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Selective Separation of Arsenic Species from Aqueous Solutions with Immobilized Macrocyclic Material Containing Solid Phase Extraction Columns

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Abstract

A combination of solid phase extraction (SPE) columns was used for selective separation of water-soluble arsenic species: arsenite, arsenate, monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA). The SPE columns, namely AnaLig TE-01 (TE-01), AnaLig AN-01 Si (AN-01) and AnaLig As-01 PA (As-01), contain immobilized macrocyclic material as the sorbent and commonly known as molecular recognition technology (MRT) gel. The retention, extraction and recovery behavior of the MRT gel SPE columns were studied at pH 4–10. Fortified deionized water spiked with 100 µM of arsenic species were treated at the flow rate of 0.2 mL min⁻¹. HNO₃ (1.0 and 6.0 M) was used as eluent to recover the retained arsenic species from TE-01 and AN-01 SPE columns. Arsenic species retained in the As-01 column were eluted with HNO₃ (0.1 M) followed by NaOH (2.0 M). Likely interference from the various coexisting ions (Na⁺, K⁺, Ca²⁺, Mg²⁺, Cl⁻, NO₃⁻, CH₃COO⁻, PO₄³⁻, SO₄²⁻, ClO₄⁻) (10 mM) were negligible. Quantitative separation of As(III), As(V), MMA and DMA was achieved based on the differences in extraction and recovery behavior of the MRT gel SPE columns with pH for different arsenic species. Complexation between arsenic species and MRT gel is the core phenomenon of the proposed technique as the complexation of MRT gels is expected to be stronger than the resin-based separation processes. MRT gel SPE columns are advantageous as compared with other reported SPE columns in terms of its performance with As(III). Effortless regeneration and unaltered separation performance of the sorbent materials for more than 100 loading and elution cycles are other sturdy characteristics to consider the MRT gel SPE columns for sensitive and selective arsenic species separation.

Keywords: Solid phase extraction; Molecular recognition technology gel; water-soluble arsenic; selective separation; pH
1.0 Introduction

Arsenic, a ubiquitous toxic trace element, has raised a major toxicological and environmental concerns (WHO, 2001). The concentration levels, oxidation and binding states, ionic and molecular forms and metabolic pathways of As vary strongly in different environmental compartments, food chains and ultimately in humans (Mandal and Suzuki, 2002). Widespread human exposure to high levels of As is reported to occur via drinking water and contaminated water irrigated food causing both cancerous and non-cancerous health effects (Karim, 2000; Rahman et al., 2008).

Arsenite (oxidation state + III), arsenate (oxidation state + V), monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) are common water-soluble arsenic species existing in natural water systems-a major pathway of arsenic ingestion to humans (Smedley and Kinniburgh, 2002). Arsenic toxicity in human depends strongly on its chemical form. As(III) is 10 times more toxic than As(V) while almost 70 times more toxic than the methylated forms, MMA and DMA (Squibb and Fowler, 1983). As(III), having successive acid dissociation constants (pK_a) of 9.2, 12.2 and 13.4, is not dissociated at neutral pH and is present as a neutral species. As(V) and MMA has a wide range of pK_a values [As(V): 2.2, 6.9, 11.5; MMA: 4.1, 8.7], and exist mainly as anionic species at almost all pH. DMA with a pK_a value of 6.2 subsists as a cation in acidic medium (Committee on Medical and Biologic Effects of Environmental Pollutants, 1977). The United States Environmental Protection Agency proposed a maximum contaminant level of 10 µg L^{-1} arsenic for the community water systems (USEPA, 2002). Because of increasingly stringent environmental regulations, selective and accurate measurement of arsenic species is required for precise monitoring and understanding the extent of arsenic contamination.

In natural waters, As usually exists at trace levels and several techniques are proposed for selective quantification and speciation analysis of arsenic species at trace levels (Barra et al.,
Ion chromatography and high performance liquid chromatography separation followed by sensitive detection such as inductively coupled plasma mass spectrometry (Lintschinger et al., 1998; Bissen and Frimmel, 2000), atomic absorption spectrometry (AAS) with hydride generation interface (Hasegawa et al., 1999; Kumar and Riyazuddin, 2007) and electrospray/nanospray mass spectrometry (Pergantis et al., 1997; Ritsema et al., 1998) are some potential techniques. However, concerns related to the use of element-selective detectors to interface the chromatographic methods limit the efficiency of these techniques (Yu et al., 2003).

