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It was in late 1993 when I visited Dr. Božidar Ogorevc (National Institute of Chemistry, Ljubljana, Slovenia) in the frame of quite intensive scientific collaboration. During frequent discussions we somehow found out that students in an advanced stage (master, PhD) are, in average, not very well prepared to present their subject and to defend it at an international conference in English. That time marks, in fact, the birth of YISAC, Young Investigators' Seminar on Analytical Chemistry, which we, in consequence, organized for the first time in Ljubljana in 1994, and then for a few years alternatingly in Graz and there.

Soon we realized that our colleagues and friends had similar experience with their students, so we finally agreed to expand the circle of participants to other institutions with which we had scientific cooperation; soon Czech Republic, then Slovakia, Italy, Poland, Bosnia and Herzegovina, Croatia, Serbia and Kosovo followed, so that nowadays YISAC comprises quite a notable number of participating institutions and universities. Until now the event was organized in Ljubljana, Graz, Venice, Pardubice, Sarajevo, Zagreb, Novi Sad, Nova Gorica and Maribor, and the next venue will be the University of Lodz in Poland. The 21st YISAC 2014 was held in Pardubice.

The meeting is intended to be an international conference for young scientists in a rather advanced state of their study, usually post-graduate, such as master or PhD study. The major goal of YISAC is to prepare young analytical chemists for their future appearances at occasions, such as attendance of international symposia, representation of own research achievements, project proposals etc.

To facilitate particularly the achievement of envisaged objectives, YISAC involves a few specific characteristics:

(a) the field of presentation / research is analytical chemistry and related areas;
(b) the conference addresses particularly students (master, PhD) and their supervisors;
(c) only oral presentations are accepted;
(d) the conference is divided into individual sessions presided by a chairlady / chairman; the chairing of sessions is also done by students;
(e) particular emphasis is paid on cultural and social aspects and approaching of the participants;
(f) basically the costs for the conference should be covered by the participants and/or their home institutions; sponsorships are particularly welcome.

YISAC 2014, organized by the University of Pardubice, was a particularly commemorative event. Apart from the fact that the presentations were again on a very high scientific level, comparable to other prestige conferences, the whole meeting was dedicated to a wonderful friend, an excellent scientist and a great person: Karel Vytras who celebrated his 70th birthday. Professor Vytras was the first non-Slovenian and non-Austrian partner to participate in YISAC, and due to his relentless efforts the University of Pardubice became the institution with the highest contingent of speakers and the most prize winners of all YISAC meetings so far. All the best to you, and hopefully we will see you at YISAC still many years!

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ICP-oTOF-MS ANALYSIS OF PLATINUM IN ALGAE AND HOSPITAL WASTEWATER

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Abstract

The ICP-oTOF-MS (orthogonal time-of-flight inductively coupled plasma mass spectrometry) and ICP-OES (inductively coupled plasma optical emission spectrometry) methods were evaluated with regard to their possible use for determination of Pt in hospital wastewater and algae *Parachlorella kessleri* gained from laboratory growth experiments. A way of sample preparation of algae prior analysis was optimised. For the ICP-oTOF-MS method (internal standard Rh 10 $\mu g\,L^{-1}$), instrumental LODs (limits of detection) were obtained on isotopes $^{194\,195\,196}$Pt 0.0241–0.0338 $\mu g\,L^{-1}$, procedural LODs for algae samples 0.121–0.169 $mg\,kg^{-1}$ and for WWTP (wastewater treatment plant) samples 0.0525–0.0845 $\mu g\,L^{-1}$. The ICP-oTOF-MS method was proved to be suitable for monitoring of Pt in algae and wastewater samples. The ICP-OES (Pt 214.42 nm) procedural LOD for algae samples 2.38 $mg\,kg^{-1}$ (instrumental LOD 4.76 $\mu g\,L^{-1}$) did not meet concentrations in algae from growth experiments. Pt in the WWTP wastewaters samples was followed without any preconcentration step. Lower concentrations were found in the WWTP output than in the input. Based on Pt in algae growth experiments, BCFs (bioconcentration factors) were estimated according to Pt species presented: 440–610 ($(NH_4)_2PtCl_6$), 690–1440 (the WWTP input) and 2510–2930 (the WWTP output), all in $L\,kg^{-1}$.

Introduction

The platinum group elements (PGEs) comprise rare metals platinum, palladium, rhodium, ruthenium, iridium, and osmium, which are noble, chemically stable and occur in nature as native alloys[1]. Their diverse features are particularly the resistance to chemical corrosion, high melting point, high mechanical strength, and good ductility, which predispose the PGMs to a diverse use (glass additives, chemical, electrical, electronics and petroleum industries, jewellery, cancer treatment drugs, dental alloys, etc.). The PGEs have gained importance as industrial catalysts recently. Since the worldwide introduction of fuel catalytic converters, platinum has become a subject of interest of researchers[2].

Exhaust gases from motor cars equipped with catalytic converters are the major anthropogenic source of PGEs pollution of particulate matters, road dust, soil, water, sediments and biota[3]. The additional contamination comes from hospital wastewaters, because the platinum compound cisplatin is frequently used as an antineoplastic drug in the cancer treatment[4]. Trace concentrations of the PGEs, especially platinum and its compounds, play an important role in the environment, because of their high allergenic and cytotoxic potential[5,6]. An analysis of water draining from roads and other sites contaminated with the PGEs provides very interesting information on the bioavailability of these metals[7].

As dominant primary producers, planktonic algae are a basic element in aquatic food chains and a crucial functional group of organisms, which has the fundamental importance in a structure and function of the whole ecosystem[8]. Bednarova et al[9] studied the sensitivity of aquatic producers (*Pseudokirchneriella subcapitata* and *Lemna minor*) to platinum (as $PtCl_4$) by monitoring of the algal biomass density and the vegetative propagation and morphology of duckweed colonies. A growth inhibition confirmed an adverse effect of platinum. Shams et al[10] observed an Rh, Pd, and Pt accumulation by the marine microalga (*Chlorella stigmatophora*) in 24hours exposure experiments. Results revealed that the accumulation in the order Rh > Pd >> Pt was determined by the reactivity of the algal surface, the abundance of reactive forms of the PGEs and the nature and concentration of algal exudates that are able to bind metals from solution.

Because of very similar chemical properties, mutual interferences and typically low concentrations, the PGEs analysis (most frequent ICP-MS and ICP-OES) usually demands separation and pre-concentration steps[1]. Paraskevas et al[11] determined of Pt and Pd in exhaust particles using ion-exchange Lewatit MonoPlus TP-214 matrix separation followed by sequential voltammetry. For measuring Pd, Pt and Rh using ICP-OES, Herincs et al[12] designed a flow-injection system. After the extraction with 1,8-diamino-4,5-dihydroxy- anthraquinone and Triton X-114, Tavakoli et al[13] determined the Pd, Pt and Rh by ICP-OES. Shams et al[9] used ICP-MS in the Rh, Pd, Pt

accumulation study. Lásztity et al[14] developed a sensitive ICP-MS method without decomposition matrix and preconcentration for determining Pd, Pt and Rh in drugs. Silva et al[15] suggested a simple procedure for noble metals based on a cloud point extraction with *O*,*O*-diethyl-dithiophosphate and Triton X-114; metals in the surfactant-rich phase were determined by ICP-MS with electrothermal vaporization. Zereini et al[7] isolated and pre-enriched Pt, Pd and Rh from the air in urban and rural areas of Germany using a combination of Te and Hg co-precipitation method followed by ICP-MS isotope dilution technique.

The aim of the study is to develop the analytical method (ICP-MS or ICP-OES) for determination of Pt in algae and hospital wastewater including an optimization of a sample preparation step and to use this procedure for monitoring of biosorption / bioaccumulation processes of Pt in algae.

Experimental

Apparatus and Instrumentation

Samples were centrifuged in the Beckman GPR Centrifuge with the GH 3.7 rotor (Beckman, USA), dried in the laboratory oven UM 400 (Memmert, Germany) and digested with the Microwave system SpeedwaveTM MWS-2 with high-pressure PFA vessels DAC-70S (the maximal pressure 100 barr, the maximum total output of the microwave generator 1000 W, Berghof, Germany).

The ICP-oTOF-MS spectrometer Optimass 8000 (GBC Scientific Equipment, Australia) equipped with the concentric nebulizer MicroMist (400 μL min^{-1}) coupled to the 70mL thermostated (10 °C) cyclonic spray chamber (both Glass expansion, Australia) was used. The operating conditions of the ICP-MS analysis were adjusted to compromise a sensitivity and resolution of the instrument for ^{238}U as well as to obtain the minimal LaO^+/La^+ and UO^+/U^+ ratios: the sample flow rate 600 μL min^{-1}; plasma power 1250 W; plasma, auxiliary and nebulizer gas flow rates were 12, 0.8 and 0.98 L min^{-1}, respectively and multitiplier gain 2870 V. The other parameters are summarised in Table I. The sensitivity of 40000 counts s^{-1} for 1 μg L^{-1} (mass integrated peak) and resolution of 1500 was reached for ^{139}La. The same parameters for ^{238}U were 50000 counts s^{-1} for 1 μg L^{-1} and resolution 1600. Working isotopes have been selected with regard to possible isobaric overlaps of interfering ions with the same mass. The external aqueous calibration with the internal standard ^{103}Rh was used (Table II).

Pt in spiked algae samples was analysed with the sequential, radially viewed ICP-OES spectrometer Integra XL 2 (GBC Scientific Equipment, Australia), equipped with the concentric nebulizer (3 mL min^{-1}) and the glass cyclonic spray chamber (both Glass Expansion, Australia). The operation conditions were as follows: the plasma power 1000 W, the sample flow rate 1.5 mL min^{-1}, the plasma, auxiliary and nebulizer gas flow rates were 10, 0.6 and 0.6 L min^{-1}, respectively, the photomultiplier voltage

Table I
The working parameters of the ICP- oTOF-MS analysis

Parameter	Value	Parameter	Value	Parameter	Value
Skimmer	–1100 V	Fill bias	1 V	Reflectron	600 V
Extraction	–1150 V	Lens body	–129 V	Multiplier gain	2870 V
Z1	–580 V	Fill	–32 V	Acquisition time	5 s
Y mean	–435 V	Fill grid	–0.6 V	Replicates	3
Y deflection	0 V	Pushout grid	–464 V	Sample intro time	5 s
Z lens mean	–900 V	Pushout plate	571 V		
Z lens deflection	–50 V	Blanker	200 V		

Table II
The ICP- oTOF-MS isotopes and ICP-OES lines with related analytical performance

ICP-MS	LOD [μg L^{-1}]	LOD_A [mg kg^{-1}]	LOD_W [μg L^{-1}]	ICP-OES	LOD [μg L^{-1}]	LOD_A [mg kg^{-1}]
^{194}Pt	0.0241	0.121	0.0603	Pt I 214.423 nm	4.76	2.38
^{195}Pt	0.0249	0.125	0.0623	Pt I 265.945 nm	10.1	5.05
^{196}Pt	0.0338	0.169	0.0845			

LOD – the limit of detection, LOD_A – the limit of detection of the whole analytical method for algae samples, LOD_W – the limit of detection of the whole analytical method for WWTPe samples

600 V, the view height 6 mm, ten replicated reading on-peak 0.5 s and the fixed point background correction. The emission lines used are listed in Table II.

Reagents and Standards

All reagents used were of an analytical-reagent grade. Deionised water was further purified using the SG Ultra Clear system (SG Water, USA). 65 % (v/v) HNO_3 (LachNer, the Czech Republic) was distilled in a sub-boiling distillation equipment (BSB 939 IR, Berghof, Germany).

For an instrumental calibration and a sample spiking, commercially available single-element standard solution Pt and Rh 1.000 ±0.002 g L^{-1} (Analytika, the Czech Republic, SCP Science, Canada) were used. Single element standards containing Pt 1000, 500, 100, 50 μg L^{-1} for the ICP-OES and 1.0, 3.0, 5.0, 10, 15, 20 μg L^{-1} for the ICP-MS analysis were prepared. For the ICP-MS analysis, each of blanks, standards, spikes and samples contained an internal standard element (Rh) in the final concentration 10 μg L^{-1}. The final concentration of HNO_3 in all analysed solutions was 1.3 % v/v with the exception of the ICP-OES standards which contained 24 mL of distilled nitric acid in each 100 mL of the final volume and 12 mL of distilled nitric acid in each 50 mL of the final volume.

Samples and Preparation

Fresh water algae (*Parachlorella kessleri*) were provided by the Fycological laboratory, University of České Budějovice (the Czech Republic). The algae were cultured under artificial light in the laboratory for 30 days at the room temperature in the Bold-Basal/Bristol Medium (BBM)[16] containing essential nutrients (in 1 L: 250 mg $NaNO_3$, 50 mg $CaCl_2 . 2 H_2O$, 75 mg $MgSO_4 . 7 H_2O$, 75 mg K_2HPO_4, 25 mg NaCl, 175 mg KH_2PO_4, 5 mg EDTA, 3.1 mg KOH, 0.498 mg $FeSO_4 . 7 H_2O$, 0.1 μl H_2SO_4 (96%), 1.142 mg H_3BO_3, 8.82 mg $ZnSO_4 . 7 H_2O$, 1.44 mg $MnCl_2 . 4 H_2O$, 0.71 mg MoO_3, 1.57 mg $CuSO_4 . 5 H_2O$, 0.49 mg $Co(NO_3)_2 . 6 H_2O$) or under another conditions (see in the section "The Cultivation and Isolation of Algae").

Hospital wastewater was collected in the input and in the output of wastewater treatment plant (WWTP) at the University Hospital in Hradec Králové (in January and March 2014) always of the 2 L. After collection, the samples were divided. One part was directly used for the growth experiment the second part was acidified (10 mL distilled HNO_3 in 1 L) and stored in sterile, acid cleaned polyethylene bottles at –20 °C up to analysis. Just before analysis, the samples were filtered through filter paper (Papermill Perštejn Keseg & Rathouský, the Czech Republic).

The algae samples were prepared for analysis as follows: 50 mL of the algae samples were separated from the growth medium by centrifugation using a Beckman GPR Centrifuge 10 minutes at 10 °C, speed 3000 RPM, the rotor GH 3.7. The supernatant was removed and the algae were repeatedly washed with demineralized water. Washed algae were dried for 48 hours at 70 °C in a laboratory oven. An accurate sample weight of dried algae (about 50 mg) was inserted together with 6 mL of distilled nitric acid to the microwave oven (the first step 160 °C, 5 min, 80 % of power; the second, step 200 °C, 10 min 80 % of power and the third step 10 min without heating). The cooled decomposed samples were transferred to volumetric flasks, filled up with demineralised water (DW) to the final volume 25 mL, inserted into leached polyethylene vials and stored until analysis at –20 °C.

Samples of WWTP wastewater were decomposed using the same microwave oven programme as in the case of algae: 20 mL of the filtered sample together with 6 mL of distilled nitric acid was used. The cooled decomposed samples were transferred to volumetric flasks, filled up with DW to the final volume 50 mL, inserted into leached polyethylene vials and stored until analysis at –20 °C.

Before the ICP-MS analysis, the digested algae and wastewater samples as well as sample blanks were diluted tenfold with DW and the internal standard (Rh) was added in the final concentration 10 μg L^{-1}. For the ICP-OES analysis, samples were not further treated.

Results and Discussion

Firstly, it was necessary to gain appropriate materials, which were represented by freshwater algae grown in laboratory experiments and in the WWTP water samples. The next step was the development and validation of the methods for Pt analysis in the above mentioned materials. Finally, results of the Pt analysis in Pt enriched algae and the WWPT water samples were used for the estimation of a bioaccumulation process in water.

The Cultivation and Isolation of Algae

The fresh water algae were obtained as a suspension in the growth BBM medium. The algae were gradually grown up to an amount sufficient to carry out all experiments and preservation for other needs. Approximately 50 mL of the algae suspension (about 4 × 10^6 cells mL^{-1}) were inserted into the 500mL sterile Erlenmeyer flask and filled up to the final volume 200 mL with: (i) the growth BBM medium, (ii) the growth BBM medium with Pt (the final concentration 0.L mg L^{-1}), (iii) water from the WWTP (input or output) from the University Hospital in Hradec Králové. The Pt concentration 0.1 mg L^{-1} in the growth experiment (ii) was set up according to the publications[8,9].

As reported in the literature, the algae samples were separated usually using filtration[9,10], which was very slow. Therefore another way to obtain a solid part of an algae suspension was searched. The algae samples were separated from the growth medium by centrifugation (see the section "Samples and Preparation"). The supernatant

was removed and the algae were repeatedly washed with DW. The washing procedure was optimized. The Pt concentration in wash water (followed by the ICP-MS method) fell below 1% of the concentration in the first supernatant after the fifth centrifugation steps (e.g. after four washes with DW). This washing way was used in the whole study.

Validation of the ICP-MS and ICP-OES Methods

An applicability and validity of both the ICP-OES and ICP-MS for the study was tested and evaluated based on limits of detection (LODs), sample blanks and the recovery study. The ICP-MS instrumental LODs were calculated as the concentration related to the intensity of three times the standard deviation of the Pt standard 1 μg L^{-1} measured in ten replicates (Table II). The ICP-OES instrumental LODs were estimated as the concentration related to the intensity of three times the standard deviation of the Pt standard 50 μg L^{-1} measured in the place of the background correction in ten replicates (Table II). In Table II, there are also summarized limits of detection for the determination of Pt in algae and WWTP samples (LOD_A, LOD_W) which take into account the dilution of the sample during the preparation step. The LOD_A and LOD_W indicate the minimum detectable amount of analyte in the original material in mg kg^{-1}, mg L^{-1} respectively. The ICP-MS dilution factor was about 5000 for algae (the sample weight of algae to mineralization about 50 mg, the final volume was 25 mL, tenfold diluted before the ICP-MS analysis) and 2.5 for WWTP samples and the ICP-OES factor was 500 (about 50 mg, 25 mL, analysed without further dilution). Both the LOD_As, for the ICP-MS and the ICP-OES were suitable for the analysis of Pt in the algae samples spiked with Pt just before the mineralized step. In other cases, the less demanding and easily operated ICP-OES was not suitable. The most sensitive Pt I 214.423 nm line and ^{194}Pt isotope were used in the next part of the study.

In order to check and solve a possible contamination, a series of sample blanks was prepared including a calibration sample blank, a growth sample blank (only the BBM medium under the growth experiment condition) and a mineralization sample blank (only pure nitric acid treated with the decomposition step). The Pt content in the sample blanks was below the LODs and proved no contamination risk in the process.

The validation of the ICP-OES / MS methods was made through a recovery study using the calibration standards (ten replicates, ICP-OES 50 μg L^{-1}, ICP-MS 1 μg L^{-1}) for and a "blank" algae sample (e.g. – algae from the growth BBM medium) spiked with platinum before the mineralisation step in an amount giving 50 μg L^{-1} of Pt in the final digests (ten replicates). For the chosen calibration standard, recoveries were 93.8–98.5 % and corresponding coefficients of variance (CVs) 2.3–4.2 % for both the methods. For the Pt spiked algae sample, recoveries were 89.1–91.0 % and CVs 2.6–9.9 % for both the methods.

Platinum in Hospital Wastewater

Wastewater from multiple locations of the University Hospital in Hradec Králové comes to the WWTP where it is treated to be safe removed outside the hospital. The wastewater samples were collected in the input and in the output of the WWTP in January and March 2014 and were partly used for the growth algae experiments and partly subjected to the microwave decomposition (the conditions described in the chapter "Samples and Preparation") and then Pt was determined using the ICP-MS method. The Pt contents (all in μg L^{-1}) for both the sampling dates mutually correspond: the WWTP input 0.117 ±0.011 (January) and 0.129 ±0.014 (March) and the WWTP output 0.040 ±0.003 (January) and 0.053 ±0.005 (March). The higher Pt content in the WWTP input than in the WWTP output confirm a partial capture probably in sewage sludge. Kümmer and Helmers[17] reported Pt found in the sewage of various German hospitals and hospital departments 110–176 during daytime and 38 at night. They also calculated from annual consumption data range 110–210 (all in ng L^{-1}). They results are in a good agreement with our findings.

Platinum in Algae

There were three types of experiments dealing with the alga *Chlorella kessleri*. The first one (algae in the growth BBM medium) was used as the growth sample blank (as discussed in the section "Validation of the ICP-MS a ICP-OES Methods"). Other experiments were evaluated in order to validate the analytical methods (algae in the growth BBM medium spiked with 100 μg L^{-1} Pt) and to estimate the bioconcentration factor (algae in the spiked growth BBM medium and in the input / output WWTP wastewater). The results are summarised in Table III. The bioconcentration factor (BFC) is defined as the ratio of the concentration in organism to the concentration in water, in our case the concentration in algae to the concentration in medium: $BCF = c_{algae} / c_{medium}$ (in g L^{-1}). The BCF value is useable for the evaluation of the steady-state experiments. From Table III, it is clear, that there are differences in the BCF values. According to the Pt species, the first experiment was clearly defined. Pt was used in the form of $(NH_4)_2PtCl_6$. From the point of view of the Pt forms, the growth experiments with WWTP wastewater are questionable. In the WWTP input, there can be partly toxic cisplatin, which may be subjected to physico-chemical and biotranformation processes during the WWTP treatment. The rest of cisplatin present may also damage the algae and may be the cause of the lower BCF compared to WWTP output. The composition of the input and output WWTP wastewater must necessarily differ from the nature of the cleaning process and purpose of the WWTP. The information on the Pt species composition is not available. Bednarova et al[9] referred the strong adverse effect of Pt (chlorosis, necrosis) on producers (*Pseudokinchneriella subcapitata* and *Lemna minor*) in the

Table III
The results* of the analysis of the algae samples from growth experiments

Growth Medium	$c_{med.-before}$[a] [μg L^{-1}]	$c_{med.-after}$[b] [μg L^{-1}]	c_{algae}[c] [mg kg^{-1}]	BCF[d] [L kg^{-1}]
BBM[1] + Pt	100	61.7 ±5.1	37.9 ±4.02	610
BBM + Pt	100	34.2 ±4.2	15.2 ±1.78	440
BBM + Pt	100	42.1 ±4.1	24.2 ±2.77	570
$WWTP_{input}$[2], January	0.117 ±0.011	0.0912 ±0.012	0.131 ±0.096	1440
$WWTP_{input}$, March	0.129 ±0.014	0.110 ±0.014	0.076 ±0.006	690
$WWTP_{output}$[3], January	0.040 ±0.003	0.045 ±0.004	0.113 ±0.073	2510
$WWTP_{output}$, March	0.053 ±0.005	0.045 ±0.006	0.132 ±0.044	2930

* results are presented as arithmetic mean ±standard deviation for one sample: BBM was spiked with Pt (100 μg L^{-1}), "before" and "after" growth medium samples were analysed in 4 replicates, algae samples in 8–10 replicates depending on a final algae amount gained from growth experiments, [a] concentration of Pt in the growth medium before a growth experiment, [b] concentration of Pt in the growth medium after a growth experiment, [c] concentration of Pt in algae samples from a growth experiment, [d] bioconcentration factor, [1] Bold-Basal/Bristol Medium, [2] wastewater sample from the input of the WWTP (sampled in January or March), [3] wastewater sample from the otput of the WWTP (sampled in January or March)

aquatic environment for $PtCl_4$ using the OECD 201 method. Shams et al[10] dealt with extra and intra cellular accumulation of PGEs by the marine microalga *Chlorela stigmatophora*. Both these contributions evaluated the Pt species influence on the effect rather from the perspective of the environmental influences on the health and vitality of algae. The view of our study is more moved for the use of algae as biosorbents in water treatment technology.

Conclusion

The analytical methods using the ICP-oTOF-MS and ICP-OES for the determination of platinum in algae and hospital wastewater including the optimisation of the sample preparation step were developed. The methods were validated using the recovery study on Pt standard solutions and the algae samples spiked before the decomposition step: recoveries were 93.8–98.5 % (CVs 2.3–4.2 %) for the Pt standard solution and 89.1–91.0 % (CVs 2.6–9.9 %) for the Pt spiked algae sample. The recoveries of both the methods were in a good mutual agreement. The LOD for ^{194}Pt was 0.0241 μg L^{-1} (0.121 mg kg^{-1} for the whole method for algae samples and 0.0603 μg L^{-1} for WWTP samples) which was satisfactory for all types of analysed samples. It enabled to analyse platinum in the WWTP wastewaters without previous preconcentration and to monitore the biosorption / bioaccumulation processes in the algae. The LOD for Pt I 214.403 nm was 4.76 μg L^{-1} (2.38 mg kg^{-1} for the whole method fo algae samples) was sufficient only to the analysis of Pt the algae samples spiked before the decomposition step used for the method validation.

As mentioned above, platinum was followed without the preconcentration step in the WWTP wastewaters samples. The lower concentrations were found in the output than in the input WWTP wastewater. It reveals that platinum was partly captured during the WWTP cleaning process.

Based on the analysis of platinum in the algae from the growth experiments, it was possible to estimate the bioconcentration factors, which differed according to the Pt species presented. For algae spiked with 100 μg L^{-1} $(NH_4)_2PtCl_6$, the BCFs were 440–610, for the WWTP input 690–1440, for the WWTP output 2510–2930, all in L kg^{-1}, which draw attention to the issue of chemical forms of platinum in the WWTP environment.

