atomization processes. The sensitivity and peak area in APGD was compared for each model analyte employing either Ar or He at a flow rate of 100 ml min^{-1} for both gases. For all three analytes tested the peak area observed in Ar as discharge gas was higher than that in He. The sensitivity in He was significantly lower for As and Sb reaching only about 25% of the value observed in Ar. In case of Se, the sensitivity between both discharge gases was comparable but showing worse signal repeatability in He. As a consequence, Ar was selected as the discharge gas for further measurements with all three analytes.

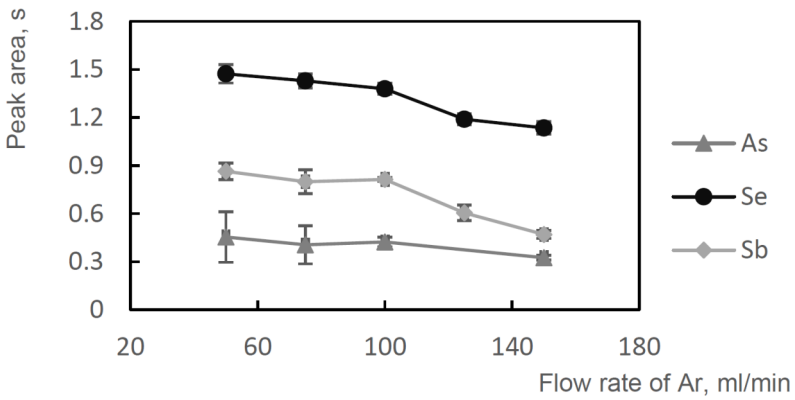

Fig. 2 Effect of Ar flow rate on peak area. Analyte standard concentration 2 ng ml^{-1} As, 10 ng ml^{-1} for Se and Sb. Power source input voltage 12 V, dryers employed for measurements with As (NaOH) and Sb (Nafion).

3.2 Effect of dryer

The losses of analyte hydride in the dryer have always to be prevented. There is no a universal dryer compatible with all hydride forming elements since each hydride has different, analyte dependent, physico-chemical properties. Based on our previous experience the dryer packed with NaOH pellets was employed for measurements with As while Nafion tube dryer was selected for experiments with Sb and Se. These analyte-dryer combinations have been previously shown not to cause analyte losses in the dryer [2, 5]. The peak area and sensitivity were not significantly affected by the presence of NaOH based dryer in case of As measurements and Nafion dryer for experiments with Sb. However, better repeatability of the measurements was observed for both elements in presence of the dryer. As a consequence, the dryers were employed in further measurements. In contrast, the signal of Se was by 25% lower in presence of the Nafion dryer. Thus, no dryer was employed for Se measurements.

3.3 Effect of Ar flow rate

The effect of Ar flow rate on analyte signal was investigated in the range from 50 to 150 ml min^{-1} Ar with the results depicted in Fig. 2. A signal plateau was observed between 50 and 100 ml/min Ar followed by signal decrease due to dilution at 150 ml min^{-1}. Impaired signal repeatability was observed at lower Ar flow rates using 50 or 75 ml min^{-1} Ar, especially for As and partially also Sb as analytes. A flow rate of 100 ml min^{-1} Ar was selected as optimum for all three analytes tested.

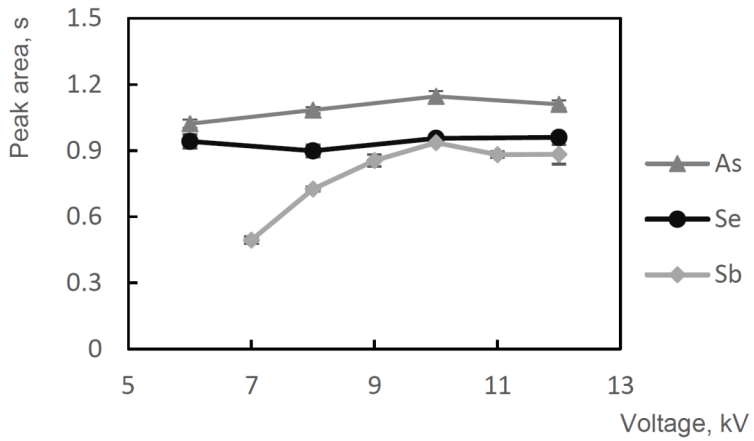

Fig. 3 Effect of input voltage on peak area. Analyte standard concentration 10 ng ml^{-1} Sb, 5 ng ml^{-1} for As and Se. Flow rate at 100 ml min^{-1} Ar, dryers employed for measurements with As (NaOH) and Sb (Nafion).

3.4 Effect of power supply source input voltage

The effect of DC input voltage delivered to the APGD power supply source was investigated in the range between 6 to 12 V and the results are depicted in Fig. 3. A stable plasma cannot be operated at input voltage lower than 6 V while DC voltage values higher than 12 V were not investigated in order to eliminate the risk of the damage of the source. Constant signal response was observed for As and Se regardless of the input voltage employed. In contrast, the signal of Sb was increasing significantly between 7 and 10 V reaching a plateau then. Input voltage of 12 V was found optimum for all model analytes.

3.5 Analytical figures of merit

Calibration curves were measured in the HG-APGD-AAS arrangement under the optimum atomization conditions determined in the APGD atomizer individually for As, Se and Sb as discussed above in sections 3.1 to 3.4. Sensitivity and LOD were quantified for each analyte with the results summarized in Table 2. For the purpose of comparison, the same analytical figures of merit, i.e., sensitivity and LOD, found previously in QTA and DBD atomizers [2], respectively, with the same hydride generation system in the same laboratory, for the same analytes are also included in Table 2.

Table 2
Basic analytical figures of merit reached for As, Se and Sb in atmospheric pressure glow discharge (APGD), quartz tube (QTA) and dielectric barrier discharge (DBD) atomizers.

Analyte	APGD		QTA		DBD	
	Sensitivity / s ng^{-1}	LOD / ng ml^{-1}	Sensitivity / s ng^{-1}	LOD / ng ml^{-1}	Sensitivity / s ng^{-1}	LOD / ng ml^{-1}
As	0.30	0.14	0.48	0.15	0.48	0.16
Se	0.22	0.11	0.53	0.15	0.32	0.24
Sb	0.48	0.25	0.36	0.14	0.46	0.15

4. Conclusions

As can be seen from Table 2 the LOD values found in APGD in this work reaching the values of 0.11 ng ml^{-1} Se, 0.14 ng ml^{-1} As and 0.25 ng ml^{-1} Sb are fairly comparable to LODs found in commonly used QTA atomizer and recently developed DBD atomizer. This indicates the potential of the APGD design proposed in this work to serve as hydride atomizer for AAS. In a near future the APGD power supply source developed within this work will be studied in detail to investigate its current-voltage characteristics in the collaborating laboratory at the Department of Plasma Physics and Technology, Faculty of Science, Masaryk University, Brno. The knowledge of the current and voltage functions delivered to the APGD electrodes is crucial for further development of APGD experimental set up. Subsequently, the HG-APGD-AAS instrumentation will be optimized for other analytically important hydride forming elements including Pb and Sn. Mechanistic studies focused on atomization efficiency and analytical applications to authentic samples will be the last part of this work.

Acknowledgments

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Optimization of Raman spectroscopy for comprehensive analysis of *ex vivo* colorectal tissue samples and diagnostics

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Keywords
classification model
machine learning
measurement optimization
Raman spectroscopy
spectra processing automation

Abstract
Early diagnosis is of paramount importance for successful treatment, yet current histological examinations are time-consuming and subjective. This study explores the potential of Raman spectroscopy as an alternative diagnostic tool that offers fast and non-destructive analysis sensitive to biochemical changes. The measurement parameters were first optimized to ensure relevant results without affecting the samples for subsequent histological evaluation. The tissues from routine preventive colonoscopies were subsequently analyzed using a portable Raman spectrometer without prior preparation. A script for automated spectra processing was developed and a classification model was created to categorize samples based on their spectra.

1. Introduction

Colorectal cancer is one of the most common oncological diagnoses in developed countries [1–4]. The prognosis of colorectal cancer is largely dependent on the stage at which it is detected. Consequently, screening programmes are underway with the objective of reducing the mortality and morbidity associated with the disease [2, 5, 6]. The final diagnosis is made histologically from tissue samples taken during colonoscopy [7]. However, histological examination of tissue is often a long and demanding process, and the precision of the diagnosis may depend to some extent on the histologist's experience [8]. Therefore, alternative diagnostic methods are being sought that could solve these problems and help the doctor

make the right decision in a timely fashion in order to begin treatment immediately [8–10].

One potential alternative would be to make the diagnosis based on a chemical analysis of the removed tissue using Raman spectroscopy. This technique can be used to detect specific molecular anomalies in biological tissues, thereby demonstrating the potential for diagnostic evaluation of tissues [11–14]. The diagnostic evaluation of tissues using Raman spectroscopy can eliminate the subjectivity of the examination and shorten the time required to make a diagnosis. The widespread use of Raman spectroscopy is constrained by several limitations, including low signal intensity, high autofluorescence, and the difficulty of distinguishing between the spectra of healthy and pathological tissues [15]. The primary challenge is the interpretation of spectral data obtained from colorectal tissues, which requires a sophisticated analysis to yield pertinent diagnostic information [16]. The analysis is conducted using machine learning methods, including K-Nearest Neighbors (KNN) [17], Random Forest [16, 17], Linear Discriminant Analysis (LDA) [8, 16], Decision tree (DT) [16], Support Vector Classification (SVC) [16-18], Bayesian methods, and neural networks [15, 17].

2. Experimental

2.1 Reagents and chemicals

In order to optimize the measurements, chicken breast muscle was used. Tissue samples from the colon were obtained from patients in the Department of Gastroenterology and Hepatology of the 4th Faculty of Medicine, Charles University, and General University Hospital in Prague using disposable biopsy forceps. Subsequently, the samples underwent a spectroscopic analysis followed later by a histopathologic evaluation at the General University Hospital in Prague. A total of 330 spectra were obtained from colorectal tissue samples collected from 155 patients. The histological examination of the samples revealed 76 spectra from healthy tissue and 254 spectra from pathological tissue. Pathological specimens included tissues diagnosed with hyperplasia, inflammatory diseases, adenomas, and carcinomas.

2.2 Instrumentation and spectroscopic measurements

The spectra were obtained using the AHURA FirstDefender RM mobile Raman spectrometer (Thermo Scientific, USA), which was equipped with an excitation laser with a wavelength of 785 nm. Following collection, colon tissue samples (1–25 mm) were immersed in a 0.9% sodium chloride solution. Prior to analysis, the samples were dried using filter paper and analyzed with metallized slides at room temperature. The laser power for the measurement was set to “high,” which

Table 1
Optimized parameters for classification methods. The selected parameters of the model with the highest accuracy are in bold.

Classification method	Optimized parameter	Tested options
K-Nearest Neighbors	Number neighbours	**1**, 3, 5, 7, 9, 11, 13
	Weights	**uniform**, distance
	Algorithm	**auto**, ball_tree, kd_tree, brute
	Metric	**euclidean**, manhattan
	Leaf size	**5**, 10, 20, 30, 40, 50, 60
Random Forrest	Number estimators	300. 500, **750**, 1000
	Max. depth	**None**, 3, 5, 10, 30
	Min. samples split	2, **5**,10
	Min. samples leaf	**1**, 4
Linear Discriminant Analysis	Solver	svd, **lsqr**, eigen
	Number components	**None**, 1, 2, 3, 4
	Shrinkage	None, **Ledoit-Wolf lemma algorithm**
	Min. significant singular value for rank	**10^{-3}**, 10^{-4}, 10^{-5}
Support Vector Classification	Scaling the regularization parameter C	0.1,1, 10, 50, 100, 200,**300**
	Kernel	linear, **poly**, rbf, sigmoid
	Degree	1, **2**, 3, 4, 5, 6
	Gamma	1/(number features X.var), **1/number features**
Decision Tree	Criterion	gini, entropy, **log loss**
	Max. depth	none, 5, 10, 20, 30, **40**, 50
	Splitter	**best**, random
	Min. samples split	1, 2, **3**, 4
	Min. samples leaf / %	0, 0.1, 0.5, 0.7, **1.0**
Gaussian Naive Bayes	Priors	None, [0.2, 0.8], [0.3, 0.7], **[0.1, 0.9]**, [0.25, 0.75], [0.15, 0.85]
	Var. smoothing	**10^{-14}**, 10^{-12}, 10^{-9}, 10^{-6}, 10^{-3}, 0.01,

corresponds to a power of 185 mW on the surface of the tissue. The measurements were conducted automatically with a spectral resolution of 7 to 10 cm^{-1}.

