Proceedings of the 19th International Students Conference "Modern Analytical Chemistry"

Prague, 14—15 September 2023

Edited by Karel Nesměrák

FACULTY OF SCIENCE
Charles University

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Preface

On 14 and 15 September 2023, we welcomed 43 participants from five countries and ten universities to the 19th international conference of PhD students of analytical chemistry. At the conference, these budding scientists had the opportunity not only to meet but also, above all, to present and discuss their research in analytical chemistry and exchange new insights and ideas for the development of this key field for humanity. Additionally, participation in the conference allowed participants to develop their presentation and language skills.

Unlike in previous years, only a small group of courageous participants took the opportunity to publish the full text of their paper in these Proceedings. Therefore, we also added a list of all the contributions presented at the conference. In this way, the reader will be able to get an idea of the scope of the conference and how modern analytical methods are being used to address various human problems.

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.

Last but not least, we are very grateful to all sponsors for their kind sponsorship making the conference possible, but for their cooperation and support in many of our other activities.

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

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Bagherimetkazini, Seyedehelahe (University of Regensburg, Germany): *Tyrosine oxidation behavior using electrochemistry, capillary electrophoresis, and mass spectrometry*

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Zelenský, Michal (Charles University, Czech Republic): *Characterization of laser treated polycrystalline boron doped diamond electrodes with various* sp^3/sp^2 *carbon ratio*

Supported liquid-solid phase extraction using wetted nanofiber discs as a simple approach for extraction of water contaminants

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Keywords
HPLC
nanofiber discs
polymer-based nanofibers
octanol
supported liquid-solid phase extraction

Abstract
The novel Supported Liquid-Solid Phase Extraction (SL-SPE) approach was developed with the use of polymeric nanofibrous discs. Sorbents were tested to extract 9 common water contaminants with a wide range of log *P* values (1.9–6.5). Three different nanofibrous polymers (polyacrylonitrile, polyhydroxybutyrate, and polylactic acid) were chosen as sorptive phases and wetted with a small amount of octanol before extraction. One-step desorption was conducted directly in an HPLC vial to eliminate time-consuming evaporation and reconstitution steps. The effects of different parameters influencing the extraction efficiency, including extraction time, ionic strength, and sample volume, were investigated and optimized. Nanofibrous discs wetted with octanol showed even a 20-fold increase of Enrichment Factor when compared to native discs. The highest enrichment factors were observed for analytes within the 3.3–4.5 log *P* range. The developed method showed good linearity for all tested analytes in the range 20–200 μg L^{-1}, and satisfactory extraction repeatability (RSD) <15%.

1. Introduction

Polymer-based nanofibers have become a hot trend in the development of new extraction sorbents. In comparison to nanostructures such as nanoparticles, nanowires, etc., nanofibers are long and continuous, can transport electrons or photons, and have excellent mechanical properties, which is crucial in many different areas [1]. Nanofibers possess many interesting features. They have a large surface area, acquire extremely high porosity, increased mechanical properties (i.e. stiffness and tensile strength), and chemical stability [2]. Moreover, they can be manufactured with good effectiveness with electrospinning process [3, 4]. Up to this day more than 50 different polymers have been

successfully electro-spun in form of nanofibers [5] and widely exploited as extraction sorbents, especially in SPE packed-cartridge [6, 7], pipette-tip [8], and several micro-extraction approaches [9, 10].

Modern trends in sorbent development moves toward designing new composite materials, where polymer-based nanofibers are often used as support for other species such as nanoparticles, metal-organic frameworks , molecularly imprinted polymers [11]. Mainly polyacrylonitrile nanofibers are employed as support in composites due to the ease of their manufacturing, good wettability and enhanced hydrogen bonding and π-π interaction of this material [12, 13]. Despite of composites big versatility, they also possess more complicated chemistries and require multi-step manufacturing. Which can lead to batch to batch differences or non-homogeneity due to loading of particles on a nanofiber support. There is still a need for simple extraction methodologies.

In our research, we wanted to combine the benefits of a highly porous nanofibrous structure as a support for low-density solvent to improve the extraction of common water contaminants. Our studies focused on the development of a simple Supported Liquid-Solid Phase extraction protocol with the use of support-free nanofiber discs. Which are wetted with a small amount of octanol right before the extraction. This methodology can be done directly in-beaker, which facilitate the application for large sample volumes. The use of a metal wire ensures the stable spinning of the discs in the bottom of the beaker. This gives higher extraction to extraction repeatability. Moreover, the elution step is made directly in an HPLC vial. This eliminates the time-consuming evaporation and reconstitution, commonly featured in SPE protocols.

2. Experimental

2.1 Reagents and chemicals

The analytical standards 2-chlorophenol, 2,5-dichlorophenol, 2,3,4,6-tetrachlorophenol, fenoxycarb and permethrin were purchased from Fluka (Germany). Bisphenol A, 2,4,6-trichlorophenol, and 4-chlorophenol were purchased from Sigma-Aldrich (Germany). *p*-Nitrophenol was purchased from Chemapol (Czech Republic). Acetonitrile and methanol for the material conditioning phase, the dissolution and elution of the analyte, and the chromatographic mobile phase in LC/MS purity grade >99.9% were supplied from Biosolve (France). 1-Octanol used for discs coating was purchased from Sigma-Aldrich (Germany). Ultra-pure water was prepared using Millipore Milli-Q Direct Water Purification System from Merck (Germany). The stock solutions of the 9 analytes were prepared at concentration 2 mg mL^{-1} in acetonitrile and stored at 4 °C. The mixed standard solution was prepared at a concentration of 50 ppm and stored at 4 °C.

2.2 Instrumentation

Chromatographic analysis was performed using the HPLC-DAD system (Shimadzu Corporation, Japan) equipped with two LC-10AD VP high-pressure pumps, FCV-10AL VP low-pressure gradient valve, DGU-14A degasser, SIL-HT A auto-sampler, SPD-M10A VP diode array detector, and CTO-10AC VP column oven. Lab Solution software was used for data evaluation. Separation of analytes was obtained with the YMC-Triart C18 ExRS column (150×4.6 mm i.d. S-5μm, 8 nm) produced by YMC (USA).

2.3 Chromatographic conditions

Acetonitrile, part A and Mili-Q water, part B, were mixed as mobile phase in the following gradient program: 0–2 min, 40% A; 2–7 min, 40–100% A; 7–9 min, 100% A; 9–10 min, 100–40% A; 10–10.5 min, 40% A. The injection volume was 10 μL and the flow rate was 1.25 mL min^{-1}. All analytes were monitored and their peak area evaluated at detection wavelength 210 nm.

2.4 Fabrication of nanofibrous materials

The fibrous sorbents were obtained from the Technical University in Liberec. Polymeric sorbents made from poly(3-hydroxybutyrate) were prepared using metblown technology Biax-380 mm PILOT (Biax-Fiberfilm, USA). Polyacrylonitrile and polylactic acid were fabricated via alternating-current electrospinning technology using Nanospider type NS 1WS500U (Elmarco, Czech Republic).

2.5 Extraction protocol

Small nanofibrous discs of 7–9 mg were cut from all materials (poly-(3-hydroxybutyrate), polylactic acid, polyacrylonitrile). Each disc was first pierced with a metal-wire as a home-made stirring device. Extraction experiments were conducted with native discs and with discs wetted with 50 μL of octanol. Before the extraction, native discs were activated by soaking in acetonitrile (polylactic acid discs were unstable in acetonitrile, so methanol was used instead) and then washed with distilled water. After activation or addition of octanol, the discs were placed in a beaker with 100 mL of water spiked with standard mix (50 ppb). All samples were placed on a magnetic stirrer and stirred for 1 hout at 600 rpm. Later, discs were removed from the sample, dried on a soft tissue, and placed directly in an HPLC vial. 1 mL of acetonitrile or methanol (polylactic acid) was added as an elution solvent and all samples were vortexed for 10 s. 10 μL of the extract was directly injected into the HPLC system (Fig. 1).

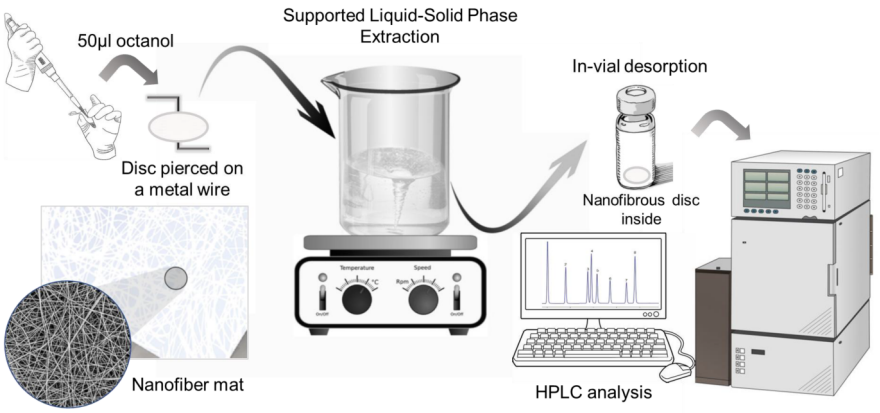

Fig. 1 Schematic representation of the extraction procedure

3. Results and discussion

Parameters such as sample volume, stirring rate, extraction time, and ionic strength were considered and optimized (Fig. 2). No significant increase in extraction efficiency was observed when the sample volume was increased by more than 100 mL or the extraction time increased by more than one hour. Faster stirring ensured better contact of the sorbent with the sample, and therefore increased the extraction efficiency. The addition of salt has been widely reported to improve the extraction of organic contaminants from the water matrix, based on salting-out effect [14]. However, we observed the decrease in extraction efficiency. When the ionic strength is increased the molecules of the electrolyte can interact with the analyte molecules, lowering their extraction.

The addition of octanol significantly improved the extraction of Bisphenol A, 2,5-dichlorophenol, 2,4,6-trichlorophenol, fenoxycarb and 2,3,4,6-tetrachlorophenol (log P = 3.3-4.5); Table 1. The highest preconcentration was obtained for 2,3,4,6-tetrachlorophenol on polyacrylonitrile wetted with octanol (Fig. 3). A volume of 50 µL of octanol was chosen as optimal, lower volumes did not give satisfactory results, and a higher amount could not be fully absorbed by the small discs. The linearity of the method was tested in the concentration range of 20–200 µg L^{-1} of standard solution mix in water. The limit of quantitation and the limit of detection were established using the regression function. Limit of detection was between 5–15 µg L^{-1}, while limit of quantitation was 15-48 µg L^{-1} for all analytes. The relative standard deviations (RSD %) from 5 extractions ranged from 3–11% for polyacrylonitrile, 8–14% polylactic acid, and 2–12% poly(3-hydroxybutyrate). The polyacrylonitrile discs wetted with octanol showed the best enrichment factors and the highest repeatability of the results. The results of the HPLC system suitability test are summarized in Table 2.

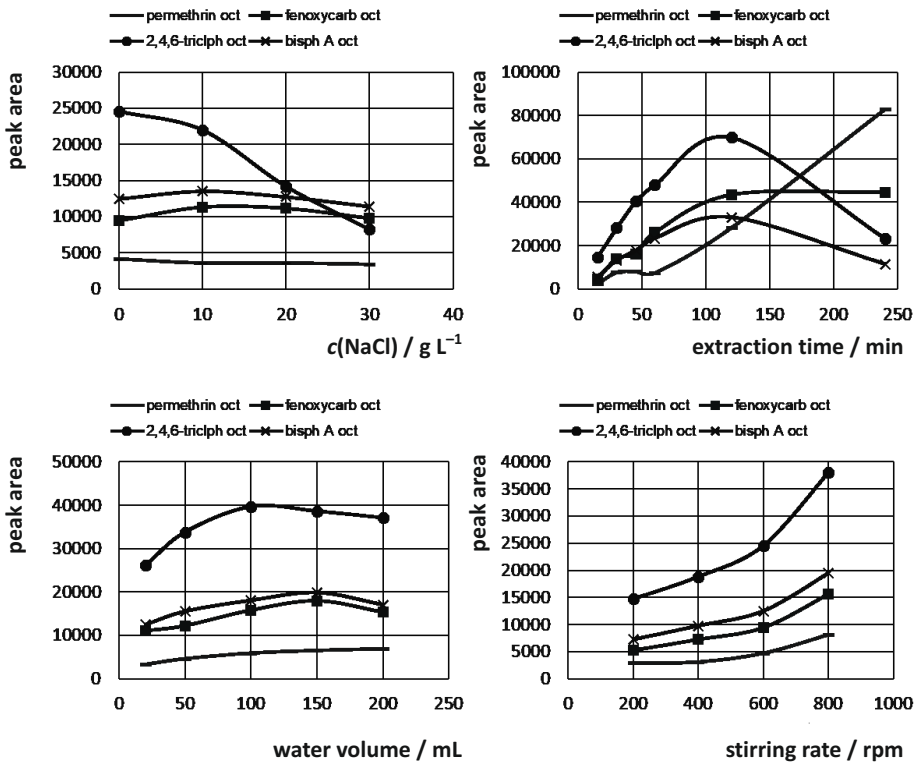

Fig. 2 Peak area of chosen analytes versus different extraction parameters. Based on the extraction results with octanol wetted poly(3-hydroxybutyrate) nanofibrous discs.