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MORPHOLOGICAL FEATURES OF MICROCELLULAR POLY(METHYL METHACRYLATE) PREPARED BY EMULSION TEMPLATING

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Abstract

Porous poly(methyl methacrylate-co-ethyleneglycol dimethacrylate) polymers were prepared by the photopolymerisation of high internal phase emulsions (HIPEs). Morphological features like cavity and interconnecting pore diameter were investigated and successfully controlled by changing the surfactant amount and pore volume ratio and up to 90 % porous monolithic samples could be obtained. Resulting polymers were studied and characterized by scanning electron microscopy (SEM). Cavity diameter could be tailored in the range between 700 nm and 65 μm and interconnecting pore diameter between 340 nm and 9.2 μm.

Introduction

Highly porous and permeable materials can be prepared in various ways and one is the polymerisation of high internal phase emulsions (HIPEs) (Fig. 1), where the internal phase exceeds 74 % of total emulsion volume[1]. When internal phase is removed after the polymerisation, porous materials with large cavities and small interconnecting pores are obtained. These materials are usually termed polyHIPEs and attracted a lot of attention due to their exceptional properties such as low density and high permeability for liquids and are therefore used for various applications[2–4].

PolyHIPEs are usually prepared in the form of monoliths taking the shape of moulds for emulsion polymerisation, while membranes[5–8], beads[9–12] or biphasic materials[13,14] can be prepared and several applications such as tissue engineering[15,16], protein separation[5,17], columns for liquid chromatography[18–20], gas storage devices[21], sensor systems[7] and others have been demonstrated. The possibility of producing chromatographic columns by direct *in-situ* polymerization makes this material attractive for the use in analytical chemistry. Despite unique morphology, polyHIPEs are usually rather brittle materials and that limits the use in application where high force is applied to the surface of the material. Convenient way to overcome poor mechanical properties is changing monomer system from most common styrene-co-divinyl benzene based to methyl methacrylate-co-ethylene glycol dimethacrylate (MMA-co-EGDMA) system since bulk poly(methyl methacrylate) (PMMA) shows significant advantages of PMMA compared to bulk polystyrene[22].

In previous work we have demonstrated that MMA can be used as an effective monomer for polyHIPE production via photopolymerisation and that mechanically stable monoliths with open porosity up to 85% can be obtained[3]. In this work we are reporting on the study of influences of surfactant content and pore volume on the morphological features of MMA based polyHIPEs.

Experimental

Materials

Methyl methacrylate (MMA; Sigma Aldrich) and ethylene glycol dimethacrylate (EGDMA; Sigma Aldrich) were passed through a layer of Al_2O_3 (Fluka) to remove the inhibitors. The surfactant Synperonic PEL-121 (Sigma-

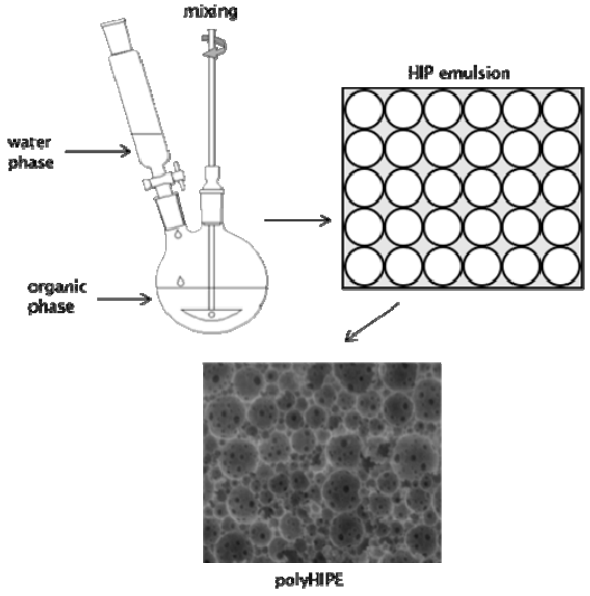

Fig. 1. **PolyHIPE preparation**

Aldrich), Irgacure 819 (I819; Ciba), calcium chloride hexahydrate ($CaCl_2 \times 6\ H_2O$) (Sigma-Aldrich) and ethanol (Merck) were used as received.

PolyHIPE preparation

Suitable amounts of MMA, EGDMA, I819 and PEL 121 were placed in a 250 ml round-bottomed three-necked reactor equipped with an overhead stirrer (IKA Eurostar 20). The aqueous phase was prepared separately by dissolving $CaCl_2$ x 6 H_2O (1,79 g) in 100 mL of degassed deionised water. The organic phase was thoroughly stirred for 2 min, after that an appropriate amount of aqueous phase was added under continuous stirring (300 rpm). After complete amount of aqueous phase was added, stirring was continued for 1 h. The emulsions were then transferred into a silicon mould and exposed to UV light for 2 min at 100% intensity (Uvitron IntelliRay 600). Resulting polyHIPEs were extracted in a Soxhlet apparatus with deionised water for 24 h and absolute ethanol for a further 24 h. After that they were dried in vacuum at 40 °C for 24 h. Compositions of emulsions are presented in Table I.

Characterization

Porous structure typical for polyHIPEs was determined using scanning electron microscope (SEM) Quanta 200 3D (FEI Company) with BSE detector at 10 kV. Samples were mounted on metal stubs using graphite tape and sputter coated with a thin layer of gold. The average cavity sizes, cavity size distributions and interconnecting pore sizes were determined from scanning electron microscopy (SEM) images, using a correction factor of $2/\sqrt{3}$ for cavity size, in order to compensate for the statistical error due to random sectioning of cavities at sample preparation[24]. Nitrogen adsorption/desorption measurements were done on a Micromeritics TriStar II 3020 porosimeter using a BET model for surface area evaluation.

Results and discussion

In order to successfully control the morphological features, influence of different polymerisation factors were studied. Since Synperionic PEL 121 has proven to be a successful surfactant in preparation of high internal phase emulsions (HIP) containing methyl methacrylate, starting formulation was taken from previous experiments with MMA polyHIPEs[23]. DVB as a cross-linker and thermal initiation was first tested but did not produce solid monoliths after polymerisation. Therefore EGDMA as a cross-linker and photopolymerisation were used in further experiments.

First the influence of the surfactant amount was studied by varying the content from 5 to 16 % based on monomer volume. The lowest concentration of surfactant did not produce typical interconnected polyHIPE

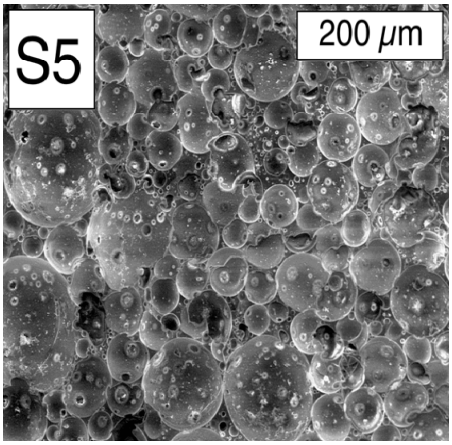

Fig. 2. **SEM image of sample S5**

Table I
Compositions of emulsions*

Sample	Internal phase [%]	Surfactant content [%]	D[a)] [μm]	d[b)] [μm]	s[c)] [μm]	BET [m^2/g]
S5	80	5	56.7	5.9	6.0	0.3
S10	80	10	19.2	1.2	2.7	17.1
S13	80	13	15.9	1.2	2.0	18.2
S16	80	16	11.5	1.1	0.9	18.3
X10	85	10	15.9	1.9	2.3	16.0
X16	85	16	11.6	1.7	1.7	27.5
Y10	90	10	28.1	4.8	3.3	18.8
Y16	90	16	7.7	1.5	1.7	25.0

*monomer ration MMA/EGDMA was 75/25 in all cases and initiator I819 was used in all emulsions, [a)]average cavity diameter, [b)]average interconnecting pore diameter, [c)]standard deviation of cavity diameter

morphology; larger cavities with a low degree of connectivity are observed (Fig. 2).

The use of 10 % of surfactant resulted in a formation of typical polyHIPE structure but rather inhomogeneous (hierarchical) morphology with cavities diameter ranging from 2.4 to 65.0 μm and with interconnecting pores in diameter from 510 nm to 3.2 μm. With increasing the amount of surfactant to 13%, a more homogenous

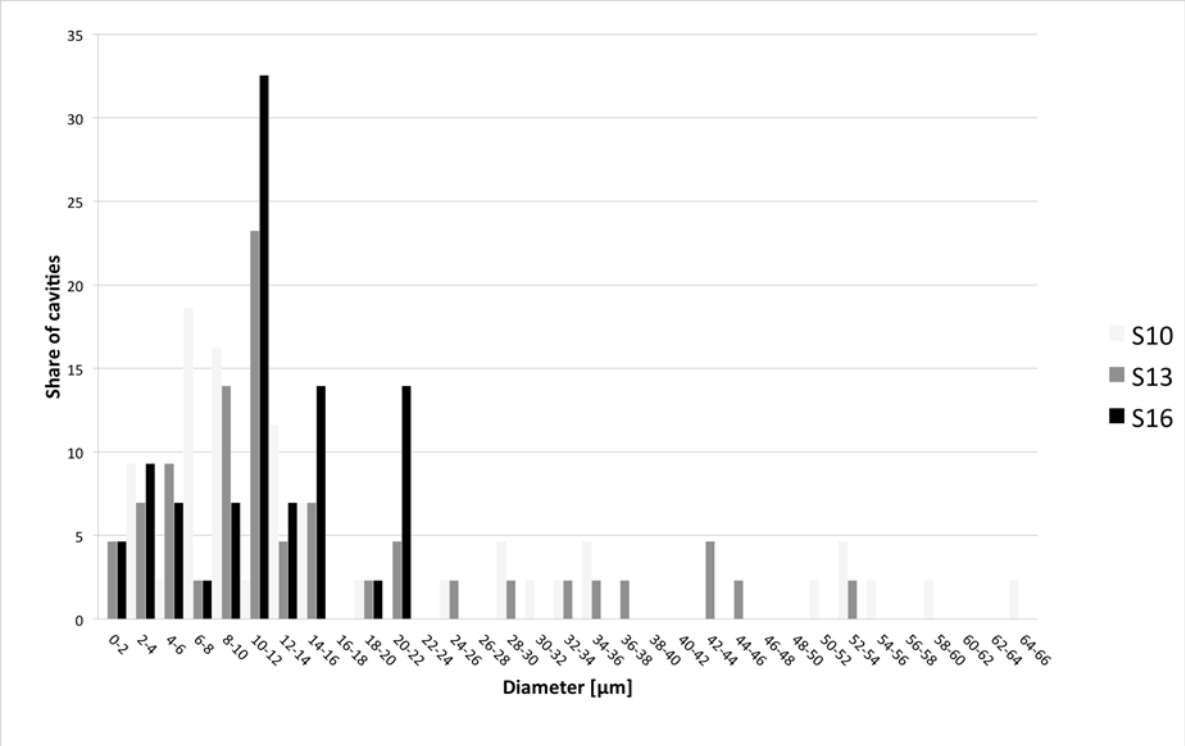

Fig. 3. **Pore size distributions of samples S10, S13 and S16**

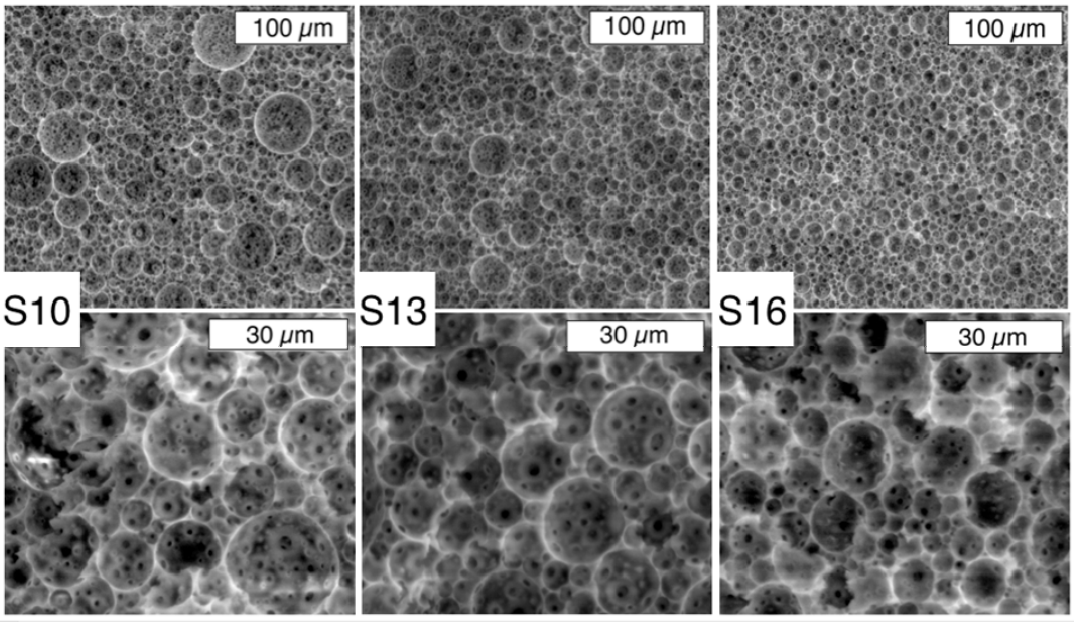

Fig. 4. **SEM images of samples S10, S13 and S16 with varied surfactant content**

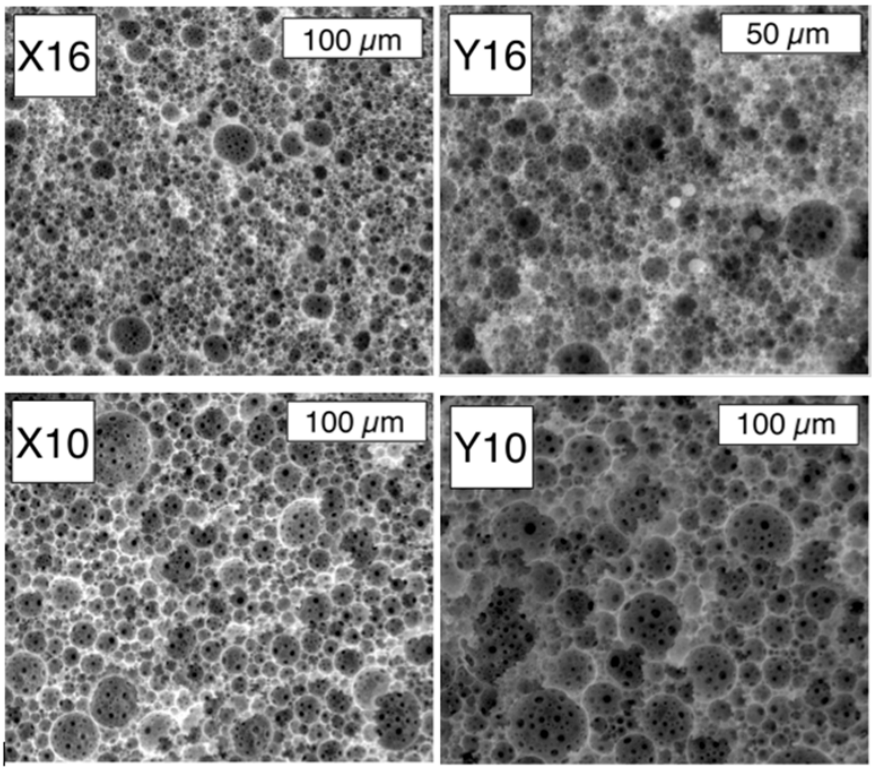

Fig. 5. **Influence of the pore volume and surfactant content**

interconnected structure with slightly smaller cavities (diameter from 1.4 to 52.1 μm and interconnecting pores in diameter from 770 nm to 2.8 μm) was obtained. Higher surfactant concentrations (16 %) resulted in an open porous morphology with a narrower pore size distribution (Fig. 3). The interconnectivity was, with the increasing amount of surfactant, practically not affected (Fig. 4).

Since surfactant proved to be an important factor for the optimization of polyHIPEs' morphology, its influence was further studied in combination with the influence of pore volume content. Pore volume was gradually changed from 80 % to 90 % (total emulsion volume). With the increasing pore volume, produced emulsions were more viscose and after the polymerisation a more interconnected structure was obtained. Such a trend has already been observed for other MMA polyHIPEs[1].

Furthermore, with the increasing pore volume, the influence of surfactant content became more obvious. With the increasing amount of surfactant concentration, the cavity size decreased and interconnectivity increased (Fig. 5). A higher concentration of surfactant in the oil phase could stabilize a higher surface and therefore an emulsion with smaller droplets was formed. Due to fast polymerisation of the continuous phase less droplet coalescence and Ostwald ripening takes place than in comparable thermally cured systems, which results in the final polyHIPE material with smaller cavity sizes. This effect becomes more pronounced within emulsions with a very high fraction of droplet phase.

Conclusion

We have shown that MMA/EGDMA system can be used for the preparation of polyHIPEs with tuneable morphology. Both surfactant content and pore volume proved to be important factors in the optimization of morphology of highly porous materials (up to 90 %) where cavity diameter could be tailored in the range between sub-micron and 70 μm. The most important influence on interconnecting pore diameter was obtained by simultaneously increasing the surfactant and pore volume ratio, which resulted in increased number but decreased diameter of interconnecting pores. Demonstrated possibility of influencing pore size and pore size distribution and relative ease of preparation, make this material attractive for further studies in the field of separation technologies.

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CHEMICAL CHARACTERISATION OF ACID MINE DRAINAGE FROM AN ABANDONED GOLD MINE SITE

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Keywords: acid mine drainage, sulphide, sulphate, metals, contamination, ICP-MS, IC

Abstract

Acid mine drainage (AMD) is a well-known environmental problem arising from both working and abandoned mines. This process happens when minerals, especially sulphide minerals, are exposed to the environment's atmospheric or water oxidation. The oxidation causes sulphide ions to oxidize to sulphate ions whereby their generation causes water pH decrease and therefore the releasing of toxic metals is increased from a mine site. AMD can cause high concentrations of metals and other pollutants in water. The most common sulphide mineral used during the mining of valuable metals such as Cu, Pb, Zn or Au is pyrite – FeS_2. The gold bearing form of this mineral was used during mining activities at the biggest gold deposit in Bosnia and Herzegovina in the Bakovici mine. The chemical parameters that were selected for research and for determining of the AMD process in water were: pH, electrical conductivity, oxidation-reduction potential, concentrations of sulphates, and the amounts of Fe, Na, Si, Al, Cr, Cu, Pb, Au, As, Zn, Cd and Ag. Additionally, standard parameters for water quality were measured for five samples taken from the River Gvozdanka which passes the gold mine site, one sample of drinkable water – underground source, and three water samples that flowed directly from the mining body. The results showed that the River Gvozdanka and water source of drinkable water were uncontaminated by AMD products but water from the mining body was highly contaminated, especially by sulphates, iron and aluminum. Interestingly gold was still present within the concentration range from 0.5 to 1.0 μg L^{-1}.

Introduction

Acid mine drainage is one of the biggest environmental problems concerning the mining industry. The process of acid mine drainage (AMD) is related to both working and abandoned mining sites[1,2]. The mining of certain minerals, including the minerals of gold, copper, lead, zinc and nickel is associated with acid drainage problems that can cause long-term impairment to water flow and biodiversity[3]. Acid mine drainage causes decreasing of pH, increasing acidity, high sulphate content. Very low pH value (1–4) can cause the releasing of metals from soil and minerals in water streams, causing contamination by heavy metals. Furthermore, some effluents generated by the metals mining industry contain large amounts of toxic substances such as cyanides and heavy metals[4]. Acid mine drainage is a process that occurs naturally but with human activities is much faster. The process that lasts from 100, 000 – 200, 000 years in nature is speeded up to several hundreds of years. Acid mine drainage happens when sulphide ore is exposed to atmospheric air and water, and the sulphide ion is oxidized to sulphate ion. One of the better known sulphidic ores is pyrite (FeS_2). In addition to pyrite there is large number of sulphidic ores that are used in the mining industry such as: arsenopyrite (FeAsS), chalcopyrite ($CuFeS_2$). Oxidation of pyrite is the most investigated from amongst all of the sulphidic ores as pyrite produces large amounts of acid (sulphate ion) during the process of acid mine drainage.

The reactions of sulphide ores with oxygen and water result in the emergence of iron sulphate solution and sulphuric acid. Fe^{2+} ions can be further oxidized to Fe^{3+} and thus produce additional acidity. In the intact natural systems, the oxidation process is very slow. However, when the ore is exposed to an oxidative environment this process becomes much faster. Sulphide ore which is oxidized produces H^+ ion (acidity), sulphate anions and metal ions, is shown in Equation 1.

$$2\ FeS_2(s) + 7\ O_2 + 2\ H_2O \rightarrow 2\ Fe^{2+} + 4\ SO_4^{2-} + 4\ H^+ \quad (1)$$

The reactions can be significantly accelerated due to the presence of bacteria, particularly Thiobacillus ferrooxidans[5,6]. It is important to note that the summary of all the responses of these processes to the final oxidation of pyrite produces four moles of H^+ per mole of FeS_2 (2). From this it follows that the pyrite is mostly responsible for the formations of acid from all known sulphide minerals.

$$FeS_2 + 15/4\ O_2 + 7/2\ H_2O \rightarrow Fe(OH)_3 + 2\ SO_4^{2-} + 4\ H^+ \quad (2)$$

Ultimately acid production processes can be divided into two parts. The first is the oxidation of sulphide ores by oxygen and ferric ions (Fe^{3+}), and the second is the hydrolysis of Fe (III) compounds and the formations of

iron oxyhydroxides and oxyhydroxide sulphates.

Acid mine drainage is characterized by several chemical parameters. The typical parameters of AMD are: low pH – high acidity, high conductivity, high concentrations of metals (especially iron and aluminum), high sulphate, and high total dissolved solids[7,8].

Site description

The Gold Mine in Bakovici is the biggest gold deposit in Bosnia and Herzegovina. According to some sources in this area of Bakovici metals were mined in the Roman era, and it is certain that the Romans in the first century were washing gold in the River Vrbas (source of which is near Bakovici). Intensive excavation of ore began in the 1880s. The digging for ore stopped in 1940 because of World War II. By 1939 the mine had produced 150,000 tons of ore containing 2000 kg of gold and 7,500 kg of silver[8]. The primary ore consisted of gold – bearing pyrite, siderite, and quartz. 13 grams of gold could be found in one tonne of extracted ore. After removal, the ore was crushed, ground into powder, mixed with lime in a container and then the process of cyanation started with usage of sodium cyanide (NaCN). The maximum coefficient of extraction was 82 % (ref.[9]).

Due to the intense mining of gold – bearing iron ore, the occurrences of increased concentrations of heavy metals (Fe, Pb, As, Cd, etc.) within the vicinity of the mine is very possible. The contamination of the environment is also possible because waste material from the Bakovici gold mine was not properly conserved[10]. The problems of mine waters and the contamination of the environment from working and abandoned mines in Bosnia and Herzegovina were not investigated sufficiently. Contamination of the environment from Bakovici has never been investigated until now. This work is the first investigation of the abandoned Bakovici gold mine regarding contamination of the environment (heavy metals, sulphates and other contaminants).

Experimental

Apparatus and instrumentation

In this research work analysis of the metals was made using an ICP-MS (Perkin-Elmer, ELAN DRC-e, USA), for analysis of sulphate anions an Ion Chromatograph using Dionex CD20 detector and Varian Pro Star pump and software (Varian Pro Star, Canada) was used. Analyses of other anions were made with a spectrophotometer (HACH 2000, USA). Measurements of pH were realized with pH meter MP 220 (Mettler Toledo, Switzerland), analysis of electrical conductivity was made with analyzer MC 126 (Mettler Toledo, Switzerland). Oxidation reduction potential was determined using ORP tester HI 98201 (HANNA, Romania) and analysis of acidity and alkalinity was made with an automatic titrator (Mettler Toledo G20, Switzerland).

Procedures

Sampling

Sampling of groundwater was done within the closed area of abandoned gold mine site in Bakovici, Bosnia and

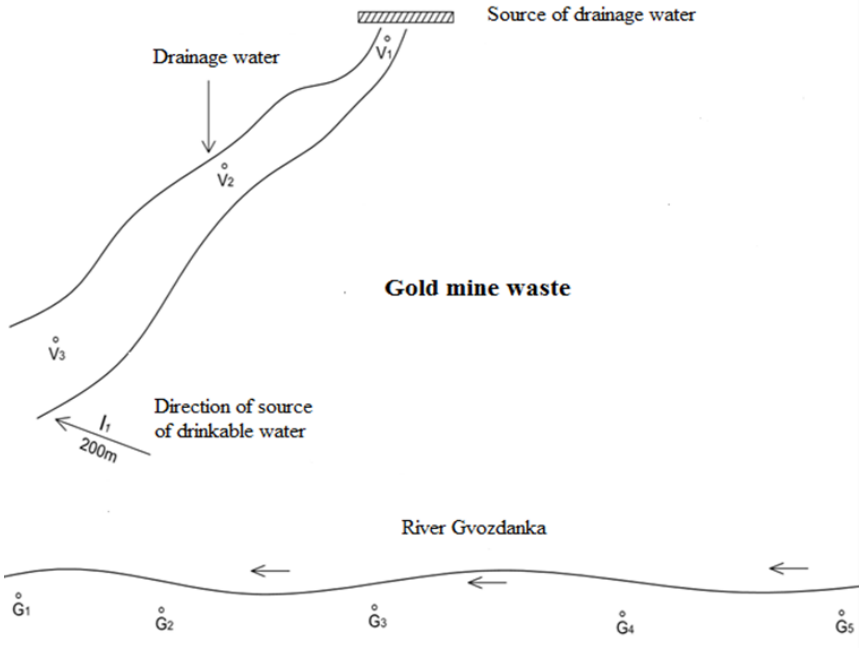

Fig. 1. **Scheme of a sampling site**

Herzegovina. During this work three types of samples were taken: river Gvozdanka, drinkable water, and drainage water. All samples except for those samples for analysis of dissolved oxygen were taken in polyethylene bottles (V = 1000 mL), and kept in a cooler at 4 °C. Samples for determination of dissolved oxygen were taken in Winkler`s bottles with defined volumes. The samples used for analysis of free cyanide were conserved in NaOH until a pH value of approximately 10 was achieved, and the samples for analyzing metals were conserved in 0.1 mol L^{-1} HNO_3 (ref.[11,12]).

