

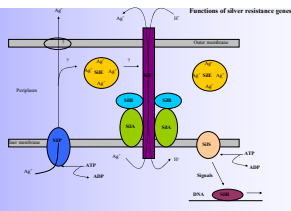
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INTRODUCTION

Silver ions come to the environment, first of all, from industry. The highest amounts of silver are used in photographic industry (more than 40 %), in electrochemical industry (more than 30 %), in electrochemical plating (production of jewellery, more than 22 %), in chemical industry (more than 6 %) and in health service (silver amalgam, more than 2 %). That is why these industries produce the most of silver waste. In addition, amount of silver ions in waste waters represents the greatest source of their entering to food chain. Moreover, marked toxicity of silver ions are published in number of publications. It is common knowledge that silver ions belongs to one of the most toxic elements causing acute toxicity of organisms e.g. fifty percent living organisms die after exposition of 10 µg l⁻¹ Ag⁺ per 96 hours. Recently number of studies concerned with influence of silver ions on quality of water, sediments, and with their toxicity on aquatic animals. Moreover about influence of silver ions on plants is known less. It clearly follows from the obtained results that presence of the silver ions improves organogenesis. Here, we aimed on optimisation of the high performance liquid chromatography coupled with electrochemical detector (HPLC ED) using for the determination.

Silver is one of the oldest anti-microbial agents that could affect more than 650 microbes. Therefore silver ions have been using for a development of new anti-microbial technologies. On the other hand, the mechanism of the toxic effect is still unclear in spite of that Ag(I) has been using for a centuries. In addition it has been published that massive leaking of protons from a membrane were observed after treatment of *Vibrio cholerae* (cholera) by silver ions, which leads to disruption of metabolism of bacterial cell.

The resistance of *Salmonella* against Ag(I) were found out in DNA of its plasmids. The plasmids' DNA resistance fragment is consisted from nine genes and three transcriptional units. The system were called as SiIRS: peri-plasmatic binding protein-Ag(I) - SiIE; two ATP pumps - SiIP; proteins participating in chemi-osmotic exchanging system RND Ag₂(OH)⁺ - SiIC, SiIB and SiIA; regulating proteins SiIS and SiIR.



MATERIAL AND METHODS

Chemicals

Silver nitrate and sodium acetate was purchased from Sigma Aldrich (St. Louis, USA). Acetic acid was purchased from Fluka (USA). All reagents used were ACS purity. Stock standard solutions were prepared by ACS water (Sigma-Aldrich, USA) and stored in the dark at the temperature of -20 °C. Working standard solutions were prepared daily by dilution of the stock solutions. All solutions were filtered through a 0.45 µm Nylon filter discs (Millipore, Billerica, Mass., USA) prior to HPLC analysis. The pH value was measured using WTW inoLab Level 3 with terminal Level 3 (Weilheim, Germany), controlled by the personal computer program (MultiLab Pilot, Weilheim, Germany). The pH-electrode (SenTix-H, pH 0-14/3M KCl) was regularly calibrated by set of WTW buffers (Weilheim, Germany).

High-performance liquid chromatography with electrochemical detection

An HPLC ED system consisted of solvent delivery pump operating in range of 0.001-9.999 ml min⁻¹ (Model 583 ESA Inc., Chelmsford, MA, USA), a guard cell (Model 5020 ESA, USA), a reaction coil (1 m) and/or a chromatographic column, and an electrochemical detector. The electrochemical detector (ED) includes one low volume flow-through analytical cells (Model 5040, ESA, USA), which is consisted of glassy carbon working electrodes, palladium electrodes as reference electrodes and auxiliary carbon electrode, and Coulochem III as a control module. The sample (5 µl) was injected manually. The obtained data were treated by CSW 32 software. The experiments were carried out at room temperature.