Table 1
Analytical characteristics of the tested nanofibrous materials in linearity range 20–200 μg L^{-1}.

	p-nitro-phenol	2-chloro-phenol	4-chloro-phenol	Bisphenol A	2,5-dichloro-phenol	2,4,6-trichlo-rophenol	fenoxycarb	2,3,4,6-tetra-chlorophenol	Permethrin
	Polyacrylonitrile								
Enrichment factor[a]									
Native	0.00	0.00	0.00	0.72	0.67	0.71	2.63	0.58	9.62
With octanol	1.02	2.02	3.27	8.53	8.23	10.16	9.23	12.90	11.68
RSD[b] / %	3.06	8.11	8.23	3.65	5.03	6.40	6.78	5.99	11.14
	Polylactic acid								
Enrichment factor[a]									
Native	0.00	0.49	0.72	0.42	1.11	0.90	1.96	0.62	3.66
With octanol	1.39	2.52	3.41	7.17	7.65	8.66	9.70	8.85	3.21
RSD[b] / %	14.03	11.97	11.31	11.66	12.05	9.18	8.76	8.15	11.67
	Poly(3-hydroxybutyrate)								
Enrichment factor[a]									
Native	0.00	0.00	0.00	0.73	0.83	0.93	5.10	1.02	14.28
With octanol	1.22	1.86	3.72	5.54	6.00	6.51	6.29	6.50	2.52
RSD[b] / %	11.57	9.61	9.07	10.59	7.84	8.92	12.05	2.12	10.02

[a] Calculated as a peak area ratio of SLSE-HPLC method versus without SLSE.
[b] RSD of five extractions of water spiked with analytes mix (50 μg L^{-1}).

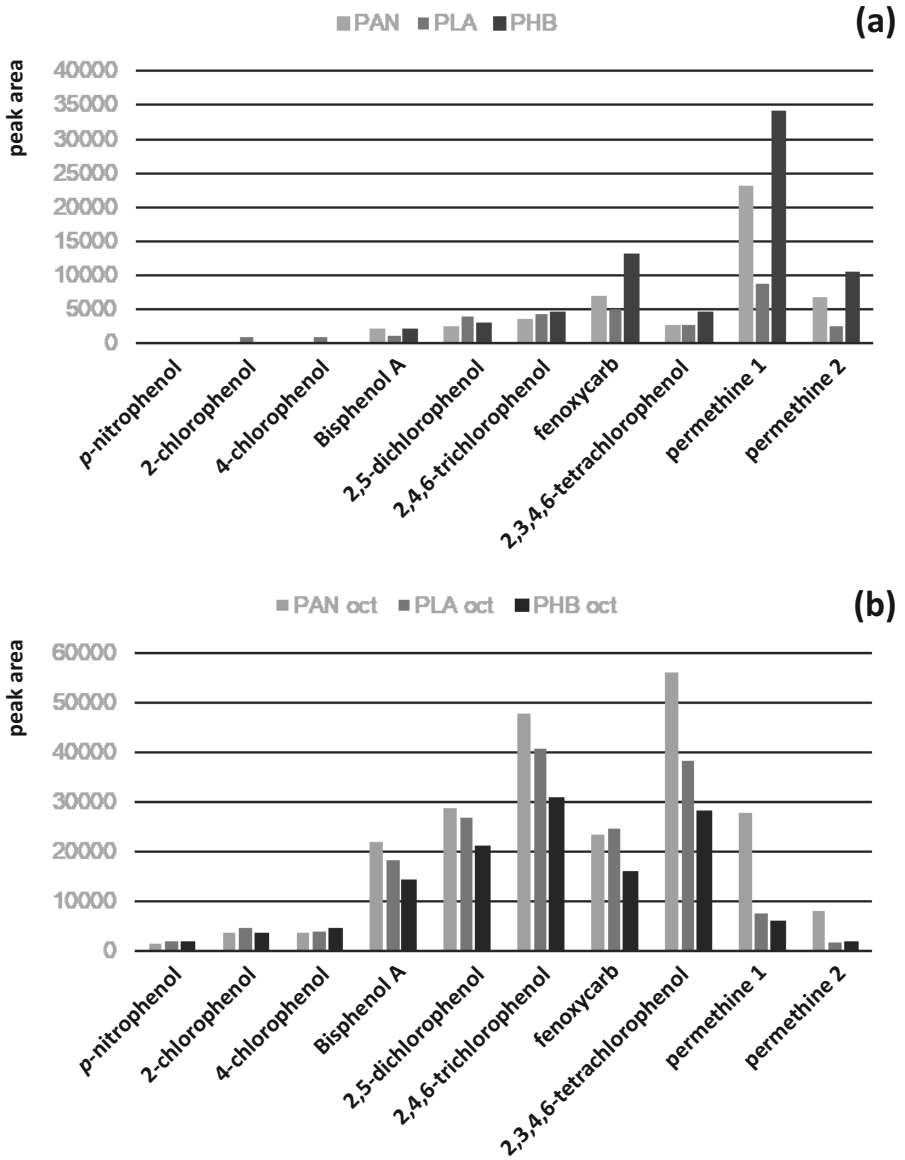

Fig. 3 Peak areas of analytes after 1 hour extraction with polyacrylonitrile (PAN), polylactic acid (PLA) and poly(3-hydroxybutyrate) (PAB) nanofiber discs (7–9 mg). Comparison of (a) native discs and (b) discs with addition of 50 μL of octanol.

Table 2
Results of the HPLC system suitability test.

Analyte	t_r /min	Tailing factor[a]	R	Peak capacity[b]	Peak areas repeatability[c] RSD / %
p-nitrophenol	3.23	1.25	–	46.06	2.40
2-chlorophenol	4.39	1.19	6.56	67.45	2.86
4-chlorophenol	4.61	1.22	1.58	73.92	2.94
Bisphenol A	4.88	1.21	2.12	80.55	1.88
2,5-dichlorophenol	5.68	1.25	6.82	87.07	2.17
2,4,6-trichlorophenol	6.67	1.23	8.94	88.50	7.26
fenoxycarb	6.90	1.23	2.15	93.11	9.68
2,3,4,6-tetrachlorophenol	7.13	1.05	1.99	80.55	8.81
permethrine	9.30	1.21	18.40	79.95	1.21

[a] Tailing Factor calculated by Lab Solution software (the entire peak width divided by twice the front half-width)
[b] Peak capacity expressing efficiency of method (gradient elution) calculated as Pc = (gradient time/average peak width at 5% height) + 1
[c] RSD calculated from six injections of the standard mixture at concentration levels 1 mg L^{-1}.

4. Conclusions

This work focused on the development of novel and simple Supported Liquid-Solid Phase extraction using wetted polymer nanofibrous discs. The addition of a small amount of octanol proved to be a promising approach to increase the extraction of moderate-lipophilic analytes. The best preconcentration was observed for analytes with log P in a range 3.3-4.5. An even 20-fold increase in preconcentration was observed for 2,3,4,6-tetrachlorophenol after extraction with octanol wetted polyacrylonitrile in comparison to native nanofibrous disc. Only 50 µL of octanol was sufficient for extraction. No need of any sorbent cartridge was required. In-beaker extraction and in-vial desorption limit the unnecessary, time-consuming steps, which simplifies the sample preparation protocol. Discs can be easily prepared in different sizes without the need of any special equipment. This methodology also shows satisfactory reproducibility of the extraction results.

Acknowledgments

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Voltammetric determination of 2-aminoindane in oral fluid

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Keywords
2-aminoindane
electropolymerization
graphite
square-wave voltammetry
thiophene

Abstract
2-Aminoindane belongs to new psychoactive substances and is an analogue of amphetamine. The unavailability of rapid screening tests to promptly detect new psychoactive substances outside the laboratory is a current challenge. Electrochemical sensors modified with polymer layers might be used as an alternative to laboratory determination of new psychoactive substances. Polymeric layers derived from thiophene and its methoxy derivative (3-(4-methoxyphenyl)-thiophene) were deposited onto the surface of graphite electrodes using cyclic voltammetry. The modified electrodes were tested to detect 2-aminoindane with square-wave voltammetry. The electrode modified with a 3-(4-methoxyphenyl)-thiophene-derived polymeric layer demonstrated the optimal characteristics for detection of 1×10^{-4} mol dm^{-3} of 2-aminoindane in oral fluid at +0.85 V (*RSD*: 1.08–1.73 %, recovery 98–100%).

1. Introduction

2-Aminoindane is a representative of new psychoactive substances that are used as an alternative to known drugs. The necessity of 2-aminoindane monitoring is explained by its similarity to amphetamine. Sensors using electrochemical principles provide perspectives for the development of in-field analysis. Moreover, the miniaturization of sensor systems and modification of electrode surface with a selective layer allows to improve their selectivity and crime scene applicability. Recently, it has been shown that the thiophene-derived polymeric layer (Th) is suitable for new psychoactive substance detection containing amino groups [1]. This work compares the recognizing capability of polymeric layers derived from unsubstituted thiophene (Th) and 3-(4-Methoxyphenyl)-thiophene (ThM) toward 2-aminoindane in oral fluid using square-wave voltammetry.

2. Experimental

2.1 Reagents and chemicals

2-Aminoindane hydrochloride (97%), tetrabutylammonium tetrafluoroborate (99%) and thiophene (98%) were obtained from Sigma-Aldrich (USA). 3-(4-Methoxyphenyl)thiophene was synthesized by Tomáš Tobrman according to [2]. Inorganic substances used for electrode pretreatment and electrochemical measurements were either from Lach-Ner or Penta (Czech Republic). The solutions were prepared using redistilled water.

2.2 Instrumentation

Voltametric measurements were carried out using Palmsens 3 (PalmSens BV, Netherlands) with three-electrode set up: a platinum plate (81 mm^2) as the counter electrode, Ag/AgCl (3 mol dm^{-3} KCl) as the reference electrode and a graphite electrode (GE; Elektrochemické detektory, Turnov, Czech Republic) as the working electrode. Before the modification, graphite electrodes were polished with $CaCO_3$, soaked in 1 mol dm^{-3} HCl for 30 minutes and finally cleaned by cycling potential in 1 mol dm^{-3} HCl in potential range from –0.3 to +1.8 V, scan rate 50 mV s^{-1}, 15 scans.

2.3 Electrochemical modification of the electrode surface

The electrochemical modification of the graphite electrode was carried out using cyclic voltammetry (CV) from a polymerization mixture containing: 0.05 mol dm^{-3} tetrabutylammonium tetrafluoroborate and monomer (0.4 mol dm^{-3} thiophene or 0.01 mol dm^{-3} 3-(4-methoxyphenyl)thiophene) dissolved in acetonitrile. The potential was scanned in range from –0.1 to +1.8 V with scan rate 50 mV s^{-1} for 10 scans.

2.4 Electrochemical measurement

The assessment of the electrochemical properties and the detection of 2-aminoindane on the experimental graphite electrodes was carried out with square-wave voltammetry. The working parameters were as follows: potential range from –0.2 to +1.2 V, step potential of 5 mV s^{-1}, amplitude 25 mV, frequency 10 Hz. The 2-aminoindane concentration was changed by adding 0.02 mol dm^{-3} standard solution to the supporting electrolyte (0.1 mol dm^{-3} KCl + 5×10^{-3} mol dm^{-3} $[Fe(CN)_6]^{3-/4-}$; V = 25 mL) in range from 1×10^{-6} mol dm^{-3} to 1170×10^{-6} mol dm^{-3}.

2.5 Preparation of oral fluid

Real oral fluid samples were collected according to [3]. Then, 1 mL of oral fluid was mixed with the appropriate weight of $[Fe(CN)_6]^{3-/4-}$ and KCl and diluted in the volumetric flask (V = 10 mL). We processed the voltametric signal of 1.10×10^{-4} mol dm^{-3} 2-aminoindane (S_X) measured in oral fluid with two methods for two G/PThM electrodes. The first method was based on subtraction of the signal obtained for the unspiked matrix 2-aminoindane (oral fluid + 0.1 mol dm^{-3} KCl + 5×10^{-3} mol dm^{-3} $[Fe(CN)_6]^{3-/4-}$; S_s). The second method was based on subtraction of the signal obtained for spiked matrix (oral fluid + 9.00×10^{-5} mol dm^{-3} 2-aminoindane + 0.1 mol dm^{-3} KCl + 5×10^{-3} mol dm^{-3} $[Fe(CN)_6]^{3-/4-}$; S_0).

3. Results and discussion

3.1 Electrochemical modification of graphite electrodes

Figure 1 presents the cyclic voltammograms obtained at the deposition of the PTh and PThM polymeric layer on the graphite electrode. For PTh, two reversible peaks were observed (anodic peak E_a =+1.10 V and cathodic peak E_c = +0.55 V). For PThM, the voltammograms revealed two anodic peaks (E_{a1} = +1.10 V, E_{a2} = +1.48 V) and one cathodic peak (E_c = +0.90 V). There are several reasons for the shift of cathodic peak to more positive potentials: i) heterogeneous electron-transfer kinetics; ii) polymer layer porosity; iii) decrease in conductivity as a result of the polymer thickness [4]. Additionally, the PThM polymeric layer had a characteristic green-blue colour [5].

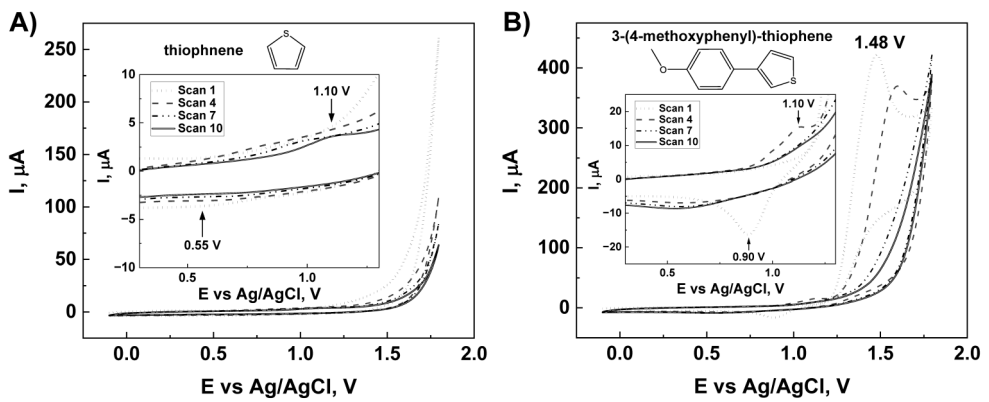

Fig. 1 Cyclic voltamogram obtained at electrochemical oxidation of (A) thiophene, and (B) 3-(4-methoxyphenyl)thiophene carried out on graphite electrode.