2.3 Data processing

To process the measured spectra, a script was created in Python programming language using open-source libraries, including NumPy, Pandas, Scikit-Learn, Matplotlib, Seaborn, and Imblearn. The range of 505–1800 cm^{-1} was selected from the raw spectrum. Subsequently, the spectra were smoothed using a Savitzky-Golay filter with a window size of seven points and a polynomial order of two.

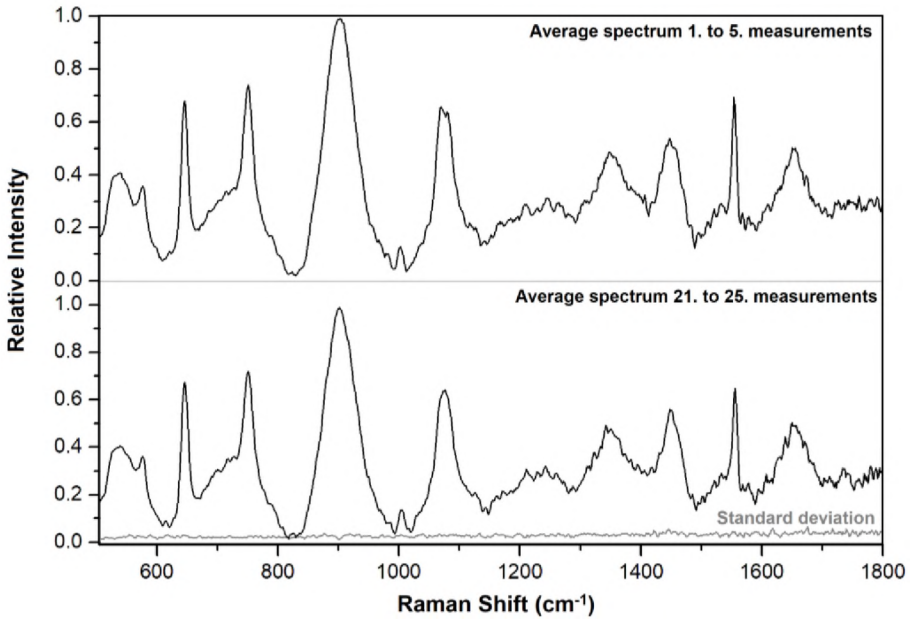

Fig. 1 Raman spectra of chicken pectoral muscles. Average Raman spectrum of 1 to 5 measurements (top), average Raman spectrum of 21 to 25 measurements (bottom), standard deviation of all 25 measurements (grey)

For baseline correction, a Savitzky-Golay filter with a window size of 91 points and a polynomial order of one was used to create a background, which was then subtracted from the smoothed spectrum. Min-Max normalization was applied, adjusting each spectrum so that the minimum value was zero and scaling it so that the maximum value was one.

Following preprocessing, the data was split into two groups, a training set and a testing set, in a ratio of 80:20. This resulted in 264 samples assigned for training and 66 samples for testing. The dataset was then exported for further analysis. A range of classification algorithms from the Scikit-Learn library was used for the classification, including KNN, Random Forest, SVC, Gaussian Naive Bayes and 1D Convolutional Neural Networks (1D CNN). To achieve the most optimal classification of samples, the most suitable parameters were selected using GridSearchCV. The optimized parameters are listed in Table 1.

3. Results and discussion

To ensure that the measurements did not affect the samples and thereby avoid any potential misdiagnosis, an evaluation of the possible denaturation that might occur after repeated exposure of the tissue to the excitation laser was conducted. Chicken pectoral muscle tissues were subjected to 25 excitations of the laser at high power settings, with spectral changes monitored between the initial and

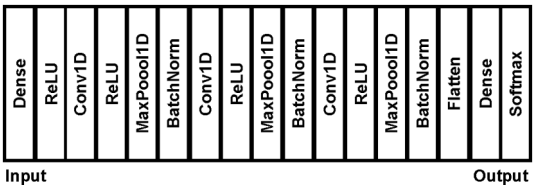

Fig. 2 Architecture of 1D Convolutional Neural Network

Fig. 3 Confusion matrix for 1D Convolutional Neural Network

subsequent exposures. No significant alterations in the tissue spectrum were observed even after 25 exposures (Fig. 1).

Once it had been established that the measurement process was safe, the laser power was optimized. Three power levels were tested: low (45 mW), medium (85 mW) and high (185 mW). The results of the analysis indicated that the highest power setting yielded the most favorable outcomes. Lower settings required the accumulation of more data, thereby extending the analysis time. This could present challenges for direct implementation in a clinical setting.

The results of the classification algorithms evaluation indicated that the DT algorithm yielded the highest values of training accuracy. However, the results of the test subset indicate that the model is overfitted. The highest testing accuracy of 77% was achieved with 1D CNN, more accurate architecture of which is depicted in Fig. 2. Classifying samples into individual groups using the 1D classification model CNN is shown in Fig. 3. The outcomes of the 1D CNN training dataset were found to be highly correlated with the results provided by the same algorithm on the testing dataset. The outcomes of all methodologies are presented in Table 2.

4. Conclusions

This study demonstrates the efficacy of machine learning in spectral data classification, emphasizing the significance of algorithm selection and parameter tuning. Despite challenges such as the dataset size and the class imbalance affecting the overall accuracy, the 1D CNN demonstrated promising performance.

Table 2
A comparison of the achieved accuracy of the tested classification methods.

Method	Accuracy	
	Training	Testing
K-Nearest Neighbors	0.87	0.73
Support Vector Classification	0.65	0.58
GaussianNB	0.68	0.73
Decission tree	1.00	0.61
Random Forest	0.96	0.71
1D Convolutional Neural Networks	0.75	0.77

Future endeavors will focus on expanding the dataset to address class imbalance and implementing transfer learning techniques to enhance model accuracy and robustness. These steps are intended to enhance the practical applicability of our classification approaches.

Acknowledgments

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Voltammetric behaviour of oxysterols and development of HPLC method for their separation

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Keywords
boron-doped diamond electrode
cholesterol
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oxysterols
voltammetry

Abstract
This study investigates the voltammetric behavior of oxysterols using a boron-doped diamond electrode in acetonitrile with perchloric acid as a dehydrating agent. The oxysterols exhibited irreversible anodic responses with specific oxidation potentials. The research explores the impact of water content and acid concentration in the supporting electrolyte on oxysterol response. Additionally, a high-performance liquid chromatography method was optimized for separating cholesterol, its precursors, and oxysterols, considering water content in the mobile phase and the use of strong acid for dehydration. The study demonstrates the potential of electroanalytical methods for oxysterol detection and the effectiveness of dehydration in facilitating their separation.

1. Introduction

Oxidized forms of sterols, oxysterols, are mainly formed during cholesterol metabolism in humans [1]. The structure of cholesterol consists of steroid nucleus, a hydroxyl group on the C3 carbon and a Δ^5 double bond, which makes it subject to oxidation reactions. Oxidation reactions can also occur on the side chain located at carbon C17 [1, 2].

Detecting oxysterols in physiological matrices is a complex yet crucial task, as they can serve as markers for various diseases. Monitoring their presence in food is also essential due to their adverse effects on the human body. The methods employed for detection include advanced instrumental techniques, such as gas chromatography of derivatives of these compounds coupled with mass detection or liquid chromatography with tandem mass spectrometry [3, 4]. The analytical challenges arise from the oxysterols' cholesterol-like structure, which coexists in matrices at concentrations at least a thousand times higher.

Electrochemical methods have limited possibilities, because steroid compounds, lacking an aromatic ring or conjugated double bonds, can only be oxidized at high positive potentials, preferably in non-aqueous media [5]. This approach was used to determine oxysterols in oxidatively modified low-density lipoprotein cholesterol using semi-micro HPLC coupled with electrochemical detection on a glassy carbon electrode [6].

In our group, an innovative electrochemical detection approach for steroid compounds involves activating them through dehydration of the steroid skeleton. This process, inspired by the Liebermann-Burchard reaction of cholesterol with sulfuric acid, acetic acid, and acetic anhydride introduces double bonds and other structural changes, enabling subsequent electrochemical oxidation of the resulting products within the potential window of common unmodified electrodes [7–10]. This two-step process – dehydration followed by direct oxidation – has been successfully applied to voltammetric determination of primary bile acids in serum [8, 9], cholesterol in dairy products [7], and determination of 7-dehydrocholesterol, as a marker for Smith-Lemli-Opitz syndrome [9].

This study aimed to investigate the potential of electroanalytical methods for detection of various oxysterols (7α- and 7β-hydroxycholesterol, 25- and 27-hydroxycholesterol, and 7-ketocholesterol) using a boron-doped diamond electrode (BDDE) in acetonitrile with a strong acid as a dehydrating agent.

2. Experimental

2.1 Reagents and chemicals

The studied substances were 7α-hydroxycholesterol, 7β-hydroxycholesterol, 25-hydroxycholesterol, 7-ketocholesterol, lanosterol, lathosterol (Avanti Polar Lipids, Alabama, USA), cholesterol, 27-hydroxycholesterol (Sigma, Prague, CZ). Other used chemicals were acetonitrile for HPLC, sodium perchlorate (Honeywell, Germany); perchloric acid 70%, phosphoric acid 85% (Penta, Chrudim, CZ), sulfuric acid 96%, nitric acid 65%, hydrochloric acid 35% (all Lach-Ner, Neratovice, CZ) and alumina (0.5 μm particle size, Electrochemical Detectors, Turnov, CZ). All chemicals were of analytical grade.

2.2 Instrumentation

For voltammetric measurements, the Eco-Tribo polarograph with MultiElchem 3.2.0 software (Eco-Trend Plus, Prague, CZ) was used. A boron-doped diamond electrode (BioLogic, Seyssinet-Pariset, France) with a disk diameter of 3 mm (A = 7.07 mm^2) was used as the working electrode. The auxiliary electrode was a platinum electrode (Electrochemical Detectors, Turnov, CZ), and the reference electrode was a non-aqueous electrode according to Pleskov, which consists of

a silver wire immersed in a solution containing $1 \cdot 10^{-2}$ mol L^{-1} silver nitrate and 1 mol L^{-1} sodium perchlorate in acetonitrile. Before each measurement, the surface of the BDD electrode was activated by polishing. Voltammetric measurements were performed by cyclic voltammetry with a scan rate of 100 mV s^{-1} and by differential pulse voltammetry with a scan rate of 20 mV s^{-1}, pulse height of ±50 mV and pulse width of +80 ms.

An Agilent 8453 spectrophotometer (Agilent Technologies, Santa Clara, USA) was used for spectrophotometric measurements. Chromatographic measurements were conducted using a Merck-Hitachi instrument (Germany) equipped with a D 7000 control unit and an L-7400 UV detector, managed by Chromatography Data Station Software version 4.0. Amperometric detection, positioned downstream of the spectrophotometric detection, was achieved with a three-electrode wall-jet arrangement controlled by an ADLC2 potentiostat (Laboratorní přístroje, Prague, CZ). Separation was carried out on a Hypersil GOLD™ C18 column (Thermo Scientific) with dimensions of 100 × 4.6 mm and a particle size of 5 µm. The injection volume was 50 µL, and the mobile phase flow rate was 1 mL min^{-1}. Spectrophotometric detection was performed at 200 nm, and electrochemical detection at +2.0 V.

