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Thin-layer chromatography combined with diode laser thermal vaporization inductively coupled plasma mass spectrometry for the determination of selenomethionine and selenocysteine in algae and yeast

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ABSTRACT

In this work we present a simple and cost-effective approach for the determination of selenium species in algae and yeast biomass, based on a combination of thin-layer chromatography (TLC) with diode laser thermal vaporization inductively coupled plasma mass spectrometry (DLTV ICP MS). Extraction of freeze-dried biomass was performed in 4M methanesulphonic acid and the selenium species were vaporized from cellulose TLC plates employing a continuous-wave infrared diode laser with power up to 4 W using a simple laboratory-built apparatus. Selenomethionine and selenocysteine were quantified with limits of detection 3 $\mu g\,L^{-1}$ in a Se-enriched microalgae *Chlorella vulgaris* and yeast certified reference material SELM-1. Results delivered by TLC-DLTV ICP MS were consistent with those obtained by a routine coupling of high-performance liquid chromatography (HPLC) to ICP MS. In addition, the TLC approach is capable of analyzing extract containing even undiluted crude hydrolysates that could damage HPLC columns.

1. Introduction

Selenium speciation, together with speciation of As, has dominated the literature about elemental speciation in previous years. [1]. Selenium is an essential element with major nutritional and biological roles for higher organisms, although the range between deficiency and toxicity is quite narrow. For humans, levels below 0.1 mg of Se per kg of body weight are considered insufficient while the consumption of more than 1 mg of Se per kg of body weight may cause serious health risks. This micronutrient is of potential use in the prevention and treatment of many diseases and it is also a key element in cancer prevention, where it acts as a stimulant for antioxidant enzymes. [2,3]

Bioavailability, toxicity and nutritional status of selenium is highly dependent on its concentration and chemical form. [3]. Selenium is found in biological samples in the form of non-volatile inorganic selenium, selenite and selenate and organic selenium species, such as selenoaminoacids, selenopeptides, selenoproteins and selenolipids [4]. Microalgae can take up inorganic forms of selenium and convert them mostly to protein-bound selenocysteine and selenomethionine, which have lower toxicity and higher bioavailability in higher organisms compared to the inorganic forms. [5]. In this regard, microalgae is probably the simplest plant-like Se-enriched biomass for nutritional purposes. [6] In order to better understand the metabolism of selenium, the development of analytical methods for speciation of selenium is of great importance as they provide information on the individual species.

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Se speciation is traditionally achieved by coupling a high resolution separation technique, such as various modes of liquid chromatography (LC) [4,7–14], gas chromatography (GC) [15–18] or capillary electrophoresis (CE) [19–21] with a sensitive detection technique. Perhaps the most routinely used system is the coupling of high performance liquid chromatography (HPLC) to inductively-coupled plasma mass spectrometry (ICP MS) [22].

Speciation applications which focus on a few biologically important species do not necessarily require highly efficient separation techniques, such as HPLC or CE; simpler and more cost-effective approaches can be sufficient here. In this regard, coupling of thin-layer chromatography (TLC) with ICP MS is a potential method of choice as TLC is convenient, easy to perform in ambient conditions, utilizing inexpensive materials and requiring only a minimal amount of mobile phase solvents. TLC allows analysis of even unknown crude samples that could potentially damage more expensive chromatography columns or cause severe memory effects. This is completely eliminated in TLC since a new stationary phase is always used. Multiple TLC separations can be performed at the same time, archived and analyzed independently at a different time and place. Thus, with planning and careful experimental design, high-throughput screening experiments can be carried out. Furthermore, TLC can be easily coupled to existing ICP MS using laser ablation (LA) or diode laser thermal vaporization (DLTV) [23–27]. In these dry desorption modes, the ICP discharge stability and ionization efficiency is not negatively affected by the introduction of organic or saline mobile phases to ICP.

DLTV allows cost effective sampling for ICP MS, based on pyrolysis of a suitable substrate by diode lasers [28,29]. An 808 nm diode laser with power from 0.2 to 1.2 W was used for DLTV from common filter paper or cellulose TLC sheets overprinted with black ink. The ink absorbs the laser irradiation heating up the substrate and finally a pyrolysis-generated aerosol is flushed out of the vaporization chamber into the plasma. The DLTV ICP MS was successfully employed for analysis of submicroliter volumes of complex liquids, e.g. blood [29] and coupled to thin-layer chromatography for speciation of cobalamins [27]. DLTV offers an easy operation, convenient sample handling, archiving and transportation and optional use of prearranged multi-elemental calibration sets.