Table 1
Comparation of sensitivities obtained by square-wave voltammetry on un- and modified electrodes with addition of 2-aminoindane.

Polymer layer	Properties	E_1 = +0.20 V	E_2 = +0.85 V	E_3 = +1.30 V
G[a]	Sensitivity / μA decade^{-1}	–5.1314	–	–
	Linear calibration range / 10^{-6} mol dm^{-3}	3–10.9	–	–
	R^2	0.9912	–	–
G/PTh[b]	Sensitivity / μA decade^{-1}	8.1548	0.7123	–
	Linear calibration range / 10^{-6} mol dm^{-3}	7.9–1170	40.4–812	–
	R^2	0.9810	0.9959	–
G/PThM[c]	Sensitivity / μA decade^{-1}	6.2614	1.6320	4.9026
	Linear calibration range / 10^{-6} mol dm^{-3}	3–627	40–812	7.9–812
	R^2	0.9929	0.9833	0.9853

[a] With the unmodified graphite electrode, no subtraction was made.
[b] At calibration of the Th-modified electrode, the current response of the supporting electrolyte was subtracted from every calibration point.
[c] At calibration of the ThM-modified electrode, the current response measured for 10^{-6} mol dm^{-3} 2-aminoindane was subtracted from every calibration point.

3.2 Electrochemical behaviour of 2-aminoindane

The electrochemical behaviour of 2-aminoindane was studied using the square-wave voltammetry technique with three working electrodes: unmodified (G), G/PTh and G/PThM modified electrodes (Table 1). Non-specific adsorption should occur between 2-aminoindane and G-electrode (Fig. 2A). Combination of non-specific adsorption and specific interactions is expected between 2-aminoindane and G/PTh or G/PThM modified electrodes (Fig. 2B). The voltametric signal for the specifically sorbed 2-aminoindane was anticipated to appear in the potential window characteristic for amine group oxidation (+0.80 to +1.44 V) [2, 6].

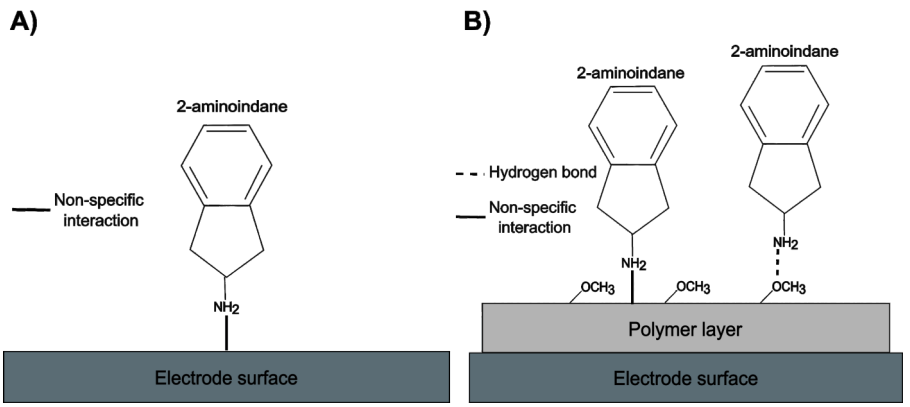

Fig. 2 Proposed mechanism of interaction of 2-aminoindane with (A) unmodified, and (B) modified electrode surface.

In contrast to PTh- and PThM- electrodes, unmodified G-electrode showed only one peak that corresponds to $[Fe(CN)_6]^{3-/4-}$. The peak E_1 = +0.20 V, that is characteristic for the redox couple $[Fe(CN)_6]^{3-/4-}$, was observed at square-wave voltammograms for all tested electrodes. The peak E_2 = +0.85 V, which is attributed to the expected oxidation of amino group, was present for both G/PTh and G/PThM electrodes and increased in the concentration range (40.4–812)×10^{-6} mol dm^{-3} 2-aminoindane. It should be noted that the sensitivity of the G/PThM electrode to 2-aminoindane was twice as high. For G/PThM electrode, an additional peak E_3 = +1.30 V was observed, which might denote a specific interaction between 2-aminoindane and methoxy group of PThM polymer layer. The G/PThM electrode was chosen and tested for the 2-aminoindane determination in oral fluid.

3.3 Determination of 2-aminoindane in oral fluid

The analysis of samples in biological matrix requires elimination of the matrix effect that influences the surface electrode properties and subsequently the sensitivity of determination. Therefore, it was necessary to choose the potential for quantification, to optimize the sample preparation, and the processing of the square-wave voltammetry signal. The peak E_2 = +0.85 V was optimal for the determination of 2-aminoindane.

We processed the voltametric signal of 1.10×10^{-4} mol dm^{-3} 2-aminoindane (S_X) measured in oral fluid with two methods for two G/PThM electrodes (Fig. 3). It was found that the second method yielded better results and the result of 2-aminoindane determination did not depend on the intensity of signals (compare G1/PThM *versus* G2/PThM) (Table 2).

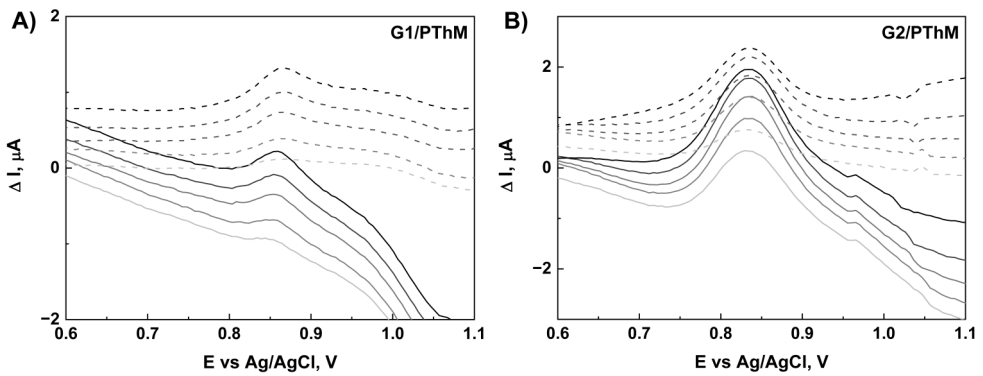

Fig. 3 Comparation of square-wave voltammograms and processing of voltammetric signal obtained for two electrodes modified with methoxy derivate of thiophene at determining of 1.10×10^{-4} mol dm^{-3} 2-aminoindane (S_X) in oral fluid. Processing of voltametric signal: solid lines correspond to S_X–S_S, dashed lines correspond to S_X–S_O (see section "2.5 Preparation of oral fluid").

Table 2
Results of 2-aminoindane determination in oral fluid after the optimalization of sample preparation and signal processing.

Electrode	c(2-aminoindane) / mol dm^{-3}		*RSD* / %	Recovery / %
	Introduced	Determined		
G1/PThM	1.10×10^{-4}	$(1.10\pm0.01)\times10^{-4}$	1.08	100
G2/PThM	1.10×10^{-4}	$(1.08\pm0.02)\times10^{-4}$	1.73	98

On the next step, the determination of 1.10×10^{-4} mol dm^{-3} 2-aminoindane in oral fluid was repeated after electrodes regeneration in supporting electrolyte (G1/PThM) or distilled water (G2/PThM) for two hours. The experimental results showed that the reproducible determination is provided through regeneration in distilled water.

4. Conclusions

The electrochemical properties of 2-aminoindane were investigated by square-wave voltammetry using three graphite electrodes: unmodified, modified with thiophene or its methoxy derivative. The oxidation of 2-aminoindane was recorded around +0.85 V on both modified electrodes, this potential is characteristic for amine oxidation. Based on the obtained voltammetric signal, G/PThM electrode was chosen for determination of 2-aminoindane in oral fluid. To eliminate the matrix effect, it was optimal to subtract the voltammetric signal for the supporting electrolyte and simulated concentration of 2-aminoindane. The determination of 2-aminoindane (c(2-aminoindane) = 1.10×10^{-4} mol dm^{-3}) in oral fluid with G/PThM was possible with *RDS* in range of 1.08 and 1.73% and recovery between 98 and 100%.

Acknowledgments

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Bisphenols and their derivatives in baby diaper samples

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Abstract
Many common products contain and leach hazardous chemicals, including endocrine-disrupting chemicals such as bisphenols that are harmful to human health. For toddlers, this dangerousness is higher because of their not fully developed detoxification system. Due to this, bisphenols content in products, such as baby diapers, should be monitored. Baby diapers not only remain in close contact with the skin, but are also used from the first hours of life. Baby diaper samples were prepared by ultrasound assisted solvent microextraction of porous membrane-packed solid sample (UASE-PMSS) and extracted analytes were determined by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Obtained recoveries value ranged from 60% to 115%. Bisphenol A was determined in 81% of the tested samples with a concentration in the range of 5.0–520 ng g^{-1}, while bisphenol A bis(3-chloro-2-hydroxypropyl)ether in 15% of the samples with a concentration ranged from 6.8 to 530 ng g^{-1}.

1. Introduction

Endocrine disrupting chemicals are nowadays widely investigated group of compounds due to their harmful properties for wildlife and humans. They can be characterized by structural similarities to naturally synthetized hormones in human organism. This property gives them possibility to react as natural hormones and in result may cause dysfunction of endocrine system such as cancer disease [1]. Not fully developed detoxification mechanism of newborns and infants may have even more dangerous effects in their organisms due to this exposure.

One of the first synthetic substances which was described as endocrine-disrupting chemical was bisphenol A (BPA) [2]. It is widely used in products made from polycarbonate plastic and epoxy resin coatings [3]. Several studies have highlighted that early exposure to BPA can cause many health effects such as

thyroid disrupting properties [4]. It has also been reported, that maternal exposure to BPA may influence to the offspring causing, e.g., genital malformations, testicular abnormalities, impairment in fertility or sexual functions [5]. Because another bisphenols have similar structure to BPA, it is highly possible to suppose, that they can cause similar health effects.

Due to the fact that the presence of bisphenols and its derivatives were reported in products such as tampons and pads [6] it seems highly recommended to monitor their content in disposable baby diaper. The aim of that project was to determine bisphenols and their derivatives in disposable baby diaper samples to estimate safety of everyday products dedicated for newborns and infants.

2. Experimental

2.1 Reagents and chemicals

Analytical standards of bisphenol A (BPA, CAS 80-05-7), bisphenol BP (BPBP, CAS 1844-01-5), bisphenol C (BPC, CAS 79-97-0), bisphenol F (BPF, CAS 620-92-8), bisphenol FL (BPFL, CAS 3236-71-3), bisphenol G (BPG, CAS 127-54-8), bisphenol M (BPM, CAS 13595-25-0), bisphenol P (BPP, CAS 2167-51-3), bisphenol S (BPS, CAS 80-09-1), bisphenol Z (BPZ, CAS 843-55-0), racemic mixture of bisphenol F diglicydyl ether (BFDGE, CAS 2095-03-06), bisphenol F bis(2,3-dihydroxypropyl) ether (BFDGE·$2H_2O$, CAS 72406-26-9), bisphenol F bis (3-chloro-2-hydroxypropyl) ether (BADGE·2HCl, CAS 4809-35-2), bisphenol A diglycidyl ether (BADGE, CAS 1675-54-3), bisphenol A (3-chloro-2-hydroxypropyl) glycidyl ether (BADGE·HCl, CAS 13836-48-1), bisphenol A bis(3-chloro-2-hydroxypropyl) ether (BADGE·2HCl, CAS 4809-35-2), bisphenol A (2,3-dihydroxypropyl) glycidyl ether (BADGE·H_2O, CAS 76002-91-0), bisphenol A bis(2,3-dihydroxypropyl) ether (BADGE·$2H_2O$, CAS 5581-32-8) and bisphenol A (3-chloro-2-hydroxypropyl)-(2,3-dihydroxypropyl) ether (BADGE·H_2O·HCl, CAS 227947-06-0) were purchased from Merck–Millipore, Germany. Internal standards, that is ^{13}C-labeled bisphenol A (CAS 263261-65-0) and d_{10}-labeled BADGE (CAS 1675-54-3), were purchased from Cambridge Isotope Laboratories Inc. (Cambridge, UK). Potassium chloride (CAS 7447-40-7) was bought from VWR, Poland, hypergrade purity methanol (CAS 67-56-1) from Merck (Germany) and 25% ammonia solution (CAS 1336-21-6) from Merck–Millipore, Germany. Salt solutions were prepared with ultrapure water, cleaned by HPL5 system (Hydrolab, Poland) equipped with an EDS-Pak cartridge (Merck–Millipore, Germany). For the extraction polypropylene membrane sheets purchased from the GVS Filter Technology (Rome, Italy) were used. Syringe filters filled with 0.2 μm pore size nylon core were obtained from Thermo Fisher Scientific, Poland.