Separation and preconcentration of contaminant ions using solid sorbent materials, known as solid phase extraction (SPE) systems, have increased in popularity since the 1980s (Hosten and Welz, 1999). The technique has been developed as a cost- and time-saving alternative to the traditional extraction techniques featuring the capability to interact with a variety of metal ions including the fairly specific selectivity to a particular ion (Nickson et al., 1995; Ghaedi et al., 2008). Ion exchange resins (Leal et al., 2004; Jitmanee et al., 2005), silica gel bonded with octadecyl functional groups (Pozebon et al., 1998), yeast immobilized on controlled pore glass (Koh et al., 2005), activated alumina (Karthikeyan et al., 1999), open tubes knotted reactors (Yan et al., 2002; Herbello-Hermelo et al., 2005), polytetrafluoroethylene turnings-packed micro-columns (Anthemidis et al., 2010) have been employed as SPE sorbent material. One group of SPE materials includes the macrocyclic chelants, such as crown ethers, immobilized on a silica or polymer support (Hosten and Welz, 1999). Ion-selective behavior of SPE-type systems with immobilized macrocyclic materials has been mentioned for preconcentration and separation of metals (Bradshaw et al., 1988; Izatt et al., 1994; Hasegawa et al., 2010). SPE techniques have been applied for the quantitative analysis/speciation/separation of various trace elements including arsenic (Yalcin and Le,
2001; Yu et al., 2003; Liang et al., 2004; Long et al., 2006; Sanchez et al., 2009). Reports on
the retention behavior of different arsenic species with some SPE systems (silica-based or
resin-based) at pH 5.5 (Yalcin and Le, 2001) and pH 5.6 (Yu et al., 2003) were available. It
was observed that the hydrophobic interaction of the arsenic species with the SPE materials,
\( pK_a \) values and ionic characters are important factors which may control the retention
efficiency of the SPE columns (Yu et al., 2003). Though quantitative retention was achieved
with the SPE columns for the water-soluble arsenic species (As(III), As(V), MMA and
DMA), elution of the retained species was quiet difficult or sometimes unachievable for some
species particularly with As(III) (Yalcin and Le, 2001; Yu et al., 2003).

The objective of the work is to investigate the feasibility of an ion-selective immobilized
macroyclic material attached to a solid phase, commonly known as a molecular recognition
technology (MRT) gel, for the selective separation of As(III), As(V), MMA and DMA from
aqueous solutions followed by graphite furnace AAS determination. We used following MRT
gel SPE columns: AnaLig TE-01, AnaLig AN-01 Si and AnaLig As-01 PA. Specific MRT
gel SPE columns have the advantage of the selective retention of the mentioned arsenic
species followed by quantitative recovery. Most importantly, As(III) was quantitatively
retained and recovered with the AnaLig As-01 PA SPE column.

2.0 Experimental

2.1 Instruments

A PerkinElmer model AAnalyst 600 AAS (PerkinElmer, Massachusetts, USA) including
the AS-800 autosampler equipped with a transverse-heated graphite atomizer with integrated,
pyrolytic graphite coated platform (THGA) and longitudinal Zeeman-effect background
corrector was used. End-capped THGA tubes were used for better sensitivity and improved
precision. An electrodeless discharge lamp (EDL) powered by EDL System II operated at
380 mA was employed as light source. The wavelength was set at the 193.7 nm resonance line and the monochromator spectral bandpass at 0.7 nm. Baseline offset correction time was set to 2.0 s and the read delay at 0.0 s. Argon was used as purge gas and the flow rate was set to 250 mL min\(^{-1}\). A temperature program was performed and the different steps were: first and second dry at 110 and 130 °C, ashing at 1200 °C and atomization at 2000 °C held at 30, 30, 20 and 5 s respectively. After a calibration with 5 standards (0.5–2.5 μM), 20 μL of sample and 10 μL of Pd–Mg matrix modifier were introduced in the graphite furnace with three replicates of each measurement. The pH of the sample solutions was measured with a Navi F-52 pH meter (Horiba Instruments, Japan) and a combination electrode.

2.2 Reagents and materials

Analytical grade commercial products were used. Stock solutions (10 mM) of As(III), As(V), MMA and DMA were prepared from sodium arsenite (NaAsO\(_2\)) (Kanto Chemical, Japan), sodium arsenate heptahydrate (Na\(_2\)HAsO\(_4\)·7H\(_2\)O), monomethylarsonic acid (CH\(_3\)AsO(OH)\(_2\)), dimethylarsinic acid sodium salt trihydrate (C\(_2\)H\(_6\)AsNaO\(_2\)·3H\(_2\)O) (Nacali Tesque, Japan). Working standards of metal solutions in the range of μM to mM were prepared by dilution on a weight basis. Deionized water prepared with a Barnstead E-Pure systems was used to prepare all solutions and is referred to as EPW hereafter.