The River Gvozdanka flows just under the abandoned gold mine site. It is anticipated that the River Gvozdanka is contaminated by AMD products. In order to avoid contamination we did upstream sampling. We took five samples: 100 m downstream (G_1), 50 m downstream (G_2), below the abandoned gold mine site (G_3), 50 m upstream (G_4) and 100 m upstream (G_5). The source of drinkable water is 200 m away from abandoned gold mine site, and was used as some kind of control value. Therefore one sample of this source was taken (I_1). Drainage water is water flow that comes from an abandoned gold mine‘s body. This flow is about 50 m long. We took three samples: at the beginning of the drainage water flow (V_1), 15 meters from the beginning (V_2) and 30 m from the beginning (V_3).

Analytical methods

ICP-MS

For the amounts of metal we used inductively-coupled plasma mass spectroscopy (ICP-MS). Calibration was carried out according to certified analytical standards. The concentration for first standard was 0.01 mg L^{-1}, second 0.05 mg L^{-1} and third 0.1 mg L^{-1}. The standard reference material (SRM) was SRM 1640a. Analyses of the samples were made on two parallels, and the concentration of each sample was taken twice. In every sample we added rhodium as an internal standard. The concentration of internal standard was 10 μg L^{-1}. Detection limit for most of the elements was 0.2 μg L^{-1}.

IC

Ion chromatography was used for measuring the concentrations of sulphates in the water samples. Several multiple standards (sulphate, chloride, nitrate) were used for calibration. The concentration range of the standards ranged from 20 to 80 mg L^{-1}. In larger numbers of samples we had to dilute the samples with miliQ water. The LOQ value for sulphates was 5 mg L^{-1}.

Other methods

Determining the concentrations of several anions and turbidity were conducted with UV-VIS spectrophotometry. Anions that are present in sample solution react with added reagent to form stable color. Determination of free cyanide was made by HACH spectrophotometer with pyridine-pyrazolone method (HACH 8027). Concentrations of nitrites and nitrates were carried out by spectrophotometry according to HACH methods 8507 and 8192. Measurements of pH were carried out with pH-meter which was calibrated with several buffer solutions before experiment; pH was measured by EPA 130.2 method. Before measurement of electrical conductivity in the water samples, conductivity meter was calibrated with standard solutions. Oxidation reduction potentials were obtained from Pt vs Ag/AgCl electrodes, and according to APHA method. The oxidation reduction meter was calibrated with standard solutions before measurement of oxidation reduction potential in the water samples. Automatic titration was used to determine acidity and alkalinity in the water samples, according to EPA methods 305.1 and 310.1. For titrants we used 0.100 M NaOH and 0.100 M HCl. Dissolved oxygen was measured by Winkler`s method, by volumetric titration of iodine.

Results and discussion

Parameters of acid mine drainage

The parameters that describe acid mine drainage are: pH value, total dissolved solids, sulphates, amount of metals (especially iron and aluminum). The results for water samples, and for measurements of parameters of acid mine drainage (River Gvozdanka, source of drinkable water and drainage water) are shown in Table I.

It can be observed that very low pH values were found in samples of drainage water (V) (average value was 3.7). Low pH value (below 4) is characteristic of the AMD process, and it can cause releasing of other contaminants

Table I
Results for analysis of acid mine drainage parameters

Parameter	Water samples									
	G_1	G_2	G_3	G_4	G_5	I_1	V_1	V_2	V_3	MAC
pH	7.6	7.7	7.8	7.8	7.8	6.6	**3.8**	**3.6**	**3.7**	6.5–8.5
TDS, mg L^{-1}	92.0	106.0	100.0	96.0	135.0	111.0	**628.0**	**631.0**	**636.0**	500
Sulphates, mg L^{-1}	36.1	35.0	33.5	32.4	40.2	59.8	**364.0**	**392.0**	**386.0**	250.0
Fe, mg L^{-1}	0.3	0.3	0.2	0.2	0.2	0.2	**28.4**	**26.6**	**26.9**	0.3
Al, μg L^{-1}	5.3	24.7	5.6	6.9	6.5	2.6	**237.3**	**280.4**	**306.3**	50

into the water flow, especially heavy metals. The results of pH for drainage water show values below the maximum allowed pH (MAC –Maximum allowed concentration – Environmental Protection Agency, 2009)[13]. The results indicate that there is a formation of AMD in the drainage water, probably due to the existence of sulphide minerals (pyrite) and the non-existence of limestone. Low pH value could be attributed to rapid oxidation of sulphidic ores that are exposed to atmospheric oxidation (air and water). At the gold mine site there were sufficient amounts of sulphidic ores to be oxidized and low amounts of basic minerals (limestone).

The River Gvozdanka (G) and the source of drinkable water had pH values above the 6.5, and they did not show significant change of pH. The results for the next step regarding total dissolved solids were studied and the results are presented in Table I. The samples of the drainage water had amounts of total dissolved solids (TDS) which were above the MAC. The mean of TDS (631.7 g L^{-1}) exceeded the maximum permitted value (500 mg L^{-1}). High TDS showed a general level of pollution, and is one of parameters that is characteristic of acid mine drainage. The amount of dissolved solids was several times lower in the samples of the River Gvozdanka (106.8 mg L^{-1}) and the sample of drinkable water (111.0 mg L^{-1}). High concentrations of sulphate in the samples of drainage water came from the process of acid mine drainage (Table I). High sulphates correspond with low pH values. Concentrations of sulphate in the drainage water were higher than the maximum allowed concentrations (MAC) for drinkable water. High amounts of sulphate exceeded the maximum permitted value of 250 mg L^{-1} and the average was 380.7 mg L^{-1}. Concentrations of sulphate in the samples of The River Gvozdanka where much lower (average 35.5 mg L^{-1}).

If we consider the pH and oxidation-reduction potential (pH-Eh diagrams), we can say that iron was, in $FeO(OH)_{(s)}$ form, in the drainage water samples and was precipitated on the stream‘s bottom. Iron was the main metal in the water samples because in the Bakovici gold mine pyrite (FeS_2) was one of the main mined ores. The results for drainage water (Table I) showed very high concentrations of iron (average value of the samples was 27.32 mg L^{-1}, 92 times higher than MAC). This very high concentration of iron in the drainage water comes from watering of the mine‘s body in which pyrite was mined. The River Gvozdanka and the source of drinkable water were uncontaminated by iron. The average value of the iron in The River Gvozdanka was below the maximum allowed value of 0.3 mg L^{-1} (0.26 mg L^{-1}). High concentrations of aluminum and iron encapsulate the process of acid mine drainage. High aluminum levels are derived at by dissolving alumosilicates. The concentrations of aluminum found in the samples of drainage water were also higher than the MAC value (5.5 times). Concentrations of aluminum in the River Gvozdanka were around 10 μg L^{-1}.

Basic quality parameters

Basic quality parameters are used to determine contamination of water. Results for determination of basic quality parameters are shown in Table II.

Very high acidity of samples was found in those of

Table II
Results for basic quality parameters of water samples

	Water samples								
	G_1	G_2	G_3	G_4	G_5	I_1	V_1	V_2	V_3
Temperature, °C	12.0	12.5	12.5	12.5	12.5	12.0	13.0	13.0	13.0
pH	7.6	7.7	7.8	7.8	7.8	6.6	**3.8**	**3.6**	**3.7**
Electrical conductivity, μS cm^{-1}	185.6	186.6	189.0	188.4	197.0	113.2	839.0	849.0	848.0
Oxidation-reduction potential, V	0.3	0.3	0.3	0.3	0.3	0.3	0.6	0.6	0.6
Turbidity, FTU	0	0	0	0	0	0	**100**	**77**	**100**
Acidity, mg $CaCO_3$/L	52.9	43.7	72.1	43.5	44.9	54.8	**402.0**	**369.5**	**340.4**
Alkalinity, mg $CaCO_3$/L	96.2	118.4	105.7	102.1	89.8	52.1	pH <4.5	pH <4.5	pH <4.5
Total hardness, mg $CaCO_3$/L	104.0	104.0	100.5	98.2	103.9	52.1	189.8	182.2	179.8
Total hardness, °dH	5.8	5.8	5.6	5.5	5.8	2.9	10.6	10.2	10.1
TDS, mg L^{-1}	92.0	106.0	100.0	96.0	135.0	111.0	**628.0**	**631.0**	**636.0**
Dissolved oxygen, mg O_2/L	11.1	9.8	10.0	6.9	6.7	5.6	**0.1**	**0.1**	**0.4**
Free cyanides, mg L^{-1}	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Nitrites, mg L^{-1}	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	0.01	0.01
Nitrates, mg L^{-1}	<0.1	1.8	<0.1	1.4	<0.1	0.2	<0.1	0.1	0.2

drainage water, average value was 370.6 mg $CaCO_3$/L. Acidity in the samples from the River Gvozdanka (average value was 51.4 mg $CaCO_3$/L) and samples of drinkable water (59.1 mg $CaCO_3$/L) were much lower (Table II). Electrical conductivity is a useful measurement of the general level of chemical pollution (average value of electrical conductivity in the drainage water was 845.3 μS cm^{-1}). The results of electrical conductivity are presented in Table II. The results for drainage water showed much higher electrical conductivity, which means high ion concentrations and therefore pollution. Electrical conductivity in the samples of River Gvozdanka and the source of drinkable water were much lower (average value in River Gvozdnka was 189.3 μS cm^{-1}, and in the sample of drinkable water 113.3 μS cm^{-1}).

As the next parameter oxidation reduction potential was studied and the results are shown in Table II where the samples of drainage water had much higher oxidation reduction potential. The oxidation reduction potential was several times higher in the drainage water samples (average value +0.61 V) than in the samples of the River Gvozdanka (average value was +0.29 V). All the samples were oxidative medium (positive values of potential), it means that oxidation was the favored process in the water samples, especially in the drainage water. Results for the total hardness are shown in Table II. The total hardness of water showed higher results in the samples of drainage water (average value was 183.3 mg $CaCO_3$/L). Lower results were found in the samples of the River Gvozdanka (average value was 102.1 mg $CaCO_3$/L). The lowest results were in samples of drinkable water. The River Gvozdanka is very rich with dissolved oxygen (average value was 9.0 mg O_2/L) The drainage water had almost no dissolved oxygen, indicating that the quality of drainage water was poor and aquatic life is impossible in that medium.

The results for free cyanides, nitrites and nitrates showed no contamination. Concentrations of these anions in a large number of samples were below the limit of quantification. The process of mining at the Bakovici mine included cyanisation, so measurable concentrations of free cyanides were to be expected. However, as the area of the mine is very acidic and has low pH value, cyanides probably evaporate as HCN. Cyanides in the water medium are stable only if the pH value was high (approximately 10).

Analysis of other metals

A very important part of the study was the analysis of metals, especially heavy metals, as they present general and real environmental problems[14]; this was the fact also in the studied area. If we compare the average concentration values of metals in the River Gvozdanka, regarding drinking and drainage waters it was evident that the drainage water had different chemical composition. The average values of heavy metals were higher in samples of drainage water than in samples of the River Gvozdanka and the source of drinkable water. This is because of the formation of AMD products in the drainage water. The concentration values were compared with the maximum allowed concantrations of contaminants presented by EPA in 2009.

The concentrations of other metals (Na, Si, Cr, Cu, Pb, Au, As, Zn, Cd and Ag) were also higher in the drainage water but the concentrations did not exceed the MAC values. It is significant that all the samples contained traces of gold. The average concentration of gold in the samples of the drainage water was 0.62 μg L^{-1}, and in the samples of the River Gvozdanka (average value was 1.01 mg L^{-1}). Gold was the only analyzed metal that showed higher concentrations in the River Gvozdanka than in the samples of drainage water. It seems that dissolved gold in the River Gvozdanka also came from another and unknown gold deposit, which has been proven now for the first time.

Table III
Results for analysis of metals in water samples

Metal	Water samples									
	G_1	G_2	G_3	G_4	G_5	I_1	V_1	V_2	V_3	MAC
Na, mg L^{-1}	1.6	2.2	1.8	2.1	1.7	3.5	4.4	5.5	4.1	–
Si, mg L^{-1}	0.3	0.3	0.2	0.3	0.6	0.7	0.2	0.2	0.1	–
Cr, μg L^{-1}	3.2	4.4	3.5	3.6	9.9	3.6	5.3	4.8	3.7	100
Cu, μg L^{-1}	2.8	3.7	1.9	4.5	1.7	1.3	6.2	9.8	6.8	1300
Pb, μg L^{-1}	1.2	2.2	1.1	2.8	1.5	0.8	9.6	7.4	2.3	15
Au, μg L^{-1}	1.0	1.2	1.0	0.8	1.4	0.8	0.9	0.5	0.4	–
As, μg L^{-1}	0.4	0.3	0.2	0.3	0.2	<0.2	0.7	0.8	0.3	10
Zn, μg L^{-1}	<0.2	5.5	<0.2	12.7	<0.2	<0.2	19.7	15.5	14.3	5000
Cd, μg L^{-1}	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	0.7	0.8	0.3	5
Ag, μg L^{-1}	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	0.9	<0.2	–

Conclusion

The area of around the closed Bakovici gold mine in Central Bosnia and Herzegovina was selected for the study of acid mine drainage and several water samples were collected, analyzed, and discussed. There were large differences in water quality between different types of water samples. From the presented results of the analysis of the River Gvozdanka, it is evident that the river is chemically very clean and there are no signs of contamination because of mining activity. The concentrations of all the studied contaminants were below the prescribed values. The only critical parameter that was close to the maximum permissible limit was iron. According to pH and Eh values (pH-Eh diagrams), iron was in the samples of the River Gvozdanka in $Fe(OH)_2$ form. Analyses of the samples of drinking water also indicated that the source was not contaminated. All measured parameters were below the prescribed values. The process of acid mine drainage was proven by pH value and with sulphate content that was higher than in the samples of the River Gvozdanka. The drainage water flow coming out of the ore body clearly showed the presence of a high degree of contamination. According to the classification of mine waters[15], this water belongs to the acidic mine waters. Analysis of the drainage water showed: high amounts of sulphate, low pH, high conductivity, and some other parameters indicated the presence of the process of acid mine drainage. Acid mine drainage is still active and will continue in the future. The drainage water was highly contaminated with iron and aluminum. According to the pH and Eh values iron was mainly in the $FeO(OH)_{(s)}$ form, and aluminum was mainly in the $Al^{3+}_{(aq)}$ form. The concentrations of other measured metals were also higher than expected. Future work will or can be focused on plant, animal material, sediment analysis, as no data are available.

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DETERMINATION OF Pb(II), Zn(II), Ni(II) and Co(II) IN FLY ASH EXTRACTS USING STRIPPING VOLTAMMETRY

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Keywords: fly ash, leaching, stripping voltammetry and chronopotentiometry, mercury, amalgam and screen-printed electrodes, organic matter

Abstract

The paper presents a report on the application of different working electrodes (hanging mercury, silver amalgam and *in-situ* plated lead film screen-printed electrode) for the stripping voltammetric and stripping chronopotentiometric determination of Ni(II), Co(II), Zn (II) and Pb(II) in fly ash extracts. The methods of eliminating dissolved organic matter and its influence on the sensitivity of trace metal determination is discussed. Voltammetric studies are complemented with optical and scanning microscopic observations.

Introduction

According to Regulation (EU) no. 305/2011, buildings must be designed and constructed in such a way that they will not be a threat to the health and safety of workers, occupants or neighbours over their entire life cycle[1].

Concrete plays a key role in construction industry and it is a basic material for all types of construction. Cement is the most important ingredient in the production of concrete, acting as the binder that glues sand and aggregates together. The construction industry consumes large amounts of raw materials such as aggregates, gravel, sand as well as clay, shale and limestone used for cement production. The use of alternative raw materials reduces the need for quarrying by replacing non-renewable resources with waste. The two types of waste successfully exploited in cement production are granulated blast furnace slag, a by-product of the iron manufacturing process, and fly ashes, one of the residues generated from the combustion of coal. The applications of fly ash are not only limited to cement and concrete production, but also include fill materials for structural applications and embankments, components in road bases, sub-bases, and pavement, and mineral filler in asphalt[2]. Fly ash contains trace metals that can have environmental consequences when this material is used in geotechnical applications. To assess the impact of fly ash containing material on soil and groundwater, the content of water-soluble constituents – primarily inorganic substances (metals and salts) – should be estimated. The passage of water or an aqueous solution through the porous material (e.g. concrete, solidified soil, road base, etc.) along with the diffusion of contaminants through pore water is regarded as the main mechanism of the release of toxic substances to the environment. To evaluate the potential of a material to release contaminants to the environment, extraction (leaching) tests are used. The material is exposed to a leachant (water, acetic buffer or diluted nitric acid) and the amount of contaminant in the extract is measured and compared to a relevant standard (e.g. Directive 999/31/EC).

The indicative list of regulated dangerous substances possibly associated with construction products is very long and contains more than 70 organic substances which may be released into soil, surface water and groundwater[3]. Heavy metals such as Cr, Cu, Co, Mo, Se, V, Zn, Tl, Pb, Cd, Ni, Hg, Sn, Sb, As and Ba are included in the list. In the case of fly ash, the predominant elements that are released are Ca and S (as part of SO_4^{2-}), followed by Cl, Na and K, and then a large number of trace elements leached at levels that are generally below 1 mg kg^{-1}. The spectrometric methods (ICP-MS, ICP-OES) routinely used for the analysis of trace elements in natural water may not be appropriate for the analysis of extracts containing a high load of soluble salts. The salt reach matrix causes the clogging of the nebulizer and sampling orifice, which results in a gradual decrease in the signal intensity, and requires the instrument to be shut down for maintenance. Simple sample dilution (the total content of dissolved solids must be below 0.1 %) is often insufficient to mitigate the signal suppression and, furthermore, the low content of target element limits the allowable degree of dilution. To overcome matrix and spectral interferences, it is often necessary to perform internal standardization and laborious standard additions method coupled with an internal standard. Moreover, spectrometric methods are unsuitable for on-site screening because of equipment size, cost and the time required to perform the analysis.

Electrochemical techniques are very sensitive analytical methods that may be used for the detection of toxic metal ions in environmental samples. The most sensitive techniques such as stripping voltammetry and potentiometry enable the simultaneous determination of several elements in low- or high-ionic-strength solutions without or after minimum sample modification. A high content of soluble salts does not hamper voltammetric

determination, because the dissolved salts can play the role of supporting electrolyte and minimize the contribution from migration as well as lower the solution resistance.

The aim of this project is the application of stripping techniques, such as stripping voltammetry (SV) and stripping chronopotentiometry (SCP), for the assessment of the release of the selected toxic metals from fly ash used as a component of building materials into the environment. Aside from the conventional hanging mercury electrode (HMDE), the more environmentally friendly amalgam film electrode (Hg(Ag)FE)[4–7] and mercury-free screen-printed electrodes were also applied.

Experimental

Apparatus and instrumentation

Electrochemical measurements were performed using a μAutolab (GPES 4.9 software) potentiostat (Ecochemie, Netherlands) with a standard three-electrode configuration. As a counter electrode served a platinum wire and an Ag/AgCl electrode (3 M KCl) (Metrohm, Switzerland) was used as the reference one in connection with mercury electrodes. The following working electrodes were applied: a) control growth mercury drop electrode (1.9 mm^2 area, MTM, Poland), b) "home-made" silver amalgam electrode (2 mm silver wire of 1 mm diameter) and c) screen-printed graphite electrode plated *in situ* with lead film[8] (4 mm diameter, DRPC-110 Dropsens, Spain). The surface of the Hg(Ag)FE was refreshed mechanically before each measurement; namely, the silver wire electrode inside was pulled up across the mercury chamber and then pushed back outside the electrode body. Before recording voltammograms by means of mercury electrodes, oxygen was removed from the examined solution by flowing argon (7 minutes). Photolysis was performed using a UV digestion system with a 150 W lamp (Mineral, Poland).

Instrumental parameters

1. The procedure used for the HMDE
a) for Zn and Pb determination – DP-ASV in solution containing 0.1 M accetic buffer (pH 4.5) instrumental parameters were as followed E_{acc}=–1.2 V, t_{acc}=30 s (for Zn) or 60 s (for Pb), t_{eq}=10 s, ΔE=50 mV;
b) for Ni and Co determination – DP-AdSV in solution containing 0.1 M ammonia buffer (pH 9.2) and $5 \cdot 10^{-5}$ M nioxime instrumental parameters were as followed E_{acc}=–0.85 V, t_{acc}=30 s, t_{eq}=10 s, ΔE=50 mV.
2. The procedure used for the Hg(Ag)FE
a) for Zn and Pb determination – CC-ASCP in solution containing 0.1 M acetic buffer instrumental parameters were as followed: E_{cond}=–1.0 V, t_{cond}=10 s, I=1 μA.
b) for Ni and Co determination – CC-AdSCP in solution containing 0.1 M ammonia buffer, $5 \cdot 10^{-5}$ M nioxime and 0.36 M $NaNO_2$ (in the case of Co) instrumental parameters were as followed: E_{cond}=–1.35 V, t_{cond}=20 s, E_{acc}=–0.8 V, t_{acc}=60 s, t_{eq}=5 s, E=2 mV, I=20 μA
3. The procedure used for the PbF-SPE
Only for Ni and Co determination – SW-AdSV in solution containing 0.2 M ammonia buffer (pH 8.2), $4 \cdot 10^{-5}$ M Pb(II), $1 \cdot 10^{-5}$ M DMG instrumental parameters were as followed: E_{cond}=–1.4 V, t_{cond}=20 s, E_{acc}=–0.8 V, t_{acc}=120 s, t_{eq}=5 s, f=50 Hz.

SEM images of the samples were obtained by employing the Nova NanoSEM 200 (FEI, USA) scanning electron microscope (SEM) with an elemental energy dispersive X-Ray microanalyser. A representative portion of fly ash sample was characterized by randomly selecting 3–4 fields of view and examining several particles observed within the selected fields.

Reagents

All solutions were made using deionized water with a resistivity of 18.2 MΩ (Millipore Simplicity UV, USA). The following reagents (analytical grade, POCH, Poland) were used without further purification: 30% H_2O_2, CH_3COOH, $CH_3COONa \cdot 3\,H_2O$. Nioxime, dimethylglyoxime (DMG) and $NaNO_2$ (analytical grade, POCH, Poland) were crystallized from ethanol and water, respectively. A 2 M ammonia buffer (pH 8.2) was prepared by mixing the corresponding amounts of NH_4Cl and ammonia solution (both Suprapur, Merck). NH_3 or HCl were added to obtain the required pH of the supporting electrolyte. 0.01 M DMG and nioxime solutions were prepared by dissolving an appropriate amount in NaOH (0.1 M, analytical grade). A standard solutions of Ni(II), Co(II), Pb(II) and Zn(II) at a concentration of 1 g L^{-1} were obtained from Merck and diluted as required. Amberlite XAD-7HP resin and fumed silica (99.8%) were delivered by Sigma, USA.

Procedures

Extraction Procedure

The extraction procedure was performed according to EN 12457-2:2002 (ref.[9]). During this procedure the samples are leached with distilled water (room temperature) at a 1:10 sample-to-solvent ratio. The mixture was sealed in polypropylene extraction bottles and placed in a rotary agitator (36 rpm, bottles placed at an angle of 45 degrees to the axis of rotation) for 24 h. After mixing, the samples were filtered, and the pH and conductivity were measured. The fraction of the extract used for voltammetric determination was acidified to pH 2 using concentrated HCl Suprapur (Merck).

Sample characteristics

The fly ash samples were collected from coal-based thermal power stations located in Poland. The sample labelled 'I' originated from the electrostatic precipitator of

a fluidized-bed, lignite-fired boiler. Sample 'II' was obtained from an electrostatic precipitator attached to a bituminous-coal-fired boiler.

Elimination of Organic Matter and Voltammetric Procedure

Prior to the voltammetric quantification of Zn, Pb, Cd, Ni and Co, it was necessary to perform photolytic decomposition of dissolved organic matter (DOM) or its adsorption on the Amberlite XAD-7HP resin[10,11] or fumed silica[12]. The acidified extract samples were spiked with 30% H_2O_2 (1 µl perhydrol per 1 ml of sample) and exposed to UV light in the digesting system for 2 h or more, if required. After digestion, the cooled samples were diluted with the supporting electrolyte and examined.

The Amberlite XAD-7HP resin was cleaned (at least four-fold) using deionized water and dried at 50 °C prior to analysis. Before the voltammetric determination 0.5 g of the resin was added to the vessel containing 10 ml of the examined solution and the mixture was stirred for 5 minutes. The mixture was not filtered; the voltammograms of the sample and the sample after the addition of standards were recorded with the resin in the vessel.