3. Results and discussion

The voltammetry in the media of 0.1 mol L^{-1} perchloric acid in acetonitrile revealed that studied oxysterols exhibit irreversible anodic responses on the BDDE with the following oxidation potentials: 7α-hydroxycholesterol and 7β-hydroxycholesterol at approximately +0.8 V, 25-hydroxycholesterol and 27-hydroxycholesterol at approximately +1.5 V, and 7-ketocholesterol at approximately +1.9 V (*vs.* $Ag/AgNO_3$ in acetonitrile). The dehydration of the steroid core of 7α- and 7β-hydroxycholesterol under these conditions possibly results in electrochemically active products, which was previously described for primary bile acids [5, 8, 10]. In addition, the study explored how water content and acid concentration in the supporting electrolyte affect the oxysterol response. The measurements using supporting electrolyte composed of sodium perchlorate in acetonitrile were conducted, which yielded responses at highly positive potentials. Furthermore, the limit of detection for all five oxysterols studied was determined using differential pulse voltammetry based on calibration dependence measurements, and it falls within the micromolar concentration range.

Another part of this work focused on the development and optimization of an HPLC method with spectrophotometric and electrochemical detection for the separation of cholesterol, its precursors, and oxysterols, with two factors studied. The first factor examined was the influence of water in the mobile phase, consisting of a mixture of acetonitrile containing 50 mmol L^{-1} sodium perchlorate, on sterol retention. The most suitable environments for sterol separation were evaluated to be those with 0% and 6% water content (Fig. 1).

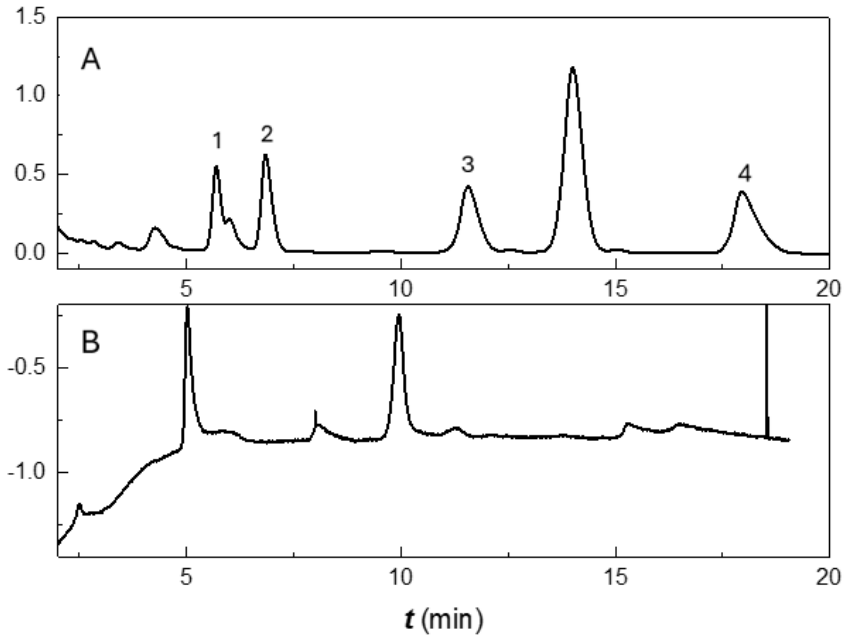

Fig. 1 Comparison of chromatograms of sterol separation at a concentration of $1 \cdot 10^{-4}$ mol L^{-1} with (A) 6% and (B) 0% water content in the mobile phase. (1) 7-ketocholesterol, (2) 27-hydroxycholesterol, (3) 7α- and 7β-hydroxycholesterol, (4) cholesterol and lanosterol. Electrochemical detection at +2.0 V on BDDE (*vs.* $Ag/AgNO_3$), flow rate of the mobile phase 1 mL min^{-1}, injection volume 50 μL.

The second tested factor was dehydration using strong acid, which affects the separation of sterols in the mixture. Perchloric acid was chosen for dehydration, as it activates the steroid skeleton during voltammetric measurements. UV-VIS spectroscopy data indicated that 27-hydroxycholesterol, 25-hydroxycholesterol and 7-ketocholesterol, in the presence of perchloric acid in acetonitrile with 0.55% water content, do not undergo significant structural changes, and their voltammetric behavior remains unchanged. The same conclusion was reached for cholesterol and lanosterol. However, 7α-hydroxycholesterol and 7β-hydroxycholesterol showed a change in the absorption maximum wavelength immediately after the addition of perchloric acid, indicating structural changes.

The sterols, after eventual dehydration, were individually measured in a mobile phase containing 0% and 6% water (Fig. 1), both with and without the addition of perchloric acid in acetonitrile prior to injection, to verify whether dehydration leads to the formation of new products. After dehydration, only the retention times of 7α-hydroxycholesterol and 7β-hydroxycholesterol changed. For both compounds, dehydration resulted in a single major peak with a different retention time than the original peak. This change was utilized to separate 7α-hydroxycholesterol and 7β-hydroxycholesterol from other oxysterols, with which they had very similar retention times.

4. Conclusions

This study investigated the voltammetric behavior of various oxysterols using a boron-doped diamond electrode in acetonitrile with a strong acid as a dehydrating agent. The oxysterols exhibited irreversible anodic responses with distinct oxidation potentials. The study also examined the effect of water and acid concentration on the oxysterol response and determined the detection limit within the micromolar concentration range. Additionally, an HPLC method with spectrophotometric and electrochemical detection was optimized for separating cholesterol, its precursors, and oxysterols. The influence of water content in the mobile phase on sterol retention was assessed, with 0% and 6% water content providing the highest resolution. Dehydration using perchloric acid was found to affect only 7α-hydroxycholesterol and 7β-hydroxycholesterol, resulting in structural changes that facilitated their separation from other oxysterols.

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Human scent analysis on fired cartridge cases from simulated crime scene

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Keywords
cartridge case
gas chromatography
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olfactronics

Abstract
Shot cartridge cases are often present on crime scenes connected with a shooting and could be useful during the investigation. However, the dactyloscopy prints that usually appear on the cartridges are only partial so there are not enough minutiae for individual identification. In this pilot study, we compare the human scent remaining on cartridge cases after shooting with scent samples from different volunteers to find out who loaded the gun before firing. In this experiment, simulated crime scene was prepared and one of our volunteers loaded the gun. Analysis of the scent remain on cartridge cases was carried out using two different methods, i.e., olfactronics and olfactorics.

1. Introduction

Forensic olfactronics is a new field of forensic analysis whose aim is to analyze the scent samples using advanced analytical chemistry techniques [1–3]. Unlike olfactorics, that uses specially trained canines, this method is objective. On the other hand, the olfactory system of a canine is more sensitive than current devices usable for human scent analysis. This is why both methods should be using for these purposes in the future even during investigations, so they can complement each other and be a desirable chain of evidence to present before the court. Cartridge cases are often one of the traces that can appear on the crime scenes usually connected with a shooting, mainly in cases of very serious crimes. Unfortunately, fingerprint is often partial, thus there are not enough minutiae for comparing it with the dactyloscopy database. In a previous study [4], it was found that the less volatile substances that seem to be significant for individual identification of human scent [5], resist high temperatures for a short time. That led to a conclusion that at least the part of the human scent remaining on the

cartridge after loading the gun and lingering there even after the gun fire would be significant for an individual identification [4].

2. Experimental

2.1 Reagents and chemicals

Firstly, the glass beads used as a sorbent for the human scent were cleaned in a chromosulfuric acid (prepared at the UCT Prague) and washed in deionized water, ethanol (for UV–VIS spectroscopy, min. 99.8%, Penta, CZ) and hexane (quality for GC-MS, Sigma-Aldrich, USA). After drying (270°C, 90 min), the glass beads were stored in a desiccator. Based on a certificated and published methodology [6], the sampling procedure was carried out on both sorbents: glass beads as well as cartridges. The sampled glass beads were then extracted into ethanol and concentrated to 70 µl, based on previous study published by Pojmanová et al. [7]. Helium (purity 5.5, Linde CZ) was used as a carrier gas for the gas chromatography. For the olfactoric line-ups, commercial fabric Aratex® (70% cotton, 25% viscose, 5% polyester, 280 g/m2, purchased from CHLUM-TEX, CZ) was utilized.

2.2 Instrumentation

For this experiment, only one gun type was used (Sauer 38H, Sauer Sohn Germany, caliber 7.65 Browning, ammunition, type FMJ 73 grs, produced by Sellier Bellot, Czech Republic).

The samples were measured by a 7980B GC two-dimensional gas chromatograph (Agilent, USA) coupled with Pegasus® 4D-C time-of-flight analyzer mass spectrometer (LECO, USA). The columns were connected in reverse order, the primary column was a semi-polar Rtx-200MS (30 m + 2 m pre-column, Restek, USA) and the secondary column was a non-polar TG5-HT (1.1 m, Thermo Fisher Scientific, USA). The diameter of the columns was 0.25 mm, and the thickness of the stationary phase was 0.25 µm. The sample was injected in a volume of 1 µl in splitless mode (2 min) at a temperature of 280 °C. The temperature gradient on the primary column started at 40 °C held for 2 min and ended at 320 °C held for 10 min. The temperature increased at a rate of 5 °C/min. The temperature on the secondary column was always 5 °C higher than on the primary column. The modulator between the two columns always had a temperature 15 °C higher than the secondary column. The cryogenic modulation at –80 °C with three modulation periods of 6, 8 and 10 s were used. The carrier gas flow rate was 1.5 ml/min. The interface between GC and MS was heated to 280 °C. The mass detector used electron ionization with an energy of 70 eV ionizing electrons, the temperature of the ion source was 250 °C. Data collection took place in TIC (Total Ion Current) mode, with a mass interval of 29–800 (*m/z*), data collection took place at a speed of 200 spectra/s. The time required to elute the solvent was set to 500 s. To

increase the detection sensitivity, a 200 V higher voltage was set on the detector compared to tuning. The measured data were firstly aligned by reference peaks that were occurring in all the samples. These references were used as some kind of anchor points for aligning all the peaks from different chromatograms. After the data alignment, only the substances that occurred at least in one of four "unknown" samples from the simulated crime scene and at least in one of all the samples from every volunteer were used. This approach was chosen to focus the analysis on substances the origin of which is the human scent and not the background of the sorbent itself or contaminants etc. However, the substances themselves are not significant for individual identification; what is important are the ratios of the peak's areas. All area ratios were then compared in all samples and ordered from the most stable to less stable. Hence, only those that are stable in time can be chosen and more probably are behind the genetic determined part of the human scent. The results were statistically evaluated by preview methods, i.e., Cluster Analysis and Principal Component Analysis.

The same set of unknown samples from simulated crime scene was used for comparing it with 19 different volunteers by trained canines. Their results are normally used in police practice as non-direct evidence before court. The line-ups were carried out in special rooms designed for odorology comparison. Every line-up was executed double-blindly. A special fabric for odorology (Aratex®), which was exposed to the cartridge case for at least 30 minutes (30 minutes, 1 hour, 6 hour and 1 day), was used for sniffing samples. This fabric, utilized as a secondary sorbent for the scent samples, was used due to the canines training. In each line up, there were five samples from the volunteers and the canine compared them with the sample from the simulated crime scene. When it finds the match, it marks the jar with the specific sample by laying down before it. The random interest control was executed before every comparison.

In this experiment we also compared the difference between cartridge cases collected from different surfaces, i.e. plastic, wooden palette and concrete. The scent samples from our simulated crime scene were collected 15 minutes after the gun was fired and then compared with volunteer samples by two separate methods: olfactronics using two-dimensional gas chromatograph and olfactoric using specially trained police canines. For the first method, 12 samples from four different volunteers were sampled and compared with the scent extracted from the cartridge cases to find out which one of the volunteers loaded the gun.

3. Results and discussion

From the olfactronic point of view, the results show successful individual distribution between different volunteers on the glass beads. However, the system could not link the human scent extracted from the gun-fired cartridge cases to one of the volunteers. This could be due to the different kind of background that is provided by glass beads and cartridge cases, despite the fact that data editing was

done in an attempt to eliminate these differences. Nevertheless, the surface differences from which the unknown trace were sampled at the crime scene seem to not play such a role. This is important for further investigation, as the cartridge cases remaining at the crime scene could lay on many kinds of surfaces and the analysis of the evidence must not depend on this fact. The inconsistency of the result from the previous experiment [4] where the cartridge cases were successfully linked to the volunteer that loaded the gun before the shooting could be based on the fact that another analyzer (Pegasus® 4D-C) with new commercial software was used for this experiment. The software having issues with processing the chromatogram may contribute to the inconsistency.