Plant material and cultivation conditions

ESEs clone of the blue spruce (*Picea pungens* Engelm.) designated as PE 14 were used in our experiments. The cultures were maintained on a semisolid (Gelrite Gellan Gum, Merck, Germany) half-strength LP medium with modifications. The concentration of 2,4-dichlorophenoxyacetic acid and N⁶-benzyladenine was 4.4 and 9 µM, respectively. The pH value was adjusted to 5.7-5.8 before autoclaving (121°C, 100 kPa, 20 min). The organic part of the medium, excluding saccharose, was sterilized by filtration through a 0.2 µm polyethylenesulfone membrane (Whatman, Paradise 25 AS). The stock and experimental cultures were maintained in a cultivation box in the dark at a temperature of 23±2°C. Cultivation medium was modified with an addition of silver chelate (Ag-EDTA) in concentrations of 0, 250, 500 and 1000 µM. A stock solution of Ag-EDTA was prepared by mixing AgNO₃ with ethylene diamine tetra-acetic acid (EDTA) in a 1:1 molar ratio and stirred at 50°C for 1 h. The filter-sterilized Ag-EDTA complex was added to the autoclaved culture medium.

Computer image analysis

We used a charge-coupled device (CCD) camera for observation of growth of spruce ESEs culture. The images of ESEs clusters were recorded at the beginning of the cultivation and in certain intervals according to the end of the cultivation. The data were converted to digital image in the Grab-IT (version 1.3) program. The area size of ESEs clusters in digital images was calculated by program Image-Pro Plus, (Sony, ver. 1.3). The data were processed in Excel (Microsoft). *Photography of ESEs in bright field*

ESEs (about 0.1 mg) were harvested by a scalpel and transferred on slide microscopic. The ESEs were spread and superimposed by cover glass. Then, the sample was placed to microscope (Olympus AX 70, Japan). The images were forty times magnified by digital camera Olympus 4040 and converted to digital image in the Grab-IT (version 1.3) program.

Electrochemical measurements

Electrochemical measurements were performed with AUTOLAB Analyser (EcoChemie, Netherlands) connected to VA-Stand 663 (Metrohm, Switzerland), using a standard cell with three electrodes. The working electrode was a hanging mercury drop electrode (HMDE) with a drop area of 0.4 mm². The reference electrode was an Ag/AgCl/3M KCl electrode and the auxiliary electrode was a graphite electrode. The supporting electrolyte was prepared by mixing buffer components. For smoothing and baseline correction the software GPES 4.4 supplied by EcoChemie was employed.

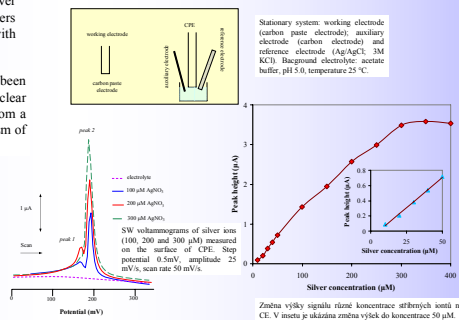
Adsorptive transfer stripping technique (ATDS) coupled with differential pulse voltammetry (DPV) Brdicka reaction

The Brdicka supporting electrolyte containing 1 mM Co(NH₃)₆Cl₃ and 1 M ammonia buffer (NH₃(aq) + NH₄Cl, pH = 9.6) was used; surface-active agent was not added. ATDS DPV Brdicka reaction parameters were as follows: an initial potential of -0.6 V, an end potential -1.6 V, a modulation time 0.057 s, a time interval 0.2 s, a step potential of 1.05 mV/s, a modulation amplitude of 250 mV, E_{ads} = 0 V. Temperature of supporting electrolyte was 0 °C.

RESULTS

A detection of ions in drinking, tap, service and other kinds of waters belongs to important tasks of analytical chemistry to solve. Therefore it is necessary to suggest new methods and techniques used for analysis of the mentioned samples, which will be ease-to-use, sensitive, low cost and computerizable. The computerizable of analysis enables to continual monitoring of the analyte in waterworks and others. Moreover ions such as heavy metals can markedly influenced organisms and, therefore, monitoring of their "movement" in the environment is necessary for suggestion of precautions leading their elimination.