The fumed silica was calcinated at 900 °C for 2 hours in an oven, and then stored in a desiccator. The required amount was added directly to the voltammetric vessel.

Results and discussion

Although stripping analysis provides a highly sensitive way of measuring trace metal content, its widespread utility is restricted. The main problem is the accumulation of undesirable substances on the surface of the working electrode. The adsorption of electroactive and

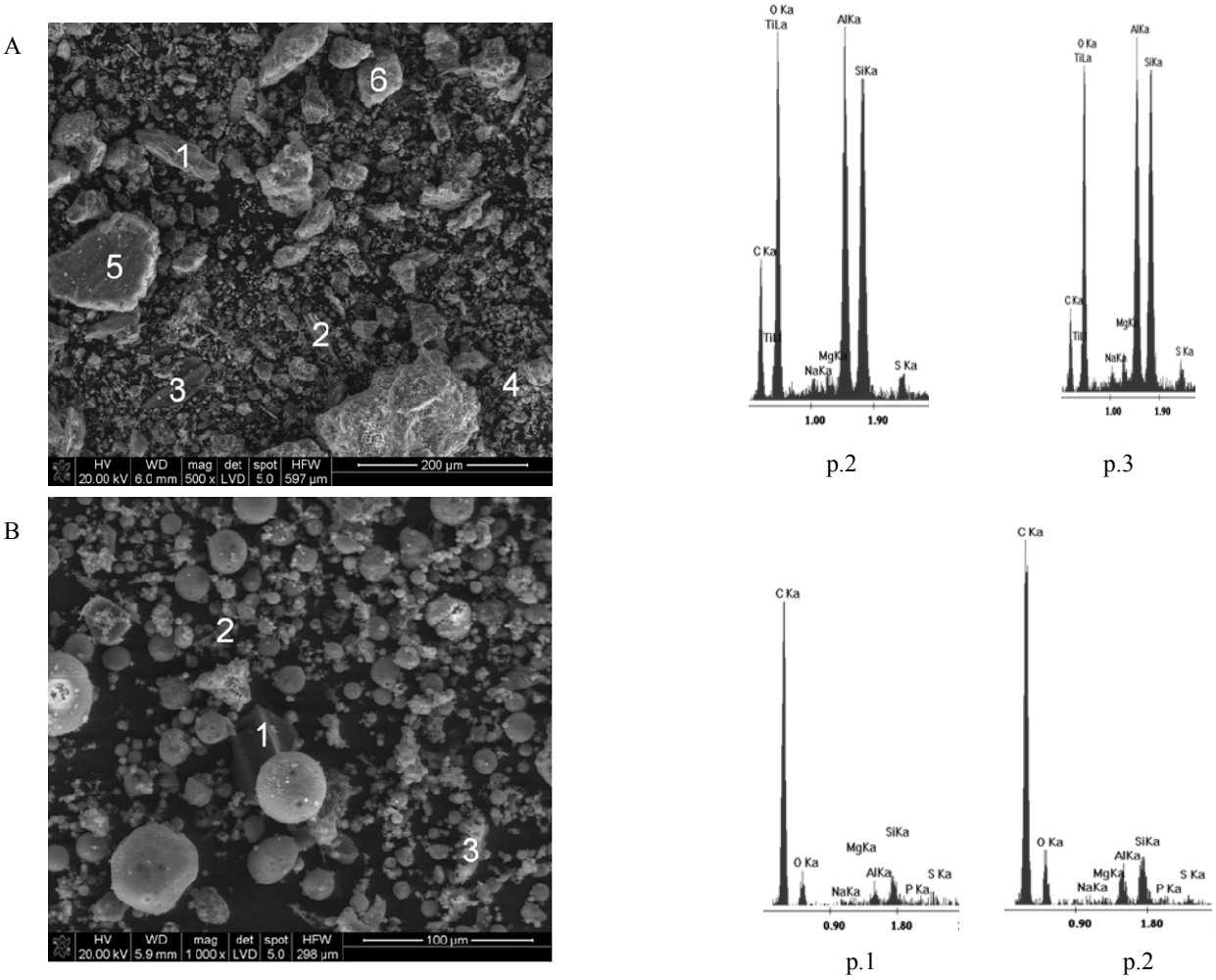

Fig. 1. **SEM images of fly ash samples: (A) fly ash 'I' (fluidized bed) and (B) fly ash 'II' (conventional burner); right column: SEM/EDS spectra**

non-electroactive species has complex effects on the voltammetric observations. These species can interfere via an overlapping response and/or competition at adsorption sites. The accumulated organic species retain their redox characteristics and can produce voltammetric signals originating from their reduction or oxidation processes. The adsorptive accumulation may also result in a substantial depression of double-layer capacitance and cause a higher background response due to adsorptive/ desorptive tensammetric peaks.

The examined fly ash samples contain some amount of carbon–rich compounds, which originate from unburned carbon. Examples of X-ray fluorescence spectra of fly ash samples shown in Fig. 1, derived from locations marked 1-3, demonstrate the presence of carbon. In the case of sample II (fly ash from a conventional burner), carbon is the predominant element in the examined locations. This could hinder the determination of trace metals.

During the preliminary voltammetric studies involving the hanging mercury drop electrode (HMDE), it was found that dissolved organic matter present in the fly ash extract was so persistent that is was impossible to eliminate its influence by simple means such as adjusting the accumulation potential or the pH of the supporting electrolyte. It was necessary to adsorb the organics using the Amberlite resin or fumed silica, or, alternatively, to apply UV irradiation to break it down. Both adsorption and photolysis approaches were efficient, as shown in Fig. 2, but the latter one was less laborious. Before use, it was necessary to condition both the resin and the silica to remove impurities. The Amberlite resin was cleaned by washing and drying in 50 °C to dryness, which took as much as 24 hours, while the silica was calcinated at 900 °C for 2 hours. The presence of the relatively large volume of the resin in the voltammetric vessel impaired the stirring and reduced the efficiency of analyte accumulation.

To avoid such difficulties it was necessary to separate the cleaned sample from the resin and transfer it to another vessel. To simplify sample handling and minimise the risk of sample contamination during introductory preparation, photolysis was applied in all subsequent voltammetric experiments.

In addition to investigating stripping voltammetry, the usefulness of the constant current stripping chronopotentiometry (CC-SCP) for the determination of trace metals in fly ash extracts was also tested.

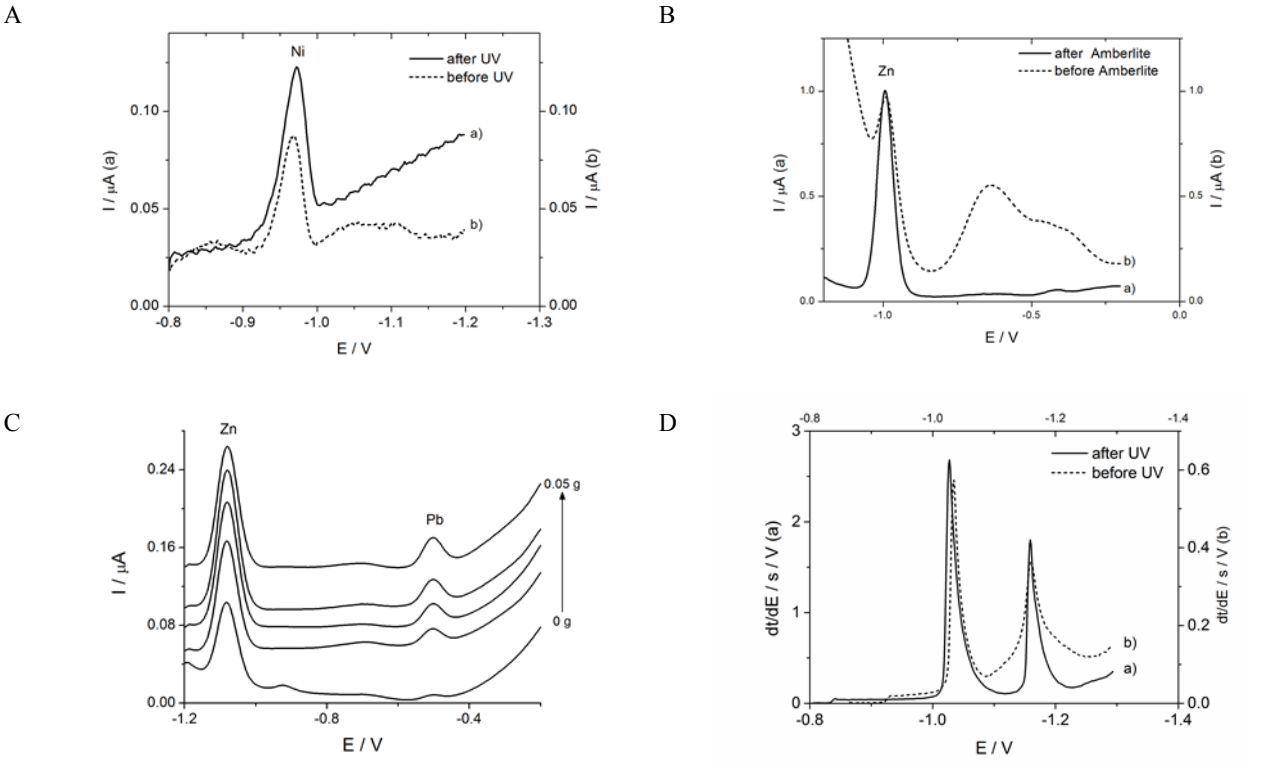

Fig. 2. **(A) DP-AdSV and (B,C) DP-ASV curves recorded at the HMDE for natural and treated fly ash extracts** (A – UV irradiation, B – Amberlite XAD-7HP resin or C – fumed silica). A – 20 times diluted extract 'I' before (a) and after 2 hours of UV photolysis (b); B – 2 times diluted extract 'II' in the absence (a) and in the presence (b) of Amberlite XAD-7HP (0.5 g of resin added to 10 ml of solution). Electrolyte: 0.1 M acetic buffer (pH 4.5). C – 2.5 diluted extract 'I' in the absence (a) and in the presence (b) of (0.01, 0.02, 0.03, 0.04 and 0.05 g) fumed silica. D – CC-AdSCP potentiograms recorded at Hg(Ag)FE for 5 times diluted fly ash extract 'I' after (a) and without UV treatment (b)

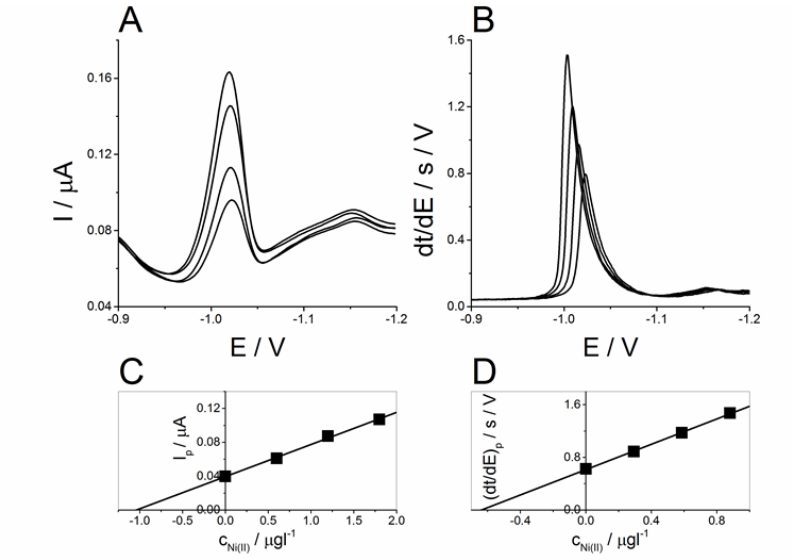

Fig. 3. **(A) DP-AdSV voltammograms and (B) CC-AdSCP potentiograms recorded for fly ash extract ‚I' without and after Ni(II) standard additions.** Examined solution: (A) sample diluted 2.5 or (B) 4 times. **Calibration plots: (C) for DP-AdSV and (D) for CC-AdSCP.** Electrodes: HMDE (A, C) and Hg(Ag)FE (B, D)

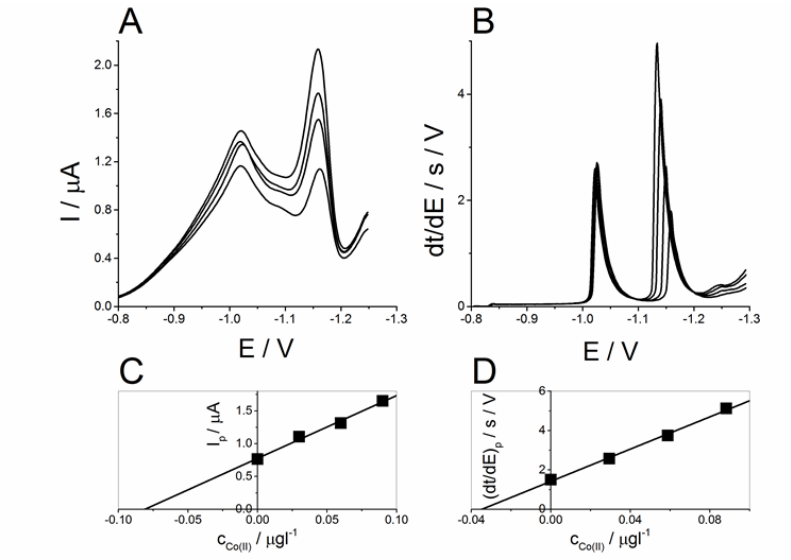

Fig. 4. **(A) DP-AdSV and (B) CC-AdSCP curves recorded for fly ash extract 'I' without and after Co(II) standard additions.** Examined solution: sample diluted (A) 2.5 or (B) 5 times. **Calibration plots: (C) for DP-ASV and (D) for CC-AdSCP.** Electrodes: HMDE (A, C) and Hg(Ag)FE (B, D)

Additionally, to meet the environmental requirements, the classic HMDE electrode was replaced with the non-toxic Hg(Ag)FE (ref.[13,14]). The two tested techniques do not differ with respect to the accumulation step, but the signal obtained during CC-SCP experiments represents potential in a function of time (as opposed to the voltammetric signal representing current in a function of potential). The potential at any time is determined by an oxidation or reduction process, and it therefore remains virtually constant until a particular analyte is depleted. Due to the differences between stripping chronopotentiometry (SCP) and stripping voltammetry (SV), it is likely that surface active compounds (SAS) affect the two techniques in different ways. However, there is conflicting evidence in the literature as to the extent to which organic matter and surface-active compounds can interfere in CC-SCP measurements. To obtain firsthand information about feasible differences between signals recorded using DP-SV

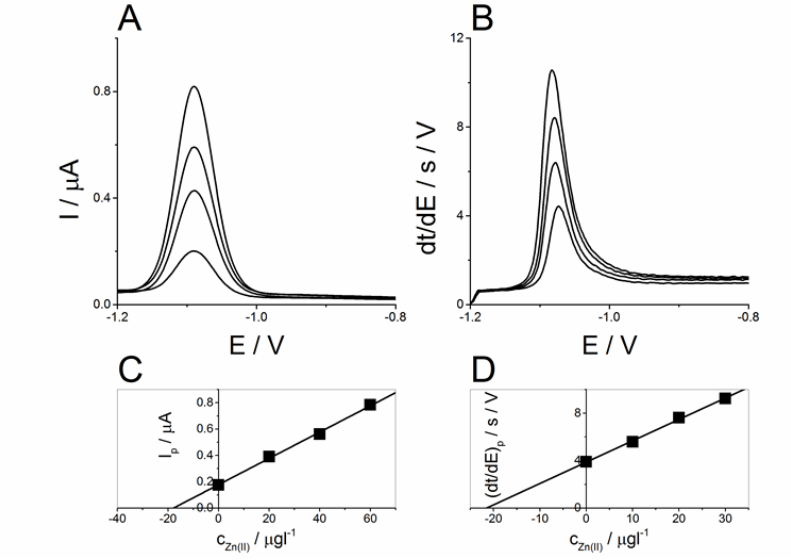

Fig. 5. **(A) DP-ASV and (B) CC-ASCP curves recorded for fly ash extract 'I' without and after Zn(II) standard additions.** Examined solution: sample diluted (A) 2.5 or (B) 3 times. **Calibration plots: (C) for DPV and (D) for PSA.** Electrodes: HMDE (A, C) and Hg (Ag)FE (B, D)

Table I
Comparison of the half peak width ($w_{1/2}$), peak separation (DE_p) and peak-to-background ratio ($I_p/I_{background}$) for DP-SV and CC-SCP signals shown in Figure 3–5

	Signal enrichment phenomenon	$W_{1/2}$ / V		DE_p / V		$I_p/I_{background}$	
		DP-SV	CC-SCP	DP-SV	CC-SCP	DP-SV	CC-SCP
Ni	adsorption	0.039	0.015	0.138*	0.132*	1.6	14.4
Co	adsorption and catalysis	0.035	0.015			2.0	15.4
Zn	electrolysis	0.063	0.040	0.589**	0.587**	5.5	6.3
Pb	electrolysis	0.063	0.035			1.2	1.6

* the peak potential separation $DE_p = E_{pNi(II)} - E_{pCo(II)}$, ** the peak potential separation $DE_p = E_{pZn\ (II)} - E_{pPb(II)}$

and CC-SCP, experiments involving UV-irradiated and non-irradiated fly ash extract samples were performed.

As shown in Fig. 2D, the CC-AdSCP signals are less affected by SAS than voltammetric curves. The signal of Ni(II) ions is almost the same in both the UV-treated and the 'natural' sample, while that of Co(II) is two times narrower after photolysis. The relative insusceptibility to SAS is not the only asset of CC-SCP. The results shown in Figs. 3–5 and Table I also indicate the advantages of either the adsorptive CC-AdSCP or anodic CC-ASCP over DP-SV with respect to the half peak width and peak-to-background ratio (Figs 3,4). The predominance of CC-SCP over DP-SV is especially striking in the case of Ni and Co determination.

The results presented above show that both the hanging mercury drop electrode and the amalgam electrodes may be successfully applied for the determination of trace metals in fly ash extracts. Unfortunately, the HMDE is inconvenient for on-site applications due to its bulky body, relatively brittle glass accessories, and – most importantly – because of mercury's toxicity and issues related to its disposal. Furthermore, keeping in mind the fact that the environment management policies call for total mercury elimination and promotion of mercury-free replacement products, screen-printed electrodes (SPE) were tested. A screen-printed graphite layer covered by *in-situ* plated lead film (PbF-SPE) was used as the working electrode[8]. The reproducibility of signals recorded in a fly ash sample covered *in-situ* with the lead layer was satisfactory (RDS = 7 %). The concentration of Co(II) in the previously tested extract I was too low to be determined using SPE;

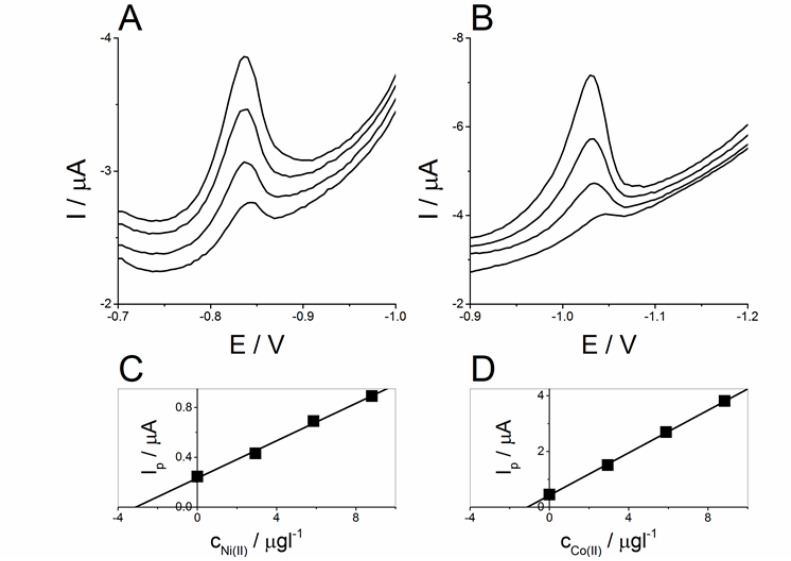

Fig. 6. **SW-AdSV voltammograms recorded in fly ash extract 'II'** (9 ml of extract in 10 ml of analyzed solution). Electrodes: PbF-SPE

Table II
Leachate characterization for the ash sample I and II (the recovery of metals ions spiked to the leached solution ranged from 96% to 101%)

	Fly ash I		Fly ash II
	DP-SV (HMDE) [μg l^{-1}]	CC-SCP Hg(Ag)FE [μg l^{-1}]	SW-SV (SPE) [μg l^{-1}]
Zn(II)	54.9 Inert	53.8 Inert	–
Pb(II)	2.0 Inert	2.4 Inert	–
Ni(II)	2.6 Inert	2.9 Inert	3.5 Inert
Co(II)	0.2 Non-regulated	0.17 Non-regulated	1.2 Non-regulated

Legend: Inert, non-regulated – Council decision of 19 December 2002 establishing criteria and procedures for the acceptance of waste at landfills pursuant to Article 16 of Annex II to Directive 999/31/EC (2003/33/EC)

consequently, a new extract of fly ash containing a higher concentration of Co(II) was used. Examples of determination of Co and Ni in fly ash samples are shown in Fig. 6. The summary of the obtained results and fly ash classification is presented in Table II.

Conclusion

The release of dangerous substances is a problem of great economic significance, as under Regulation (EU) no 305/2011 the leaching assay is among the basic requirements that construction works must satisfy. The introduction of alternative fuels may, however, impair boiler performance, and the amount of unburned matter in fly ash (and thereby in its water extracts) may be elevated. In consequence, it is expected that the level of organic matter present in the fly ash water extracts will increase. The performed experiments proved that UV pretreatment and the use of either the Amberlite XAD-7HP resin or fumed silica were sufficiently effective at eliminating the interfering dissolved organic matter. Both stripping voltammetry and striping chronopotentiometry are suitable techniques for evaluating the release of dangerous metals such as Zn, Pb, Ni and Co from fly ash. Stripping chronopotentiometry is less susceptible to SAS

interferences and it surpassed DP-SV with respect to the half peak width and peak-to-background ratio. The superiority of SCP over SV is especially striking in the case of Ni and Co determination.

The voltammetric procedure coupled with environmentally mercury-free screen-printed electrodes enabled the determiantion of Ni(II) and Co(II). Their application will further shorten and simplify the voltammetric determination of targeted metals.

Financial support from the Polish National Science Centre (Project No. 011/01/B/ST8/07794) is gratefully acknowledged.

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AMPEROMETRIC TYROSINASE CARBON PASTE-BASED BIOSENSOR IN FOOD ANALYSIS

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Keywords: amperometry, antioxidant, biosensor, carbon nanotubes, carbon paste electrode, polyphenols, tyrosinase, Vitamin E, Trolox

Abstract

The biosensor described in this contribution is based on a carbon paste electrode modified by carbon nanotubes and further, by the enzyme tyrosinase immobilized on the electrode surface through the Nafion conductive copolymer. The use of the enzyme biosensor to determine the total concentration of phenolic compounds was optimized and tested in model samples containing up to 16 % of ethanol. This nutrition value is expressed as a mass equivalent of water-soluble analogue of vitamin E, known as Trolox. Thus, the amperometric procedure monitoring the current response could substitute or alternate the commonly used spectrophotometric evaluation. The biosensor was tested to determine the Trolox equivalent in wine and, generally, it can be recommended to the same application in food analysis for samples containing low contents of vitamin C such as wines, beers, teas, or selected fruits to monitor their freshness or to control technological processes.

Introduction

Mushroom tyrosinase is metalloenzyme with catecholase and cresolase activity[1] which is presented in plants, bacteria or fungi[2]. This enzyme is capable to insert another hydroxyl group in *ortho* position of hydroxybenzene (cresolase activity) and oxidizes these two hydroxyl groups (catecholase activity); therefore tyrosinase catalyzes oxidation of polyphenols by air oxygen[3], which can be exploited in construction of biological analytical device (biosensor). Quinoid compounds are formed during oxidation of polyphenols presented in sample and they can be easily electrochemically reduced at constant working potential during amperometric detection[4].

Polyphenolic compounds are significant antioxidants[5], because negative effect of free radicals is eliminated by the reduction properties of polyphenols, thus the determination of total phenolic content (TPC) is important in food analysis. TPC can give valuable information of freshness, authenticity, and nutritional value of foodstuffs. TPC can be expressed as mass equivalent of water soluble analog of vitamin E, which is known as Trolox[6] – Trolox equivalent antioxidant capacity (TEAC).

Immobilization of tyrosinase on the surface of carbon paste electrode (CPE) or glassy carbon electrode (GCE) as electrochemical transducers using conductive copolymer Nafion[7] represents a simple way of developing relatively economically advantageous sensor for measuring of TPC or direct TEAC. Biosensor applications in food analysis and possibilities of using carbon nanotubes[8] (CNTs) to increase the sensitivity of the biosensor to polyphenols such as tocopherols were investigated in this paper, as well as its mechanical stability and shelf-life. It is important to know, how many measurements can be done by one sensor and which kinds of food samples could be analyzed. Matrix of samples has usually negative influence on selectivity and sensitivity of biological sensors. For this reason, influence of different content of ethanol on current response of catechol was evaluated.