For the second method, specially trained police canines performed four double blind line-up procedures with four kind of cartridge cases (same as for the first olfactronics one). In every line, five different scent samples from five different volunteers were compared with the sniffing sample. These samples in each line-up were compared by four different canines with two different handlers. The results showed that the canine is able to detect the human scent remained on the cartridge case and successfully link it to the volunteer that loaded the gun. In two cases, the canine did not mark the volunteer on the first attempt passing the line-up without marking any of the sample. This could be based on the fact that these were the samples that were exposed to the cartridge cases only for 30 minutes and one hour. This leads to the conclusion, that the time of the exposition of the traces to the Aratex fabric should be longer.

4. Conclusions

Two methods for comparing four traces from the simulated crime scene with 22 different volunteer samples were used. The aim was to find the one volunteer that loaded the gun with eight identical cartridges. Four samples of cartridge cases were then subjected to the olfactronic analysis. These were collected from three different surfaces (concrete, plastic and wooden palette). Four identical samples were compared also by the trained police canines. Both the methods showed that the surface from where the traces were collected does not play significant role for the identification. The results from the forensic olfactronic method showed the ability to distinguish each volunteer sampled on the glass beads, however, it was not possible to link the fired cartridge cases to any of the volunteers yet. This problem could be solved in the future by target analysis focused only on the substances that are genetically determined or by solving the problems with processing the peaks by the commercial software that comes with the two-dimensional gas chromatograph.

Acknowledgments

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Abstracts

Real-time monitoring of acetaminophen transformations using advanced Raman spectroscopy techniques

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Keywords
acetaminophen
large scale substrates
photochemistry
plasmon catalysis
surface-enhanced Raman scattering

The surface-enhanced Raman scattering (SERS) technique, which has been known for 50 years, offers numerous advantages. However, interpreting SERS spectra accurately remains a challenging aspect, particularly when dealing with special cases. These cases involve the diverse effects of chemical enhancement, which may arise as a result of the transformation of chemical species induced by interaction with incident radiation. While such behavior has been reported multiple times, the range of molecules undergoing such transformations appears to be vast and includes medicinally important molecules as well. In this study, we document the observation of photochemical reactions of acetaminophen adsorbed on Ag, Au, and Cu enhancing substrates. By employing various excitation wavelengths spanning from the visible (457, 532, 633 nm) to the near-infrared spectral region (785 and 1064 nm), we investigate this issue, revealing a pronounced tendency of acetaminophen to undergo transformation due to incidental radiation. Through multiple sequential measurements, we captured numerous potential pathways that acetaminophen molecules adsorbed on the substrates could undergo. These results were then compared with density functional theory (DFT) calculations, which proved helpful in explaining at least some of the

observed effects. To obtain a deeper understanding of the behavior of acetaminophen on plasmonic substrates, scanning electron microscopy (SEM) was used to provide detailed information about the structure and morphology of the substrate, which was formed by electrochemical deposition of platinum targets. Given the medicinal importance of acetaminophen, we believe that the findings presented here could be applied to its proper SERS spectrochemical analysis. Furthermore, our results could help to clarify some of the questions arising in this field, thereby contributing to the broader area of plasmon-assisted photochemistry.

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Chemical enhancement of surface-enhanced Raman scattering: formation of surface complexes, (photo)chemical reactions, and implications for chemical analysis

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Keywords
analytical applications
chemical enhancement
molecule-metal complexes
(photo)chemical reactions
surface-enhanced Raman scattering

The formation of surface complexes remains one of the most challenging aspects of surface-enhanced Raman scattering (SERS) spectroscopy, even after five decades of research. Not only does it significantly contribute to the overall enhancement, but it also influences the appearance of SERS spectra. Furthermore, the formation of molecular complexes on enhancing substrates can alter molecular absorption spectra, making chemisorbed molecules more prone to light-induced transformations. These aspects can affect the results and reliability of analytical methodologies, as the species present on the plasmonic surface may differ from the substances initially deposited. Here, we present several examples of these phenomena with an emphasis on chemical analysis.

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Formation of molecule/metal surface complexes and their effect on SERS-spectra in the systems of amphetamine-based drugs and colloidal nanoparticles

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Keywords
amphetamines
enhancement
SERS

Surface-enhanced Raman scattering (SERS) spectroscopy emerges as a promising technique for addictive substances analysis. It overcomes limitations of standard Raman spectroscopy, albeit with potential signal variations caused by molecule-surface interactions. This study explores the potential of SERS for the detection of amphetamine-based addictive stimulants, with a particular focus on possible surface-complex formation and its effect on the resulting SERS spectra's intensity and profile. To extract the maximum amount of information from the obtained spectra, we used density functional theory computations. Our results show that in cases where molecule-metal complexes are formed, the SERS signal is much higher and more specific than in the opposite cases. We believe that the presented findings could prove useful when considering SERS spectroscopy as a tool for amphetamine-based drug analysis and from a physico-chemical perspective, where insight into the chemical enhancement mechanism is provided.

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Monitoring pharmaceuticals and personal care products in surface water samples on the territory of the Czech Republic and Slovakia by LC-MS/MS method to estimate their potential health risk

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Keywords
direct injection
personal healthcare products
pharmaceuticals
surface water
UHPLC-MS/MS

A multi-residue UHPLC–MS/MS analytical method, previously developed for monitoring 52 pharmaceuticals in drinking water and wastewater, was used to analyse these pharmaceuticals in surface water sampled on the territory of the Czech Republic and Slovakia. Of the 29 surface water samples analysed by the validated UHPLC-MS/MS, each sample contained at least one quantifiable analyte. This study reveals the prevalence of several different drugs at high concentrations; median concentrations of 126.35 ng L^{-1} of caffeine, 50.81 ng L^{-1} of diclofenac, 134.22 ng L^{-1} of gabapentin, 137.76 ng L^{-1} of iohexol, 177.57 ng L^{-1} of iomeprol, 102.32 ng L^{-1} of iopamidol, and 81.87 ng L^{-1} of iopromide, were present. These comprehensive findings contribute valuable insights about the presence of pharmaceuticals in aquatic ecosystems and the environment, which together with the already published literature, gives a more complete picture of the burden on the aquatic environment.

Effect of side chain functional groups on nitrile imine cross-linking in peptide gas-phase ions

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Keywords
cross-linking
gas-phase ions
mass spectrometry

Photochemical cross-linking is a widely adopted method for studying protein-protein and protein-nucleic acid complexes. A phototag attached to the studied compound undergoes photodissociation, forming a transient reactive intermediate that rapidly reacts with the proximal functional groups to form a covalent bond. Nitrile imines, produced by dissociation of 2,5-diaryltetrazole tags have been shown to be effective cross-linkers in gas-phase peptide ions. The tetrazole group attached to the peptide C-terminus readily dissociates upon UV light irradiation, forming a nitrile imine moiety that selectively cross-links to the N-terminal peptide chain residue and forms a cyclic peptide structure.

To investigate the effects of side chain functional groups on cross-linking, we varied the N-terminal residues of tetrazole-peptide conjugates and studied the gas-phase ions using tandem mass spectrometry, cyclic ion mobility, Born-Oppenheimer molecular dynamics (BOMD) and density functional theory (DFT) calculations. To gain further insight into the reactivity of N-terminal amide and side chain groups, we introduced specific modifications: esterification of the Asp and Glu side chain carboxyl groups, and methylation of the Lys amine group. Furthermore, exhaustive hydrogen-deuterium exchange and accurate mass measurement provided valuable information regarding the fragment identity.

The conjugates were subjected to UVPD dissociation in the MS^2 step, forming $-N_2$ fragments with up to 41% efficiency. The $-N_2$ fragments were isolated and further fragmented by CID-MS^3 with cross-linking yields ranging from 40% to 89%, demonstrating a high efficiency of the cross-linking process. Several fragmentation pathways were identified alongside the linear peptide chain fragmentation. Conjugates with N-terminal basic amino acid residues showed a prevalent loss of phenylhydrazine followed by a loss of neutral internal residues, indicating cyclization of the peptide. The atypical loss of the N-terminal residue side chain and subsequent internal amino acid neutral losses pointed toward cross-linking at the N-terminal amide. Losses of internal neutral residues within the peptide chain confirmed cyclization. Cyclic ion mobility measurements and BOMD and DFT calculations provided complementary information regarding the gas-phase conformation of the conjugates and reaction thermodynamics.

Our multimodal approach allowed us to investigate the effects of side chain functional groups on nitrile imine cross-linking of peptide gas-phase ions, probing the applicability of this novel method.

Development of microfluidic optical sensing technology for the detection of H_2O_2 in cell-based bioassays

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Keywords
hydrogel
hydrogen peroxide
microfluidic device
optical sensor

Hydrogen peroxide is a reactive oxygen species that can damage cells and tissues. At cellular levels, hydrogen peroxide concentrations between 2 and 200 µM may lead to oxidative stress and free radical production. Elevated levels of hydrogen peroxide (120 to 150 µM) can induce a temporary growth arrest, while higher concentrations can lead to apoptosis. Therefore, this study aims to develop a simple pump-driven microfluidic chip containing a sensor cocktail embedded in a hydrogel to monitor cell-released analytes, particularly hydrogen peroxide. The sensor exploits the enhancement in luminescence intensity of the hydrogen peroxide probe europium tetracycline when exposed to various concentrations of the analyte using excitation at 405 nm and emission at 615 nm. Key research goals are the optimization of the sensor cocktail and its integration into a pump-driven microfluidic system, including the optical setup and downsizing in scale. Experiments with D4 polyurethane hydrogel and europium tetracycline in 96-well microtiter plates indicate that 15% (w/w) D4 in EtOH/H_2O (9:1) and 0.25% (w/w) europium tetracycline yield the optimal cocktail for quantitation of low mM concentrations of hydrogen peroxide. By switching to a microtiter plate with a polymethylmethacrylate bottom, the microfluidic chip material was evaluated. Optimum results were achieved with a cavity diameter of 1.5 mm and minimizing the volume of the sensor cocktail to 1 µL. The integration of the sensor system into a microfluidic chip and aligning it with a fiber-optic showed promising first results, but a black cover on top of the microfluidic chip is needed for the reduction of light scattering. We expect to establish an online sensing system to monitor hydrogen peroxide by the optimization of the measurement protocol, refining of the fluorimeter setup, and precise adjustment of flow parameters, respectively.

Molecular imprinted polymer coated laser-induced carbon nanofibers as a high-performing enzyme-free sensor

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Keywords
glucose
molecularly imprinted polymers
laser-induced carbon nanofibers

Glucose plays a crucial role in biological processes, and monitoring its levels becomes indispensable when a correlated disease such as diabetes is diagnosed. Therefore, enzyme-based electrochemical glucose sensors have been extensively studied and commercialized due to their excellent sensitivity and selectivity. However, enzymes have inherent disadvantages, including high material and fabrication costs, instability during sterilization, and vulnerability to changes in temperature and pH. To overcome these limitations, enzyme-free approaches, such as the catalytic oxidation of glucose by metal-based electrodes, are highly promising. Laser-induced carbon nanofibers (LCNFs), with their high effective surface area, ease of mass production, and uncomplicated modification using metal nanoparticles, serve as an optimal sensor platform for this purpose. However, the catalytic conversion of glucose on metal oxides lacks selectivity, as it reacts with other saccharides and organic substances. Another promising enzyme-free approach involves sensing with affinity-based recognition elements, such as molecularly imprinted polymers (MIPs). Despite their advantages, including high chemical stability and selective recognition of target molecules, MIPs suffer from limited sensor performance. A combination of both methods could therefore mitigate each other's disadvantages and capitalize on their respective advantages. Here, we investigated experimental conditions to enable glucose imprinting on LCNFs via electro-polymerization using cyclic voltammetry

(CV). Various kinds of functional monomers were studied in which ortho-phenylenediamine was the most promising candidate. Furthermore, studies on electrode cleaning process, number of CV cycles for electro-polymerization, and various template removal strategies were conducted. Ultimately, the successful MIP coating on LCNFs will facilitate enzyme-free glucose sensor with high selectivity and superior sensitivity at low cost.

