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Speciation of selenomethionine and selenocystine using online micro-column containing Cu(II) loaded nanometer-sized Al_2O_3 coupled with ICP-MS detection

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ABSTRACT

A flow injection online speciation procedure by using micro-column packed with Cu(II) loaded nanometer-sized Al_2O_3 coupled to inductively coupled plasma mass spectrometry (ICP-MS) for the separation and determination of selenomethionine (SeMet) and selenocystine ($SeCys_2$) has been developed. The main factors affecting the separation and preconcentration of SeMet and $SeCys_2$ including pH value, sample flow rate, eluent concentration, eluent volume and flow rate, and interfering ions have been investigated. It was found that $SeCys_2$ could be selectively retained by micro-column packed with Cu(II) loaded nanometer-sized Al_2O_3 at pH 4.0, and the retained $SeCys_2$ could be eluted by 1.0 mol L^{-1} HNO_3, while SeMet was not retained and passed through the micro-column directly at this pH. Both SeMet and $SeCys_2$ could be quantitatively adsorbed by the micro-column at pH 9.0, and the retained SeMet and $SeCys_2$ could be easily eluted with 1.0 mol L^{-1} HNO_3. The content of SeMet was obtained by subtracting the $SeCys_2$ from the total content of seleno amino acids. With the enrichment factor of 7.8 and 7.7, the limits of detection (LODs) for SeMet and $SeCys_2$ were found to be 24 pg Se mL^{-1} and 21 pg Se mL^{-1}, respectively. The relative standard deviations (RSDs) for $SeCys_2$ and SeMet with seven replicate determinations of 1.0 ng mL^{-1} SeMet and $SeCys_2$, were 2.1% and 1.6%, respectively, the sampling frequency of 8 h^{-1} was obtained. The proposed method was applied to the speciation of SeMet and $SeCys_2$ in selenized yeast, human urine and serum with satisfactory results.

1. Introduction

Selenium (Se) is an essential nutrient in a very narrow concentration range [1]. Dietary studies have shown that selenium in amino acids is absorbed more readily by the body than its inorganic species. Organo seleno compounds, mainly seleno amino acids, were widely existed in biological samples [2]. One of the seleno amino acids, selenocysteine (SeCys), the 21st essential amino acid, is considered to be biologically active, in contrast to the other major form, selenomethionine (SeMet), which can replace methionine to participate in the synthesis of protein [3]. The biological and toxicological effects of selenium are strongly dependent on its chemical forms ("species"). Therefore, interest in the differentiation of selenium species (inorganic and organic) is increasing, either in foodstuff and supplements, or in body fluids such as serum and urine.

The general methods for the selenium speciation are based on combining a very efficient separation technique with a sensitive detection technique. In order to avoid the interference of sulfur-containing amino acids due to their similarity to seleno amino acids, elemental specific detectors, such as atomic emission spectrometry (AES) [4], atomic absorption spectrometry (AAS) [5], atomic fluorescence spectrometry (AFS) [6] and inductively coupled plasma mass spectrometry (ICP-MS) [7] have been commonly used to detect seleno amino acids. Of all these elemental specific detection methods, ICP-MS has been increasingly widespread in selenium speciation because of its several advantages for selenium speciation analysis over more traditional detectors, including multi-element and multi-isotope detection and high sensitivity with a wide linear dynamic range.

Numerous separation methods including liquid chromatography (LC) [8–11], gas chromatography (GC) [12,13], capillary electrophoresis (CE) [14,15] and solid phase extraction (SPE) [16–19], have been utilized for speciation of seleno amino acids. Analysis of seleno amino acids by GC requires derivatization of the carboxylic and amino groups in order to increase their volatility using some derivatization reagents, such as chloroformate, etc. [12,13]. HPLC in conjunction with various elemental-selective detectors (especially ICP-MS) is one of the most used techniques for the speciation of seleno amino acids, and the limits of detection (LODs) are typically at the levels of nanogram per milliliter or sub nanogram per milliliter. CE is a good alternative for the speciation of seleno amino acids, but it suffered from the insufficient sensitivity when it was used for real world sample analysis. Compared to

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Table 1
Optimum operating conditions for ICP-MS.

Plasma	
Rf power	1200 W
Plasma gas flow rate	15 L min^{-1}
Carrier gas flow rate	1.08 L min^{-1}
Sampling depth	7.0 mm
Sampler/skimmer diameter orifice	Nickel 1.0 mm/0.4 mm
Time-resolved data acquisition	
Scanning mode	Peak-hopping
Dwell time	0.1 s
Points per spectral peak	1
Isotopes	^{77}Se, ^{82}Se

chromatographic methods, SPE possessed some special merits like easy to operate, time saving, reduced solvent utilization and low cost. Unfortunately, SPE methods for separation/preconcentration of organo seleno compounds are scarce. Preconcentration of four seleno amino acids (SeMet, selenoethionine (SeEth), selenocystine ($SeCys_2$), selenocystamine (SeCysta)) on a porous graphitic carbon column was obtained by Abbas-Ghaleb et al. [20], however, the separation of seleno amino acids in this method was achieved by HPLC. Latva et al. [19] separated SeMet from inorganic selenium with metal-loaded activated charcoals. Huang et al. [16] have developed a novel, fast, and cheap non-chromatographic method for speciation of dissolved inorganic and organic selenium species in environmental and biological samples by flow injection (FI) dual-column preconcentration/separation on-line coupled with ICP-MS determination. These results demonstrated that SPE was a good alternative for separation/preconcentration of seleno amino acids. Besides, considering the same functional groups (amino and carboxyl groups) existing in seleno and conventional amino acids, some other methods for preconcentration conventional amino acids are supposed to be adopted for seleno amino acids.

