Speciation of Arsenic Ions (As^{3+}/As^{5+}) with the Yeast-Immobilized Column

Janghan Koh and Yong-Nam Pak*

Department of Chemistry Education, Korea National University of Education, Cheongwon 363-791, Korea
**E-mail: pakyn@cc.knue.ac.kr*
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The speciation study of trace metals in analytical science is getting more attention in these days because different toxicity and chemical properties are shown depending on their oxidation states and elements combined with.[1] Arsenic has been widely used in industrial and agricultural products for a long period. After being absorbed in bio-organisms, it could be converted in various species such as monomethyl and dimethyl As. Inorganic arsenic species is more toxic than organic species and As^{3+} shows at least 10 times higher toxicity than As^{5+}.[2] Consequently, investigations have been performed on arsenic oxidation states as well as organic species.[3-10]

Gas Chromatography (GC)[11,12] and High-performance liquid chromatography (HPLC)[10,13-15] have been successfully used for the separation of arsenic species. Anionic[16] and cationic[17] ion pairing, ion exchange,[10] and other modes of separation have been used.[18] Recently, ICP-MS has become a very powerful technique for arsenic speciation study combined with HPLC. Several researchers[19-23] have extensively used the HPLC ICP-MS system for arsenic speciation studies.

It is quite interesting that biological substrates show preconcentration and speciation abilities. Proteins and carbohydrates of cell wall provide good sites for a metal binding. Algae,[24] yeast,[25] bacteria,[26] plant derived material,[27] and erythrocytes[28] have been reported for showing good capabilities in preconcentration and selective sorption of trace metals. However, most of studies were focused on either a simple metal preconcentration or speciation with a classical batch mode. We now report for the on-line method of speciation using yeast-immobilized column in ICP-AES (Inductively Coupled Plasma-Atomic Emission Spectrometry).

Contrary to a batch mode, the on-line process with an immobilized substrate can provide low resistance in flow, high sample throughput, high binding efficiency and easy recovery of trace metals. In our work, *Saccharomyces cerevisiae* is covalently bonded on CPG (Controlled Pore Glass) and used for the selective preconcentration of As^{5+} over As^{3+}. Though yeast has been studied before,[29] the study was using a simple batch mode. Interestingly, the result showed quite different characteristic of retainment for As species.

A block diagram of the experimental set-up is shown in Figure 1. After the column was flushed with a strong acid, rinsed with water and conditioned with a buffer, sample was injected into the column using an injection valve. Arsenic could be eluted with an inorganic acid. A chromatographic pump (Analytical P2000, Spectra-Physics Analytical Inc., CA) was used to provide enough pressure for the packed column (4.7 cm length × 0.45 cm diameter). The efficiency of arsenic separation was tested for different columns. Pure silica, yeast immobilized on silica, yeast immobilized on CPG, columns were prepared and compared. The results are shown in Figure 2. All the experimental conditions for elution reagent, flow rate, pH were the same. The experimental conditions for the separation and the column are listed in Table 1.

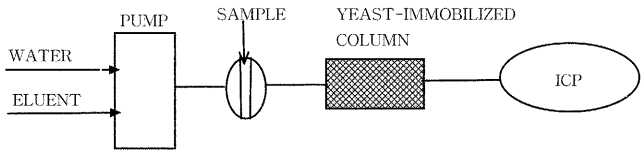

Figure 1. Schematic diagram of the experimental set-up.

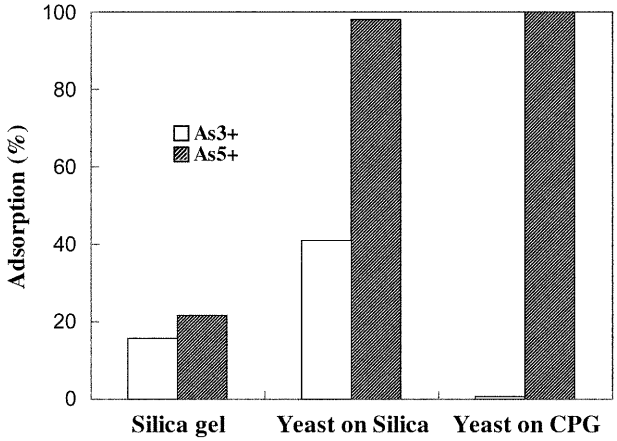

Figure 2. Adsorption efficiency depending on substrates. Yeast-immobilized on CPG shows the best efficiency.