Activity of mushroom tyrosinase upon the different substitution of hydroxyl groups on benzene ring was studied using isomers of diphenols with the aid of cyclic voltammetry and amperometry in batch configuration and in phosphate buffer solution (pH 7.0). Neutral value of pH is optimal for activity of used enzyme[9]. Recognition of individual polyphenols at tyrosinase biosensor is not possible without the use of preceding chromatographic technique. The biosensor is only suitable for determination of TPC or total antioxidant capacity (TAC) for samples with low concentration level of chemical compounds with reduction properties (non-phenolic substances).

Experimental

Chemicals

N,*N*-Dimethylformamide (DMF), Nafion (5 % m:m solution in lower alcohols), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), benzene-1,2--diol (catechol), benzene-1,3-diol (resorcinol), benzene-1,4--diol (hydroquinone), and mushroom tyrosinase (ex. *Agaricus bisporus*, EC 1.14.18.1, 3130 U mg^{-1} solid) were from Sigma-Aldrich. Ultrapure water (18.3 MW cm, Milli-Q system, Millipore) was used for preparing all the solutions. All other chemicals were of the analytical grade purity.

Carbon powder CR-2 with average particle size of 2 μm was from Maziva Týn nad Vltavou., s.r.o., Czech Republic. Paraffin oil for spectrometry was bought from

Merck, Darmstadt, Germany. Vienna, Austria. Multi-walled carbon nanotubes (MWCNTs, diameter 10–30 nm, length 5–15 μm and specific surface area 40–300 $m^2 g^{-1}$) and single-walled carbon nanotubes (SWCNTs, diameter < 2nm, length 5–15 μm and special surface area > 400 $m^2 g^{-1}$) were pursed from Shenzhen Nanotech Port Co., Ltd., Shenzhen, China.

Apparatus and instrumentation

A conventional three-electrode cell was used for all electrochemical experiments. The working compartment consisted of glassy carbon electrode (Radiometer, Denmark) or carbon paste electrode, both with diameter 3 mm, with immobilized CNTs covered by Nafion film with tyrosinase, a reference electrode (Ag|AgCl|3 M KCl) and an auxiliary electrode (platinum metal wire). A miniature potentiostat EmStat (PalmSens, The Netherlands) operated by the corresponding software (PSTrace, version 2.4.2) was used for electrochemical measurements. All potentials discussed in this work are referred to the above mentioned reference electrode.

Preparation of electrodes

Bare carbon paste electrode with resistance[10] ~10 Ω was prepared via thorough mixing of 0.5 g carbon powder with 130 μL of paraffin oil and subsequent pressing into the Teflon holder with conductive inner piston. Glassy carbon electrode was polished in alumia slurry with particle diameter of 0.5 μm, then sonicated in water and finally washed in water and ethanol. Carbon nanotubes were immobilized at the surface of bare CPE and GCE by casting 20 μL of 2.0 mg mL^{-1} CNTs suspension in DMF and drying one day under laboratory conditions.

Biosensor preparation

Nafion solution (40 μL), neutralized to pH 7 using 8 % ammonia, enzyme water solution (150 μL, 500 μg mL^{-1} of enzyme), and pure water (60 μL) were mixed in small vial. An aliquot (10 μL) of this mixture was pipetted on the surfaces of electrochemical transducers and left to dry under laboratory conditions for one hour. If not in use, the biosensors were stored in a refrigerator at temperature of 5 °C.

Procedures

Cyclic voltammetry (CV) and hydrodynamic amperometry in batch configuration were used as electrochemical techniques. All experiments were performed in 10 mL of 0.01 mol L^{-1} phosphate buffer (PB) pH 7.0 at temperature 25 ± 1 °C. Potential range for CV was set from –0.5 to 1.3 V, scan rate 0.05 V s^{-1}, and potential step 5 mV. Usually 10 μL of 0.01 mol L^{-1} solution of corresponding analyte was pipetted into 10 mL PB in voltammetric glass cell. Amperometric measurements were carried out also in the same glass cell containing 10 mL PB with constant stirring of 400 rpm. If not stated otherwise, the detection potential of –0.24 V was applied to the working electrode.

Results and discussion

Stability of Nafion membrane

Nafion membrane with soluble tyrosinase did not exhibit any visible signs of cracking during the drying step at GCE surface. Problems with cracking of membrane were observed only in case of immobilization using Nafion on the surface of CPE. Carbon paste is by nature a plastic electrode material comparing to rigid surface of glassy carbon. Therefore, Nafion membranes are expected to have better mechanical properties on GCE rather than on CPE surface. The membrane is not dissolved in mixed water-ethanolic solutions where the amount of ethanol is lower than ~50 % v/v. Such observation was confirmed in repetitive electrochemical measurements of catechol because tyrosinase with dissolved membrane goes out during the changing of supporting electrolyte for next analysis and any current response is not obtained in this measurement.

Effect of Nafion content in membrane

Peak potentials of catechol were shifted depending on increasing content of Nafion in membrane layer. They were shifted to more positive or more negative potentials due to increasing thickness and compactness of the layer, thus blocking the transfer of studied chemical compound to active sites of electrode. Peak currents for oxidation (I_p^a) and reduction (I_p^c) processes were suppressed with increasing content of Nafion in membrane to reach the limit value at 1 % v/v (Fig. 1). The current levels remained almost constant for contents of Nafion beyond this value.

Influence of CNTs on sensitivity

A catalytic effect of CNTs was observed when comparing I_p^a/I_p^c ratio at bare CPE to I_p^a/I_p^c ratio obtained at CPE/CNTs using cyclic voltammetry of Trolox. Carbon paste modified with CNTs exhibited pronounced cathodic signal for Trolox reduction. No significant difference between the use MWCNTs and SWCNTs was noticed. Similar electrochemical behavior was recorded when tyrosinase was used as biocatalyst in cyclic voltammetry of Trolox (Fig. 2). Resulting cathodic current response of Trolox was more than five times higher at CPE/CNTs than at bare CPE, which can be attributed to enlarged surface area and enhanced electron transfer at carbon nanotubes. Tyrosinase biosensor without the presence of CNTs was not found sensitive for Trolox detection; therefore it was not suitable for determination of total phenolic content expressed as TEAC value. At biosensor without the carbon

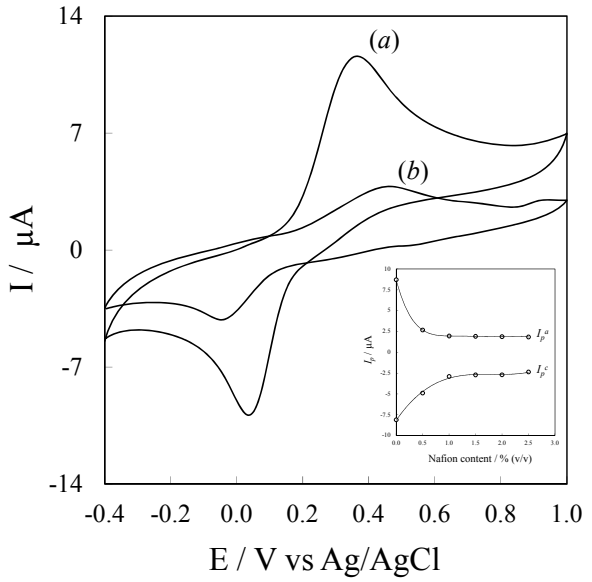

Fig. 1. **Cyclic voltammetry of $5 \cdot 10^{-4}$ mol L^{-1} catechol at GCE without (*a*) and with 2.5 % Nafion coating (*b*) in 0.1 mol L^{-1} PB (pH 7.0), scan rate 0.05 V s^{-1}.** Inset: dependence of oxidation (I_p^a) and reduction (I_p^c) peak current responses on Nafion content (v/v) in membrane

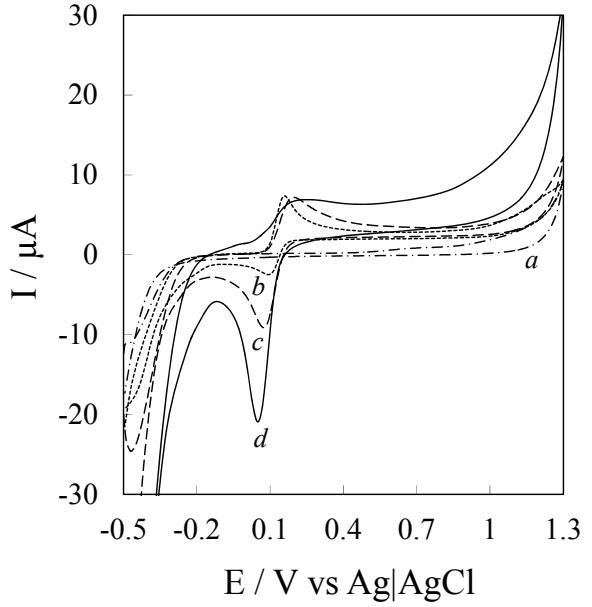

Fig. 2. **Cyclic voltammograms of blank (*a*) and $0.5 \cdot 10^{-3}$ mol L^{-1} Trolox at bare CPE (*b*), CPE/SWCNTs (*c*) and CPE/SWCNTs/tyrosinase/Nafion (*d*) in 0.01 mol L^{-1} PB (pH 7.0) at scan rate 10 mV s^{-1}**

nanotubes as electrode modifier, the TPC value can be estimated as mass equivalent of other phenolic compound such as hydroquinone or derivates of catechol, which easily undergo redox reactions at carbon electrodes.

Selectivity of tyrosinase biosensor to various benzenediols

Three benzenediols isomers were used as substrates to elucidate the influence of structure of analyzed compound, i.e. the positions of hydroxyl groups on benzene ring, on the activity of tyrosinase. Variously substituted benzene rings are possible to find in chemical structure of many important antioxidants such as flavonoids, stilbenes, phenolic acids, and tocopherols. In this study, lower current responses for benzene-1,3-diol (resorcinol) and benzene-1,4-diol (hydroquinone) were measured comparing to benzene-1,2-diol (catechol) at CPE/tyrosinase/Nafion biosensor. Such observation is in accordance with catecholase activity of enzyme tyrosinase[11].

Influence of ethanol in samples

Wines and beers are alcoholic drinks with ethanol content usually less than 15 %. These samples have certain concentration level of polyphenolic compounds. As it was observed during experiments, the sensitivity of tyrosinase biosensor decreased with increasing content of ethanol in the sample. The influence of ethanol content in supporting electrolyte on amperometric response is shown in Fig. 3. Linear behavior between current response and ethanol content was found; the dependence was ascertained for concentration of catechol $1 \cdot 10^{-5}$ mol L^{-1}, using GCE/tyrosinase/Nafion biosensor speed of stirring 400 rpm, and detection potential −0.2 V. The following equation of linear regression I (μA) = 0.1263 (μA V_{water} $V_{ethanol}^{-1}$) − 1.9641 (μA) with R^2 = 0.9963 was calculated. The limit amount of ethanol, which could be presented in the sample and still not completely diminished the current response of biosensor, was determined as 16 % ($V_{ethanol}$ / V_{water}). Concentration levels of polyphenols in wines or beers are usually higher than 10 μmol L^{-1}, therefore the biosensor is suitable for measurement of TPC in these samples.

Application of tyrosinase biosensor

Tyrosinase biosensor is suitable for measuring of TEAC in sample with low concentration level of ascorbic acid such as wines, beers, selected fruits, and vegetables at CPE(or GCE)/CNTs/tyrosinase/Nafion, because ascorbic acid can seriously interfere the current response due to its easy oxidation at relatively low positive potentials. Proposed biosensor can be useful e.g. in monitoring of right procedure in winemaking; there might be up to 40 % losses of polyphenols, which have significant influence of final taste, when the wine is filtrated. Analysis of real sample of selected red wine (Rulandské modré) using

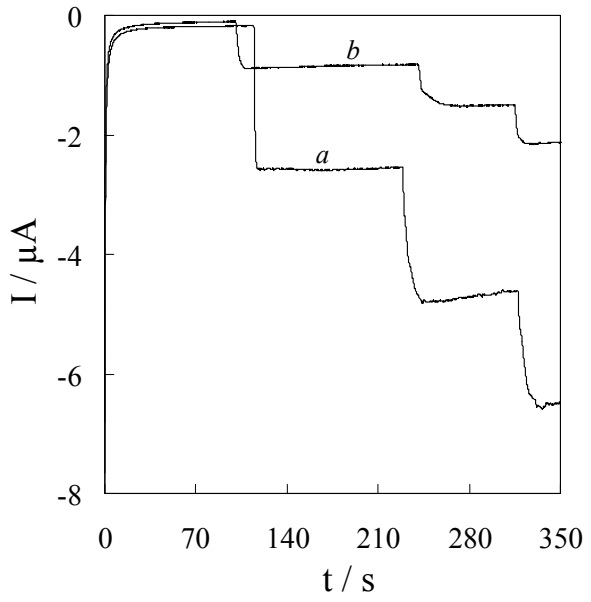

Fig. 3. **Hydrodynamic amperometry of $1 \cdot 10^{-5}$ mol L^{-1} catechol at GCE/tyrosinase/Nafion biosensor in 0.1 mol L^{-1} PB (pH 7.0) without (*a*) and with 10 % (*b*) ethanol.** Three standard additions of $1 \cdot 10^{-5}$ mol L^{-1} catechol, working potential –0.2 V, speed of stirring 400 rpm

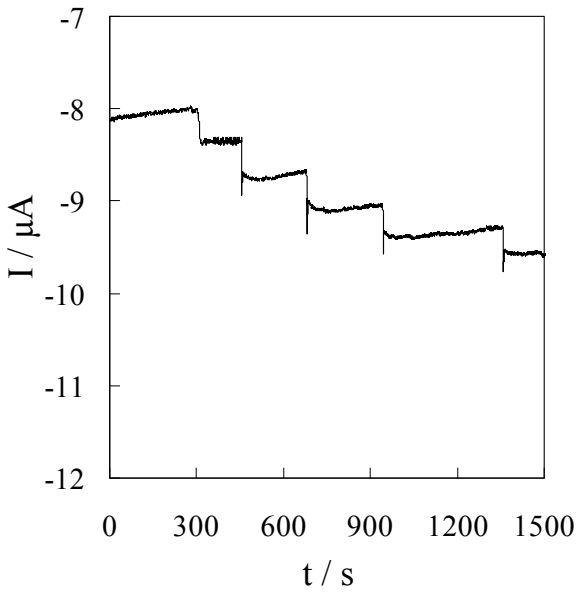

Fig. 4. **Typical analysis of Rulandské modré wine using CPE/MWCNTs/tyrosinase/Nafion biosensor.** 0.5 mL of sample and four individual additions of 0.5 mL 0.01 mol L^{-1} Trolox standard to 9.5 mL of 0.1 mol L^{-1} non-deareated PB (pH 7.0), working potential –0.24 V, stirring speed 400 rpm

CPE/MWCNTs/tyrosinase/Nafion biosensor is shown in Fig. 4. It can help to detect the authenticity of wine or assay the food freshness as well.

Lifetime of biosensors

Freshly prepared sensors provided constant current response for the equal additions of substrate during amperometric measurements. The currents started to decrease after one week from the preparation of particular sensor due to the limited lifetime of used enzyme, which loses its activity in time or might be leached from the membrane. Moreover, the Nafion coating is sensitive to drying; however, it can be prevented by storing the sensor in a vial with phosphate buffer.

Conclusion

Biosensor based on carbon paste or glassy carbon electrode and modified with carbon nanotubes and tyrosinase immobilized using Nafion membrane was found suitable for direct amperometric determination of TEAC in different kinds of foods. The presence of carbon nanotubes at the surface of electrode is inevitable for the feasible electrochemical monitoring of Trolox. The biosensor with GCE/CNTs has better mechanical properties than its analog with CPE. It is planned to use a screen-printed carbon electrode with immobilized CNTs as a low cost and disposable electrochemical transducer for future application of tyrosinase biosensor in flow injection system.

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PILOT RESEARCH ON EXPANDING SLOVENIAN NEWBORN SCREENING PROGRAMME FOR INHERITED METABOLIC DISORDERS DETECTABLE BY TANDEM MASS SPECTROMETRY

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Keywords: newborn screening, tandem mass spectrometry, inborn errors of metabolism, C5OH-Carnitine

Abstract

Slovenia has not yet incorporated tandem mass spectrometry (MS/MS) into its newborn screening (NBS) programme for inborn errors of metabolism (IEM). In our pilot study we will use MS/MS to screen newborns for IEM with the aim to evaluate a possible expansion of Slovenian NBS programme. To detect IEM we quantified 28 acylcarnitines and 12 amino acids in dried blood spots. So far we have screened 1000 newborns and found one clearly abnormal elevation of C5OH-Carnitine. The follow-up tests gave normal results so we concluded that it was a false-positive case.

Introduction

Inborn errors of metabolism (IEM) are rare diseases; the incidences are in the range of 1 per several thousand[1]. They are caused by genetic defects that lead to deficient or absent enzymes or defective proteins[2]. Early detection of IEM can help in making correct and timely clinical decisions that can prevent serious consequences of the diseases or even death[3,4].

Screening methods are very important in preventive medicine. Their goal is detecting disorders before or at the beginning of the disease to prevent the disease or to lessen its symptoms. They are used on a larger population of people who show no signs of the disease. Individuals, who have a risk for a specific disease, are searched for[5].

NBS for IEM started in 1962 in Massachusetts, USA with a bacterial inhibition test for the screening for phenylketonuria[6]. NBS soon started in other countries and was also expanded with additional IEM[7–10]. Introduction of tandem mass spectrometry (MS/MS) in the field of NBS allowed a major expansion of the number of screened IEM and is now widely used in most of the developed world[7,11]. MS/MS allows an accurate quantification of amino acids and acylcarnitines in dried blood spots (DBS) in one short run[12,13]. This enables the screening for up to 30 IEM with a single test[11].

In Slovenia we currently routinely screen newborns for two IEM, phenylketonuria and congenital hypothyroidism[14]. MS/MS is not yet used in Slovenian NBS programme. In our research we have screened newborns for IEM with MS/MS and it will be the first time that the incidences of other inborn errors of metabolism, besides phenylketonuria and congenital hypothyroidism, will be evaluated in the Slovenian population.

Experimental

Samples

Analysis of amino acids and acylcarnitines: DBS were collected for screening for phenylketonuria and congenital hypothyroidism[14]. Blood samples were collected on Whatman 903 filter paper from the third to the fifth day after birth and dried at room temperature. We have quantified the analytes from 3 mm disks, which were punched out of DBS with a hand punch. The study protocol was approved by the Slovene Medical Ethics Committee.

Analysis of urine organic acids: Urine samples.

Apparatus and instrumentation

Analysis of amino acids and acylcarnitines: PerkinElmer Series 200 HPLC System with a 3200QTRAP ABSCIEX tandem mass spectrometer.

Analysis of urine organic acids: Agilent 7890A Gas Chromatograph with an Agilent 5975C mass detector (GC/MS). Agilent Ultra2 25 m × 200 μm × 0.33 μm column was used.

Procedures

Analysis of amino acids and acylcarnitines: For the analysis we use "MassChrom® Amino Acids and Acylcarnitines from Dried Blood" reagent kit, which includes control samples with low and high levels of measured analytes. Sample preparation is as described in the instruction manual for sample preparation with filter plates. The main steps are:

1. Punch a 3 mm dried blood spot disk out of the filter

paper card. We used a hand punch.
2. Extract the analytes with the extraction buffer with added internal standard mix.
3. Derivatise the analytes to butyric esters with the derivatisation buffer at 60 °C.
4. Evaporate of the derivatisation reagent, after evaporation reconstitute the eluate in the reconstitution buffer.

Analysis of urine organic acids: Urine organic acids were measured with an in-house method.
1. Pipette 1 ml of urine into a glass tube.
2. Add 20–30 mg *O*-ethylhydroxylamine, incubate 15 min at room temperature.
3. Add 2-phenylbutyric acid according to the concentration of creatinine in urine (100 mmol 2-phenylbutyric acid/mol creatinine).
4. Add 100 μL 4N HCl.
5. Saturate the solution with NaCl.
6. Add 2 mL ethyl acetate, mix and centrifuge 5 min at 3500 rpm.
7. Transfer 1 mL of supernatant to a clean glass tube and evaporate ethyl acetate with purging with N_2.
8. Add 50 μL pyridine and 200 μL BSTFA (*N*,*O*-bis (trimethylsilyl)trifluoroacetamide) and carefully mix.
9. Transfer the solution into a glass vial to analyse with GC/MS.

Analysis

Analysis of amino acids and acylcarnitines: Autosampler injects 25 μL of the sample, flow of the mobile phase is constant at 0.10 mL min^{-1}, run time is 1.7 min. Analytes are measured with tandem mass spectrometer in MRM mode (Table I and Table II). Mass transitions for the analytes are given in the Chromsystems Instructional Manual for LC-MS/MS Analysis for MassChrom Amino Acids and Acylcarnitines from Dried Blood and were later adjusted to our analytical system.

Table I
Mass transitions for amino acids analysed stated in the form x – y, where x is m/z value of precursor ion and y m/z value of product ion. Mass transitions of the internal standards were appropriately corrected for their isotope mass

x – y	Analyte	Internal standard
146.2 – 44.1	Alanine	Alanine-D4
246.3 – 144.3	Aspartic acid	Aspartic acid-D3
260.3 – 158.2	Glutamic acid	Glutamic acid-D5
188.4 – 86.1	Leucine	Leucine-D3
206.2 – 104.2	Methionine	Methionine-D3
222.2 – 120.1	Phenylalanine	Phenylalanine-D5
238.2 – 136.2	Tyrosine	Tyrosine-D4
174.2 – 72.1	Valine	Valine-D8
231.3 – 69.9	Arginine	Arginine-D7
232.2 – 113.1	Citrulline	Citrulline-D2
132.2 – 76.1	Glycine	Glycine-^{13}C2-^{15}N
189.2 – 70.1	Ornithine	Ornithine-D6

Table II
Mass transitions for free carnitine and acylcarnitines analysed stated in the form x – y, where x is m/z value of precursor ion and y is m/z value of product ion. Mass transitions of the internal standards were appropriately corrected for their isotope mass

x – y	Analyte	Internal standard
260.2 – 85.0	C2-Carnitine	C2-Carnitine-D3
274.3 – 85.0	C3-Carnitine	C3-Carnitine-D3
288.4 – 85.0	C4-Carnitine	C4-Carnitine-D3
304.4 – 85.0	C4OH-Carnitine	C4-Carnitine-D3
374.3 – 85.0	C4DC-Carnitine	C4-Carnitine-D3
302.2 – 85.0	C5-Carnitine	C5-Carnitine-D9
300.2 – 85.0	C5:1-Carnitine	C5-Carnitine-D9
318.3 – 85.0	C5OH-Carnitine	C5-Carnitine-D9
388.4 – 85.0	C5DC-Carnitine	C5DC-Carnitine-D6
316.3 – 85.0	C6-Carnitine	C6-Carnitine-D3
344.3 – 85.0	C8-Carnitine	C8-Carnitine-D3
342.2 – 85.0	C8:1-Carnitine	C8-Carnitine-D3
372.3 – 85.0	C10-Carnitine	C10-Carnitine-D3
370.3 – 85.0	C10:1-Carnitine	C10-Carnitine-D3
400.5 – 85.0	C12-Carnitine	C12-Carnitine-D3
428.5 – 85.0	C14-Carnitine	C14-Carnitine-D3
426.3 – 85.0	C14:1-Carnitine	C14-Carnitine-D3
444.3 – 85.0	C14OH-Carnitine	C14-Carnitine-D3
465.5 – 85.0	C16-Carnitine	C16-Carnitine-D3
472.4 – 85.0	C16OH-Carnitine	C16-Carnitine-D3
454.4 – 85.0	C16:1-Carnitine	C16-Carnitine-D3
470.4 – 85.0	C16:1OH-Carnitine	C16-Carnitine-D3
484.5 – 85.0	C18-Carnitine	C18-Carnitine-D3
500.6 – 85.0	C18OH-Carnitine	C18-Carnitine-D3
482.4 – 85.0	C18:1-Carnitine	C18-Carnitine-D3
498.6 – 85.0	C18:1OH-Carnitine	C18-Carnitine-D3
480.2 – 85.0	C18:2-Carnitine	C18-Carnitine-D3

Analysis of urine organic acids

Samples were injected with an autosampler. We used a splitless injection of 1 μL, He flow was constant at 1 mL min^{-1}. The temperature in the temperature program was first kept at 70 °C for 2 min, then heated with the rate 3.5 °C min^{-1} till 270 °C, 270 °C was kept for 2 min.

Table III
Abnormal concentration of C5OH-Carnitine in our patient and the reference values we use for this metabolite

Metabolite	Concentration [μM]	Lower reference value [μM]	Upper reference value [μM]
C5OH-Carnitine	0.92	0.06	0.32

Results and discussion

For our pilot research of IEM in Slovenia we quantify the analytes listed in Table I and Table II. We wanted to minimise any additional stress to newborns because of our research. For this reason we have used DBSs taken for routine NBS in Slovenia for phenylketonuria and congenital hypothyroidism.

Concentrations of measured analytes were compared to the reference values for healthy newborns. When one or more analytes deviated from the reference values the first step we took was repeating the measurement in the same DBS. Upon another abnormal result additional steps had to be taken.