2.2 Instrumentation

Experiment was conducted using ultra-performance liquid chromatograph Schimadzu Nexera X2 (Japan) coupled to Shimadzu LC-MS-8060 (Japan) tandem mass spectrometer. Separation of analysed substances took place in Kinetex® 1.7 µm EVO C18 100 Å, 100×2.1 mm chromatography column purchased from Phenomenex (Germany). Additionally UHPLC precolumn purchased from Phenomenex (Germany) was used. Moreover, instruments such as Mettler Toledo XP504 analytical balance (Poland), Z6667 impulse sealer (Poland), stainless steel net (Poland), ultrasonic bath UM 4-Badelin (Sonorex , Germany) and TurboVap LV evaporation system (Caliper LifeSciences, USA) were used.

2.3 Sample preparation

All samples of disposable baby diaper were purchased in local stores in Gdańsk, Poland. Firstly, they were homogenized by machinery cutting to small pieces and each sample was divided into absorbent core and supporting wings. Afterward weighted 0.1 g of sample was placed in prepared before polypropylene membrane bag. 20×10^{-3} mol dm^{-3} potassium chloride solution and two internal standards of concentration 10 µg mL^{-1} were then added to weighted sample. Prepared like that membrane bag was sealed by impulse sealer and placed in 15 mL vial, where 7 mL of methanol was added. Because of floating observed at the first moments of sample preparation cleaned stainless steel net was introduced to immobilize membrane bag inside of extractions solvent. Vials prepared this way was then placed in the beakers on ultrasonic bath were the ultrasound assisted solvent microextraction of solid samples contained in a porous membrane (UASE-PMSS) took place for 20 minutes at 25 °C . Subsequently, the membrane and stainless steel net was removed from extract. Afterward, it was ready to evaporate the methanol under gentle nitrogen stream on TurboVap LV evaporation system. Subsequently, 1 mL of methanol was added to dry residue of extract the analytes. Prepared solution was mixed on vortex, filtered by nylon syringe filters and placed in chromatographic vial. To exclude possible contamination blank samples were also prepared and analyzed.

2.3 Bisphenols determination

Obtained solution with internal standars and extracted analytes were analysed by UPLC-MS/MS. Calibration curve, necessary to quantify the amount of compounds in disposable baby diaper was prepared by dissolving stock solution of analytes and internal standars. During analysis internal standrads concentration was kept at 10 ng mL^{-1} each while analytes were prepared in concentration equal 0.5, 1, 2, 5, 10, 20, and 50 ng mL^{-1}. All analytes were detected and analysed in positive and negative ionization by multiple reaction monitoring mode. Separation took place

Table 1
Parameters of weighted regression, standard deviation of the slope of calibration curve, standard deviation of intercept of calibration curve, limit of detection, limit of quantification, and recoveries at three concentration levels.

Analyte	Regression equation	s_a	s_b	r	*LOD* / ng g^{-1}	*LOQ* / ng g^{-1}	Recovery / % ± SD (n = 9)		
							c = 20 ng g^{-1}	c = 50 ng g^{-1}	c = 100 ng g^{-1}
BPA	$y = 0.002317\,x + 0.00085$	0.000023	0.00011	0.9985	1.5	4.4	88.9 ± 5.5	60.6 ± 4.7	60 ± 12
BPS	$y = 0.04192\,x - 0.0084$	0.00037	0.0018	0.9990	1.4	4.1	45.8 ± 8.2	52 ± 12	58.6 ± 7.6
BPP	$y = 0.002852\,x + 0.00107$	0.000023	0.00011	0.9992	1.2	3.7	109.8 ± 7.6	105 ± 10	100.7 ± 2.2
BPG	$y = 0.002052\,x + 0.00196$	0.000033	0.00010	0.9946	1.5	4.5	103.3 ± 7.2	87.5 ± 2.1	90.8 ± 7.0
BPM	$y = 0.003364\,x + 0.000895$	0.000013	0.000064	0.9995	0.6	1.8	115 ± 14	103.7 ± 4.2	106.0 ± 4.6
BPBP	$y = 0.002485\,x + 0.00169$	0.000022	0.00010	0.9989	1.3	4.0	102 ± 13	66 ± 10	60 ± 13
BPZ	$y = 0.004991\,x + 0.001233$	0.000020	0.000096	0.9997	0.6	1.9	89 ± 14	73.3 ± 8.8	71 ± 19
BPFL	$y = 0.01013\,x + 0.0607$	0.00045	0.0021	0.9933	8.2	25	70 ± 13	70.9 ± 8.9	78.5 ± 7.9
BPC	$y = 0.0013883\,x + 0.000806$	0.0000034	0.000016	0.9992	0.4	1.2	111 ± 13	69.0 ± 3.6	71.4 ± 7.5
BPF	$y = 0.002831\,x + 0.001144$	0.000018	0.000088	0.9995	1.0	3.0	64 ± 20	72.6 ± 9.6	81.5 ± 6.9
BADGE·HCl	$y = 0.038057\,x + 0.00182$	0.000086	0.00041	0.9999	0.4	1.1	102.6 ± 9.5	85.1 ± 5.7	86.8 ± 4.0
BADGE	$y = 0.10232\,x + 0.0323$	0.00035	0.0017	0.9998	0.5	1.6	106.5 ± 5.2	90.0 ± 3.4	85.6 ± 7.5
BFDGE·2HCl	$y = 0.02598\,x + 0.01042$	0.00012	0.00057	0.9996	0.7	2.1	106.0 ± 2.7	87.2 ± 2.4	90.0 ± 5.7
BADGE·H_2O·HCl	$y = 0.03294\,x + 0.06268$	0.00016	0.00079	0.9997	0.8	2.4	105.1 ± 6.1	93.2 ± 7.8	92.8 ± 3.8
BFDGE	$y = 0.04814\,x + 0.00392$	0.00011	0.00051	0.9999	0.3	1.0	107.7 ± 3.6	91.7 ± 1.3	91.4 ± 5.7
BADGE·2HCl	$y = 0.004048\,x + 0.003677$	0.000011	0.000053	0.9987	0.4	1.3	80.8 ± 5.9	75 ± 10	83.7 ± 6.7
BADGE·H_2O	$y = 0.05415\,x + 0.01673$	0.00020	0.00097	0.9998	0.6	1.7	93.2 ± 3.8	86.0 ± 9.6	87.8 ± 1.5
BADGE·2H_2O	$y = 0.07478\,x + 1.3493$	0.00068	0.0032	0.9992	1.4	4.2	99 ± 14	81.8 ± 7.3	84.6 ± 7.5
BFDGE·2H_2O	$y = 0.05255\,x + 0.0124$	0.00024	0.0012	0.9996	0.7	2.1	87.8 ± 4.1	77.5 ± 6.6	85.0 ± 7.3

by using two separate chromatographic method. For determination of BPA, BPS and BPA-C13 isocratic mode with water (component A) and methanol (component B) as mobile phases were used (55% of water and 45% of methanol) while for diglycidyl ethers with other bisphenols 0.01% ammonia solution in water (component A) and methanol (component B) were used.

3. Results and discussion

Obtained calibration curves were linear in tested concentration range. Limit of detection, limit of quantification and correlation coefficient were calculated by using weighted regression. Obtained data are shown in Table 1 together with recovery values. Results obtained for real samples analysis are shown in Table 2. BPA was quantified in the vast majority of tested samples (81%) with the concentration ranged between 5.0 ng g^{-1} to 520 ng g^{-1}. During the experiment also BADGE·2HCl was characterized by high concentration in the range from 6.8 to 530 ng g^{-1}, but it is frequency of determination was incomparably lower (15% of tested samples). The frequencies and bisphenols content in this research is comparable to determination of bisphenols in feminine hygiene products [6] and infant clothing [7]. Bisphenol A was quantified in infant clothing in 82% of tested samples [7] while in pads, panty liners and tampons frequency ranged from 69 to 92% [6].

Table 2
Concentrations and frequencies of determination bisphenols and their derivatives in disposable baby diaper samples (ND –not detected, NA – not analyzed).

Analyte	Concentration found / ng g^{-1}							
	Absorbent core				Supporting wings			
	Min	Median	Max	Frequency / %	Min	Median	Max	Frequency / %
BADGE·$2H_2O$	12	13	15	4.2	5.3	30	79	13
BADGE·2HCl	6.8	15	530	13	12	21	69	17
BFDGE	17	39	66	13	6.1	10	220	26
BADGE·H_2O·HCl	6.3	8.6	13	4.2	8.5	13	33	8.7
BADGE	20	49	96	17	4.9	43	98	17
BADGE·HCl	15	25	36	8.3	6.2	17	43	13
BPF	5.2	11	53	13	43	52	55	4.3
BPC	ND	ND	ND	ND	8.2	13	18	4.3
BPZ	8.6	11	18	4.2	ND	ND	ND	ND
BPBP	5.2	5.4	5.6	4.2	ND	ND	ND	ND
BPG	5.5	6.1	8.6	8.3	ND	ND	ND	ND
BPA	5.1	9.7	200	75	5	8	520	87
BPS	NA	NA	NA	NA	NA	NA	NA	NA
BPP	ND	ND	ND	ND	ND	ND	ND	ND
BFDGE·$2H_2O$	ND	ND	ND	ND	ND	ND	ND	ND

4. Conclusions

Bisphenol A was found in the vast majority of tested samples (81%). Other bisphenols were determined not more often than in 26% of tested samples. Although bisphenols and their derivatives were quantified in disposable baby diapers, it is impossible to judge if they can be harmful with such doses. To make sure about safety of personal care products dedicated for children, estimation of dermal exposure should be done and compared with present regulations.

Acknowledgments

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Investigation of burdock tea: Spectroscopic and electrochemical study

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Abstract
The aqueous extract of burdock root exhibits variability in color development over time. Spectrophotometric and electrochemical characterization of these changes in the aqueous extracts of burdock root were examined in distilled water, very hard water, phosphate buffers pH = 7.0, 7.5, 8.0, and Britton-Robinson buffer pH = 8.0. Furthermore, the effects of EDTA and inorganic ions in the aqueous extracts of burdock root were investigated.

1. Introduction

Tea plays a vital role in most individual's daily routines, making it an integral part of their lives. Whether used for its pleasant taste or its numerous health benefits, tea is one of the most consumed beverages worldwide [1]. Two prominent characteristics of tea as a beverage are its taste and color. The composition of tea is influenced by various factors, such as the region in which they are cultivated and the time of harvesting while the antioxidant activities of tea infusions are influenced by their preparation methodologies [2–3]. Previous studies have shown that a correlation between the quality of tea and multiple factors, including the environment, the variety of tea plants, the cultivation practices employed, and the technology used during processing, collectively influence the fundamental characteristics of tea, such as color, aroma, and taste [4].

Arctium lappa L., commonly called burdock, is a medicinal plant from the Asteraceae family known for a diverse range of biological activities and has been widely used in folk medicine to treat different conditions such as skin diseases, digestive and genitourinary tract disorders [5]. Moreover, root extracts have shown hepatoprotective effects in murine models of liver injuries, preventing mucous injuries caused by alcohol [6]. Arctium species have many scientifically proven biological properties related to phenolic compounds, including a wide range of benefits including reduction of inflammation, antitumor activity, antidiabetic, antimicrobial, antifungal, and antioxidant. The primary metabolites found in burdock extracts include some carbohydrates, such as inulin, galactose,

rhamnose, glucose, mannose, and fructans. Polysaccharides used as an inulin source are present mainly in burdock roots [6–7]. In addition to its traditional medicinal uses, burdock has also found its utility in the food industry and the cosmetic industry, particularly in shampoos and conditioners, due to its anti-dandruff properties and hair health benefits. [8].

The aqueous extract of Burdock root exhibited variability in color development over time, the color changing from golden dark to bright green. While this manifestation was not studied before, in order to understand the cause of this transformation and the qualities of burdock root tea, spectroscopic and electrochemical studies were carried out.

2. Experimental

2.1 Reagents and chemicals

Deionized water, potassium dihydrogen phosphate (Lach-Ner), ethanol 96% (Lach-Ner), potassium phosphate anhydrous (J.T. Baker), magnesium chloride hexahydrate (Lachema), manganese chloride tetrahydrate (Lachema), calcium chloride anhydrous (Penta), sodium bicarbonate (Lachema), sodium hydroxide (Penta), barium chloride (Lachema), potassium chloride (Penta), acetic acid 99% (Lach-Ner), methanol ≥99% (Honeywell), sodium acetate (Sigma), magnesium sulfate (Sigma), calcium sulfate (Sigma), ethylenediaminetetraacetic acid (Penta).

2.2 Sample preparation

2.2.1 Preparation of buffers

For the preparation of infusions, several types of phosphate buffers were used, with a pH of 7.0, 7.5, and 8.0, which were prepared by mixing respectively 9.343 g L^{-1} K_2HPO_4 and 6.309 g L^{-1} KH_2PO_4; 12.813 g L^{-1} K_2HPO_4 and 3.598 g L^{-1} KH_2PO_4; 16.282 g L^{-1} K_2HPO_4 and 0.8878 g L^{-1} KH_2PO_4.

Very hard water was prepared, by mixing the exact amount of $NaHCO_3$ (0.384 g), $CaSO_4$ (0.24 g), $MgSO_4$ (0.24 g), KCl (0.016 g) in distilled water.

Britton-Robinson buffer was prepared by measuring 1.16 mL CH_3COOH (0.04 mol L^{-1}), 1.35 ml of H_3PO_4 (0.04 mol L^{-1}), 1.2366 g of H_3BO_3 (0.04 mol L^{-1}). Next, a 0.2 mol L^{-1} NaOH solution was prepared. A pH = 8.0 buffer was prepared by mixing appropriate amounts of Britton-Robinson buffer and NaOH solution into a 250 mL flask. The pH values were always checked using a pH meter with a glass electrode.