LC-ICP-MS analysis of anti-obesity peptides labeled with lanthanide mass-tag (utilizing a new type of mobile phase compatible with argon plasma)

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Keywords
anti-obesity peptides
1,2-hexanediole
lanthanide mass tags
LC-ICP-MS

Analogs of neuropeptide prolactin-releasing peptide (PrRP31) are very promising in the development of new drugs for the treatment of obesity and related diseases. This peptide becomes a more stable entity in the human body after lipidization with a fatty acid, in our case palmitic acid, providing palm^{11}-PrRP31. This analog reduces food intake and body weight in preclinical obese rodent models after chronic peripheral administration. However, the mechanism of action of this lipopeptide remains unclear. In order to shed light on it, we have labeled this analog with a ClickZip lanthanide mass tag that allowed us to better study the fate of palm^{11}-PrRP31 under in vivo conditions. Using ICP-MS, the metal content can then be determined at very low concentrations, thereby indirectly quantifying the peptide. The exceptional stability of the ClickZip tag allows independent quantification from unchelated lanthanides that may be present in the sample. We used reversed-phase (RP) liquid chromatography coupled with ICP-MS (LC-ICP-MS) to validate the chemical form of the tag. Herein, a major obstacle is the incompatibility of commonly used organic solvents in RP-LC, such as methanol or acetonitrile, with the argon plasma of ICP-MS. We used 1,2-hexanediole instead, which has a high elution strength in RP chromatographic mode and is compatible with ICP-MS. We optimized the composition of the mobile

phase and found that the composition of 5% of 1,2-hexanediole in H_2O containing 2% formic acid provided the best results. The whole LC-ICP-MS method was validated according to EURACHEM. Validated parameters were the limit of detection (LOD), linearity, accuracy, and precision. All of them met the criteria of the EURACHEM validation guideline. In conclusion, we developed and validated new LC-ICP-MS method suitable for the determination of anti-obesity lipopeptides labeled with an extremely stable ClickZip lanthanide mass tag.

Methods of extraction and stabilization of silver nanoparticles in cosmetics

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Keywords
nanoparticles
sample preparation
silver
single particle mode ICP-MS

The use of nanoparticles engineered by humans and their range of use in the consumer products is rapidly increasing. After the most frequently used titanium dioxide nanoparticles, the second commonly used type of nanoparticles in cosmetics are silver nanoparticles due to their antimicrobial, antifungal, anti-inflammatory and other properties. Even though the results of the silver nanoparticles dermal toxicity test determined it safe for topical application, the fate of the silver nanoparticles after releasing them into the environment is largely unknown. Thus, innovative analytical approaches are necessary to determine the presence of the nanoparticles. Among these approaches, inductively coupled plasma mass spectrometry in single particle mode can be included. Sample preparation for conventional solid sample elemental analysis using ICP-MS is usually done via nitric acid decomposition and transferring the analytes into a solution. However, this traditional digestion using acids likely leads to dissolution of most nanoparticles. While determining the nanoparticles with single particle mode ICP-MS, matrix solubilization is needed, yet the nanoparticles contained in the sample have to stay preserved, which means, sample preparation is a key for achieving the correct results. Several problems from the preparation, such as incomplete extraction, aggregation, dissolution or insufficient stability, may appear. If not carefully avoided, these problems lead to a misrepresentation of results, both in particle diameter and number concentration. This work focuses on optimization of the sample preparation for the determination of silver nanoparticles in cosmetics using single particle mode ICP-MS. Several methods of stabilization of silver nanoparticles in various agents and different time ranges were examined. Together with the possibilities of stabilization, methods of extraction using

diverse agents were explored along with other aspects possibly affecting the extraction yield. The optimal stabilization and extraction agent, and also the optimal sample preparation procedure were selected.

Development of liposomes as diagnostic reagent for complement-related diseases

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Keywords
complement system
liposomes
liposome ligands

The complement system is part of the innate immune system and comprises more than 30 soluble or membrane-bound proteins that play an important role in host defense and inflammation. Excess, deficiency, or dysfunction of specific complement components can lead to diseases, with more than fifty diseases currently known to involve the complement system. A novel liposome platform is currently being developed to serve as cell biomimic to assist in unraveling the role of specific complement proteins and their interactions with cell surfaces. Liposomes can easily be synthesized from desired lipid mixtures, can encapsulate desired marker molecules and hence enable full control over their characteristics including size, surface charge, surface chemical groups and stability in human serum. Here, strategies were investigated to couple ligand molecules to the liposome surface. Possibilities include the addition of the ligands already in the lipid mixture prior synthesis, post-insertion into the lipid bilayer once liposomes are formed, or chemical covalent coupling through, e.g., EDC/NHS chemistry. Liposomes are characterized using inductively coupled plasma optical emission spectroscopy (ICP-OES) for lipid concentration, dynamic light scattering for their size, zeta potential for surface charges and ligand binding assays for the identification and quantification of specifically introduced groups. As the liposomes are stable in serum, real-world analyses can be performed hence demonstrating these liposomes as a novel diagnostic platform technology for complement protein analyses.

Development of novel sample preparation strategies for nucleic acid detection at the point of care

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Keywords
DNA extraction
sample preparation
zwitterionic nanofibers

New solutions and platform technologies for nucleic acid-based, rapid and sensitive detection of pathogens in resource limited settings are needed as most common strategies depend on laboratory equipment and settings. Much attention has been paid toward nucleic acid amplification and detection in portable, often microfluidic sensors, however, innovation is also needed for the first, critical step in which the nucleic acids are extracted from their biological matrices such as blood, saliva, urine. Research focused on the design, fabrication, characterization and application of nanofibers for this purpose as their general characteristics such as an easy production process, tunable chemical properties, lightweight and high surface-to-volume ratio make them a desirable material for on-site nucleic acid extraction. Prior research had demonstrated that electrospun nylon doped with the cationic poly(allylamine hydrochloride) and the anionic poly(acrylic acid) resulted in zwitterionic nanofiber mats in which surface charges could be tuned through the pH of the surrounding solution. High adsorption (over 95%) and elution (75%) yields demonstrated their general capability in a proof-of-concept assay. However, when applied to real samples such as serum, those proteins also electrostatically attach to the nanofibers and co-elute with the nucleic acids. This can easily be overcome by diluting serum samples to 1%, but further design studies are on-going in which lowered adsorption pH, adjusted polymer doping, the addition of proteinases or mere denaturing through heat may lower interfering effects. In the end, the nanofiber-based nucleic acid isolation can easily be integrated in lateral-flow assay concepts of microfluidic-based strategies.

Tagging of HRP-entrapping liposomes with proteins to enable highly sensitive photometric immunoassays

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Keywords
click chemistry
horseradish peroxidase
liposomes

Liposomes are lipid bilayer vesicles that are widely used in the fields of pharmaceuticals, food, cosmetics and bioanalytics. In the latter, they often serve as signal amplification systems and are tagged on their outside with biorecognition elements. Here, we study the encapsulation of the enzyme horseradish peroxidase in liposomes to generate highly sensitive liposomes enabling a simple, photometric readout through the addition of 3,3'5,5'-tetramethylbenzidine as substrate along with hydrogen peroxide. While the horseradish peroxidase encapsulating liposomes are very promising, it was quickly found that liposome surface modification via 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide/*N*-hydroxysulfosuccinimide (EDC/sNHS) chemistry inactivated the encapsulated enzyme. Alternative strategies now focus on click chemistry, specifically studying the strain-promoted azide-alkyne cycloaddition (SPAAC). Thus, liposomes were modified with DPPE-DBCO via post-insertion of the lipid into the liposomal bilayer. As a model protein, streptavidin was modified with an amine-reactive azide. The conditions of the click chemistry were optimized using sulforhodamine B encapsulating liposomes as a control system to remain independent of possible effects on the activity of horseradish peroxidase. The coupling efficiency via SPAAC click chemistry was compared to EDC/sNHS chemistry using heterogeneous binding assays and found to be very similar. The impact on horseradish peroxidase activity, liposome stability and long-term storage are currently under investigation.

Development of liposome surface chemistries to study the complement system

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Keywords
complement assay
complement system
liposome
surface chemistry

The complement system, an integral part of the human immune system, is comprised of over 30 proteins that play a key role in defense against pathogens, being triggered via three major activation pathways, the classical, alternative and lectin pathways, respectively. Each pathway activity may differ from individual to individual, each pathway is triggered by specific cell surface properties, and most importantly, the pathways are increasingly recognized to play a major role in acquired and hereditary diseases such as age-related macular degeneration, atypical hemolytic uremic syndrome and C3 glomerulopathy. To determine complement activity for diagnosis and therapy control, either an ELISA is used which quantifies complement proteins, or a hemolysis assay, which determines the functionality of the classical and alternative pathway using animal erythrocytes. Also, liposomes have been established to serve as biomimetics and determine the classical pathway activity via a colorimetric assay. Only the liposomal approach bears characteristics that would lend itself for affordable high-throughput or even rapid analysis. Thus, we investigated and studied a new liposome concept with the goal to differentiate between the three pathways, determine their overall complement activity and functionality, and enable a time-resolved as well as endpoint analysis. Initial effort was put toward the liposomes stealthiness in human serum in the absence of complement trigger molecules on their surface. Also, assay formats for the different readout strategies were optimized. Currently, the performance of the liposomes toward the pathway specific activation is being investigated and will be tested using cohorts of healthy patient sera.

Studying aptamer-modified liposomes as control reagent in lateral flow assays

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Keywords
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bioassays
his-tag
lateral flow assays
liposomes

Aptamers have long been heralded as possible biorecognition element in bioassays, but typically their specificity and sensitivity does not rival that of good antibodies. Intriguing, however, is the possibility of their use as non-natural biorecognition element, generated against difficult analytes and the non-animal product-derived synthesis. Here, we investigated an aptamer targeted against the his-tag of recombinant proteins for its use as a universal tag for liposomes. Liposomes can be used as signal amplification means for bioassays and in theory, aptamers can easily be attached to the phospholipid membrane. The optimization of aptamer-coupled liposomes was studied through three different conjugation strategies, either by post insertion via cholesterol modifications, by non-covalent bonding via biotin-streptavidin interactions or by covalent coupling via EDC-sNHS chemistry to achieve efficient binding to the target molecules. Aside from coupling efficiency, important design criteria include the preservation of the relevant aptamer secondary and tertiary structure and continued integrity of the liposome. Initial experiments were carried out in microtiter plate assays, where both, the post-insertion approach and biotin-streptavidin-based coupling resulted in functional aptamer-coupled liposomes. In contrast, covalent binding via EDC-sNHS inhibited the binding ability of the aptamers with the targets as it likely unfolded during the reaction conditions needed. The optimum buffer conditions and secondary structure properties of the aptamer could be investigated with circular dichroism measurements, revealing hairpin motifs in HEPES-

and Tris-based buffers responsible for binding. The aptamer-coupled liposomes were applied in a lateral-flow assay demonstrating efficient binding to a his-tag protein control line. In the future, this system can be used for nucleic acid-based, antibody-based, and competitive structured lateral-flow assays as a universal control.

Capillary electrophoresis analysis of interactions between lipophilic active pharmaceutical ingredients and liposomes

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Keywords
capillary electrophoresis
interactions
liposomal electrokinetic chromatography
liposomes

Liposomes are small lipid-based vesicles with amphiphilic character, meaning various substances of different properties can be incorporated either into the core or into the membrane of the liposome. They hold strong potential for targeted drug delivery and therapeutic applications and their composition makes them suitable as a model system for controlled transport of bioactive substances and drugs through organisms. This study aims to develop a capillary electrophoresis method suitable for examining the interactions between selected active pharmaceutical ingredients and liposomes. Those interactions can influence the kinetics of analytical separations, causing changes in peak shape and/or mobility. This study should enhance our understanding of the complex interplay between liposomes and active pharmaceutical ingredients and it holds significant implications for drug delivery optimization and their formulation. Liposomes at a total lipid concentration of 5 mg ml^{-1} were prepared by lipid film hydration method in 10 mM sodium phosphate buffer at pH = 7.10. As a background electrolyte, we used sodium phosphate buffer at 10 mM ionic strength with the addition of liposomes into the background electrolyte. Furthermore, we investigated the active pharmaceutical ingredient-liposome interactions under different physiological conditions, such as various temperatures during separation and different pH of the background electrolyte.