Yeast immobilized on CPG column showed the best retainment for As^{5+} and selectivity over As^{3+}, which was satisfactory for the speciation study. The most important factor for controlling sorption of ions unto the column was pH, which was controlled using $NaHCO_3/Na_2CO_3$, strong acid, and acetic acid/acetate buffer. The sorption was the most efficient at pH 7 and slowly decreased thereafter.

To elute As^{5+}, different concentrations (0.3 M, 0.5 M, 1-6 M) of HCl, HNO_3 and H_2SO_4 were examined and the results are shown in Figure 3. Both HNO_3 and H_2SO_4 showed good

Table 1. The optimum conditions of speciation of As with the yeast-immobilized column

Yeast immobilized on	Controlled Pore Glass
sample concentration	1 μg/mL As
sample volume	0.3 mL
pH	7.0
carrier	1 M nitric acid
eluent flow rate	1.5 mL/min

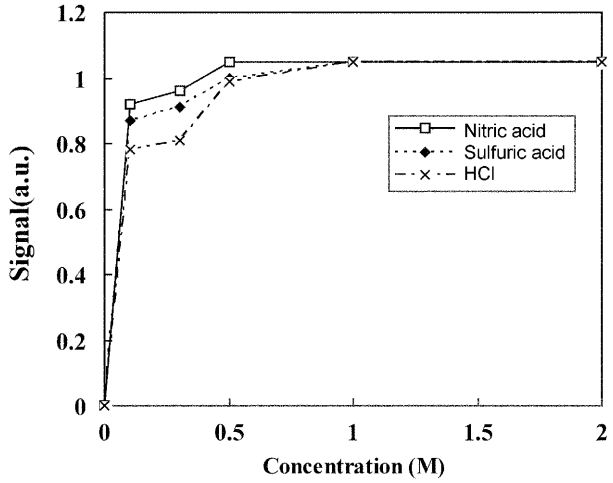

Figure 3. Relationship between the concentration and type of eluents *vs*. signal.

results but the precision was better for nitric acid. The optimum flow rate of carrier was sought. At low flow rates, the peak was spread wide especially at below 0.3 mL/min. Over 2.0 mL/min, insufficient sorption occurred because of the lack of equilibrium. The best result could be obtained at 1.5 mL/min. Even though the flow rate of eluent did not give much effect on signal, it should match for the nebulization in ICP-AES at the same time.

A test solution was prepared by mixing equal volume of As^{3+} and As^{5+} (1 μg/mL for each) and was injected into the column. As^{3+} was not adsorbed at the column while As^{5+} was retained strongly and then eluted out by a strong acid. The result is exhibited in Figure 4, which shows a good ability of As speciation with yeast immobilized column. The

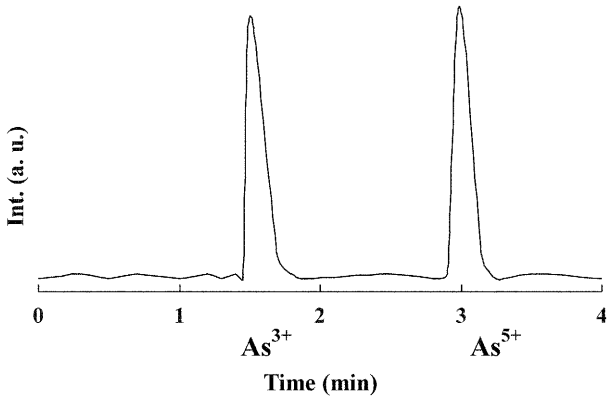

Figure 4. Separation of Arsenic ions (As^{3+}/As^{5+}) with the yeast-immobilized CPG column.

analysis could be done within a few minutes and the ratio of peak area is 1 : 1, which shows a good recovery for both species.

This work demonstrates that biological substrates could be immobilized unto the glass surface and used for the metal speciation study. The speciation of As was achieved using the yeast, which is covalently bonded unto the surface of glass bead and then packed into a column. The on-line technique provides a simple, fast, and effective separation over a batch method. To increase the sensitivity, a hydride generation cell could be connected after the column, which is under investigation in our laboratory.

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