2.2.2 Preparation of EDTA solutions and inorganic salts

EDTA solutions were prepared by mixing a certain amount of loose disodium EDTA salt (0.05 g, 0.1 g, 0.2 g, 0.3 g, 0.5 g, 0.8 g, 1 g) with 20 ml of burdock extract

solution in phosphate buffer pH = 8.0 into glass flasks with closable lids. For control, a blank experiment with only burdock extract solution without the addition of EDTA was prepared.

Furthermore, 0.2 mol L^{-1} and 0.1 mol L^{-1} EDTA solutions were prepared in 250 mL flasks. The solutions were diluted with distilled water to the required concentrations (0.05 mol L^{-1}, 0.033 mol L^{-1}, 0.025 mol L^{-1}, 0.05 mol L^{-1}, 0.005 mol L^{-1}, 0.0005 mol L^{-1}), then 1 mL of each concentration was mixed in a glass flask with a closable lid with 20 mL of burdock extract in phosphate buffer pH = 8.0. A blank was prepared in the same way, but distilled water was used instead of the EDTA solution. Chlorides (Mn^{2+}, Mg^{2+}, Ca^{2+}), and sulfates (Mg^{2+}, Ca^{2+}), were accurately weighed on analytical balances and transferred to a 100 mL flask to make up a final concentration of $MgSO_4$ 0.5 mol L^{-1} and $CaSO_4$ 1.84×10^{-2} mol L^{-1}. Furthermore, solutions with a concentration of 0.25 mol L^{-1}, 0.33 mol L^{-1}, 0.1 mol L^{-1} $CaSO_4$ and solutions with a concentration of 9.2×10^{-3} mol L^{-1}, 3.07×10^{-3} mol L^{-1} were prepared in the same way.

2.3 Instrumentation

Spectrophotometric measurements were conducted using the Shimadzu UV-VIS UV-2600 instrument. Samples were measured in quartz cuvettes (10 mm). The measurement range was set to 800–450 nm. The measurements were performed at a scanning speed of "medium" with a sensitivity of 0.1 nm.

Both freshly prepared extracts and extracts stored in darkness for 24 hours were measured. The results were processed using UVProbe software version 2.7 (Shimadzu).

All electrochemical measurements were carried out in a conventional three-electrode system. It consisted of one working electrode, carbon paste electrode; saturated calomel electrode as the reference electrode, and Pt-plate as the counter electrode. This electrode setup was connected to a potentiostat/galvanostat Autolab PGSTAT101 operated via the Nova software (version 1.11; Metrohm). The measurements were conducted in a glass voltammetric cell containing 16 mL of the sample under investigation. Prior to each measurement, it was necessary to renew the surface of the carbon paste electrode by polishing it for 10 seconds to remove any residual water and surface contaminants.

Changes in color for samples were measured by the UltraScan VIS spectrophotometer (Hunter Associates Laboratory, USA) in a reflectance mode against a white tile. The color was expressed as CIELAB color space values L^* [dark (0) to light (100)], a^* [red (+) to green (−)], b^* [yellow (+) to blue (−)].

3. Results and discussion

3.1 Effect of pH

At first, it was necessary to find out whether the pH values influence the color of the burdock extract, and in that case, several burdock extracts were prepared and monitored under different conditions with three types of water and buffers. The results show that the pH values of freshly prepared solutions and solutions after 24 hours differ for some extracts. The burdock extract in demineralized water and the burdock extract in very hard water experienced a rise in pH value after 24 h from 6.1 to 6.3 and 7.3 to 7.4 respectively. Furthermore, for some extracts, the pH value decreased after 24 hours, for the burdock extract in Britton Robinson buffer pH = 8.0, where the pH value dropped to 7.7, and for the burdock extract in phosphate buffer pH = 7.0, where the pH value decreased from 6.9 to 6.8. It is possible that these minimal changes in pH values after 24 hours may affect the stability of extracts, especially for extracts with unstable pH; therefore, only phosphate buffer (pH = 8.0) was used in further experiments.

Table 1 shows the color parameters of various infusions, where the L^* value ranges from 59.9 to 87.9. The burdock extract in phosphate buffer with pH = 8.0 showed a significantly darker color ($L^* = 76.05$). After 24 hours, there was a significant decrease in L^* for the extract in very hard water (a decrease of approximately 25 units) and for the extract in phosphate buffer pH = 8.0 and Britton-Robinson buffer pH = 8.0 there was a decrease of 10 units.

The burdock extract in very hard water with a value of 59.9 and the burdock extract in phosphate buffer pH = 8.0 with a value of 65.3 are significantly different and are closer to the middle of the scale. The parameter a^* (transition between green and red color) was positive for the freshly prepared solution of burdock root in deionized water, while the other fresh extracts were slightly greener. After 24 hours, the a^* value decreased, and the samples were greener. The greatest change was observed in the very hard water extract (change of 25 units) and in the extract in phosphate buffer pH = 8.0 (change of 10 units).

Parameter b^* (transition between yellow and blue color) was for all samples in the positive quadrant, where the yellow color prevailed in the samples. It can be seen from the table that the samples in very hard water and phosphate buffer pH = 8.0 were more yellow than the other samples. After 24 hours, the value of the b^* parameter was lower (less yellow) in these samples.

According to the C^* parameter, all samples, except for the extract in Britton-Robinson buffer, are close to 100, indicating a small representation of gray color and high saturation. The position on the standard color wheel, expressed as the h value, usually ranges between 22.9 and 49.2. The extract in Britton-Robinson buffer pH = 8.0 is in a completely different region (89.7 to 98.1). The h value was the highest for freshly prepared Britton-Robinson buffer pH = 8.0 but it decreased by 10 degrees over time. The burdock extracts prepared in very hard water and

Table 1
Measured pH, color values (expressed as CIE color space values: L^* for perceptual lightness, a^* and b^* for the four unique colors of human vision: red, green, blue and yellow, C^* for chroma, and h for hue), and the actual color of the sample detected according to the parameters $L^*a^*b^*$. Values expressed as the arithmetic mean ± standard deviation.

Burdock extract prepared in	pH	L^*	a^*	b*	C^*	h / °	Color
Deionized water	6.1	87.9±3.4	1.8±0.5	23.9±4.4	87.9±4.4	23.9±1.7	
Deionized water after 24 h	6.3	85.85±1.5	–2.25±0.1	22.5±1.4	85.9±1.4	22.6±0.5	
Very hard water	7.3	85.1±2.4	6±1.4	37.8±1.9	85.1±1.7	38.3±2.4	
Very hard water after 24h	7.4	59.9±3.1	–30.5±2.4	23.7±4.3	59.9±1	38.9±7	
Phosphate buffer pH = 8.0	8.0	76.05±2	–4.5±2.3	48.9±3	76.1±2.9	49.2±2.8	
Phosphate buffer pH = 8.0 after 24h	8.0	65.3±1.1	–14±2.3	42.1±1.4	65.3±1.2	44.4±3.1	
Phosphate buffer pH = 7.5	7.3	84.3±0.4	3.9±0.4	34.8±0.1	84.4±0.1	35±0.6	
Phosphate buffer pH = 7.5 after 24h	7.3	81.2±0.4	–5.2±0.7	37.6±0.4	81.2±0.9	38±1.2	
Phosphate buffer pH = 7.0	6.9	82.9±1.3	–2.2±0.5	35.7±2.2	82.9±2.2	35.8±1.1	
Phosphate buffer pH = 7.0 after 24h	6.8	83.5±2.1	–0.9±0.8	40.1±3	83.5±3	40.1±1.3	
Britton-Robinson buffer	8.0	81.9±3.5	–4.6± 0.7	32.6 ±1.6	32.9 ±1.5	98.1± 1.6	
Britton-Robinson buffer after 24h	7.7	71±9.4	0.1± 2.7	36.4± 1.8	36.4 ±1.8	89.7 ±4.3	

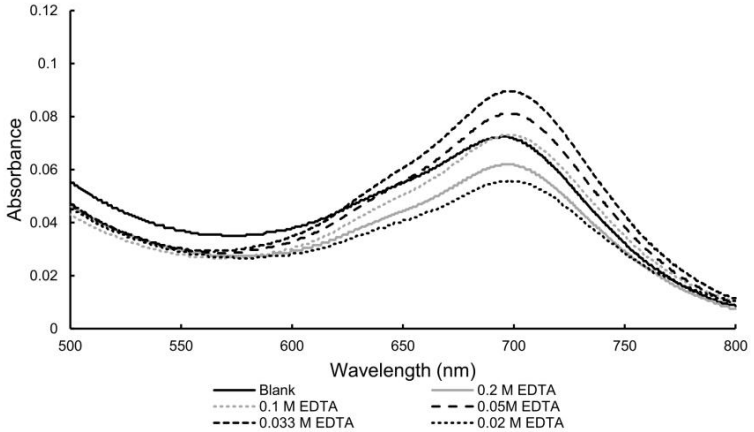

Fig. 1 Effects of the addition of EDTA solutions in different concentrations on burdock extract in phosphate buffer pH= 8.0.

phosphate buffer with pH = 8.0 differed the most, where their values significantly decreased compared to other samples. There was a change in the parameter a^* where from yellow completely changed to green. Since the greatest color changes occurred with burdock extract in very hard water and phosphate buffer pH = 8.0, the influence of pH can be assumed for these samples. Therefore, further experiments were used to monitor the effect of the addition of EDTA and metal ions.

3.2 Effects of addition of EDTA and inorganic ions

The change in the color and spectral properties of burdock extract in very hard water and in phosphate buffer pH = 8.0 can be caused by the formation of a complex of phenolic substances with some mineral substances. Assuming that the changes in the color of the burdock extract are due to the presence of a complex, it can be expected that the addition of a chelating agent may decompose the complex and lead to the loss of the green color. This assumption applies if the stability of the complex present in the burdock root extract is less than the stability of the complex of mineral substances with EDTA. In the first experiment, different amounts of EDTA were added to freshly prepared burdock root extracts in phosphate buffer pH = 8.0.

After the addition of EDTA solutions (Fig. 1) there was a decrease in absorbance at 699 nm, indicating the possibility that the original unknown complex was disrupted and a new complex with EDTA was formed. Unfortunately, no direct dependence on the EDTA concentration has been demonstrated. Originally, a clear connection between the concentration of EDTA and the change in absorbance was expected (the higher the concentration, the greater the change), this fundamental discrepancy suggests that the possible formation of a complex in the burdock root

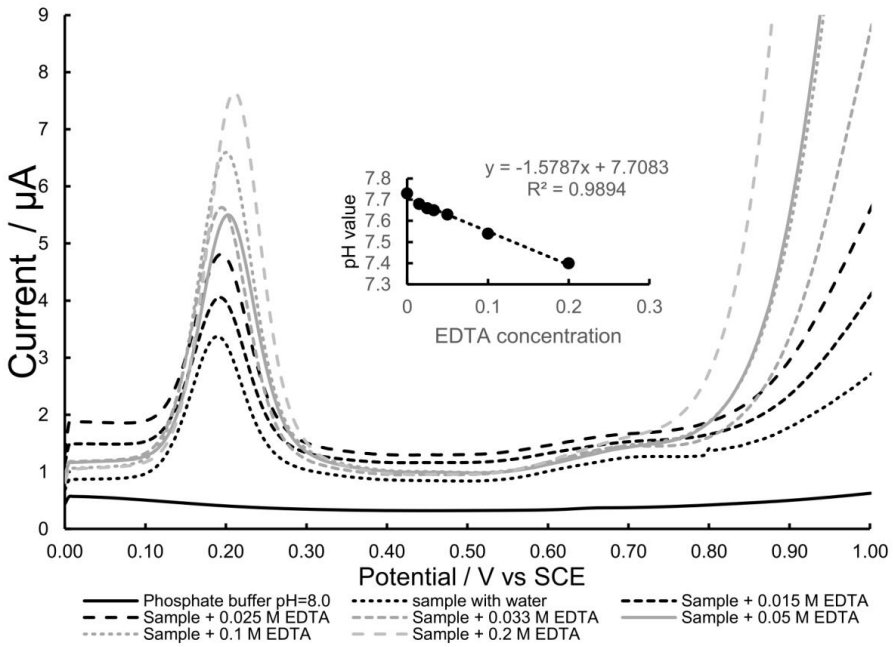

Fig. 2 Voltammetric measurement of the addition of EDTA in different concentrations with burdock extract in phosphate buffer pH = 8.0 and the dependence of EDTA added concentration on the pH of the solutions.

extract may not be related only to the concentration of mineral and organic substances present. It is possible that the color formation of the complex occurs in connection with other unknown factors, such as the concentration of oxygen, carbon dioxide, etc.

After selecting the working electrode and working conditions (initial potential (0.0 V), end potential (+1.2 V), frequency (10 Hz), amplitude (25 mV s^{-1}), and step potential (5 mV)), voltammetric measurements of the same infusions as the previous experiment were performed (Fig. 2). All extracts with the addition of an EDTA solution showed higher current yields with a potential of 0.2 V compared to the extract in distilled water. As the EDTA concentration decreased, so did the current value. The highest current was measured in the extract with the addition of 0.2 mol L^{-1} EDTA (7.64 µA), the lowest current was measured in the extract with the addition of water (3.31 µA). At a potential of 0.20 V, almost no significant shift was observed. The pH values of the solution range from 7.4 to 7.68.

Figure 2 also shows the overall dependence of the concentration of added EDTA on the pH of the solutions, and the result is that the higher the concentration of EDTA in the extract, the greater the pH value decreased from the original 8.0 to 7.4 Although this difference may appear to be negligible, previous experiments have shown that simply changing the pH of the extract from 8.0 to 7.5 resulted in a significant change in color.