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Spiciness of hot peppers – determination using voltammetry

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Keywords
capsaicin
dihydrocapsaicin
voltammetry

There are many varieties of peppers in the world, including hot peppers, which contain several capsaicinoids belonging to secondary plant metabolites. These compounds have gained popularity in the scientific world due to their numerous pro-health properties. One of the most important of them is analgesic effects. The mechanism of action of capsaicinoids involves activation of TRPV1 receptors, located in the membranes of neurons of the peripheral nervous system leading to a sensation of burning pain. However, with prolonged action of capsaicinoids on these receptors, they go into a state of inactivation, resulting in the cessation of pain sensation. Research also confirms the beneficial effect of capsaicinoids on the treatment of many diseases, including obesity, cardiovascular diseases and diabetes. Additionally, capsaicinoids have a positive effect on the body's thermoregulation. Furthermore, these compounds show anticancer potential (induce apoptosis of small cell lung cancer), bactericidal properties (e. g., for *Helicobacter pylori*) and inhibit the growth of pathogenic fungi (e. g., *Candida albicans*). Capsaicin (8-methylo-*N*-vanillyl-*trans*-6-nonenamide) and its structural analogue – dihydrocapsaicin are the capsaicinoids with the highest concentration in hot peppers and are mainly responsible for their spiciness. One of the most popular ways to determine the pungency of peppers is the Scoville test. Appropriately prepared samples are administered to specialized testers whose task is to assign the Scoville heat units (SHU). However, this test is a subjective way to determine

the heat level of peppers. In this research spiciness of peppers (Hellboy, MAMP Blackberry GUM, Primotali Red, Pockmark Orange, Big Mama Red), which have similar spiciness on the Scoville scale, reaching approximately 1 million SHU, was checked with electrochemical methods. Spiciness, in this aspect, is understood as the sum of two capsacinoids with the highest concentration in hot peppers: capsaicin and dihydrocapsaicin.

Study of the electrochemical behaviour of selaginpulvilins differing in the presence of a hydroxyl group

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Keywords
cyclic voltammetry
electrochemical impedance spectroscopy
oxidation
selaginpulvilins
UV-Vis spectroelectrochemistry

Selaginpulvilins are natural compounds containing a polyarylated fluorene skeleton, found as secondary metabolites in plants of the genus *Selaginella*, exhibiting anti-inflammatory and antioxidant effects. This study focuses on the electrochemical properties of newly synthesized selaginpulvilins differing in the presence of a hydroxyl group, using cyclic voltammetry, UV-Vis spectroelectrochemistry, and electrochemical impedance spectroscopy. The stability of the hydroxylated derivative in acetonitrile was studied spectrophotometrically and compound was stable at least for 3.5 hours. Both studied derivatives provided oxidation responses in the potential range up to +2 V (*vs.* Ag | AgCl | 1M LiCl) in the media of a tetrabutylammonium hexafluorophosphate in acetonitrile. Electrochemical impedance spectroscopy of hydroxylated derivative confirmed the presence of a chemical step following electron transfer. These partial results of this study provide important insights for proposing the oxidation mechanism of selaginpulvilins.

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Monitoring of platinum drug-loaded liposome changes by CE-ICP-MS/MS

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Keywords
anticancer drug
human serum
liposomes
tandem mass spectrometry

Cancer is one of the most dangerous diseases in the world. One method of treatment, chemotherapy, is required by more than half of diagnosed patients. Despite the frequent use of chemotherapy, it causes side effects. Drug carriers such as liposomes should be introduced to reduce the frequency of side effects and increase the effectiveness of therapy. Liposomes are biocompatible carriers that can selectively deliver the drug to cancer cells. Several techniques have been used to study changes in liposome systems. However, these techniques have drawbacks, such as the ability to observe only one element during analysis and the presence of interferences. Due to the shortcomings of the techniques used so far, a new method based on applying combined capillary electrophoresis with inductively coupled plasma tandem mass spectrometry (CE-ICP-MS/MS) was proposed. This technique enables quantitative and qualitative analysis. It allows for the calculation of the drug's encapsulation efficiency and the assessment of the polydispersity of the carriers and their stability. During the study, the effect of the lipid composition and the concentration of the drug on encapsulation efficiency were analyzed. The first stage of the research was to optimize the CE-ICP-MS/MS method, where appropriate separation conditions do not cause changes in the liposome systems. On the other hand, tandem mass spectrometry enables simultaneous quantitative analysis of phosphorus, platinum, and sulfur. Another advantage of the CE-ICP-MS/MS technique is the determination of protein interactions with liposomes. This makes it possible to determine whether a protein corona is formed and predict the carriers' stability under in vivo conditions.

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Forced oxidative degradation of pharmaceuticals: an electrochemical approach

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Keywords
boron-doped diamond electrode
forced degradation tests
oxidation
pharmaceutical
salicylic acid

Forced oxidative degradation tests are a crucial part of the comprehensive stability studies that are a mandatory requirement for all newly developed pharmaceuticals. These tests are conducted in accordance with guidelines issued by international regulatory authorities, such as the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), the European Medicines Agency (EMA), and the Food and Drug Administration (FDA). The objective of forced oxidative degradation tests is to preliminarily assess the resistance of a pharmaceutical to oxidation, to determine the structure of the degradation products formed, and to develop a suitable analytical method for their determination before the initiation of accelerated and long-term tests. The optimal rate of pharmaceutical degradation is between 5 and 20% of the original amount. This interval represents a balance between a reasonable degradation time and a sufficient quantity of degradation products to allow for reliable analytical detection, without the formation of undesirable secondary degradation products. In this study, electrochemical oxidation was employed as a promising, accelerated, and more environmentally friendly alternative to chemical oxidation, which primarily uses 0.1 to 3.0% hydrogen peroxide. The oxidation process was conducted in novel cells fabricated by 3D printing. Boron-doped diamond was selected as the working electrode material, as it exhibits high mechanical strength, corrosion resistance, and suitability for the oxidation of organic compounds. The optimization of the degradation conditions was conducted using salicylic acid as a model active pharmaceutical ingredient. The ultra-high performance liquid chromatography-mass spectrometry method was employed for the detection of degradation products. The work includes a detailed description of the design of the oxidation cells and the process of optimization of the degradation parameters.

Analysis of liquids by LA-ICP-MS

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Keywords
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ICP-MS
LA-ICP-MS
oil analysis

Elemental analysis of samples with high levels of organic solvents using inductively coupled plasma mass spectrometry (ICP-MS) faces challenges such as plasma instability and non-spectral interferences. Laser ablation coupled with ICP-MS (LA-ICP-MS) is an analytical method currently used for the analysis of solid samples. In this method, only a small amount of sample in the gaseous state enters the plasma, reducing the impact on plasma conditions. Therefore, LA-ICP-MS should be suitable for elemental analysis of organic solvents, though its use for liquid samples is atypical. This work aims to innovate the use of LA-ICP-MS for liquid samples, especially oils. As the analysis of liquids by LA-ICP-MS is uncommon, it is necessary to develop a completely new methodology, including the selection of suitable organic solvents considering their volatility, choosing the sample pad surface, designing it to achieve an appropriate contact angle of sample droplets, and optimizing the ablation process. Polytetrafluoroethylene was selected as the sample pad material due to its ability to achieve the highest contact angle for most liquids and its resistance to all types of organic solvents. To predict the behavior of a liquid droplet in the ablation cell, a mathematical model was developed. This model describes the evaporation time of the sessile droplet under ablation conditions (He flow 0.8 L min^{-1}, room temperature). LA-ICP-MS parameters were optimized, selecting a fluence of 1 J cm^{-2} for satisfactory signal intensity and minimal risk of splashing. The optimal number of laser pulses was determined to be 50 at a frequency of 2 Hz, ensuring a stable signal. Additionally, it was found that the ablation process is independent of the volume of the sessile droplet.

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Electroanalysis of thymoquinone

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Keywords
carbon paste electrode
square wave voltammetry
thymoquinone

Thymoquinone is a chemical compound of plant origin. It occurs naturally in the seeds of the plant *Nigella sativa*, also known as black cumin. Thymoquinone has many proven health benefits, the most important of which is its antioxidant activity. Several methods for determining this compound in various real samples have been documented in the literature, the most popular being those based on high-performance liquid chromatography. The aim of this work was to develop a first voltammetric method for the determination of thymoquinone using a carbon paste electrode as a working electrode. During the study, the optimal measurement conditions, such as pH and composition of the supporting electrolytes, the optimal way to refresh the electrode, as well as the optimal parameters of the square wave voltammetry technique, such as frequency, potential step, and amplitude, were developed. After the optimisation stage of the method, the linear correlation between the thymoquinone peak currents and its concentration was determined. Calibration curves were determined using three different signal processing methods. The results were statistically processed by determining the limit of detection and the limit of quantification for each method. The final stage of the research was to use the most optimal signal processing method to analyse the results on a real sample. The results obtained were compared with those of the reference method based on high performance liquid chromatography.

UV-Photochemical vapor generation of silver: study of conditions

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Keywords
inductively coupled plasma mass spectrometry
photochemical vapor generation
silver

The first UV photochemical generation of silver was performed by Guo et al. in 2004. Their conclusion was that silver appears to be amendable to the UV-photochemical vapor generation method as a sample introduction system, however the very noisy signals achieved suggested that the compounds created were unstable. Our work addresses the search for optimum conditions for the generation of volatile species of silver and its mechanism. We found that the reduction of silver is relatively simple, but it is reduced to its metal form rather than volatile species. The most direct and rapid transport in the system is required for successful generation of volatile species, so the emphasis has been on the experimental setup, including shortening and reducing the number of connection points. The flow injection mode was used in conjunction with ICP-MS detection, and silver was detected at mass 107 in time-resolved analysis. The obtained signals were mainly dependent on the type of low molecular weight acid used, its concentration, the sample and carrier gas flow rate and, and the positioning of the carrier gas inlet. Attention was also paid to the construction of the gas-liquid separator. We confirmed that the response is due to volatile species and not to aerosol, and that the volatile species arise due to action of UV radiation and not due to heat transmission. For a better understanding of the mechanism of UV-photochemical vapor generation generation, signals obtained with a solution of ionic standard were compared to signals acquired with a solution containing silver nanoparticles.

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Application of GC-MS/MS method for quantification short-chain fatty acids in biological samples

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Keywords
gas chromatography
inflammatory bowel disease
mass spectrometry
short-chain fatty acids

Inflammatory bowel disease is a group of diseases characterized by repetitive episodes of gastrointestinal tract inflammation caused by an inappropriate immune response to the intestinal microbiota. The most common inflammatory bowel disease are Crohn's disease and ulcerative colitis. Short-chain fatty acids may be potential markers of inflammatory bowel diseases. Short-chain fatty acids include acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, and isovaleric acid. Lactic acid is a precursor in the synthesis of short-chain fatty acids, so it is also an important marker. The aim of this research was to develop a method for the determination of short-chain fatty acids using gas chromatography coupled with tandem mass spectrometry with a triple quadrupole (GC-MS/MS). The multiple reaction monitoring mode was used to increase the sensitivity and selectivity of a new method. Application of the highly polar capillary column enabled the analysis of short-chain fatty acids without the need for derivatisation. The new analytical method was optimized and validated following the ICH Q2 requirements. The application of the developed GC-MS/MS method allowed the determination of seven organic acids. The limit of quantification of the analytes were 0.50 μg mL^{-1} except for lactic acid, where the value was 2.5 μg mL^{-1}. The developed method is linear in the range 0.50–10.0 μg mL^{-1}, except for lactic and valeric acids, where the range was appropriately 2.50–25.0 μg mL^{-1} and 0.50–7.50 μg mL^{-1}, correspondingly. The new GC-MS/MS method is characterised by good precision (2.21–9.70% of CV). The developed GC-MS/MS method shows potential for the determination of short-chain fatty acids in biological samples from patients with inflammatory bowel disease.