As of now we are still at the beginning of our research. So far we have screened around 1000 newborns. One newborn had a clearly abnormal result, where the measured analyte was markedly elevated. In this patient an abnormal concentration of C5OH-Carnitine was measured (Table III). On repeated analysis of the same DBS the concentration of C5OH-Carnitine was the same. Elevations of C5OH-Carnitine are associated with different IEM. These are 3-methylcrotonyl-CoA carboxylase deficiency, 3-OH-3-methylglutaryl-CoA lyase deficiency, holocarboxylase deficiency, biotinidase deficiency and 3-oxothiolase deficiency[15]. As different IEM were possible we performed additional tests. The first step that is commonly taken when abnormal concentrations of any metabolite in DBS is determined is repeating the analysis from a new DBS[16]. In the case of elevated concentrations of C5OH-Carnitine after analysis of the new DBS, analysis of organic acids in urine is helpful in determining a possible metabolic disorder[17].

At the patient's visit to the doctor at about 6 months of age both blood and urine samples were taken. We also took the urine samples besides the DBS to speed up the follow-up tests in the case of a repeated elevation of C5OH-Carnitine in the new DBS. For the follow-up we first repeated the analysis of amino acids and acylcarnitines in the new DBS. To help with interpretation we also measured organic acids in urine by GC-MS, a method also used for selective screening of Slovenian paediatric population[18]. At repeated analysis of the new DBS spot the concentration of C5OH-Carnitine was normal. The analysis of organic acids in urine did also not show any deviations from normal concentrations of organic acids. As the results of both tests came back negative we concluded that this case was a false-positive.

There are two main reasons for a false-positive when measuring C5OH-Carnitine concentration in a DBS taken from a newborn. One reason is that C5OH-Carnitine that was elevated in the newborn was due to maternal elevation of C5OH-Carnitine, which was no longer show in the newborn at the time we did the follow-up tests[17]. The second possible reason is that our upper reference values are too low for this metabolite and have to be corrected in the future as more newborns will be screened. Albeit we listed two reasons for the false-positive, the aim of MS/MS NBS for IEM is to detect all affected newborns. Some false positives are to be expected and are seen in established NBS programmes[19].

The marked elevation of C5OH-Carnitine in the patient we presented was not the sole abnormal result we found. There were also elevations of other metabolites, which were not so markedly elevated as C5OH-Carnitine in the described patient. C4-Carnitine, the elevation of which does not pinpoint a single disease, was also elevated in a few patients (Table IV) [20,21].

For the follow-up tests analysis of urine organic acids is useful as it can differentiate between the three disorders associated with isolated elevation of C4-Carnitine; isobutyryl CoA dehydrogenase deficiency, short-chain acyl-CoA dehydrogenase deficiency (SCADD) and ethylmalonic encephalopathy. The incidences of these diseases are around 1 : 100000 (ref.[16]). Genetic analyses of causative genes are available for all disorders and can be helpful as a confirmation test[16,22]. Based on the rarity of the diseases it is highly unlikely that 3 patients in the 1000 screened are positive for one of the three disorders. For the follow-up we will first sequence the *ACADS* gene, associated with SCADD (ref.[23]). On the basis of molecular analysis results further diagnostics steps will be done.

Table IV
Elevated concentrations of C4-Carnitine at first measurement in DBS. The reference values for healthy newborns are between 0.11 and 0.68 μmol L^{-1}

Patient	Concentration [μM]
Patient 1	1.03
Patient 2	0.96
Patient 3	0.90

Conclusion

The marked elevation of C5OH-Carnitine in the patient that we presented was not the only case of abnormal concentration of metabolites in the 1000 patient

we screened. All deviations from the reference values need to be evaluated and if necessary the follow-up tests need to be done. By screening more newborns we will be able to better set the reference values for the Slovenian newborn population to get as little false-positives as possible. Future work will also help to evaluate expansion of newborn screening programme in Slovenia.

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DETERMINATION OF FATTY ALCOHOL ETHOXYLATES IN THE LABE AND CHRUDIMKA RIVERS

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Keywords: fatty alcohol ethoxylates, solid phase extraction, reversed phase liquid chromatography, water samples

Abstract

A method for determination of fatty alcohol ethoxylates in water samples employing reversed phase liquid chromatography has been developed. Fatty alcohol ethoxylates were isolated and preconcentrated using solid phase extraction with the octadecyl silica gel-based sorbent and the mixture of methanol:dichlormethane (9:1 (v/v)) as an elution solvent. After extraction step, isolated alcohol ethoxylates were derivatized using phenyl isothiocyanate reagent with addition of triethylamine as a catalyst. The quantitative parameters, limit of detection and quantification were determined. The mean values of limits of detection and quantification were 20.4 ng mL^{-1} and 67.9 ng mL^{-1}, respectively. The method has been applied for the analysis of water samples from the rivers Labe and Chrudimka. The determined concentrations of alcohol ethoxylates were in the range of 1–8 μg L^{-1} with the highest concentrations attributed to nonethoxylated alcohols followed by alcohols with low degree of ethoxylation.

Introduction

Fatty alcohol ethoxylates (FAEs) belong to a group of nonionic surfactants. They are composed of the alkyl chain (hydrophobic tail) and the ethoxylated chain (hydrophilic head). The most commonly used FAEs contain alkyl chain with average length of 12–18 carbon atoms and with average number of ethoxy units 0–18. Alcohol ethoxylates are prepared by the reaction between aliphatic alcohol and ethylene oxide. The reaction is usually catalyzed by sodium hydroxide or potassium hydroxide[1,2].

FAEs are often used as a substitution for highly toxic nonylphenol ethoxylates. They are widely applied in laundry detergents, cleaning agents, cosmetics, herbicides etc. Their toxicity is related to the length and structure of an alkyl chain and to the length of ethoxylated chain. Linear alcohol ethoxylates are more chronically toxic than branched ones. Toxicity of FAEs is increasing with increasing length of an alkyl chain and decreasing number of ethoxy units. Fatty alcohol ethoxylates could be relatively highly toxic to algae, invertebrates and fish in a different range of median effective concentrations (EC 50 from 0.05 mg L^{-1} to 100 mg L^{-1}). Therefore, it is important to control FAEs concentration in the water environment[3].

Experimental

Apparatus

The water samples were filtered through 0.2 μm Nylon 66 membrane filters (Supelco, Bellefonte, USA). For solid phase extraction, the Supelclean™ ENVI™-18 tubes (6 mL, 0.5 g) (Supelco, Bellefonte, USA) were used. The separation of fatty alcohol ethoxylates was realized on a modular liquid chromatograph (Shimadzu, Kyoto, Japan) with UV detection. The liquid chromatograph consisted of degasser DGU 3014, two pumps LC-20AD XR involving binary gradient of mobile phase composition, mixer, six port injection valve with 5 μL sample loop (Rheodyne, USA), column thermostat LCO 102 (ECOM, Prague, Czech Republic) and UV detector SPD-20A. Alcohol ethoxylates were separated using Ascentis Express C18 column (150 mm × 4.6 mm) packed with porous shell 5 μm particles.

Chemicals

Methanol, acetonitrile and phenyl isothiocyanate were purchased from Sigma Aldrich (Steinhein, Germany), dichloromethane was from Merck (Darmstadt, Germany) and triethylamine from Lach-ner (Neratovice, Czech Republic). Deionized water was prepared using Demiwa 5-ROI (Watek, Ledeč nad Sázavou, Czech Republic) and SG UltraClear apparatus (SG, Hamburg, Germany). Acetic acid used as a mobile phase additive was from Penta (Chrudim, Czech Republic). Technical sample of alcohol ethoxylates Slovasol 247 was kindly provided by Sasol Slovakia (Nováky, Slovakia).

Liquid chromatography conditions

Mobile phase consisted of acetonitrile and deionized water, both with the addition of acetic acid at the concentration 0.1 % (v/v). The samples were separated using gradient elution mode with linear gradient profile

0 min 70% ACN/water – 30 min 100% ACN followed by reequilibration of the column. The mobile phase flow rate was 0.4 mL min^{-1}. Column was termostated at the temperature 35 °C. Derivatized alcohol ethoxylates were detected at the wavelength 280 nm.

Statistical evaluation of the method

Calibration solutions were prepared from derivatized technical sample Slovasol 247 in the range of total concentration 3.76–120 μg mL^{-1}. Each calibration solution was analyzed in triplicate and the mean values of the peak areas corresponding to the individual alcohol ethoxylates were calculated. The concentration of individual alcohol ethoxylate (c_i) used for construction of calibration curves was calculated from the overall concentration (c_t) of the sample by the equation:

$$c_i = \frac{\left(A_i * c_t * 100\right)}{\sum A_i} \quad (1)$$

where A_i is the peak area of individual alcohol ethoxylate. The calibration curve for each alcohol ethoxylate constructed as a dependence of peak area or height on the concentration, c_i, was fitted using a least square linear regression. The Correlation coefficients of every calibration curve were higher than 0.9970.

Limits of detection (LOD) and quantification (LOQ) were calculated for every individual alcohol ethoxylate by the equations:

$$LOD = \frac{3 * h_s}{k} \quad (2)$$

$$LOQ = \frac{10 * h_s}{k} \quad (3)$$

where h_s is standard deviation of baseline noise and k is a slope of calibration equation for individual alcohol ethoxylates. Recovery of SPE of individual alcohol ethoxylates was calculated by the equation:

$$R(\%) = \frac{c_i(2)}{c_i(1)} * 100 \quad (4)$$

where $c_i(2)$ and $c_i(1)$ are the concentrations of individual alcohol ethoxylates determined after extraction and prepared by spiking of distilled water with Slovasol 247 sample before extraction, respectively.

Sample preparation

Water samples (1 l of each sample) were collected from the rivers Labe and Chrudimka during May and June 2014; temperature of the water was measured. The samples were immediately transported to the laboratory and adjusted into three 250 mL glass bottles. The bottles were stored in a dark at two temperatures (25 °C and 4 °C) for 24 hrs, 48 hrs and 72 hrs. Last portion of the water sample (250 mL) was immediately prepared for the separation by liquid chromatography.

First, the sample (250 mL) was filtered through nylon filter. The filter was washed with 10 mL of methanol. After filtration, 250 mL of the sample was passed through SPE column and alcohol ethoxylates were sorbed on the solid phase. The mixture of methanol and dichloromethane (9:1 v/v) was used as an elution/extraction solvent. The elution was done consecutively three times with 1 mL of elution mixture. The extract was completely dried using gentle stream of nitrogen. Derivatization reagent (6 μL of phenyl isothiocyanate) and catalyst (6 μL of triethylamine) were added to dried extract. The reaction mixture was kept in sealed vial at 100 °C for 90 min. The sample of derivatized alcohol ethoxylates (phenyl isothiourethanes) was diluted by 1 mL of acetonitrile and injected to liquid chromatograph after filtration with syringe filter.

Results and discussion

The chromatographic separation of technical sample 247 is shown in Fig. 1. The sample contains alcohol ethoxylates with 12, 14 and 16 carbons in an alkyl chain. The degree of ethoxylation was in the range from 0 to 19. This sample was used as a calibration standard and according to the Eqs. (*2*) and (*3*) the quantification parameters were calculated for individual oligomers. Limit of detection was in the range 20.33–20.43 ng mL^{-1} and limit of quantification was between 67.78 ng mL^{-1} and 68.09 ng mL^{-1}.

To evaluate the accuracy of sample preparation method, the recovery of solid phase extraction of alcohol ethoxylates was calculated. Recovery calculated using Eq. (*4*) decreases with increasing length of the alkyl chain in the surfactant molecule, except of nonethoxylated alcohols (Fig. 2).

Further, the degradation of alcohol ethoxylates during storage at different conditions (25 °C, 4 °C) was studied. In the Fig. 3A,B the comparison of degradation of alcohol ethoxylates during storage at 25 °C for 0, 24, 48 and 72 hrs is presented. After 72 hrs at laboratory temperature, the surfactants were almost decomposed yielding partly nonethoxylated alcohols and other low molecular weight compounds. For the detailed study of the degradation products, it would be necessary to employ different type of detection (e.g. mass spectrometry). The rate of degradation is changing with degree of ethoxylation (Fig. 3A,B). Therefore, it is necessary to analyze the samples of alcohol ethoxylates immediately after collection to provide accurate results. Another possibility would be to use suitable preservative for improvement of the sample stability, which is the subject of ongoing studies.

After optimization of the sample preparation, derivatized river water samples were analyzed. Fig. 4 shows the comparison between the separation of derivatized technical sample and water sample collected

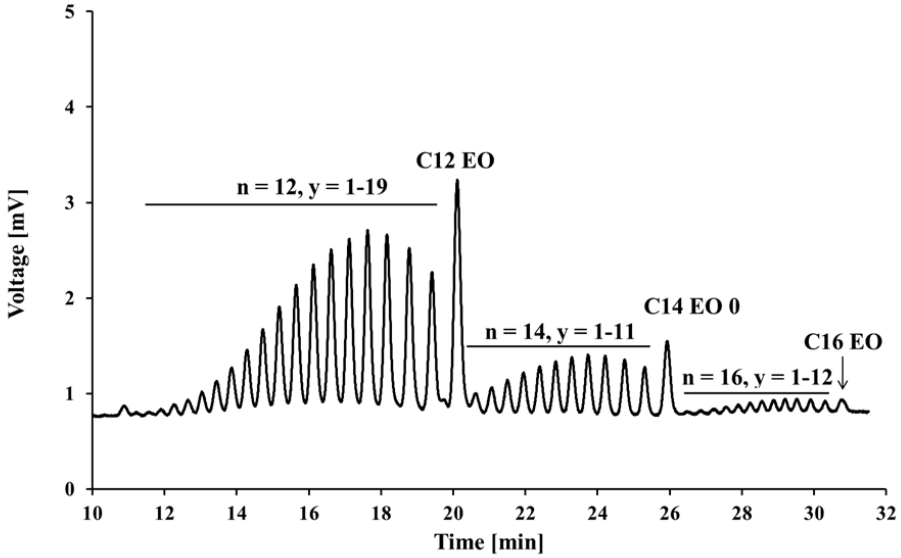

Fig. 1. **Separation of derivatized technical sample Slovasol 247 with the concentration 15 μg mL^{-1}.** Separation conditions: Ascentis Express C18 column (150 × 4.6 mm, 5 μm), mobile phase flow 0.4 mL min^{-1}, gradient 0 min 70% ACN with 0.1 % (v/v) acetic acid/ water with 0.1 % (v/v) acetic acid – 30 min 100% ACN with 0.1 % (v/v) acetic acid, temperature 35 °C, detection UV at 280 nm

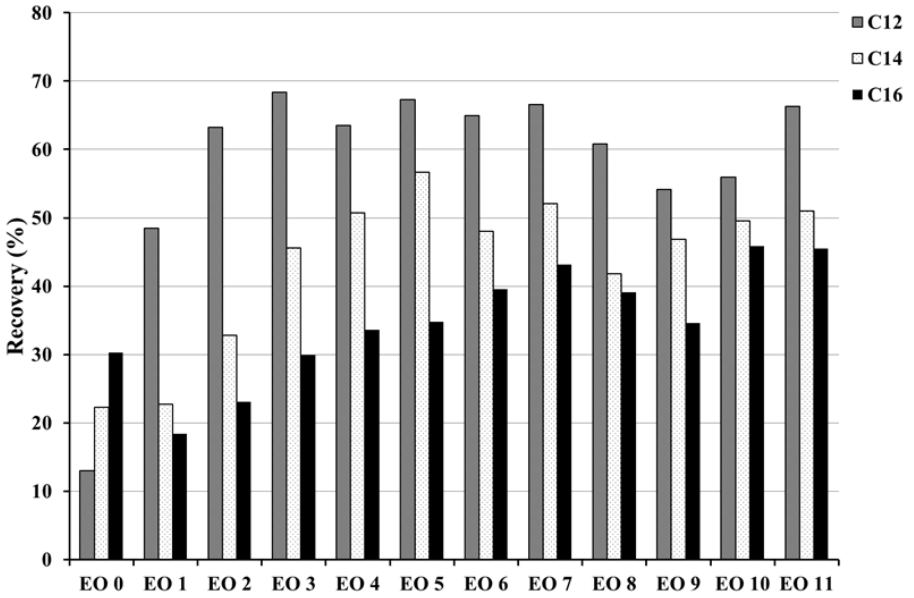

Fig. 2. **Recovery of the solid phase extraction for individual alcohol ethoxylates**

from the river Labe. The dodecanol, tetradecanol and hexadecanol ethoxylates mostly with low degree of ethoxylation were identified in the river water sample based on the comparison of retention times. Results of analyses of water samples from Labe and Chrudimka rivers (treated and analyses immediately after collection) are summarized in Fig. 5. As seen in this figure, all samples contain mostly nonethoxylated and low ethoxylated fatty alcohols.

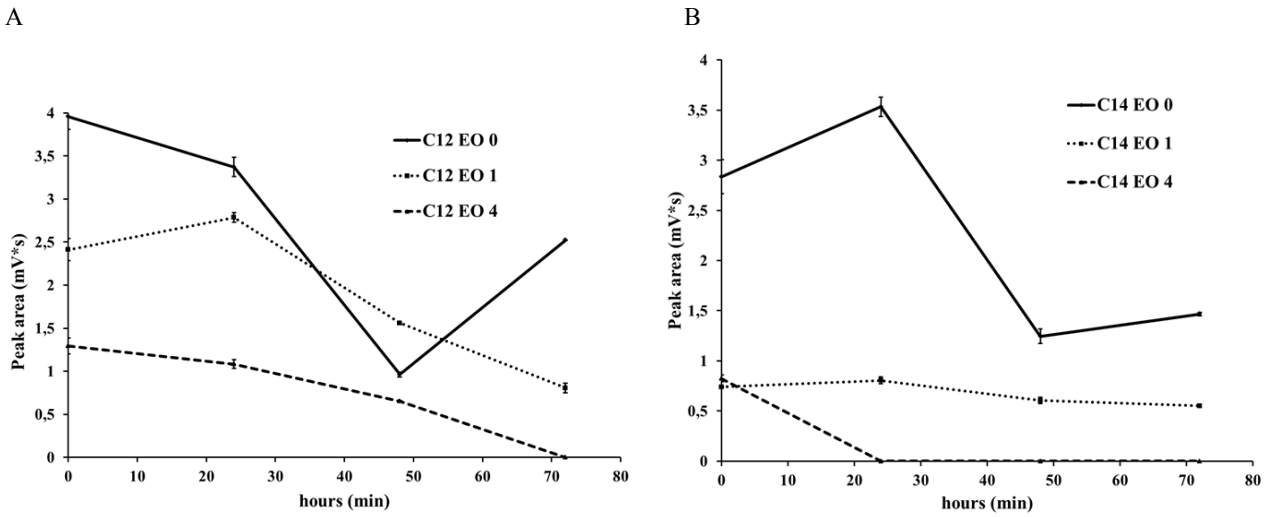

Fig. 3. **Degradation of fatty alcohol ethoxylates at 25 °C;** A – dodecanol and dodecanol ethoxylates, B – Tetradecanol and tetradecanol ethoxylates

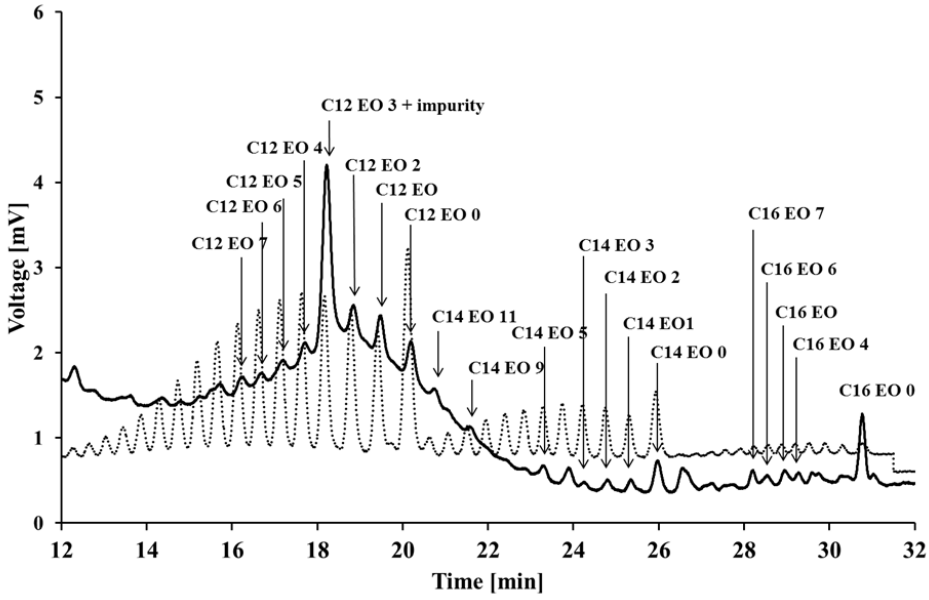

Fig. 4. **Separation of derivatized technical sample (dotted line) and derivatized real water sample took from the river Labe, May 12th, 2014, 15.0 °C (full line).** For separation condition see Fig. 1

Conclusions

A method for determination of alcohol ethoxylates using RP-liquid chromatography after derivatization with phenyl isothiocyanate was developed. Technical Slovasol 247 containig dodecanol, tetradecanol and hexadecanol ethoxylates with degree of ethoxylation between 0 and 19 was used as the calibration standard. Water samples were collected from the rivers Labe and Chrudimka. Samples were prepared immediately after transport to laboratory and after storing at 25 °C and 4 °C (dark, no preservative, no air). River water samples could not be stored without using any efficient preservative because of decradation of FAEs. In the both rivers, dodecanol, tetradecanol and hexadecanol ethoxylates with low degree of ethoxylation were detected and quantified. The concentration of alcohol

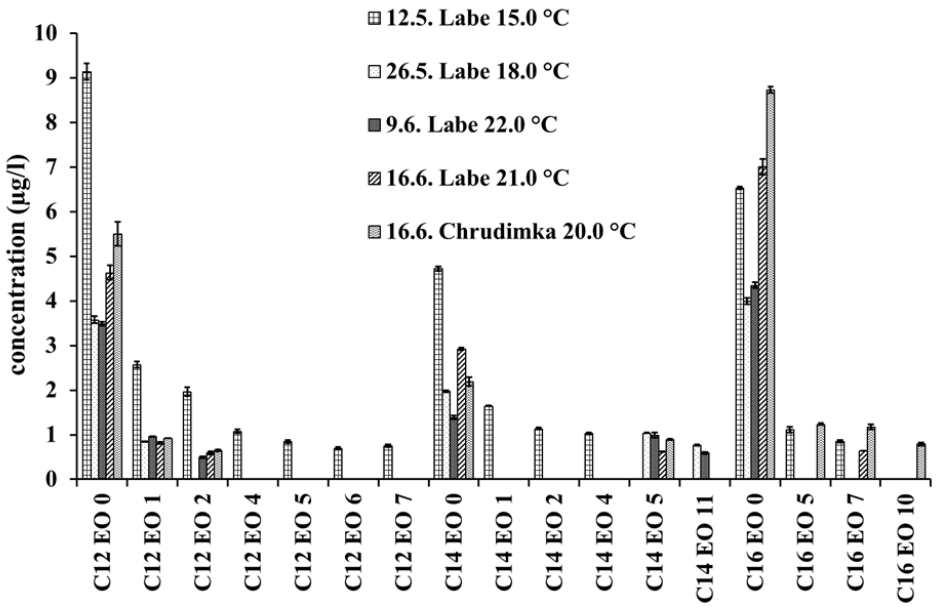

Fig. 5. **Concentration (µg L^{-1}) of individual alcohol ethoxylates found in the rivers Labe and Chrudimka**

ethoxylates found in the rivers Labe and Chrudimka was below the median effective concentration that could cause acute toxicity to water organisms.

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DEVELOPMENT OF MICELLAR ELECTROKINETIC CHROMATOGRAPHIC METHOD FOR DETERMINATION OF POLYPHENOLS IN ELDER INFUSIONS

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Keywords: micellar electrokinetic chromatography, phenolic acids, flavonoids, elderflower

Abstract

A method for determination of 18 flavonoid aglycones, glycosides and phenolic acids using micellar electrokinetic chromatography has been developed. Sodium decyl sulphate was used as pseudostationary phase at the concentration of 36 mmol L^{-1} and addition of cyclodextrins up to the 5 mmol L^{-1} was tested for improvement of the separation of isomeric compounds. In the electrolyte containing heptakis(2,6-di-*O*-methyl)-β-cyclodextrin, three isomers of chlorogenic acid (chlorogenic, neo- and cryptochlorogenic acid) were successfully baseline separated. The quantitative parameters of the method were determined including limits of quantification, which were in the range of 0.15–1.4 μg mL^{-1}, together with the linearity of calibration curves. The method was successfully applied for the analyses of water infusions of elderflower samples and the statistically significant differences were found between the concentrations of polyphenols depending on the sample harvest location.

Introduction

Micellar electrokinetic chromatography is a powerful tool for separation of mixtures of charged and neutral molecules. Separation of neutral molecules is possible due to their different distribution coefficients between micelles and a running buffer[1]. Micelles of a surfactant (at higher concentration than the critical micelle concentration) used in micellar electrokinetic chromatography form pseudostationary phase. Micellar electrokinetic chromatography is usually carried out in non-coated fused silica capillaries with sodium decyl-, dodecyl- and tetradecyl sulphate as a micellar agent. In this work, micellar electrokinetic chromatography with sodium decyl sulphate was used for the quantification of antioxidants in elderflower infusions.