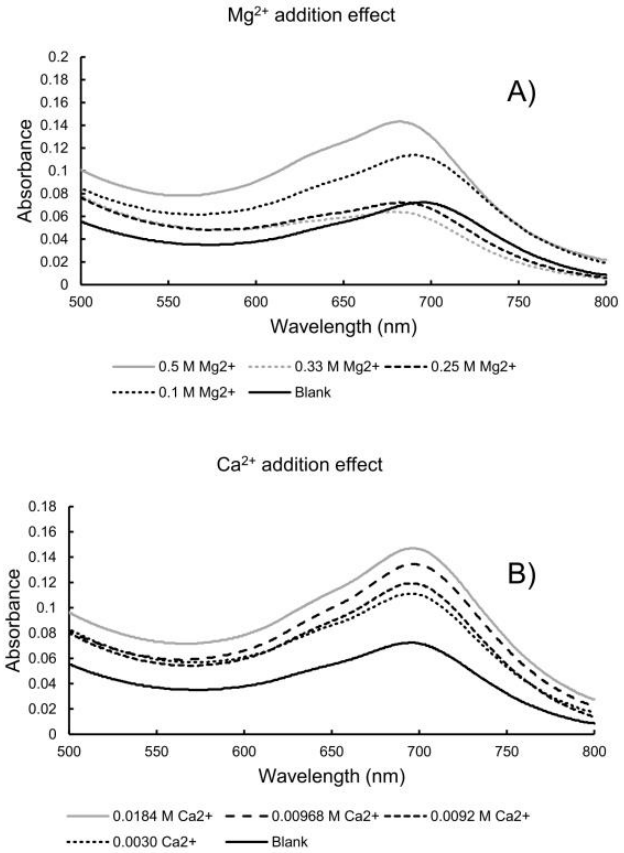

Fig. 3 Addition effect of (a) $MgCl_2$, and (b) $CaCl_2$ solution in different concentrations on burdock extract.

As shown in Fig. 3, $MgSO_4$ and $CaSO_4$ solutions were used to test their effect on the extract. It was found experimentally that Mg^{2+} and Ca^{2+} sulfate cations have an effect on the extract. The addition of different concentrations of cations caused an increase in absorbance compared to the blank. This means that Mg^{2+} and Ca^{2+} ions can influence the formation of a colored complex in the extract. In this case, even using a lower solution concentration increased the absorbance. The most significant increase in absorbance was observed for the Mn^{2+} cation, for which a maximum value of 0.44 was reached. The Ca^{2+} cation follows with a maximum of 0.275. These observations confirm the assumption that the color change of burdock extract in phosphate buffer (pH = 8.0) can be caused by complexes with some divalent ions.

Figure 4 shows a clear influence of the current response on cation concentration. The blank current value is 11.57 μA while the current value for Mg^{2+} ions is 14.92 μA for 0.005 mol L^{-1} and 21.43 μA for 0.0005 mol L^{-1}. Even in this case, it was confirmed that a lower concentration of the ions has a greater effect on the current peaks. The potential was stable at 0.21 V. There is a large effect on the addition of Ca^{2+} cation in both concentrations. The blank current value is 11.57 μA versus 16.25 μA for 0.0005 mol L^{-1} and 16.26 μA for 0.005 mol L^{-1} Ca^{2+}. The differences in

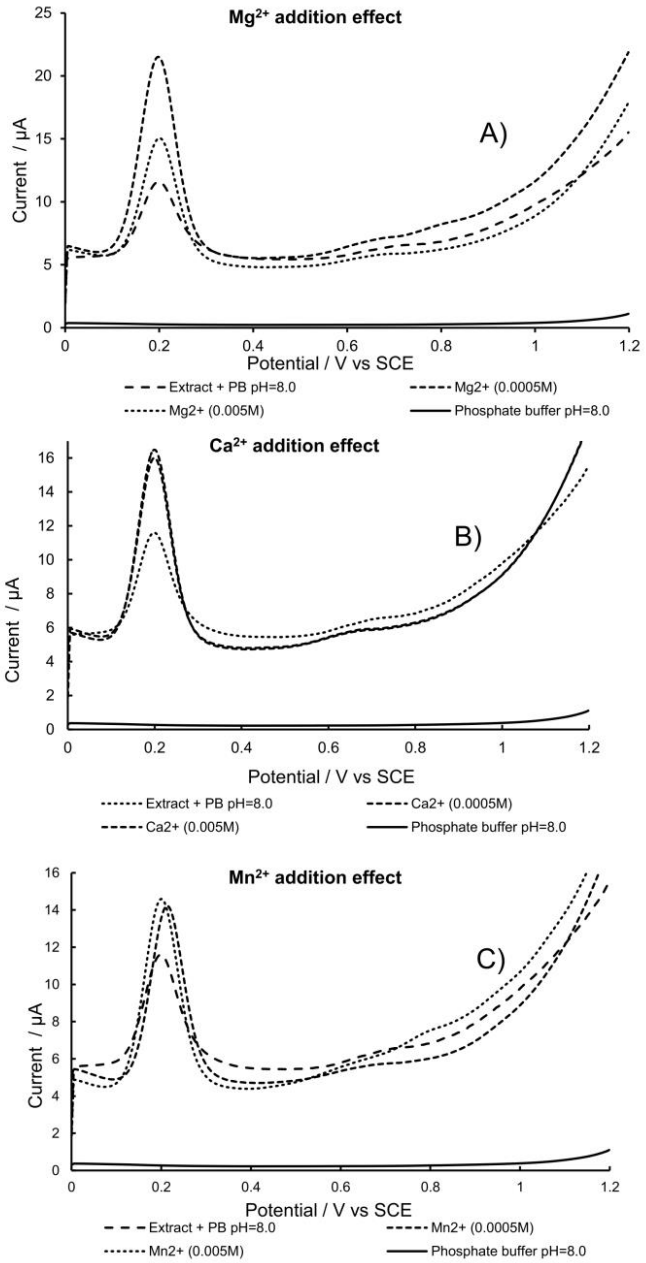

Fig. 4 Voltammetric measurement of (a) Mg^{2+}, (b) Ca^{2+}, and (c) Mn^{2+} ions addition in different concentrations of burdock extract in phosphate buffer pH = 8.0.

Mn^{2+} ion addition current yield are not significant. Thus, the current values for the blank are 11.82 µA compared to 14.11 µA for 0.0005 mol L^{-1} and 14.44 µA for 0.005 mol L^{-1} Mn^{2+}. The current yield is not related to the concentration of Mn^{2+}.

The voltammetric measurements were performed as a supplement to the UV/Vis measurement of burdock extract in phosphate buffer pH 8.0 in order to verify the results of this measurement and obtain additional information about

the electrochemical behavior of ions in the solution. It was observed that the addition of any concentration of Mg^{2+} to the burdock extract had an effect on the current response, but a linear relationship between the concentration of added Mg^{2+} and the current response was not demonstrated. The addition of Ca^{2+} ions had also a positive effect on the current yield, overall, the most significant effects were investigated at concentrations of 0.003 mol L^{-1} Ca^{2+} and 0.25 mol L^{-1} Mg^{2+}.

4. Conclusions

It can be concluded from the research that the color of the extracts is related to the resulting pH value, in the basic environment (pH = 8.0), a pronounced green color component predominates in terms of color.

Experiments showed that the addition of EDTA caused a decrease in the absorbance of the color of the extract in the 680–690 nm region (responsible for the green color), which was more related to a change in the value of pH at high EDTA concentrations (shift to the neutral region).

The electrochemical properties of burdock root extracts differed in the shift of the oxidation potential and the increase in the oxidation potential current. This effect is rather a consequence of the change in the pH of the solution. In this part of the research, it is not possible to unequivocally state that EDTA had an effect on the formation of a complex between metal and phenolic ions substances present in burdock extract.

As part of the investigation, selected cations were added and their influence on the spectral was evaluated. It was found experimentally that only Mg^{2+}, Ca^{2+}, and Mn^{2+} have a real influence on the color of the extract. The most significant increase in absorbance was observed for the Mn^{2+} addition, followed by the Ca^{2+} addition, and the smallest increase in absorbance was observed for the Mg^{2+} cation addition.

The whole experiment was accompanied by excessive variability in color development over time, which could have been caused by different sample particle sizes. Different concentrations of oxygen may also have played a role here, which must be excluded/confirmed in further experiments.

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Tyrosine oxidation behavior using electrochemistry, capillary electrophoresis, and mass spectrometry

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electrochemistry
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Abstract
The oxidation behavior of L-tyrosine was investigated at a carbon-based screen-printed electrode in ammonium acetate buffer (pH = 7.0) with an electrochemical flow cell coupled to mass spectrometry via capillary electrophoresis. Cyclic voltammetry and linear sweep voltammetry were used to examine the oxidation behavior of this compound. The migration behavior in capillary electrophoresis, mass-to-charge ratios, and the isotopic pattern of L-tyrosine oxidation products were investigated. The results showed that four different oxidation products could be detected in the potential between 0.0 V and 1.3 V. Since the measurement was carried out within a short time scale, both stable oxidation products and intermediates could be detected.

1. Introduction

Tyrosine is a non-essential amino acid related to crucial physiological events. Since its oxidation can cause beneficial or detrimental effects on biological systems, it has become one of the crucial targets of oxidation studies [1]. Additionally, this phenolic amino acid has become a topic of interest in neuroscience because of being a precursor of the neurotransmitters norepinephrine (noradrenaline) and epinephrine (adrenaline) [2]. Therefore, several methods are developed for understanding and studying the oxidation behavior of this electrochemically active material, including enzymatic oxidation of tyrosine by phenylalanine hydroxylase, and non-enzymatic oxidation by hydroxyl free radicals [3, 4].

Tyrosine is electrochemically active on different carbon-based electrodes allowing electrochemical scientists to easily study its behavior. In the most recent studies, Bao et al. [5] used carbon-based carbon black and graphene oxide co-doped electrodes for electrochemical testing and analyzing of tyrosine. Shumyantseva et al. [6] used screen-printed electrodes modified with multi-walled carbon

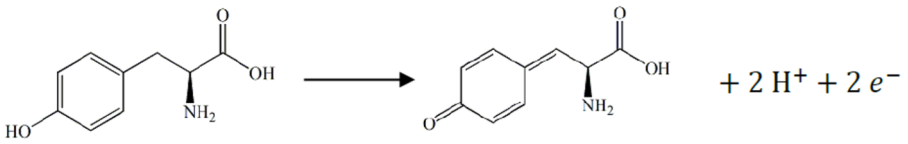

Fig. 1 Proposed oxidation reaction of electrochemical oxidation of tyrosine [6].

nanotubes and nanosized titanium oxide for oxidizing tyrosine and studying its oxidation mechanism by suggesting an oxidation reaction, in 2017. The proposed oxidation reaction is shown in Fig. 1. There are many benefits for electrochemical oxidation ranging from well-controllable parameters such as the wide variety of electrode materials, oxidation potential, reaction time, to simple setup [7]. Therefore, electrochemical oxidation is an attractive alternative to chemical oxidation processes.

Although classical electrochemical techniques represent several advantages, the limitation of not being able to identify the reaction product species, cannot be neglected. Apart from the oxidation protocols, for understanding the mechanism of the reaction and identification of the oxidation products other analytical methods needed to be hyphenated with electrochemistry [8]. In this context, coupling electrochemistry with mass spectrometry (EC-MS) is among the most powerful techniques since it allows the measurements to identify both products and intermediates of the electrochemical reactions. The versatility of MS stems from two facts; first, MS can be considered a powerful and sensitive detector for electrochemical cells, second, its compatibility with many electrochemical cells, and providing information related to the molecular weight and the isotopic patterns leads to reliable information about many target analytes. Furthermore, MS analysis can be used for structural determination based on ion dissociation [9]. Electrospray ionization (ESI), for the formation of ionic species in the gas phase, is often used as an ion source for MS analysis.

In this study, a real-time mass voltammogram was recorded by online EC-MS using an electrochemical flow cell with an integrated disposable electrode DRP-110, providing the potential dependent product profile. On the other hand, EC-CE-MS electropherograms resulting from measurements with and without electrochemical pre-treatment were investigated to study the separation behavior and the structural properties of the individual species.

2. Experimental

2.1 Reagents and chemicals

The following chemicals were used, of analytical grade or higher: acetic acid (Sigma Aldrich USA), formic acid (Merck, Germany), isopropanol (Roth, Germany), tyrosine and caffeine (Sigma-Aldrich, USA). Fused silica capillaries

(Polymicro Technologies, USA) with an inner diameter of 50 μm and an outer diameter of 360 μm were used. Disposable screen-printed carbon electrode type DS-110 was obtained from DropSens (Spain).

2.2 Instrumentation

2.2.1 Electrochemistry with mass spectrometry

The electrochemical conversion of tyrosine was performed using a modified flow cell (Metrohm DropSens, Spain) for screen-printed electrodes. Potentials were applied using a μAutolab III potentiostat, controlled by NOVA 2.0 software (Metrohm Autolab, The Netherlands). The sample solution flowed through the electrochemical flow cell by implementing a syringe pump of type UMP3 with a Micro2T pump controller (World Precision Instruments, USA) which was equipped with a 1 mL glass syringe. For MS detection, a Bruker micrOTOF mass spectrometer (Bruker Daltonics, Germany), equipped with a coaxial sheath liquid electrospray ionization interface (Agilent Technologies, Germany), was used. Sheath liquid (isopropanol:water:formic acid = 49.9:49.9:0.2; *v/v/v*) was injected using a syringe pump (Type 161553, KD Scientific, Holliston, MA, USA), equipped with a 1 mL glass syringe (ILS, Germany) at a flow rate of 8 μL min^{-1}. The MS was operated in positive mode using the following settings: m/z = 50–400, spectra rate 5 Hz, nebulizer 1.0 bar, dry gas 4.0 L min^{-1}, dry temperature 250 °C. A fused silica capillary (internal diameter 50 μm) with a length of 21 cm was used for the connection between the flow cell and the MS.