Mordant metals used in cultural heritage objects: characterisation and identification by ESI-MS/MS

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Keywords
archaeometry
coordination complex
mordant metals
natural dyes
tandem mass spectrometry

Modern analytical techniques have been applied to artwork studies, allowing their physical essence to be explored. The identification of dyes in antique textiles is a valuable source of information on past textile production technologies, enabling their age, origin or authenticity to be determined. An important element of this research is the identification of natural dyes, which were the only dyes used until the mid-19th century. Among them, mordant dyes constitute the most numerous and widely used group. The coloring matter obtained with them has a dual nature: it consists of organic coloring compounds as well as metal cations (derived from mordants, a water-soluble aluminum or transition metals salts) which mediate the bonding of these colorants with a fiber to form stable, colored complexes. Using different mordants ensures that the color range obtained from a single dye may be very wide. Although metal cations are an integral part of coloring matter, researchers of historical works have often paid little attention to them. To fully understand the nature of the coloring created with mordant dyes, knowledge of the used mordants is crucial. To fill this research gap, the development of a method for the identification of mordant metals in artworks using electrospray ionization tandem mass spectrometry (ESI-MS/MS) was undertaken, exploiting the ability to complex mordant metals (aluminum, chromium, copper, iron, tin) by various ligands (e.g., EDTA, DTPA, DCTA, oxalic acid, bromide and chloride anions). First, a possible elemental composition of the resulting complexes was determined on the basis of the acquired isotopic profiles.

Then the conditions of sample preparation and complex ionisation were optimized. Finally, the complexes were fragmented to determine the path of their decomposition and to select the transmission pairs that will be used in further studies. These preliminary results indicate the usefulness of using ESI MS/MS for the analysis of mordant metals.

Electrochemical study of adsorption properties of boron doped diamond electrodes

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Keywords
dadsorption
Anson plot
BDD electrode
chronocoulometry
sp^2/sp^3 ratio

Boron-doped diamond (BDD) electrodes are widely employed in electroanalysis due to their superior properties compared to traditional metal and sp^2 carbon electrodes. Their comprehensive characterization may help improve design for targeted applications. It involves evaluating parameters including boron concentration, sp^2 carbon content, surface roughness, and surface termination, all of which significantly influence the kinetics of heterogeneous electron transfer. In this study, an indirect method for determining the sp^2 carbon content on the surface of BDD electrodes is presented. By utilizing chronocoulometric measurements, the surface coverage (Γ) of adsorbed molecules was calculated. The sp^2 carbon content was then compared based on the calculated Γ values for different electrode types. The model electrodes used included commercial BDD electrodes (BDD_c) from BioLogic SAS, France ([B] = $1.8\times10^{20}\,cm^{-3}$), LA-MW-CVD BDD electrodes on Si substrate (BDD_{LA}) ([B] = $1.8\times10^{21}\,cm^{-3}$), and glassy carbon electrodes (GCE). Chronocoulometric measurements of estrone in $0.5\,mol\,L^{-1}$ H_2SO_4 showed that surface coverage (Γ) was the highest on the GCE and lower on both BDD electrodes, reflecting higher sp^2 carbon content. The BDDLA electrode had a slightly higher Γ value than the BDDC electrode, likely due to higher boron concentration and so on sp^2 carbon content.

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Studying the GHK-Cu tripeptide loaded liposomes using CE-ICP-MS/MS

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Keywords
capillary electrophoresis
GHK-Cu
liposomes
mass spectrometry

The cosmetics industry is constantly looking for ways to increase the ability of active compounds to penetrate the epidermis and increase their bioavailability. Nanometric liposomes can be considered a solution. Liposomes are vesicles built from a double phospholipid layer. The ability of these structures to encapsulate active compounds inside them, release them in a controlled manner, and build into the membranes of skin cells makes them promising carriers of cosmetically active compounds. Encapsulation of a cosmetically active substance inside liposomes may affect its more effective delivery to the appropriate layers of the skin. The research aims to develop a method for the effective formation of liposomes and encapsulation of GHK-Cu, which is a cosmetically active compound with an anti-aging effect. The studies allowed us to determine the influence of the total lipid content, the lipid composition of the formed liposomes, and the presence of additional steps in the forming procedure on the encapsulation efficiency of the tested active compound and the physicochemical properties of probed systems. Subsequently, the transport of such systems from simple cosmetic formulations into the skin was investigated. Capillary electrophoresis combined with inductively coupled plasma tandem mass spectrometry (CE-ICP-MS/MS) was used to monitor the encapsulation process and determine the encapsulation efficiency. This modern technique has allowed the direct quantitative analysis of the crude liposomal mixture without the use of organic solvents, which may interfere with liposome morphology. The dynamic light scattering technique (DLS) was used to determine the size, dispersity, and stability of the liposome-GHK-Cu systems.

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Determination of antibiotics using smartphone-based detection

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Keywords
fluorescamine
gentamicin
smartphone-based detection

Currently, antibiotics are an important issue in modern analysis due to their widespread use. A popular group of antibiotics includes aminoglycosides, which have a relatively wide medical use in various human diseases. It should be noted that aminoglycoside antibiotics in uncontrolled concentrations show nephrotoxic and ototoxic effects, also being the cause of antibiotic resistance. Therefore, the development of novel analytical tools and methods for their on-site determination is an emerging analytical challenge. Among such tools, smartphone-based devices are becoming used more and more widely. This is due to their accessibility, portability, and ease of use. The demand for them exists in many fields, including healthcare, food quality, safety monitoring, and the environment, where they are used, for example, as Point-of-Care or Point-of-Need devices. Today, many spectrophotometric, spectrofluorometric, or electrochemical analytical methods are being adapted for smartphone-based analysis. In the study, a smartphone-based detection system was used for the fluorimetric determination of gentamicin. The proposed method for the determination of gentamicin was based on the reaction of the amino groups of gentamicin and a fluorescamine. Preliminary studies included the selection of appropriate excitation (415 nm) and emission (489 nm) wavelengths, based on which suitable light-emitting diodes were selected for measurements using smartphone-based detection. During the study, appropriate reaction conditions (reaction time, fluorescamine concentration, pH) were selected. In addition, optimal camera and measurement settings were selected, such as software, channel (for analytical signal collection), ISO parameter, white balance, and shutter speed. The proposed method was verified

by determining gentamicin in model samples. Analytical parameters of the proposed method, such as linearity range, limit of detection and quantification, precision, and accuracy were determined. The results obtained using smartphone-based detection were compared with those obtained using the classical spectrofluorimetric method. The developed method was applied to the determination of the analyte in selected pharmaceutical samples.

Development of highly sensitive enzyme-encapsulating liposomes for the Point-of-care

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Keywords
lateral flow assay
liposomes
nanoLuciferase

Point-of-care testing has become a major part in bioanalysis, allowing rapid results and easy readout. Point-of-care tests like lateral flow assays fulfill the criteria of fast and user-friendly readout, however often lack in sensitivity as the achievable limit of detection is often order of magnitude above those needed for an analyte detection. To overcome this deficiency, signal enhancing enzymes and liposomes have long been proposed. Here, a combination of these two enhancement strategies is being investigated to truly push limit of detections of lateral flow assays into the desirable region. The enzyme of interest is NanoLuciferase, a small, highly stable luciferase enzyme demonstrating excellent performance in bioanalysis recently due to its small size, high thermal stability and bright (bio)luminescence generated solely with furimazine and no auxiliary reagents. Initial experiments used a traditional horseradish peroxidase for the optimization encapsulation into liposomes. Here the thin film hydration method was optimized at 35 °C, investigating different lipid- and buffer compositions. The binding of the biotin-containing liposomes to the test line of a lateral flow strip, utilizing the high binding affinity of biotin and poly-streptavidin, was studied. NanoLuciferase-encapsulating liposomes were synthesized with these optimized conditions. In further studies, the binding properties of the liposomes to the lateral flow assays and their bioluminescence readout will be investigated. Comparisons to other labels will be made to show the superior system of the NanoLuciferase-encapsulating liposomes.

Development of the CE-MS method for the identification of disperse dyes extracted from polyester fibers for forensic purposes

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Keywords
capillary electrophoresis
disperse dyes
forensic fiber analysis
mass spectrometry
polyester

Fibers are one of the most important groups of micro traces revealed at the crime scene. They provide valuable information such as fiber type and dye class. The main challenge in forensic fiber analysis is their extremely small size (a few millimeters in length and diameter less than 25 μm). The amount of dye contained in this type of trace is also small, estimated at 2–200 ng. Therefore, it is necessary to look for methods suitable for the analysis of fiber extracts. Capillary electrophoresis (CE) is a separation technique that allows the analysis of complex fiber extracts with minimal sample consumption. The hyphenation of CE with a time-of-flight mass spectrometer (CE-MS) additionally enhances analytical capabilities by increasing the sensitivity and providing high mass resolution, which gives the possibility of sample component identification. The aim of the study was to develop a procedure for identification of disperse dyes present in polyester fibers for forensic purposes. The procedure included extraction of dyes from fibers, analysis of extract component in the CE-ESI-TOF-MS system, and identification of dyes based on the recorded mass spectra. For identification purposes the database with accurate masses of 540 dyes was created (based on website World Dye Variety). The developed method is characterized by sufficient precision and accuracy. The validated procedure was successfully applied for the identification of dyes in 6 real samples: 1 mm length polyester threads secured from clothes. As a final stage, laboratory test was carried out to replicate the real comparative study of the fibers. The test proved the correct differentiation of samples from different sources. Thus, the obtained results show the potential of the developed procedure for implementation in forensic fiber analysis.

Research on the development of a Lab-on-Chip system as an alternative approach for psychoactive substances detection

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Keywords
electrochemistry
forensic
Lab-On-Chip
Δ^9-tetrahydrocannabinol

Δ^9-Tetrahydrocannabinol is one of the most important substance in cannabis. Currently, numerous products available in many countries, including hemp oils, supplements, drops, mostly contain cannabidiol, non-psychoactive substance, while psychoactive Δ^9-tetrahydrocannabinol is found in minimal, often undetectable amounts. Despite the legalization and therapeutic use of medicinal marijuana, the recreational misuse of marijuana remains a significant public health concern. This psychoactive substance affects psychomotor performance, and excessive doses, whether for medical or recreational use, may carry substantial risk for user and others, particularly in the context of car accidents or crime scenes. Rapid detection and quantification of Δ^9- tetrahydrocannabinol are crucial in such scenarios, enabling police and procedural authorities to take timely and appropriate actions. There is a growing demand for development of rapid, simple and portable tests that provide qualitative and semi-quantitative analyses of biological and plant samples for toxicological and forensic purposes. Lab-On-Chip technology, increasingly thriving with today's technological capabilities, introducing functional and sustainable advancements, is finding numerous applications across various scientific fields, particularly in Point-of-Care diagnostics and toxicology. Traditional forensic laboratories methods for analyzing psychoactive substance require extensive instrumental facilities, trained personnel and time. In contrary, miniaturized Lab-On-Chip systems offer an alternative for in-field applications with short time and non-specialist personnel. Moreover, that test may allow for shortening both analytical procedures, by targeting

a specific substance and the investigative process concerning the cause of the incidents. Research on development an electrochemical sensor for Δ^9-tetrahydrocannabinol analysis, using screen-printed electrodes and voltammetry as the detection method are proposed, introduces significant potential for on-site analysis of biological or plant samples in forensic contexts. Optimalization and validation of the method are crucial to provide reliable results. Lab-On-Chip technology is still developing, offering opportunities to conduct rapid, cost-effective, portable, sensitive methods for detecting psychoactive substances.

F

Electrochemical degradation of pharmaceuticals: A study of flow and static cell configurations

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Keywords
degradation
HPLC-MS
oxidation

Electrochemical degradation is an effective method for the rapid degradation of pharmaceuticals. It allows acceleration of stress studies and more accurate predicting pharmaceutical degradation. Electrochemical cell for oxidation studies can be set up in two possible configurations. The first is a batch arrangement, where the working electrolyte with the analyte is introduced into the space containing the working electrode, onto which a constant voltage is applied. The second option is a flow cell design with infusion pump, which ensures a stable flow of solution through the cell and by regulating the flow rate the contact time of the analyte with the electrode can be optimized. Batch configuration is traditionally used for its simplicity and controllability of experimental conditions, making it suitable for basic degradation studies. In contrast, flow configuration can be more easily miniaturized, controlled and allows continuous analysis, enabling realtime monitoring of electrochemical degradation. Coupled with mass detection, it is ideal for online monitoring of pharmaceuticals and their degradation products. In this study, comparison of the results of batch and flow electrochemical cell configurations on various pharmaceuticals and their impact on electrochemical degradation was investigated. The aim was to assess the efficiency of both configurations in the context of drug compound decomposition and subsequently monitor their degradation products.