Elder (*Sambucus nigra* L.) is a shrub growing in almost all Europe. Elder is rich source of flavonols, phenolic acids, anthocyanins, carotenoids, vitamins and minerals[2]. Bioactive compounds from berries and flowers of elder have potent antioxidant, anticancer, antimicrobial and anti-inflammatory properties[3]. Flowers and fruits of elderberry are used in many ways such as food and as a medicine. Extracts of elderflowers are used for treatment of cold, fever and sinusitis[4]. They are used for their diuretic and perspiratory properties. Compounds in elderflowers and berries can play an important role in the prevention of oxidation and cellular inhibition or delaying the oxidative processes[5]. Polyphenols are secondary products of plant metabolism and contain multiple phenol structural units[6]. The group of 18 polyphenols including aglycones and glycosides flavonoids and phenolic acids was used as the standards and the method for their determination in elderflower infusions has been developed in the present work.

Experimental

Chemicals

Standards of flavonoids aglycones (hesperetin, kaempferol, naringenin, quercetin), flavonoids glycosides (hesperidin, isoquercitrin, kaempferol-*3*-*O*-rutinoside, rutin), phenolic acids (caffeic acid, chlorogenic acid, cryptochlorogenic acid, ferulic acid, gallic acid, neohlorogenic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid), electrolyte components (boric acid, sodium tetraborate, sodium decyl sulphate, sodium dodecyl sulphate, β-cyclodextrin, heptakis(6-*O*-sulfo)-β-cyclodextrin and heptakis(2,6-di-*O*-methyl)-β-cyclodextrin) were purchased from Sigma Aldrich (St. Louis, MO, USA). Standards of isorhamnetin-3-*O*-rutinoside and isorhamnetin-3-*O*-glucoside were obtained from Extrasynthese (Genay, France). Sodium hydroxide and thiourea were purchase from LachNer (Neratovice, Czech Republic). Deionized ultra-pure water was prepared by Ultra CLEAR UV apparatus (SG, Hamburg, Germany).

Micellar electrokinetic chromatography

Micellar electrokinetic chromatography was carried out using Agilent CE 7100 capillary electrophoresis instrument (Agilent, Palo Alto, CA, USA) in bare fused silica capillary (50 μm i.d., 48 cm total length, 40 cm length to the detector, 150 μm extended light path cell, Agilent). The new capillary was rinsed with 0.5 mol L^{-1} sodium hydroxide and with ultra-pure water (both for 15 minutes) before the first use. Background electrolyte

contained 25 mmol L^{-1} borate buffer (pH 9.30) with addition of 36 mmol L^{-1} sodium decyl sulphate. The value of pH was measured using Metrohm 827 laboratory pH meter equipped with Unitrode electrode (Metrohm, Herisau, Switzerland). pH meter was calibrated by standard buffer solutions with pH 7.00 and 9.00. Separation was carried out at applied voltage 20 kV and the temperature 20 °C. The samples were injected hydrodynamically by applying pressure of 100 mbar for 10 s at the vial with the sample. The detection wavelengths were set to 214, 254 and 280 nm.

Calibration

Seven calibration solutions used for the quantitative analysis were prepared in the concentration range of 0.5–200 μg mL^{-1} for each compound. All calibration solutions were analyzed in triplicate. The calibration solutions with concentrations of standards 0.5, 10 and 100 mg mL^{-1} were measured five times. The calibration curves were evaluated by Statistica 10 software (StatSoft, Inc., 2011). The limit of quantification was calculated using the equation:

$$LOQ = \frac{10 * h}{s} \qquad (1)$$

where h is standard deviation of noise signal and s is slope of the calibration curves of the dependencies of zone height on the concentration of the standards.

Elderflower infusions analysis

Three series of elderflower infusions were analyzed. At first series of samples, five infusions were prepared from five elderflower tea bags from MEGAFYT Pharma. The second series was prepared by homogenization of twenty tea bags from MEGAFYT Pharma, too. The third series was prepared from air-dried wild growing elder collected in Veltruby, Czech Republic (location 50° 4.3219' N, 15° 10.8356' E) on June 2013.

The infusions were prepared by keeping the bags or dried elderflowers in 200 mL of 90 °C hot water for 15 minutes and cooling to the laboratory temperature. Before injection the infusions were filtered using syringe 0.45 μm polytetrafluoroethylene filter (Millipore, Bedford, MA, USA).

Results and discussion

First, the conditions of analysis of phenolic acids and flavonoids using micellar electrokinetic chromatography were optimized. Based on our previous results, application of sodium decyl sulphate in MEKC yielded better resolution than sodium dodecyl sulphate for the analysis of polyphenols[7]. The value of critical micelle concentration of sodium decyl sulphate in borate buffer (pH 9.30) is 25.4 ± 0.9 mmol L^{-1} (ref.[8,9]). For the separation of selected antioxidants, the concentration of 36 mmol L^{-1} was selected, which represents concentration of micelles approximately 10 mmol L^{-1}. Some pairs of compounds (isorhamnetin-3-*O*-rutinoside and kaempferol-3-*O*-rutino-

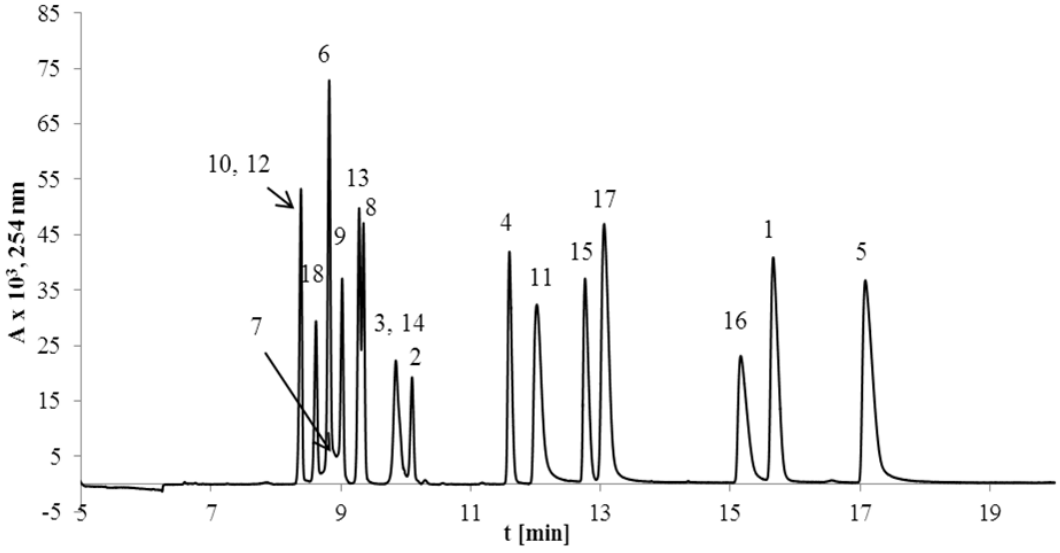

Fig. 1. **MEKC separation of polyphenols standards;** BGE: 36 mmol L^{-1} sodium decyl sulphate, 25 mmol L^{-1} borate buffer pH 9.30, for other experimental conditions see Experimental. Compounds: 1 – caffeic acid, 2 – chlorogenic acid, 3 – cryptochlorogenic acid, 4 – ferulic acid, 5 – gallic acid, 6 – hesperetin, 7 – hesperidin, 8 – isoquercitrin, 9 – isorhamnetin-3-*O*-glucoside, 10 – isorhamnetin-3-*O*-rutinoside, 11 – kaempferol, 12 – kaempferol-3-*O*-rutinoside, 13 – naringenin, 14 – neochlorogenic acid, 15 – *p*-coumaric acid, 16 – *p*-hydroxybenzoic acid, 17 – quercetin, 18 – rutin

side, neochlorogenic acid and cryptochlorogenic acid) were not resolved and pair of compounds isoquercitrin/ naringenin was partially separated under these conditions (Fig. 1).

To improve the separation selectivity, addition of cyclodextrins to the background electrolyte was tested. Cyclodextrins can selectively form inclusion complexes with hydrophobic part of analyte molecules, while the

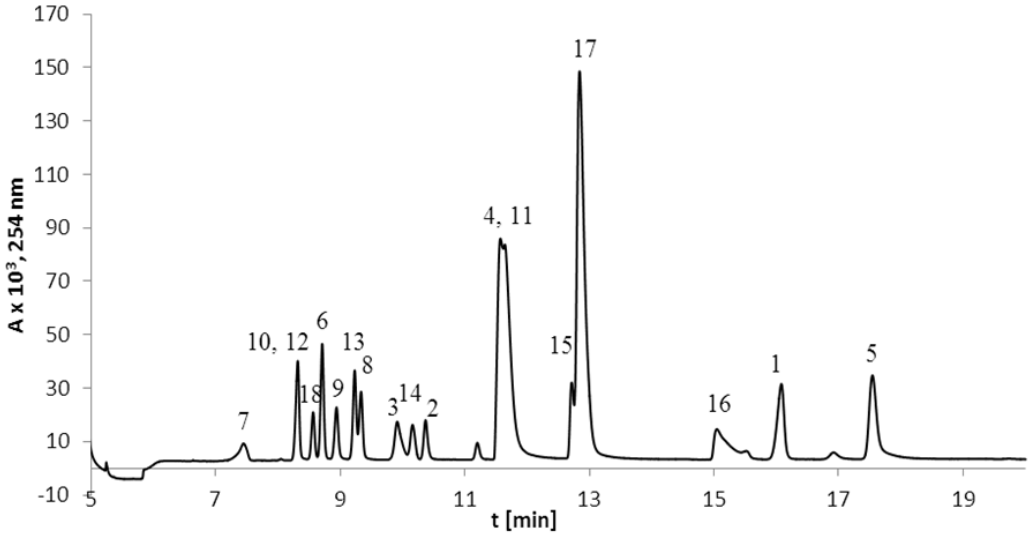

Fig. 2. **MEKC separation of polyphenols standards in electrolyte with heptakis(2,6-di-*O*-methyl)-β-cyclodextrine;** BGE: 36 mmol L^{-1} sodium decyl sulphate, 25 mmol L^{-1} borate buffer pH 9.30, 5 mmol L^{-1} heptakis(2,6-di-*O*-methyl)-β-cyclodextrine, for other experimental conditions see Experimental. Compounds: 1 – caffeic acid, 2 – chlorogenic acid, 3 – cryptochlorogenic acid, 4 – ferulic acid, 5 – gallic acid, 6 – hesperetin, 7 – hesperidin, 8 – isoquercitrin, 9 – isorhamnetin-3-*O*-glucoside, 10 – isorhamnetin-3-*O*-rutinoside, 11 – kaempferol, 12 – kaempferol-3-*O*-rutinoside, 13 – naringenin, 14 – neochlorogenic acid, 15 – *p*-coumaric acid, 16 – *p*-hydroxybenzoic acid, 17 – quercetin, 18 – rutin

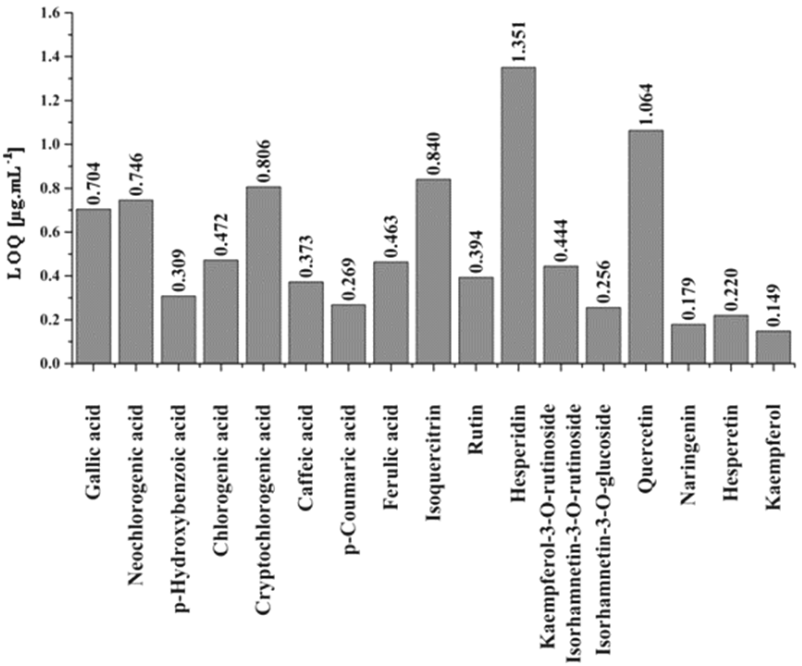

Fig. 3. **The values of the limits of quantification**

inclusion equilibrium competes with partitioning in micellar phase. Thus β-cyclodextrin, heptakis(2,6-di-*O*-methyl)-β-cyclodextrin and heptakis(6-*O*-sulfo)-β-cyclodextrin were tested as the additives to the background electrolyte. In background electrolyte with addition of heptakis(2,6-di-*O*-methyl)-β-cyclodextrin, the resolution of cryptochlorogenic and neochlorogenic acids significantly improved, however the resolution of ferulic acid and kaempferol, *p*-coumaric acid and quercetin decreased (Fig. 2). The similar results were observed for other tested cyclodextrins. According to the obtained results, the electrolyte containing 5 mmol L^{-1} heptakis(2,6-di-*O*-

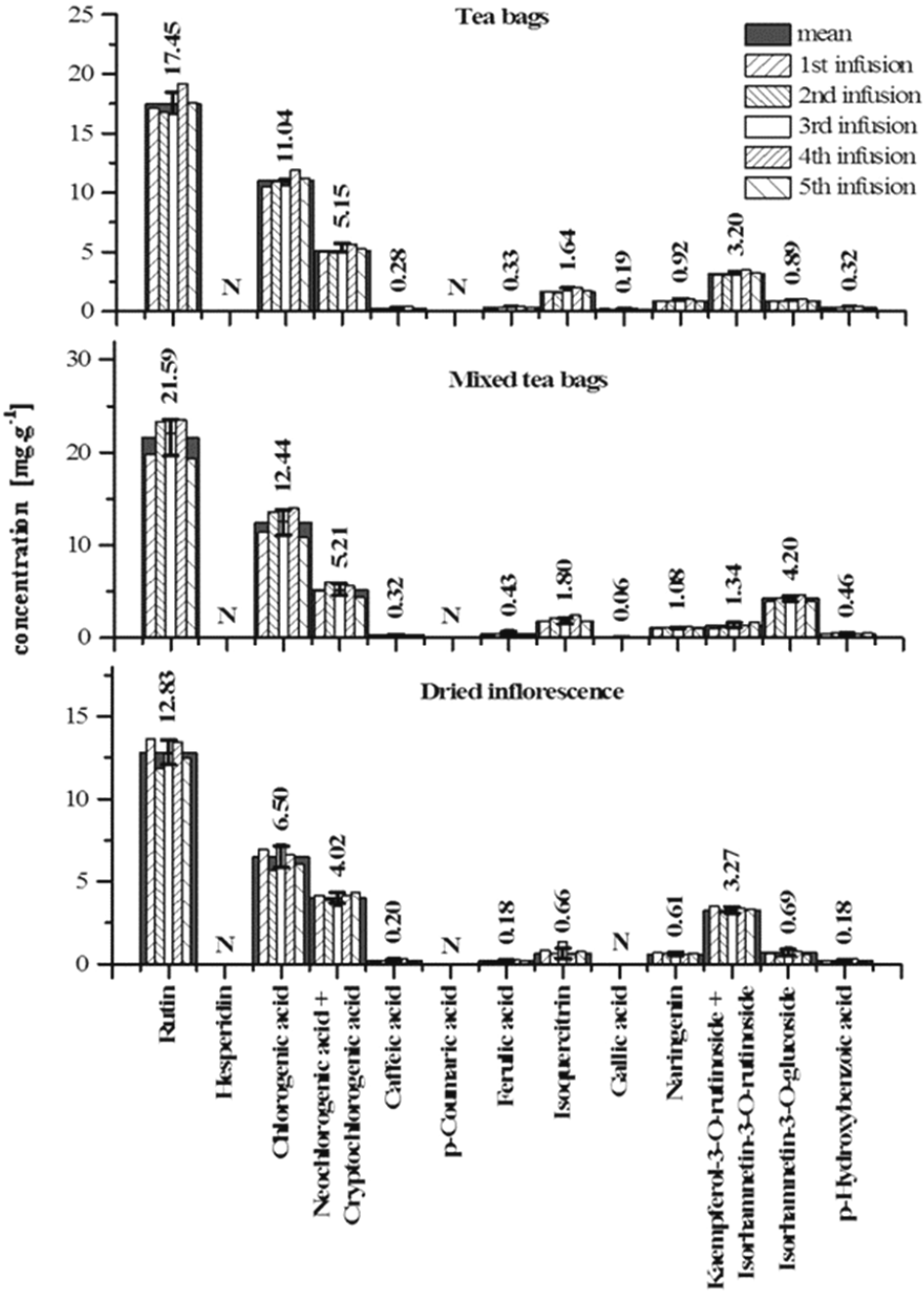

Fig. 4. **Results of the quantitative analysis of elderflower infusions;** N – the concentration of compounds is below the limit of quantification

methyl)-β-cyclodextrin is suitable for analyses of isomers of chlorogenic acid, but for the complex quantitative analyses of polyphenols in elderflower infusions, the electrolyte without cyclodextrins was used.

For the quantitative analyses of polyphenols, the calibration curves were constructed. The coefficients of determination, slopes, intercepts and limits of quantification (Fig. 3) were evaluated from the calibration curves. The coefficients of determination were higher than 0.995 for all analyzed compounds except quercetin. The coefficient of determination for quercetin was 0.9155. The developed method is suitable for quantification of polyphenolic compounds ranging from 0.15 $\mu g\ mL^{-1}$ to 100 $\mu g\ mL^{-1}$.

Results of quantitative analysis of three series of elderflower infusions are presented in the Fig. 4. Five infusions from each series of samples were analyzed, each infusion was measured three times. The values of concentration of some substances are not show because the concentration of hesperidin, *p*-coumaric acid in the samples and gallic acid in the third series of samples represented by air-dried wild-growing *Sambucus nigra* L. is below the limits of quantification. The sum of the concentration of neochlorogenic acid and cryptochlorogenic acid was determined and the sum of the concentration of isorhamnetin-3-*O*-rutinoside and kaempferol-3-*O*-rutinoside was determined.

The polyphenolic profile of all samples of elderflower infusions is similar with the highest concentration of rutin and chlorogenic acid isomers presented in the samples. There are significant differences in the concentrations of lower abundant compounds between the samples. The highest content of polyphenols was observed in mixed tea bags of commercial sample of elder tea, wild-growing elder possess the lowest concentration of polyphenols.

Conclusion

In the present work, the method for determination of 18 polyphenols including aglycones and glycosides of flavonoids and phenolic acids in elderflower infusions using micellar electrokinetic chromatography with UV detection was developed. The conditions of separation were optimized and the limits of quantification were calculated. The highest concentrations of polyphenols were found in the mixed tea bags, the lowest concentrations of polyphenols were determined in the air-dried wild-growing elderflower infusion. The content of polyphenols strongly depends on the conditions of growing such as climate, soil type, and moisture.

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VOLTAMMETRIC METHODS FOR CHROMIUM(VI) DETERMINATION IN EXTRACTS FROM COAL FLY ASH AND CEMENT

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Keywords: chromium speciation, catalytic adsorptive stripping voltammetry, gold film and gold screen-printed electrodes, fly ash, extracts

Abstract

Catalytic adsorptive stripping voltammetry with DTPA and nitrate was applied for the determination of chromium(VI) and total chromium in the extracts from fly ashes and cement. The voltammetric results of the chromium speciation study obtained in the optimal conditions, i.e. in the supporting electrolyte with a composition of 0.1 M acetic buffer (pH 6.0), 0.01 M DTPA, 0.25 M KNO_3 after 20 s of accumulation at the potential of –0.95 V, were validated by means of absorption spectrophotometry with diphenylcarbazide, and good agreement was achieved. The elaborated procedures can be used to monitor Cr(VI) content in water extracts from the raw materials and products exploited in building material technologies to ensure that they meet the EU standards. A series of additional speciation procedures were investigated, all of which were based on the electroreduction of Cr(VI) to Cr(III) in acidic media at the gold and gold film electrodes. An attempt at determining the Cr(VI) concentration in a selected extract at the gold screen-printed electrode was undertaken, and it was found that the tested gold SPE is a promising voltammetric sensor for Cr(VI) quantification.

Introduction

The most common species of chromium, Cr(III) and Cr(VI), are present in the environment mostly as the result of human industrial activity such as metal plating, leather tanning, fuel combustion and the production of steel, refractory and building materials.

The influence of chromium species on living organisms depends on their oxidation state. Chromium (III) is less toxic, and it is one of the nutrients required for the maintenance of glucose, lipid and protein metabolism[1]. There are, however, cases in which prolonged contact with materials containing Cr(III), such as tanned leather, led to skin irritation and skin diseases[2]. On the other hand, chromium (VI) is regarded as toxic for the human body, and is a contributing factor in lung cancer and skin diseases; it is also suspected of causing asthma and renal diseases [1,3–5].

The negative influence of Cr(VI) on human health is particularly noticeable in the case of people working in the building industry, who come into contact with cement or bricks. The high temperature and oxidative atmosphere during the production process induce the oxidation of chromium (III) present as an impurity in raw materials to chromium (VI)[2]. In procedures used to prepare concrete, labile chromium (VI) is released from cement and can cause an allergic contact dermatitis in employees who work with wet portland cement. Fly ashes, which are the product of burning of coal, are often used in the production of clinker, cement, concrete, and bricks as well as in road construction. The high temperature at which coal is burnt and the oxidative atmosphere cause the oxidation of Cr (III) to Cr(VI). It is therefore essential to elaborate analytical procedures of the determination of chromium, especially its Cr(VI) species, in order to monitor its concentration to reduce its negative influence on the health of the workers employed in the building industry, and to ensure compliance with the EU regulations[6].

Monitoring very low yet hazardous chromium concentrations requires the application of very sensitive instrumental analytical methods. These can be divided into two main groups[7]:

– valence-specific techniques, which enable the determination of chromium (VI) usually in the presence of a great excess of chromium (III), such as absorption spectrophotometry and especially voltammetry;
– valence-nonspecific techniques, such as atomic absorption (AAS, ET AAS), inductively coupled plasma atomic emission (ICP AES) and inductively coupled plasma mass spectrometry (ICP MS). These techniques only enable the determination of total chromium concentration. However, they may also be adapted for chromium speciation by selectively separating the two Cr species prior to quantification[8].

The commonly used voltammetric techniques are mainly based on the reduction of Cr(VI) in the form of CrO_4^{2-} or $Cr_2O_7^{2-}$ to Cr(III) or the reduction of Cr(III) to Cr(II)[9]. The most sensitive electrochemical procedures of the determination of chromium utilize the reduction of complexes of Cr(III)·L to Cr(II)·L, where L is the ligand bound to Cr(III) or Cr(II) ion. The high sensitivity of these

procedures is the result of two phenomena: an enhancement of chromium voltammetric response due to the catalytic reduction of an oxidant induced by the Cr(III)·L/Cr(II)·L redox system and the adsorptive accumulation of the Cr(III)·L complexes at the electrode surface[9].

The total analytical signal of chromium (III) „$I_{f,total}$" can thus be expressed by the equation:

$I_{f,total} = I_d \times$ catalytic enhancement $\times$ adsorptive accumulation

where I_d – faradic diffusion current of the reduction of Cr (III)·L in the absence of both the oxidant and adsorption.

Amongst many adsorptive catalytic systems, the system containing Cr(III)-DTPA (diethylenetriamine-pentaacetic acid) and nitrate is the one used most frequently for ultrasensitive catalytic adsorptive stripping voltammetric (CAdSV) quantification and speciation of chromium. The procedures based on this system have mainly been applied for the specific determination of Cr (VI) and/or the quantification of total chromium concentration after preliminary oxidation of Cr(III) species to Cr(VI) (for example[10–15]). The CAdSV speciation is based on the differing abilities of Cr species to form complexes with DTPA[7, 9,12].

Cr(VI) ions present in the solution containing DTPA are reduced at the electrode at the adsorptive accumulation potential of ca. –0.9 V to the Cr(III) ions *in-statu nascendi*. Such very active Cr(III) ions immediately form a complex with DTPA and are adsorbed on the electrode surface. After preconcentration at the electrode surface, the Cr(III)-DTPA complex is reduced to Cr(II)-DTPA during stripping at ca. –1.25 V, and then the catalytic cycle occurs in the presence of nitrate ions.

Cr(III) species present in the bulk of the solution also form stable complexes with DTPA, Cr(III)-$DTPA_{stable}$, which can also adsorb at the electrode and participate in the catalytic cycle, producing a voltammetric signal at the same potential as that for the Cr(II)-$DTPA_{active}$. However, the CAdSV signal of Cr(III)-$DTPA_{stable}$ is lower in comparison to Cr(III)-DTPA formed *in-statu nascendi*; moreover, the voltammetric activity of the former complex decreased very quickly with time. Hence, after ca. 40 min the CAdSV response of Cr(III)-$DTPA_{stable}$ almost completely diminishes. Consequently, to determine Cr(VI) in the presence of Cr(III), quantification is performed 30 to 40 min after adding DTPA to the solution.