2.2.2 EC–CE–MS

65 μL of the sample solution was applied to the central region of the screen-printed electrode DRP-110. During electrochemical pretreatment, 0.8 V oxidation potential was applied for 20 s. Afterward, the separation capillary was automatically moved into the sample drop with an injection time of 10 s. The capillary was then moved back into the buffer vial. Electrophoretic separations were accomplished by applying a high voltage of 25 kV for all measurements. The separation capillaries had a length of 35 cm. The sample solution consisted of 1.0 mM tyrosine, and 0.05 mM caffeine as the electroosmotic flow marker in 50 mM ammonium acetate (pH = 7.0). 500 mM acetic acid with pH = 3.0 was used as the buffer for the separation.

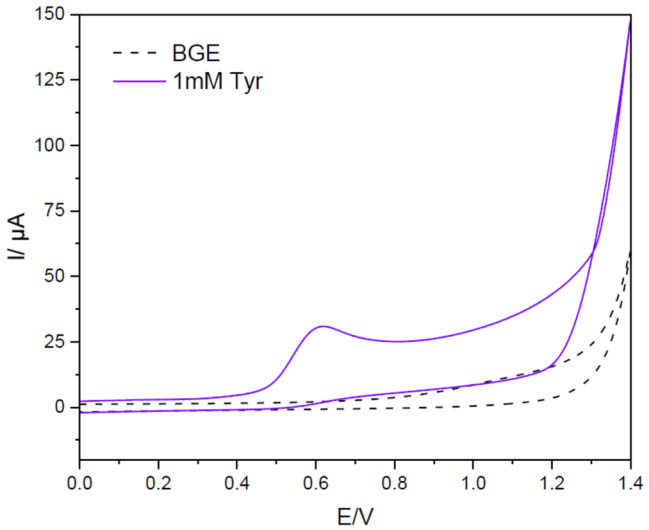

Fig. 2 Cyclic voltammogram of a 65 µL droplet of 1 mM tyrosine in 50 mM CH_3COONH_4 on a screen-printed carbon electrode DRP-110 at a scan rate of 50 mV s^{-1} (dotted line background electrolyte (BGE), solid line 1 mM tyrosine in backgroud electrolyte).

3. Results and discussion

3.1 Online EC-MS measurements

Since tyrosine is oxidizable on SPE DRP-110, all measurements were conducted using the same electrode. Figure 2 shows the cyclic voltammetry measurements of 1 mM tyrosine in 50 mM ammonium acetate pH 7.0. With the same sample solution, EC-MS measurements were performed to provide information about the relation between potential and the formation of oxidation products of tyrosine. Figure 3 shows that a mass voltammogram not only validates MS information (*m/z*, MS signal intensity) but also current-potential characteristics could be carried out at the same time. Here *m/z* indicates mass-to-charge ratios of positively charged species ($[M+H]^+$). As can be seen, after 0.4 V the intensity of the mass trace of tyrosine (*m/z* 182.08) decreases, and the intensity of the main oxidation product (*m/z* 180.06) increases and stays almost the same until the end of the measurement. Another oxidation product in the potential range of 0.6 V to 0.9 V (*m/z* 286.10) was also detected.

Two other species with mass-to-charge ratios of 270.06 and 107.04 show up in the potential range between 0.4 V to 0.9 V. The signal intensities of these two species are lower compared to the other oxidation products which makes it difficult to further investigate the details.

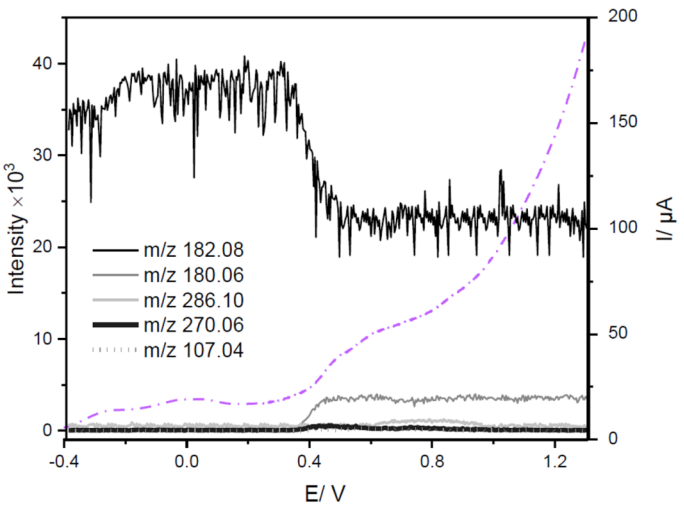

Fig. 3 EC-MS measurement of 1 mM tyrosine in 50 mM CH_3COONH_4 adjusted to pH = 7. The measurements were conducted on a screen-printed carbon electrode implemented in an electrochemical flow cell. Linear sweep voltammograms shown as dot and dashed lines were performed from 0.0 to 1.4 V at a scan rate of 10 mV s^{-1}. The flow rate of sample solutions was 16 µL min^{-1}. The inner diameter of the transfer capillary was 50 µm with the length of 23 cm.

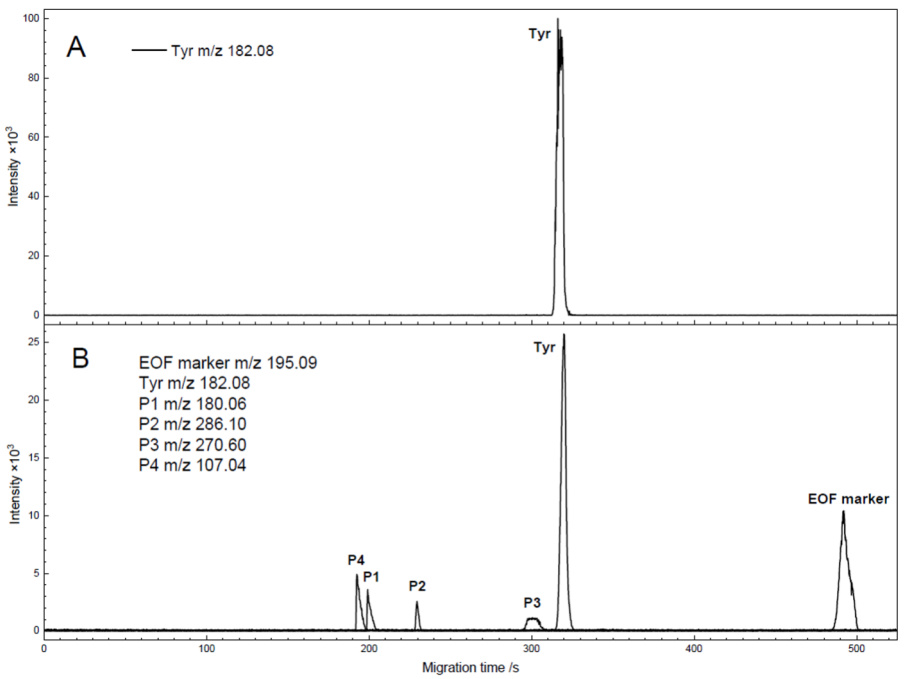

Fig. 4 Electropherograms of CE-MS measurements of 1 mM tyrosine in 50 mM CH_3COONH_4 adjusted to pH = 7.0: (A) without applying electrochemical pretreatment protocol, and (B) after applying electrochemical pretreatment protocol on an screen-printed carbon electrode DRP-110 at 0.8 V for 20 s. Capillary inner diameter was 50 µm with the length of 35 cm, 10 s hydrodynamic injection, separation voltage 25 kV.

3.2 Online EC-CE-MS measurement

Online EC-CE-MS measurements were performed to clarify the EC-MS measurement results. Figure 4A shows the electropherogram resulting from a measurement without electrochemical pre-treatment and 4B with applying electrochemical pretreatment. The electrochemical pretreatment was performed through the following protocol: a potential of 0.8 V was applied for 20 s to 65 µL of 1 mM tyrosine solution in 50 mM ammonium acetate adjusted to pH = 7.0. The migration behavior of the detected species indicates all the components are positively charged (cationic) because they all moved through the capillary and were detected before the electroosmotic flow marker (caffeine). Without applying electrochemical pretreatment no signal related to the oxidation products was detected while by applying electrochemical pretreatment 4 signals were recorded with specific migration time for each. Tyrosine (m/z = 182.08) migrates at 320 s and the signal related to the main oxidation product (m/z = 180.06) shows up at 200 s. The migration time of the other produced species with the mass-to-charge ratios of 107.04, 286.10 and 270.60 are at 190 s, 230 s, and 270 s, respectively.

The results indicate that not only does the main oxidation product forms on the surface of SPE DRP-110 but also 3 other components are forming after specific potential ranges, mainly after 1.4 V. Therefore, the application of EC-CE-MS resulted in the sufficient signal intensities to be recorded. The measured mass-to-charge ratios, the migration times, and the isotopic patterns of electrogenerated products are being studied for finding the possible chemical formula or structures. However, the provided data are still not enough for proposing possible structures for each detected chemical component. By considering such a limitation, still hyphenated techniques provide many useful information towards the identification of electrogenerated species, compared to pure electrochemical approaches.

4. Conclusions

To conclude, hyphenating electrochemistry with mass spectrometry can be very beneficial towards reaching more in-depth and detailed studies on different target analytes in comparison with pure electrochemical approaches. Coupling electrochemistry with mass spectrometry is a powerful approach to the characterization of redox products. By pure electrochemical studies, only one oxidation product of tyrosine could be detected while our approach differently claims what other oxidation products might be producing during electrochemical treatment of tyrosine in even higher potential values.

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Electrochemical visualization of latent fingerprints using polyphenazine dyes on the brass cartridges

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Keywords
brass cartridge
electrochemical deposition
latent fingerprint
polyphenazine

Abstract
The presented work is focused on the visualization of latent fingerprints left on brass unfired cartridges. Polymer films were prepared from 2 mM neutral red or 5 mM toluidine blue, respectively, using, alternatively, two different electrochemical methods (cyclic voltammetry or chronoamperometry) with relatively short polymerization time. The conditions for depositing conductive polymers poly(neutral red) and poly(toluidine blue) from a neutral medium (phosphate buffer with 0.1M KNO_3 or 0.1 M KNO_3, respectively) were optimized to preserve genetic information while producing high quality visualization of fingerprints left on brass substrates. The surface morphology and quality of the polymer films after the electrochemical deposition of both polyphenazine dyes were characterized optically. Finally, a methodology is proposed, how to apply this technique of visualizing latent fingerprints with observed details of papillary lines in forensic practice.

1. Introduction

Fingerprints, created by friction ridges placed on the palms, are used as basic evidence for identifying persons because of their uniqueness. Fingerprints can be found in visible, plastic or latent form. Latent fingerprints, which are invisible to the naked eye, are most often found at crime scenes [1, 2]. For their visualization, it is necessary to select a suitable dactyloscopic method.

Eccrine sweat glands, which are located mainly on the papillary lines, and sebaceous sweat glands, which are located on the head, contribute to the formation of latent fingerprints. Sebaceous fingerprint is largely made up of free fatty acids and their esters. A substantial part of both sebaceous and eccrine

fingerprint consists of water, which can easily evaporate during fingerprint aging [1–4]. In practice, we mainly encounter the fingerprints consisting of both of these components.

So far, there are only few techniques that successfully make fingerprint visible on metallic materials [5]. The choice of the most appropriate method depends primarily on the composition of a specific fingerprint and also on the surface on which the fingerprint is found [2, 6]. At crime scenes, there are often smooth or rough surfaces without pores, on which fingerprints settle and therefore they are easily destroyed.

The recovery rate of fingerprints from firearms and ammunition is very low, so the cyanoacrylate fumigation process is frequently used to visualize them. However, the combination of cyanoacrylate fumigation together with the application of Gun blue and fluorescent dye provides a better quality fingerprint [5, 6]. However, both of these methods are unfavorable for the health of the users, therefore, other suitable methods of visualizing fingerprint on these materials are being sought. Recently, the use of conductive polymers has been offered, because when applying a conductive polymer film, its formation and resulting thickness can be regulated in order to achieve optimal results. With the help of conductive polymers, it is possible to effectively make visible even low-quality fingerprints of cartridges [7].

Conductive polymers can be deposited on metal material either potentiostatically [7, 8] or by means of cyclic voltammetry [9]. Poly(neutral) red [9] and poly(toluidine blue) were used in our experiments. They are polyphenazine dyes that can be electrochemically deposited on various metal substrates [10]. During the application, the poly(neutral red) and poly(toluidine blue) are deposited on the metal substrate between the papillary lines of the fingerprint; this procedure creates a so-called negative image [2, 6, 8].

The goal of the work was to visualize latent fingerprint on unfired cartridges, using polyphenazine thin films, and to optimize and characterize this procedure for future application to fired cartridges from forensic practice.

2. Experimental

All commercial chemicals were used as-received without further purification. neutral red, toluidine blue, H_2SO_4, KH_2PO_4, $Na_2HPO_4.12H_2O$, KNO_3, acetone and ethanol were obtained from Lachema, Lach-ner and Penta (Czech Republic), respectively. Solutions of monomers were prepared i) in pH = 7 phosphate buffer with 0.1 M KNO_3 for 2 mM neutral red, and ii) in 0.1 M KNO_3 for 5 mM toluidine blue.

The cartridges were first chemically cleaned by soaking in a sequence of solutions: redistilled water, acetone, warm soapy water, and ethanol according to the Beresford procedure [11]. Subsequently, greasy fingerprint was applied to the dry substrate by i) washing the hands with warm soapy water, ii) allowing them to

Table 1
Conditions for the deposition of polyphenazine dyes on brass cartridges with applied fingerprints.