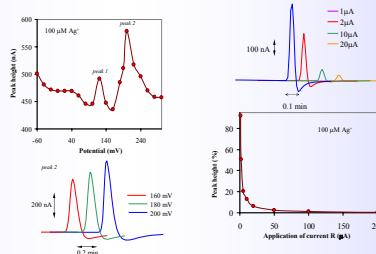
STATIONARY SYSTEM



Primarily we performed the determination of silver by stationery electrochemical analyser. V experimentu jsme použili jako pracovní uhlíkovou pastovou elektrodu (CPE). Vlastní elektrochemické měření probíhalo v prostředí acetoátového pufru pH 5.0 a laboratorní teplotě (about 25 °C). Pracovní povrch CPE byl před každým měřením mechanicky obnoven ořmením o navlhlém filtrační papír. Získané voltamogramy byly velmi dobře reprodukovatelné (R.S.D. about 5%). Typické křivky jsou ukázaný v Fig. Na voltamogramu byla pozorovatelná dvě maxima, peak 1 (about 140 mV) a peak 2 (about 240 mV). Pro analytické účely jsme zvolili peak 2. Peak 2 se lineárně měnil v závislosti na koncentraci stříbrných iontů až do koncentrace 300 µM (Fig.). Při dalším zvyšování koncentrace stříbrných iontů se však analytický signál měnil velmi pozvolně. Při těchto vysokých koncentracích stříbrných iontů, se pravděpodobně na povrchu CPE vytváří vrstva redukčních produktů, které brání prostupu elektronů přes elektrodu dvojitvrstvou.

FLOW SYSTEM

The obtained results were used for analysis of silver by a flow technique with electrochemical detection. Therefore it was necessary to optimise the basic chromatographic parameters such as working potential, flow rate and concentration of acetate buffer. The most suitable HPLC ED parameters for the determination of silver were as follows - flow rate: 0.5 ml min⁻¹, guard cell potential: 0 mV, working electrode potential: 200 mV, current: 10 µA. The most optimal mobile phase was 0.2 M acetate buffer (pH 5.0). The detection limit of silver was hundreds of nM. The technique is able to use not only for sensitive determination of silver but also for analysis of biological sample such as plant tissue, blood serum etc.

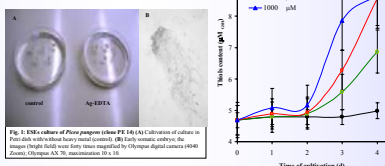


V průtokovém systému (Coulchem III) prochází mobilní fáze před vstupem na amperometrickou detekční celou guard cell. Zajímalo nás, jak potenciál, který aplikujeme na guard cell ovlivní analytický signál. Zjistili jsme, že negativní potenciál mírně signál stříbrných iontů zvyšuje, ale pozitivní potenciály na signál stříbrných iontů nemají výrazný vliv (Fig.). Data získaná detektorem jsou upravena pomocí časového filtru. Pro naše účely je nejvhodnější asi filtr s časem 1 s.

Jak jsme již zjistili ve stacionárním uspořádání pH mobilní fáze výrazně ovlivní výsledný signál stříbrných iontů. Pokud při použití acetoátového pufru pH 5.0 kolem 4.0 byla získána maximální odezva detektoru (Fig.).

V našich experimentech jsme sledovali změnu výšky signálu stříbrných iontů v závislosti na potenciálu, který byl vložen na pracovní uhlíkovou elektrodu. Získaný hydrodynamický voltamogram je ukázaný v Fig. 1 v průtokovém systému jsme pozorovali dvě maxima (peak 1 and peak 2). Peak 2 byl zvolen pro další studium chování stříbrných iontů na CE v průtokovém uspořádání. Aplikovaný proud zvyšuje senzitivitu elektroanalytického stanovení analytu. Sledovali jsme změny signálu stříbrných iontů v závislosti na aplikovaném proudu (Fig.). Avšak při používání nižšího proudu byla pozorována menší reprodukovatelnost výsledků. A v našich dalších analýzách jsme aplikovali proud 10 µA.

Vliv stříbrných iontů na somatické embrya smrku



Množství thiolů (vztažených na GSH) se u kultury somatických embryí klonu PE 14 4 rostoucí období vzroste a aplikovanou koncentraci stříbrných iontů. K výraznému vzestupu množství thiolů bylo pozorováno od druhého dne kultivace. V prvních dnech kultivace nebyly změny obsahu thiolů neapatné a byly zatíženy velkou variabilitou (5-15%).

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