Techniques similar to DLTV were reported for desorption molecules from TLC sheets, showing the increasing popularity of low cost, easy to operate, small sized diode lasers in analytical sciences. TLC-atmospheric pressure chemical ionization analysis of the phospholipids was allowed by the infrared diode laser desorption from graphite covered TLC sheets. [30,31] More recently, a 1 W 532 nm diode laser was applied to the analysis of explosives from TLC plates coupled with ion mobility spectrometry (IMS) [32] and a 1 W 445 nm laser was applied to the analysis of low molecular organic species, such as pyrazole derivatives, alkaloids and pharmaceuticals, separated by TLC using a flowing atmospheric pressure afterglow (FAPA) source. [33] In the latter case, the heating followed by evaporation of a silica gel stationary phase was initiated by a graphite mark absorbing the laser energy. Another approach using a near-infrared diode laser for desorption of analytes from a glass surface, the reverse side of which was coated with black ink, was applied for the analysis of a variety of nonvolatile species, e.g., pesticides, pharmaceuticals and explosives. [34]

The aim of this work is to develop an application of DLTV to couple TLC with ICP MS for speciation of selenium containing compounds in algae and yeast and to compare the technique performance with that of HPLC-ICP MS. Six organic and inorganic selenium species on which selenium speciation usually focuses are chosen as a model system; namely selenite (SeO_3^{2-}), selenate (SeO_4^{2-}), selenocystine (Se_2Cys_2), methylselenocysteine (MeSeCys), selenomethionine (SeMet) and methaneseleninic acid (MSeA). The content of SeCys and SeMet is quantified in selenium-enriched algae and yeast.

2. Material and methods

2.1. Chemicals and materials

Sodium selenite (SeO_3^{2-}) was purchased from Lachema a.s. (Brno, Czech Republic), sodium selenate decahydrate (SeO_4^{2-}), seleno-L-cystine (Se_2Cys_2), Se-(Methyl) selenocysteine hydrochloride (MeSeCys), seleno-D,L-methionine (SeMet), methaneseleninic acid (MSeA), dithiothreitol (DTT), mercaptoethanol (ME) and methanelsulfonic acid from Sigma Aldrich (St. Louis, USA). Ammonia, 26% solution in water, p.a. was obtained from Mikrochem (Pezinok, Slovakia). Algae (*Chlorella vulgaris,* strain R117*)* were obtained from the collection of the Laboratory of Algal Biotechnology (Institute of Microbiology, Třeboň, Czech Republic) and lyophilized selenium enriched yeast certified reference material SELM-1 was obtained from NRC-CNRC (Ottawa, Canada).

Stock solution of Se_2Cys_2 was prepared in 0.1% HCl (Sigma Aldrich) in concentration 0.5 g L^{-1}; stock solutions of each of the other species were prepared in distilled water with a concentration of 1 g L^{-1}. For individual analytical tasks, the freshly diluted solutions from the stock solutions were always prepared.

Precoated aluminum-backed 20 × 20 cm HPTLC sheets (item no. 1.16092.0001) with 100 μm layer of cellulose sorbent were purchased from Merck (Darmstadt, Germany) and 10 × 20 cm TLC silicagel sheets (item no. 02599) with aluminum support were purchased from Sigma-Aldrich (St. Louis, USA).

2.2. Biomass cultivation

The fast-growing microalga *Chlorella vulgaris*, strain R117 (*Chlorophyta*) was cultivated in a modified inorganic medium at pH 7.4 [5,35,36]. All chemicals for the preparation of the modified inorganic medium were purchased from Penta s.r.o. (Prague, Czech Republic) or Lach-Ner s.r.o. (Neratovice, Czech Republic) with a high purity for analysis (p.a.).

The cultures were grown in glass columns (working volume 330 mL; internal diameter 35–37 mm) that were submerged in a temperature-controlled water bath (28–29 °C) and mixed by bubbling with air + 1.5% CO_2 (*v*/*v*).

The cultures with a starting density 1.5 g L^{-1} were exposed to continuous illumination for 4 days. Photosynthetically active radiation (PAR) provided by a panel of high frequency cool fluorescent tubes (36 W/830 Lumilux, Osram, Germany) was measured directly inside an empty cultivation column using a quantum sensor (LI-190SA, cosine-corrected up to 80° angle of incidence) coupled to a light meter (LI-250, Li-Cor, USA). The light intensity was set to 300 μmol photons m^{-2} s^{-1} and Se was added twice a day in the form of sodium selenite (0.2 g L^{-1}) in the ratio of 8 mg Se per 1 g of starting biomass concentration [5]. After 4 days, the biomass was harvested by centrifugation (7000 × *g*, 10 min) and stored at −70 °C prior to lyophilization.

2.3. Acid hydrolysis of the biomass

The lyophilized yeast and algae were treated by methanesulphonic acid. [37–39] The published technique of acid hydrolysis was modified and adapted for micro-volume scale; 6 mg of the lyophilized powder was weighed in a glass vial and 1 mL of 4 M methanesulphonic acid added. To keep the reducing environment during the hydrolysis, 20 μL of ME or 11 mg of DTT were dissolved in the mixture prior to the hydrolysis. The mixture was vortexed and kept at 95 °C for 26 h. After the extraction, another 20 μL of

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