Sample label	N1	B2
Substrate	.45 AUTO	9 mm
Polyphenazine dye	Poly(neutral red)	Poly(toluidine blue)
Deposition technique	Cyclic voltammetry	Chronoamperometry
Parameters	Potential range = −200–500 mV	Applied potential = 500 mV
	Scan rate = 50 mV s^{-1}	Time interval = 10 ms
	Number of cycles = 8	Time of applied potential = 120 ms

dry freely, iii) the right thumb rubbing against the bridge of the nose and forehead due to the formation of a greasy film, and iv) with a slight pressure for period of 1–2 s fingerprint was applied.

Electrochemical measurements were performed with a PGSTAT-12 Autolab potentiostat/ galvanostat (Eco-Chemie, The Netherlands). The electrode cell was composed of a saturated Ag/AgCl reference electrode, a Pt large-area electrode as an auxiliary electrode, and brass cartridges as working electrodes, which were clamped with alligator clips.

The deposition of polyphenazine dyes was carried out under the conditions listed in Table 1, which were gradually optimized for the cartridges. After finishing the polymerization, the substrates were rinsed firstly in monomer-free base electrolyte and subsequently in redistilled water and allowed to air dry under laboratory conditions.

The objects were subsequently photographically documented using a Samsung S21 5G mobile phone, a Nikon SMZ1500 stereoscopic microscope with a Canon 1100D digital SLR (Japan), and a Mira 3 LMH scanning electron microscope (SEM) (Tescan, Czech Republic).

3. Results and discussion

Electrochemical methods suitable for the deposition of polyphenazine dyes on metal substrates are the cyclic voltammetry and chronoamperometry, which allow monitoring and controlling the formation of the emerging polymer layer. Figure 1 shows the deposition processes of poly(neutral red) (a) and poly(toluidine blue) (b) on brass cartridges.

During poly(neutral red) deposition, a cathodic peak around a potential of 0.07 V was observed in the first cycle. This peak shifted to a potential of 0 V as the number of cycles increased. The current value changed apparently during polymerization, which indicated the formation of a polymer film on the brass substrate.

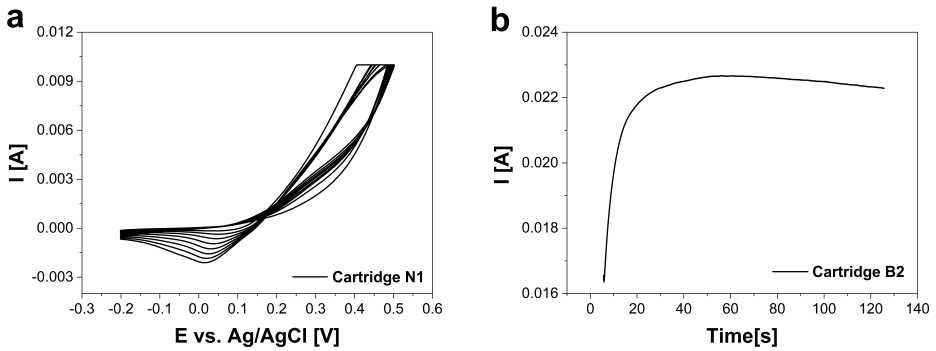

Fig. 1 Deposition of polyphenazine dyes on cartridges with fingerprint (a) cyclic voltammogram of poly(neutral red) in phosphate buffer with 0.1 M KNO_3 in the potential range –0.20–0.50 V (*vs.* Ag/AgCl) at scan rate of 50 mV s^{-1} for 6–10 cycles, (b) chronoamperogram of poly(toluidine blue) in 0.1 M KNO_3 at a potential of 0.50 V (*vs.* Ag/AgCl) for 60–120 s

At poly(toluidine blue) deposition, the current firstly rose steeply from 16 mA to approximately 23 mA and then fluctuated only slightly at this current value. The outline of these curves is very similar to the curves of electrodeposition of PEDOT on cartridges presented by Costa et al. [7]. During the electrolytic process the brass is partially dissolved and at the same time the poly(toluidine blue) polymer layer is deposited on the brass surface. However, the dissolution of the brass surface does not take place throughout the deposition, but only on its beginning, which can cause a stronger anchoring of the polymer film on the surface.

The thickness of the deposited polymer layer was not allowed to be greater than the thickness of the fingerprint grease, in order to avoid over-layering of the fingerprint, as observed by Costa et al. [7] at potentiostatic deposition of PEDOT. Regulation of the thickness of the resulting film can be achieved either by the variating of potential in certain interval or by applying a constant potential and variating the deposition time of film.

When optimizing the method, it was necessary to "soften" the polymerization conditions in order to eliminate the dissolution of brass to a minimum by choosing a narrower potential range for cyclic voltammetry, or setting lower potential values for chronoamperometry. The resulting polymer film was more homogeneous and brighter in color, as documented in Fig. 2. On the visualized fingerprint on the cartridges, the found second-level details 1–24 are marked with numbers. Some of them can be observed with the naked eye, for example terminations, bifurcation and eyelets.

The morphology of the polymer layer, fingerprint and their interface was determined using SEM. It is clear from Fig. 3 that poly(neutral red) forms fibers with a diameter of about several units m, while poly(toluidine blue) forms rather shorter oval (granular) structures. Both polymer films cover the brass surface without any defects.

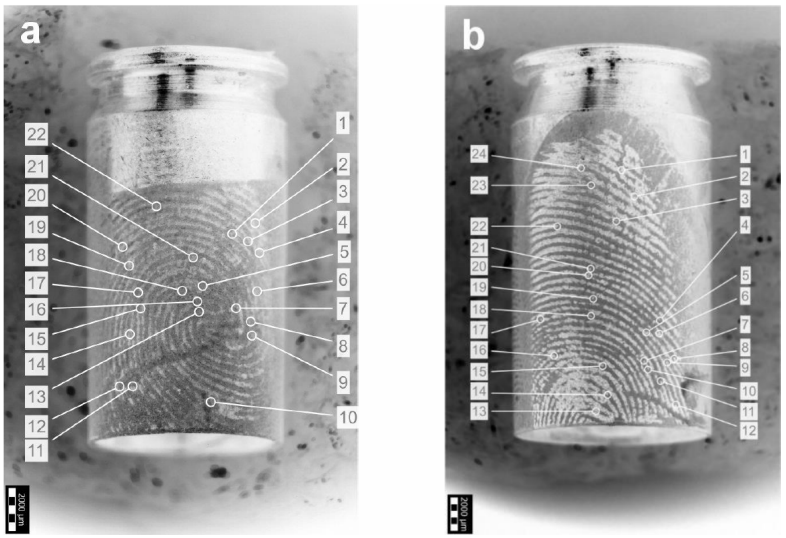

Fig. 2 Unfired brass cartridges after deposition of (a) poly(neutral red), and (b) poly(toluidine blue) with visualized grease fingerprint and associated second-level details (image taken using a stereoscopic microscope)

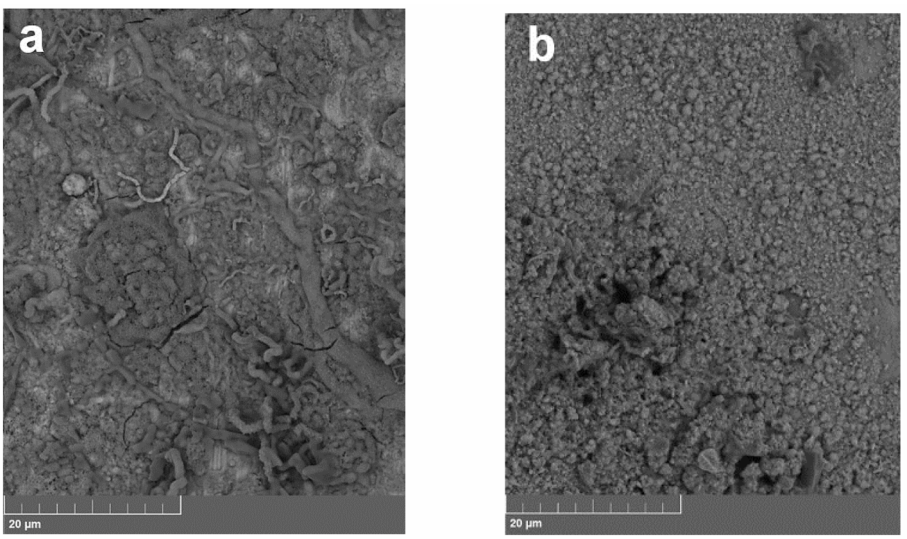

Fig. 3 Scanning electron microscope images of the morphology of polyphenazine films taken in the mode of back-scattered electrons of (a) poly(neutral red) and (b) poly(toluidine blue)

The modified brass substrates were characterized by FTIR spectroscopy and profilometry. The results will be processed in the next study.

4. Conclusions

This proposal of a simple fingerprint visualization method based on electrochemical deposition of poly(neutral red) and poly(toluidine blue) from a neutral environment, where the damage to the genetic information is minimized, could facilitate the fingerprinting of cartridges in practice. The parameters of both visualization methods (base electrolyte, potential range, number of cycles or applied potential, and deposition time) were gradually optimized until the imprint was sufficiently visible. The morphology and structure of the modified surfaces were studied with fingerprints and polyphenazine dyes. Further development of the method and, above all, the application of the method to fired cartridges, which are a key topic in forensic practice, are expected.

Acknowledgments

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Determination of bioplasticizers in water samples by a new SPME-GC-MS/MS method

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Keywords
bioplasticizers
environment
SPME
water

Abstract
Nowadays, bioplasticizers are gradually replacing fossil plasticizers in the production of plastics. However, their effects on the environment are not properly investigated. Microextraction techniques enable the extraction and concentration of analytes with minimal use of organic solvents or sample pretreatment. They are suitable for the analysis of environmental water samples. The aim of this work was the development of solid phase microextraction (SPME) for the extraction and concentration of bioplasticizers from aqueous matrices.

1. Introduction

The unsustainability of fossil resources and their impact on human health and the environment have highlighted the need to use "greener" sources of plastics. Research is turning to the development of environmentally sustainable and renewable alternatives to commercial petroleum-based plasticizers [1]. Bioplasticizers such as epoxidized vegetable oils, citrates, and isosorbide esters are mostly obtained from biomass sources. However, during the degradation of bioplastics, bioplasticizers escape into the environment and subsequently reach the surrounding soil and water, so it is necessary to closely monitor them in the environment even at very low concentrations. The goal of this work was the development of a microextraction technique for selected bioplasticizers from water matrices. Compared to the conventional extraction methods, microextractions are faster, simpler, cheaper, and more ecological [2]. Solid phase microextraction (SPME) uses a smaller sample volume and a small amount of organic solvent; therefore, it falls under the ideals of "green" chemistry.

2. Experimental

2.1 Reagents and chemicals

HPLC grade acetonitrile (Chromasolv LC-MS ≥99.9%) and ultra-pure water (LC-MS Chromasolv™) were obtained from Honeywell (Germany). Following pure standards of plasticizers from Sigma-Aldrich (Germany) and TCI Europe (Switzerland & Liechtenstein) were used: *N*-methyl-2-pyrrolidone, 1-ethyl-2-pyrrolidone, dimethyl adipate, triacetin, 1,2,3-propanetriol triacetate, diethyl adipate, trimethyl citrate, 2,2,4-trimethyl-1,3-pentanediol diisobutyrate, triethyl citrate, diisobutyl adipate, 1-hydroxycyclohexyl phenyl ketone, acetyl triethyl citrate, dibutyl adipate, *N*-butylbenzenesulfonamide, benzyl salicylate, methyl 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propionate, dibutyl sebacate, (tri)butyl citrate, tributyl acetylcitrate, bis(2-butoxyethyl) adipate, bis(2-ethylhexyl) adipate, 2-ethylhexyl diphenyl phosphate, diethylene glycol dibenzoate, and bis(2-ethylhexyl) sebacate.

2.2 Instrumentation

Direct immersion-SPME with 50/30µm DVB/CAR/PDMS fiber (Stableflex, 23Ga, Autosampler) was used for the extraction of selected plasticizers. Plasticizer analyses were performed using a Bruker 456-GC gas chromatograph coupled to a SCION triple quadrupole mass spectrometer (Bruker Daltonics, USA). A Factor-Four VF-5ms capillary column (30 m × 0.25 mm i.d., 0.25 µm) from Varian BV (Middelburg, The Netherlands) was used for chromatographic separation.

3. Results and discussion

The following parameters were optimized for the SPME extraction method: incubation time, incubation temperature, extraction time, desorption time, and desorption temperature. Another optimized factor in the extraction was also the type of the SPME fiber. The samples were firstly incubated in a heating chamber for 10 min at 70 °C (continuous stirring, 250 rpm), followed by SPME extraction with a 50/30 µm DVB/CAR/PDMS fiber, Stableflex, 23Ga, Autosampler for 60 min (continuous stirring, 250 rpm). After extraction, the fiber was transferred by autosampler to an inlet equipped with a 0.8 mm SPME glass insert for thermal desorption of analytes at 250–270 °C for 3 minutes in splitless mode. Plasticizers were detected by GC-MS/MS analysis and optimal parameters for SPME were selected by comparing the areas of chromatographic peaks of individual analytes. Headspace-SPME and Direct immersion-SPME were also compared. When implementing Direct immersion-SPME, a larger number of analytes was extracted.

4. Conclusions

This work dealt with the development of a new analytical method for the extraction and pre-concentration of bioplasticizers from water samples using SPME-GC-MS/MS in order to enable the monitoring of these substances and their degradation products in the environment at low concentration levels.

Acknowledgments

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Keyword Index

Contents

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