Application of silver solid amalgam as a substrate for the preparation of new film electrodes

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Keywords
metal film electrodes
1-nitronaphthalene
silver solid amalgam
voltammetry

In this work, the voltammetric behavior of 1-nitronaphthalene on a polished silver solid amalgam electrode was studied using DC voltammetry (DCV) and differential pulse voltammetry (DPV). It was found that the optimum volume ratio of organic (ethanolic) to aqueous phase suitable for the preparation of 1-nitronaphthalene solution with a concentration of 1×10^{-4} mol L^{-1} is 1:9. The optimum medium for the voltammetric determination of 1-nitronaphthalene was sought with pH values of Britton-Robinson buffer ranging from 2.0 to 12.0. The optimum media for DCV and DPV determination were ethanol– Britton-Robinson buffer pH = 6.0 (1:9) and ethanol– Britton-Robinson buffer pH = 11.0 (1:9), respectively, in which the repeatability of 1-nitronaphthalene determination was investigated, and the calibration dependences were measured to achieve the lowest limit of detection (*LOD*) and determination (*LOQ*), too. The 1-nitronaphthalene concentration ranges measured were 10^{-7}, 10^{-6}, and 10^{-5} mol L^{-1}, and the *LOD* and *LOQ* values obtained were 0.90 and 3.0 μmol L^{-1}, respectively, for DCV and 0.55 and 1.8 μmol L^{-1}, respectively, for DPV. The applicability of the newly developed voltammetric methods for the determination of 1-nitronaphthalene was verified on authentic samples of drinking and river water with 1-nitronaphthalene concentration ranges of 10^{-7} and 10^{-6} mol L^{-1}. In the case of this research, this is a pilot study for the development of new metal film electrodes with films consisting of intermetallic phases of silver solid amalgam and other less noble metals (e.g., Cu, Bi, or Sb). The amalgam intermetallic phase forming the surface of these electrodes should bring an increase in the sensitivity of the above methods. The first results of this phase of research will also be presented in this conference contribution.

Miniaturized UV-photochemical generator of volatile species combined with sequential injection analysis

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Keywords
atomic spectrometry
sequential injection analysis
UV-photochemical generation

UV-photochemical generation of volatile species is an alternative but rapidly developing sample introduction technique for methods of analytical atomic spectrometry, applicable to a broad range of elements. The analyte in the liquid phase is converted to its volatile species by photoinduced radical reactions and transported to the detector in the gas phase, which increases sample introduction efficiency and eliminates possible spectral interferences from the matrix. Low molecular weight organic acids are the typical sources of reducing radicals and the generated products can be hydrides, carbonyls or alkylated species, depending on the analyte and the acid used. In UV-photochemical generation of volatile species, the important parameter is irradiation time. Its optimal value depends on the absorption characteristics of the liquid medium, rate of volatile species formation and stability of the generated volatile species towards excessive UV irradiation, temperature, etc. Currently, especially flow injection systems are in use and based on bulky photoreactors, where irradiation time is controlled by a sample flow rate. In this work, we present a miniaturized UV-photochemical generator equipped with sequential injection analysis for precise control of sample irradiation time in the photoreactor and delay before analyte release to the gas phase. Other benefits are the easy automation of the procedure and a significant reduction in consumption of chemicals.

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Determination of explosives in plant matrix

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Keywords
hexogen
LC-MS/MS,
plant matrix
trinitrotoluene
wheat

Trinitrotoluene and hexogen are widely used in the military and civilian industries due to their high effectiveness and chemical stability. As a result of their widespread use and production, explosives contaminate the environment, posing a serious threat to human health and ecosystems because of their presence in the agricultural environment, where they can be accumulated by crops like wheat. The development of effective methods for detecting and extracting these contaminants from the plant matrix is crucial for monitoring and remediating contaminated sites. The study focuses on the optimization of extraction methods and the quantification of explosives from wheat grown on Knop's contaminated media. Optimization of the extraction process included various solvents: methanol, acetonitrile, and enzymatic reagents: viscozyme, alcalase, and flavourzyme. The enzymes used were responsible for hydrolyzing bonds in polysaccharides and proteins, peptide bonds within polypeptide chains, and detaching terminal amino acids from the polypeptide chain. In addition, the time, temperature, and shaking power during the extraction process were optimized. The best organic solvent was found to be methanol for trinitrotoluene and acetonitrile for hexogen, for which the extraction efficiency was 85–105%. The "green chemistry" alternative was the 7% solution of flavourzyme in water, for which extraction efficiencies ranged from 70–90% for both compounds. Extraction with organic solvents was a simple and rapid process that was carried out at room temperature in 6 minutes, while enzymatic solvents required increased temperature and 24 hours of shaking. An analytical method for the quantification was developed using liquid chromatography coupled to mass spectrometry. For trinitrotoluene and hexogen, the limit of detection was 1 ng mL^{-1}, the limit of quantification was 10 ng mL^{-1}, and the working range of the calibration curve was 10–100 ng mL^{-1} with a determination coefficient exceeding 0.99.

Study of electrochemical behaviour of primary and secondary bile acids on boron-doped diamond electrode

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Keywords
bile acids
cyclic voltammetry
oxidation
reduction

Primary bile acids (cholic and chenodeoxycholic acids representing the majority of bile acids present in the human body) are substances that are formed in the liver by the metabolism of cholesterol. These can be further transported to the intestine, where dehydroxylation at the C7 position occurs under the action of the microorganism to form the so-called secondary bile acids (deoxycholic, ursodeoxycholic, and lithocholic acids). Bile acids contain a steroid nucleus in their structure, which is electrochemically inactive. A new activation method for electrochemical oxidation of bile acids is dehydration of the steroid nucleus with a strong acid introducing double bonds. In this contribution, the electrochemical behaviour of selected primary and secondary bile acids in acetonitrile and perchloric acid with minimal water content (ca. 0.55%) on boron-doped diamond electrode was studied. Following their dehydration and electrochemical oxidation, in the region of negative potentials at ca. 0.4 V (vs. $Ag/AgNO_3$), there is an increase in the response of the primary bile acids. Secondary bile acids, which are not oxidizable, feature decrease in the current response in this region. These findings could be used for the detection of individual bile acid groups.

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An automatic and unsupervised artificial peak detection approach for preprocessing GC-MS and GC×GC-MS metabolomic data

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Keywords
comprehensive gas chromatography
data preprocessing
molecular networks

Comprehensive gas chromatography coupled with mass spectrometry (GC×GC-MS) is a powerful tool for metabolomic studies of volatile and semivolatile compounds, thanks to its sensitivity and separation efficiency. However, its inherent drawback is the detection of artificial chromatographic features. Originating usually from following sources: peak splitting due to size or shape, from column or sorbent bleeding. For that reason, they either belong to a series of homologs or to the same peak, both cases having highly similar mass spectra. Manual curation of individual chromatograms in metabolomics represents a significant workload, calling for automated processing techniques. Here, we present a new algorithm for the removal of undesired features and demonstrate its use on multiple complex samples. The individual mass spectra of detected peaks are compressed by an autoencoder. Subsequently, the similarity among the compressed mass spectra is calculated and expressed as a molecular network. Clusters are then defined in this network by Louvain community search. Within-group similarity is then evaluated using mean group transitivity. Finally, a manually optimized cutoff value serves to exclude groups of artificial peaks. The whole process described above was written as a function in R and tested on the three datasets: The training dataset composed of n-HC, Supelco 37 mix, Grob solution, and lime oil applied on a cotton pad and sampled with DHS on a thermal desorption tube, then desorbed with a thermal desorption unit into the GC×GC-MS. Human armpit odor which was desorbed in dynamic headspace and captured on a TDU tube. Termites were extracted in hexane overnight for the termite hexane extracts and injected through an SSL injector. All three datasets

were processed by either ChromaTOF or ChromatofTile commercial software. Using our algorithm on the training dataset, we achieved 88% sensitivity and 97% selectivity for artificial peak detection. When used on a real dataset of human body odor samples, we achieved a sensitivity of 80% and a selectivity of 85%. When tested on the termite dataset for tailing peak detection, we managed to detect 89% of peaks belonging to tailing peaks. We present here an unsupervised, easy, and fast way of preprocessing GC-MS and GC×GC-MS metabolomic data before statistical analysis.

Development of LC-MS method for determination of metabolic profile of 25E-NBOH in human liver microsomes, rat urine and *Cunninghamella elegans*

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Keywords
Cunninghamella elegans
25E-NBOH
human liver microsomes
LC-MS
metabolomics

N-benzylphenethylamines are a class of new psychoactive substances increasingly used as recreational drugs with a wide range of adverse effects, potentially even death. Currently, the appearance of new *N*-benzylphenethylamines far outstrips studies of their metabolism. One such compound, 25E-NBOH, has been on the market for the last five years, but still its pharmacological and toxicological effects remain unreported. To provide a platform for such studies, we investigated 25E-NBOH metabolism in three different systems: human liver microsomes, Wistar rat urine, and *Cunninghamella elegans* fungus, which contains enzymes similar to those found in mammals. Extraction methods have been developed to process all three matrices. Untargeted LC-HRMS was used to detect phase I and phase II 25E-NBOH metabolites in all systems. While there were metabolic differences between the three systems, the most abundant metabolites were found in all of them. To confirm their presence, sixteen substances were synthesized and measured under the same conditions as real samples, their identification being confirmed by comparing the proposed metabolites with the synthesized substances. Eight metabolites were identified and also confirmed so far. In addition, the primary metabolic pathways detected were hydroxylation at various positions, *O*-demethylation and *N*-demethoxybenzylation, followed by glucuronidation or *N*-acetylation. Furthermore, the specific positions of the hydroxy

moieties in the hydroxylated metabolites were determined. We believe that our metabolic profile of 25E-NBOH provides a good basis for the pharmacological and toxicological studies necessary to support diagnosis by clinicians in the field.

Determination of nicarbazin in acetonitrile-based media using differential pulse voltammetry and capillary electrophoresis with amperometric detection

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Keywords
capillary electrophoresis
coccidiostats
differential pulse voltammetry
electrochemistry
nicarbazin

Nicarbazin is a worldwide used veterinary drug to prevent and treat coccidiosis in poultry. Prevention has proven more effective than treatment, leading to its routine inclusion in animal feed as a feed additive in over 60% of cases. Moreover, nicarbazin is one of 11 coccidiostats approved to use in the European Union. Despite its benefits to livestock management, nicarbazin poses a threat to the safety of breeding industry, ecological environment and human health. Due to this, nicarbazin residues in poultry feed, enviromental objects and food of animal origin should be monitored. This contribution is aimed to investigate the electrochemical oxidation of nicarbazin on platinum ultramicroelectrode in non-aqueous media using differential pulse voltammetry and capillary electrophoresis hyphenated to amperometric detection. While differential pulse voltammetry is relatively more time-efficient, cost-effective and portable technique, capillary electrophoresis hyphenated to amperometric detection benefits from lower sample consumption and very good separation efficiency. The aim of this project is to provide complementary investigation of nicarbazin oxidation by leveraging the advantages of both separation and detection techniques. The initial phase of the research focused on finding which component (4,4'-dinitrocarbanilide or 2-hydroxy-4,6-dimethylpyrimidine) of the investigated equimolar mixture nicarbazin is electroactive and/or charged in used acetonitrile-based

background electrolyte. Then, under the optimized experimental conditions, the calibration dependencies were constructed yielding limit of detection values of 3.3 μM for differential pulse voltammetry and 2.0 μM for capillary electrophoresis hyphenated to amperometric detection. Finally, the developed methods were applied to detect nicarbazin residues in various real feed samples. Thus, this research provides not only the comparison of two different electrochemical techniques for characterizing nicarbazin oxidation at platinum ultramicro-electrode in acetonitrile-based media but also aims to develop the straight-forward and sensitive methods for monitoring nicarbazin in poultry feed of different nature.

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