It is reported that Cu(II) can form stable complexes with seleno amino acids [21] as well as can be easily adsorbed on the surface of nanometer-sized Al_2O_3 [22]. In view of that, the aim of this work was to achieve online preconcentration and separation of SeMet and $SeCys_2$ using Cu(II) loaded nanometer-sized Al_2O_3 coupled to ICP-MS and apply the proposed method to the determination of SeMet and $SeCys_2$ in real samples.

2. Experimental

2.1. Instrumentation

An Agilent 7500a ICP-MS (Agilent Technologies, Takatura, Japan) system with Babington nebulizer was used for the determination of target analytes. The optimum operation conditions were summarized in Table 1. ^{77}Se and ^{82}Se were monitored, but only isotope ^{82}Se was used for quantification in this work. The pH values were controlled with a Mettler Toledo 320-S pH meter (Mettler Toledo Instruments Co. Ltd., Shanghai, China) supplied with a combined electrode. An IFIS-C flow injection system (Ruimai Tech. Co. Ltd., Xi'an, China) and a self-made PTFE micro-column (20 mm × 2.0 mm i.d.) packed with nanometer-sized Al_2O_3 were used in the online separation/preconcentration process. A minimum length of PTFE tubing with an i.d. of 0.5 mm was used for all connections in order to minimize the dead volume.

2.2. Standard solution and reagents

All reagents used were of highest purity or at least analytical reagent grade. The stock standard solutions (1.000 mg Se mL^{-1}) were prepared by dissolving 62.1 mg of DL-selenomethionine and 52.9 mg L-selenocystine (Acros Organics, Geel, Belgium) in 25 mL of high-purity de-ionized water and 0.01 mol L^{-1} hydrochloride, respectively. Work solutions were prepared daily by stepwise dilution of their stock solutions with high-purity de-ionized water. High-purity de-ionized water obtained by Milli-Q system (18.2 MΩ cm, Millipore, Molsheim, France) was used throughout the whole experiments. Nanometer-sized Al_2O_3 was prepared in our laboratory, and the details on its synthesis and characterization were described in Ref. [23].

2.3. Column preparation and experimental procedure

50 mg of nanometer-sized Al_2O_3 was filled into a PTFE micro-column (20 mm × 2.0 mm i.d.) plugged with a small portion of degreased cotton at both ends. The schematic diagram was shown in Fig. 1, as described in Ref. [22].

Before use, 0.1 mol L^{-1} NaOH and high-purity de-ionized water were passed through the column (1 mL min^{-1}) in sequence in order to clean and condition it. Then, 1.0 mol L^{-1} $CuSO_4$ solution and high-purity de-ionized water was sequentially passed through the column (1 mL min^{-1}) to modify the surface of the nanometer-sized Al_2O_3. The sample solution was divided into two portions; one was adjusted to pH 4.0, and the other to pH 9.0. For the determination of $SeCys_2$ (C_1), sample solution with pH 4.0 was passed through the column, the retained $SeCys_2$ on the micro-column was eluted with HNO_3 and the eluent was directly introduced into ICP-MS for determination of selenium. For the total content of SeMet and $SeCys_2$ (C_2), sample solution with pH 9.0 was processed with the same procedure as mentioned above. The concentration of SeMet was calculated as the difference of C_1 and C_2.

For the regeneration of the nanometer-sized Al_2O_3 packed micro-column, 0.5 mol L^{-1} NaOH solution and high-purity de-ionized water were passed through the column, followed by passing through the column with 1.0 mol L^{-1} $CuSO_4$ solution. By this treatment, the column could be reused for at least 20 times, and no obvious reduce on the recoveries of analytes was found.

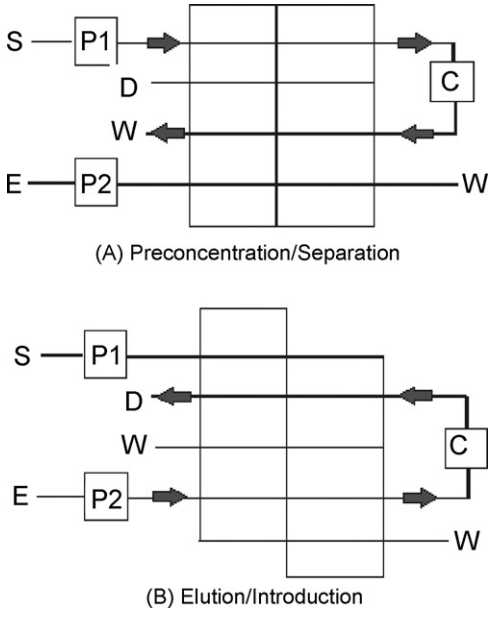

Fig. 1. Flow injection manifold and operation for on-line SPE and ICP-MS determination. (A) Preconcentration/separation step; (B) elution/introduction step. S: sample; E: elution; W: waste; C: micro-column packed with nanometer-sized Al_2O_3; P1, P2: peristaltic pumps; D: detector.