The reduction of Cr(VI) to Cr(III) was also utilized for the voltammetric determination of chromium. In strongly or moderately alkaline solutions, CrO_4^{2-} ions give well-defined, irreversible voltammograms that are suitable for the determination of low concentrations of chromium[15,16]. However, new strict regulations concerning the use of mercury forced electroanalysts to search for new conductive materials for working electrodes, which would replace mercury. For this purpose, graphite[18], carbon paste[19], and carbon modified with gold or silver nanoparticles[20–24] or with Prussian blue[25] were used. The modification of carbon with organic films allowed the preconcentration of Cr(VI) on the modified electrode surface by means of chemisorption, and made it possible to achieve a sensitivity of the determination of Cr(VI) comparable with that obtained by means of CAdSV with DTPA and nitrate[26,27].

The aim of this work was to apply voltammetric techniques for the assessment of the release of toxic chromium(VI) from fly ash and cement used as a component of building materials into the environment. As a first choice, CAdSV with DTPA and nitrate was tested and optimized. The results were validated via comparison with those obtained by means of the spectrophotometric method with diphenylcarbazide. In addition, an attempt at the determination of Cr(VI) in a selected extract at the gold film and gold screen-printed electrodes was also undertaken.

Experimental

Apparatus and instrumentation

The CAdSV experiments were carried out with a multipurpose electrochemical analyzer EA9 (MTM, Cracow, Poland). The electrochemical cell consisted of a Control Growth Mercury Drop Electrode (MTM, Poland) with a surface area of 1.9 mm^2 as a working electrode used in the hanging mercury drop electrode (HMDE) mode, silver-silver chloride (3M KCl) (Metrohm, Switzerland) as the reference electrode, and platinum wire as an auxiliary electrode.

Differential pulse voltammetry (DPV) measurements were carried out using an Autolab PGSTAT 20 apparatus (Ecochemie, Netherlands) coupled with a gold screen-printed electrode (4 mm diameter, DRP-C 220 AT, Spain). GPES 4.4 software was used to record, save, and process the collected data.

All experiments were performed at room temperature. pH measurements were performed with the multi-purpose laboratory pH meter CX-721 (ELMETRON, Poland). The digestion of extract samples was carried out in quartz tubes by means of the Mineral 8 UV Digester with a 150 W UV lamp (Mineral, Poland). The Spekol 11 spectrophotometer (Carl Zeiss) was applied for the determination of the Cr(VI) and total chromium in the selected extract samples.

All dilutions and sample preparations were performed using deionized water produced by Millipore Simplicity UV unit (Millipore, USA). A magnetic stirrer (ca 300 rpm) was used to convect the solution during accumulation and deaeration periods. All glassware was soaked in 6 M nitric acid and was rinsed several times with deionized water prior to use.

Reagents

The acetic buffer (1 M, pH 6.0) was prepared by mixing the appropriate amounts of 96% acetic acid and 25% ammonia solution (both Suprapur®, Merck). A DTPA solution (0.1 M) was prepared by dissolving the appropriate amount of the reagent (Carl Roth, Germany)

and adding 25% ammonia (Suprapur®) until a pH of 6.0 was obtained. Potassium nitrate (1 M) was prepared by dissolving an appropriate amount of the salt (POCH, Poland) in deionised water. This solid reagent was purified via the coprecipitation of the impurities on $La(OH)_3$ and crystallized from water. Cr(VI) and Cr(III) standard stock solutions (1 g L^{-1}) were obtained from Merck and diluted.

Extraction procedures

The extraction procedure for fly ashes was performed according to European Standard EN 12457-2:2002 (ref.[28]). 90 g of ash was placed in a dry polypropylene bottle and mixed with 900 mL of distilled water (room temperature). The sealed bottle was then placed in a rotary agitator (extractor) (36 rpm, bottle placed at an angle of 45 degrees to the axis of rotation) for 24 h and the solution was separated from the residue via filtration on a glass fiber filter (Whatman, GF/C).

The leaching procedure for cement was performed according to European Standard PN-EN 196-10:2008 (ref.[29]). 50 g of the cement sample was leached for 15 min with 50 mL of water; during this entire time, the suspension was stirred with a magnetic bar. The solution was then separated from the residue and transferred to a 50 mL volumetric flask.

CAdS voltammetric procedure for Cr(VI) quantification

UV-digestion procedure

The oxidation of Cr(III) present in the extract samples to Cr(VI), which is necessary for the CAdSV determination of total chromium according to the protocol proposed earlier[11], was performed by means of the UV-irradiation procedure. After setting pH to 7.2, the extract sample was irradiated for 2 h before proceeding with chromium quantification.

Spectrophotometric quantification of Cr(VI)

5 mL of the extract were mixed with 20 mL of water and 5 mL 0.25% diphenylcarbazide (DPC) dissolved in acetone, pH was fixed to the value of 2.1 and the solution was made up with deionized water to 50 mL in a volumetric flask. After 10 min absorption was measured at 540 nm. The concentration of Cr(VI) was calculated from the calibration plot.

Voltammetric quantification of Cr(VI)

0.5 mL of the extract, pre-diluted to 1:10, was mixed in a voltammetric vessel with 1 mL of 2.5 M KNO_3, 0.5 mL of 2 M acetic buffer, 0.5 mL 0.2 M DTPA and 7 mL of water. The solution was left to settle for 30 min, and was then deaereated with pure argon for 7 minutes. Quantitative measurements were performed by means of the differential pulse mode (DPV) using the standard additions procedure. Accumulation was performed by applying a potential of E_{acc} = –0.95 V for t_{acc} = 20 s with stirring and, after a resting period of 10 s, voltammograms were recorded in the differential pulse mode with polarisation in the negative direction up to –1.4 V. The other experimental parameters were as follows: step potential E_s = 2 mV, pulse potential ΔE = 50 mV, scan rate v = 25 mV s^{-1}. After each standard addition the solution was deaereated for one minute.

Results and discussion

Optimization studies

To determine the chromium redox speciation, the analytical procedure elaborated for river water[14] was adopted for chromium speciation in the extracts from fly ashes and cement. Such an approach required the optimization of the CAdSV method to the analysis of the investigated samples containing both Cr(VI) and Cr(III) species.

The preliminary experiments involved the selection of the composition of the supporting electrolyte, i.e. concentration of DTPA and KNO_3, as well as instrumental parameters such as potential and time of accumulation. The above investigations were performed in the solutions containing extracts of fly ash or cement to avoid the potential influence of the matrix on the conditions of CAdSV chromium (VI) quantification.

Figure 1 shows the dependence of the DP-CAdSV chromium response of the Cr(VI) present in the diluted extract of fly ash on the concentration of DTPA and the oxidizing agent – KNO_3. As can be seen from the presented plots, increased KNO_3 and DTPA concentrations cause the peak current to increase and then, in the case of DTPA, level off. The optimal concentrations of DTPA and KNO_3 were determined to be 0.01 M and 0.25 M, respectively.

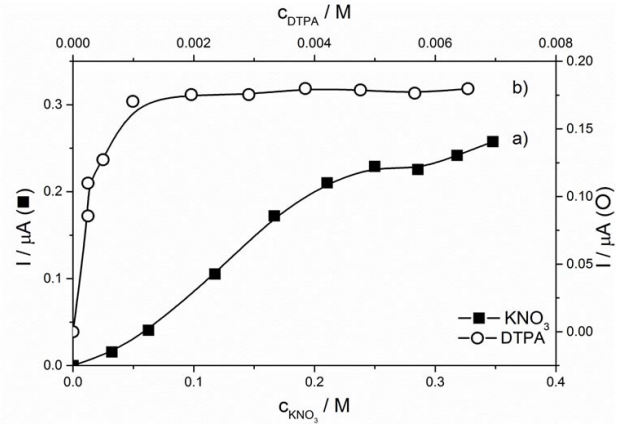

Fig. 1. **Dependence of DP-CAdS voltammetric peak current for Cr(VI) present in the 200-fold diluted extract of fly ash on KNO_3 (a) and DTPA (b),** concentration in a solution containing 0.01 M DTPA (a) or 0.25 M KNO_3 (b) in 0.1 M acetic buffer. t_{acc} = 20 s, E_{acc} = – 0.95 V

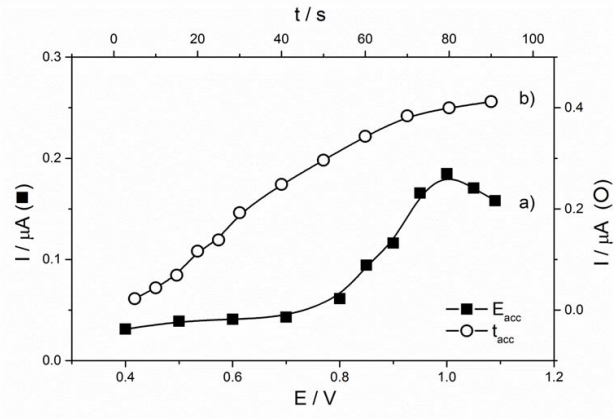

Fig. 2. **Variation in DP-CAdS voltammetric Cr(VI) peak current induced by change of accumulation potential (a) or accumulation time (b).** Examined solution: 200-times diluted extract, 0.1 M acetic buffer, 0.01 M DTPA, 0.25 $MKNO_3$. Instrumental parameters: t_{acc} = 20 s (a) and for E_{acc} = –0.95 V (b)

Figure 2 shows the effect of the accumulation potential (Fig. 2a) and accumulation time (Fig. 2b) on the Cr(VI) peak current. The maximal chromium response was observed for preconcentration potentials in the range from –0.95 V to –1.05 V. In further experiments the accumulation potential of – 0.95 V was chosen. The 20 s accumulation time also allowed highly sensitive and well-shaped chromium peaks to be obtained and was sufficient for accurate and precise quantification in the analyzed materials.

Figure 3 presents the time dependence of the CAdSV response obtained for the solution containing Cr(VI) or Cr (III) in the selected extract of fly ash. To highlight the effect of the presence of Cr(III) on the analytical signal, the Cr(III) standard at a concentration of 1 μg L^{-1} was added into the investigated solution.

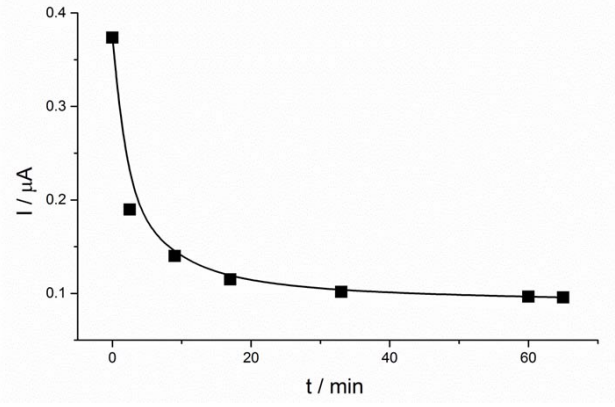

Fig. 3. **Chromium DP-CAdSV response with time in the solution containing 200-fold diluted fly ash extract additionally spiked with 1 μg L^{-1} Cr(III).** Supporting electrolyte: 0.1 M acetic buffer, 0.01 M DTPA, 0.25 M KNO_3. Instrumental parameters: t_{acc} = 20 s, E_{acc} = – 0.95 V

As can be seen from Figure 3, the CAdSV signal originating from the Cr(III) present in a ca. 4-fold excess in relation to Cr(VI) completely diminished over the 30 min starting from the moment of the solution preparation in the voltammetric vessel. The fast fall of the Cr(III)-DTPA response in time allowed the accurate determination of Cr(VI), even in the presence of some excess of Cr(III).

Analysis of fly ashes and cement extracts

Figure 4 presents examples of Cr(VI) quantification in the diluted fly ash (Fig. 4A) and cement (Fig. 4B) extracts by means of the standard additions procedure. The voltammetric curves of Cr(VI), obtained after 20 s of accumulation, were well-shaped and the calibration plots were highly linear (determination coefficient, $R^2 > 0.999$) for all the investigated extract samples. The determined values of Cr(VI) in the investigated fly ashes extracts ranged from 50 to 100 μg L^{-1}. To determine total chromium by means of CAdSV, the extracts were irradiated with UV to oxidize all Cr(III) forms to Cr(VI). The concentration of Cr(III) was calculated from the difference between the total chromium content and the Cr (VI) concentration.

To validate the results obtained with CAdSV, absorption spectrophotometry with diphenylcarbazide was used as the reference method for Cr(VI) and total chromium determination, the results obtained using the two methods were highly consistent. These results are presented in Table I. The obtained results show that the examined fly ashes could be assign to inert or non-hazardous category of waste.

Analysis of fly ash and cement extracts using gold film electrodes

The results collected in Table I show that the hanging mercury drop electrode and catalytic adsorptive stripping voltammetry may be successfully applied for the determination of Cr(VI) in fly ash extracts. Unfortunately, the HMDE is inconvenient for on-site applications due to its bulky body, relatively brittle glass accessories, and – most importantly – because of mercury's toxicity and issues related to its disposal. To overcome these obstacles, gold electrodes were tested. The Cr(VI) concentration was determined on the basis of Cr(VI) reduction to Cr(III) in acidic media, without any accumulation step. The preliminary experiments involved gold disc electrodes; unfortunately, with these electrodes it was impossible to record signals when the Cr(VI) concentration in the examined solution was lower than 100 μg L^{-1}. The results obtained using the ex-situ prepared gold film electrodes plated on a glassy carbon support appeared far more promising. After optimization of instrumental and chemical factors, a linear calibration curve was obtained

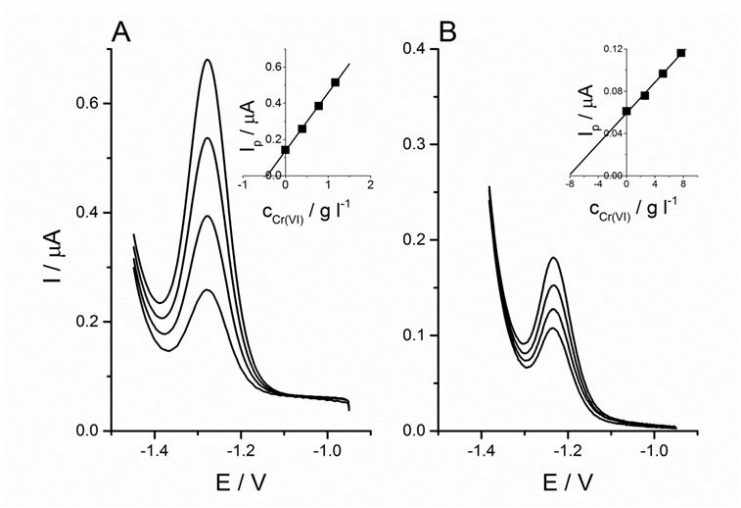

Fig. 4. **DPV voltammograms recorded in fly ash extract III (A) and cement extract (B) without and after Cr(VI) standard additions.** Examined solution: sample diluted 800 (A) times or 300 (B), 0.1 M acetic buffer, 0.025 M DTPA and 0.25 M KNO_3. Instrumental parameters: E_{acc} = –1.2 V for 20 s, t_{eq}= 5 s, E_s = 2 mV, ΔE = 50 mV. Inset: standard additions plot

Table I
Cr(VI) and total chromium contents (mean ± s) in extracts from fly ash and cement determined by means of the investigated CAdSV and spectrophotometric methods

Sample	Cr(VI) contents [mg kg^{-1}] dry substance		Cr_{total} contents [mg kg^{-1}] dry substance		Limit value [mg kg^{-1}]
	spectrophotometry	voltammetry	spectrophotometry	voltammetry	
Fly ash I*	0.81 ± 0.03	0.80 ± 0.03	1.49 ± 0.04	1.55 ± 0.03	0.5 (a)
Fly ash II*	1.1 ± 0.05	1.13 ± 0.03	1.15 ± 0.03	1.16 ± 0.05	10 (b)
Fly ash III**	0.35 ± 0.003	0.36 ± 0.01	–	0.35 ± 0.01	70 (c)
CEM III BS 32,5 R**	2.4 ± 0.008	2.55 ± 0.08	–	–	2(d)

Legend: * liquid to solid ratio (L/S) of 10 L/kg, ** liquid to solid ratio (L/S) of 1 L/kg, (a) inert, (b) non-hazardous, (c) hazardous; Council decision of 19 December 2002 establishing criteria and procedures for the acceptance of waste at landfills pursuant to Article 16 of Annex II to Directive 999/31/EC (2003/33/EC), (d) Directive 2003/53/EC

(12.5–150 µg L^{-1}, r^2 = 0.992), covering the range of Cr(VI) concentration expected in the studied fly ash extracts. While investigating the potential interfering ions, it was found that chloride ions impair the performance of the gold film electrodes. The leaching of chloride ions present in the reference electrode was minimized when a double-junction electrode filled with KNO_3 was used. The removal of chloride ions from the tested samples was more challenging. Eventually, the ions were precipitated by adding 2 g of $KAl(SO_4)_2$ per 1000 mL of extract, followed by 15 minutes of stirring. The white precipitate of calcium-rich compounds containing bound chloride (Fig. 5) was separated from a solution using a 0.22 µm syringe filter. The chloride-free extract was added to the 0.1 M acetic buffer containing 50 µg L^{-1} of Cr(VI). As seen in Fig. 6, the Cr(VI) signal faded gradually as the sample was added. Such trend indicates that not only chloride, but also other constituents present in fly ash extracts interfere with the Cr (VI) determination. To limit interferences resulting from the fly ash extract's complexity, it was necessary to dilute the sample at least 20 times, but in such solution no observable Cr(VI) peaks were detected.

Afterwards, the screen-printed gold film electrodes were tested. The experiments were performed using gold film screen-printed working electrodes arranged with either screen-printed reference and auxiliary electrodes (silver and gold, respectively), or classic reference (Ag/AgCl/3 M KCl) and auxiliary (platinum wire) electrodes.

The voltammograms obtained for increasing concentrations of Cr(VI) recorded using the two configurations, shown in Fig. 7, demonstrate that the 'classic configuration' is superior to the 'three screen-printed electrodes' version. Cr(VI) signals recorded using the classic reference and auxiliary electrodes were well-

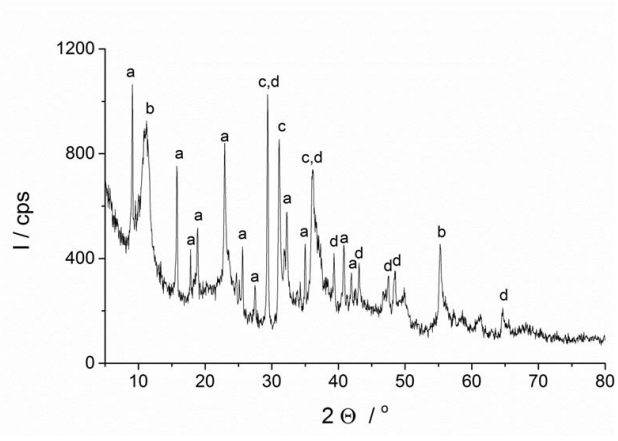

Fig. 5. **X-ray diffractogram of white precipitate separated from fly ash extract after addition of $KAl(SO_4)_2$.** Legend: (a) – ettringite ($Ca_2Al_2(SO_4)_3(OH)_3 \cdot 26\ H_2O$), (b) – calcium chloroaluminate $Ca_2Al(OH)_6Cl \cdot 2H_2O$, (c) – Friedel's salt ($Ca_4Al_2O_6Cl_2 \cdot 10H_2O$), (d) – calcite ($CaCO_3$)

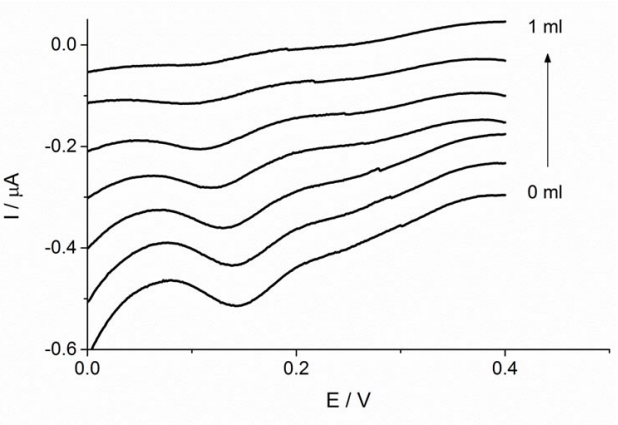

Fig. 6. **DPV voltammograms of the solutions containing 50 µg L^{-1} of Cr(VI) and increasing volumes of fly ash extract.** Volume of chloride-free extract: 0, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mL. Supporting electrolyte: 10 mL of 0.1 M acetic buffer. Plating parameters: t_{plat} = 45 s, E_{plat} = –0.45 V; plating solution: 0.4 g L^{-1} $NaAuCl_4$ in 1 M HCl

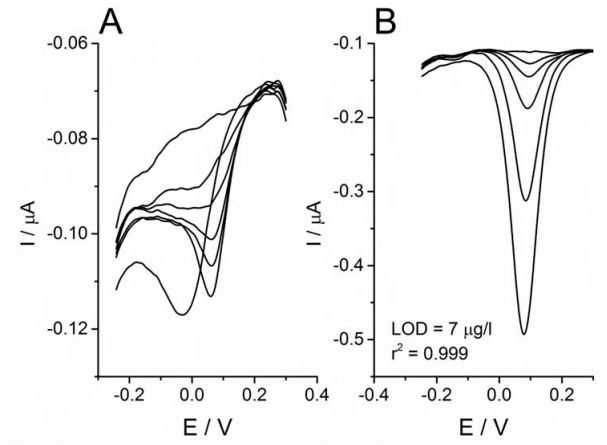

Fig. 7. **DPV voltammograms recorded at the working gold screen printed electrodes for solutions containing increasing amounts of Cr(VI).** A: screen-printed reference and auxiliary electrodes (Ag and Au, respectively), B: reference electrode – classic Ag/AgCl/3M KCl, auxiliary electrode – Pt wire; A: Cr(VI) concentrations – 0, 25, 50, 75, 100, 125, 250 µg L^{-1}, B: Cr(VI) concentrations – 0, 25, 50, 100, 250, 500 µg L^{-1}. Supporting electrolyte: 0.1 M acetic buffer. Instrumental parameters: t_{eq}= 5 s, E_s = 2 mV, ΔE = 50 mV

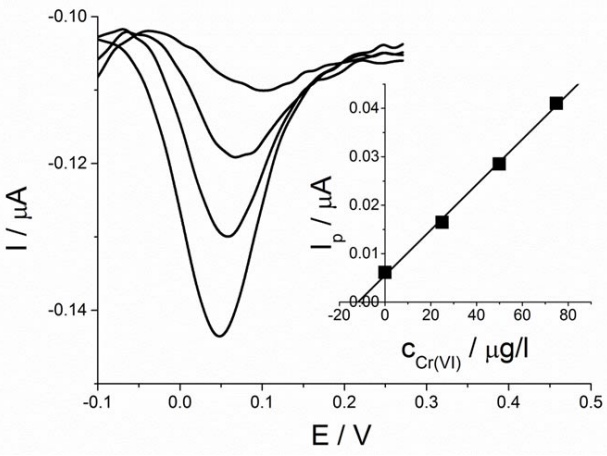

Fig. 8. **DPV voltammograms recorded for the fly ash extract without and after Cr(VI) standard additions.** Examined solution: fly ash extract diluted 5 times, 0.1 M acetic buffer. Instrumental parameters: t_{eq}= 5 s, E_s = 2 mV, ΔE = 50 mV. Inset: standard additions plot

defined, symmetrical and more clearly separated from the background than signals obtained using the 'three screen-printed electrodes' configuration. Additionally, in the case of the screen-printed Ag reference electrode, a variation in peak potential was observed due to the very low concentration of chloride ions.

Using classic reference and auxiliary electrodes, a series of voltammograms of the fly ash extract were recorded, revealing that the gold screen-printed working electrode was far less susceptible to poisoning by matrix components than the electroplated one. Eventually, the Cr (VI) concentration in the fly ash extract was determined using a 5-fold diluted extract (Figure 8). The analysis revealed that the content of Cr(VI) in fly ash amounted to 0.57±0.07 mg kg^{-1}; this value is equal to the limit value specified for this contaminant for inert waste.

Conclusion

It was demonstrated that catalytic adsorptive stripping voltammetry at the HMDE is superior for the speciation study of chromium in fly ashes and cement extracts, and it even more so for the quantification of toxic Cr(VI) in both types of materials. In the case of higher concentrations of Cr(VI), the elaborated method could be used as an alternative to the commonly applied spectrophotometric procedure with DPC (LOD and LOQ in a 1 cm cuvette are 41.5 $\mu g\, L^{-1}$ and 125 $\mu g\, L^{-1}$ Cr(VI), respectively[7]). However, due to its sensitivity, which is at least three orders of magnitude higher[7], CAdSV is indispensable for the speciation of much lower Cr(VI) concentrations.

The results shown in the last section demonstrate that gold screen-printed electrodes have a great potential for on-site determination of chromium(VI). The surface of gold screen-printed electrodes cured at a high temperature is less susceptible to poisoning by matrix constituents when compared to *ex-situ* plated gold films. Their sensitivity is sufficient to determine Cr(VI) content in fly ash extracts. Furthermore, the determination protocol is extremely simple – there is no need to remove oxygen, no accumulation step is required, and the solution only needs to be stirred for one minute before recording the voltammograms. To fully exploit the advantages of the screen-printed electrodes comprising three electrodes on one strip, further experimental studies focused mainly on the stabilization of the reference electrode response must be undertaken.

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