The experimental pH range was 4–10, and adjusted using either 1 M HCl or 1 M NaOH. MES (2-(N-morpholino) ethanesulfonic acid monohydrate, C\(_8\)H\(_{13}\)NO\(_4\)S·H\(_2\)O) (Sigma–Aldrich, USA), HEPES (N-2-Hydroxyethylpiperazine-N’-2-ethanesulfonic acid, C\(_8\)H\(_{18}\)N\(_2\)O\(_4\)S) (Nacali Tesque, Japan), and TAPS (N-Tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid, C\(_7\)H\(_{17}\)NO\(_6\)S) (MP Biomedicals, USA) were used as buffer reagents for pH 4–6, 7–8 and 9–10, respectively.

NaCl, KCl, CaCl\(_2\), MgCl\(_2\) were used as a source of cations while the Na-salt of anions (Cl\(^-\), NO\(_3\)\(^-\), CH\(_3\)COO\(^-\), PO\(_4^{3-}\), SO\(_4^{2-}\), ClO\(_4^{-}\)) (Nacali Tesque, Japan) were used to study the
effect of coexisting ions. Working solutions of 10 mM concentration were prepared in H$_2$O matrix and pH was maintained to 7.0. The final solutions were allowed to equilibrate for 24 h before use. The interference study were carried out in a non-competitive environment by applying 4 mL of fortified deionized water at the optimized flow rate with subsequent collection using appropriate eluent.

Experimental variables, e.g. sample loading flow rate, selection of eluent and eluent concentration were optimized using As(V) spiked solutions (100 µM) in H$_2$O matrix with pH maintained at 5.0. The MRT gel SPE columns were fed with 4 mL of sample solutions at varying flow rates, and the retention percentage of the As-species into the columns was determined. Different eluent (individual or combinations), 0.1–6.0 M HNO$_3$ and 0.1–4.0 M NaOH, was checked to select the most appropriate eluent or eluent combinations that were suitable for optimum recovery of the ‘captured’ species.

Certified reference materials (CRMs): BCR-713 (effluent wastewater) and BCR-610 (groundwater) from EC-JRC-IRMM (European Commission Joint Research Centre, Institute of Reference Materials and Measurements), fortified samples of ‘real’ waters: tap water sample from our laboratory in Kakuma campus, Kanazawa University (Kanazawa, Japan) and river water sample from Asano River (Kanazawa, Japan) were analyzed to validate the proposed separation process. Each of the real water samples was filtered through the cellulose membrane filter of 0.45 µm pore size (Advantec, Japan) before the analysis.

Low-density polyethylene bottles (Nalge, USA), perfluoroalkoxy (PFA) tubes and micropipette tips (Nichiryo, Japan) were used throughout. The laboratory wares were cleaned following the sequence: (a) soaking in an alkaline detergent (Scat 20X-PF, Nacali Tesque, Japan) overnight, (b) rinsed with EPW, (c) soaking in 4 M HCl overnight, and (d) rinsed with EPW. PFA tubes and micropipette tips were cleaned according to the procedure described by Sohrin et al. (1998).
2.3 Separation procedure

2.3.1 Column cleaning and conditioning

MRT gel SPE columns: AnaLig TE-01 (TE-01), AnaLig AN-01 Si (AN-01), AnaLig As-01 PA (As-01) were purchased from GL Sciences, Japan. The SPE sorbents are proprietary polymeric organic materials comprised of ion-selective sequestering property. The sorption ability of the SPE materials is based on the molecular recognition and macrocyclic chemistry. SPE materials packed in 3 mL columns were used in the experiments. Column cleaning was conducted with 8 mL of 1.0 M HNO₃ and 6 mL of EPW. Appropriate buffer solution (5 mL) was allowed to follow through the column to ensure the desired pH condition (4–10).

2.3.2 Retention, extraction and recovery of arsenic species with MRT gel columns

The work-flow sequence for the separation of As(III), As(V), MMA and DMA using MRT gel SPE columns followed by GF-AAS determination is summarized in Table 1. Sample solution (4 mL) was passed through the SPE column at the optimized pre-set flow rate of 0.2 mL min⁻¹. The pH of the sample solution was pre-adjusted with 0.1 M buffer solution (MES, HEPES or TAPS, whichever appropriate). The column effluent was collected. The MRT gel SPE columns were then washed with EPW to remove the analyte that is not captured by the immobilized macrocyclic material in SPE columns. The total analyte concentration in the column effluent and EPW wash solution represent the unretained concentration of analyte in the SPE system. The final step is the elution of analyte from the SPE systems. HNO₃ (1.0 and 6.0 M) was used to elute the arsenic species retained in TE-01 and AN-01 SPE columns, and analytes retained in As-01 column were eluted with 0.1 M HNO₃ followed by 2.0 M NaOH. The arsenic concentrations in the sample, effluents and eluent solutions were measured with GF-AAS. Three replicate measurements per sample were made in all instances. The peak height of the reported signal was proportional to the concentration of the respective arsenic species and was used for all measurements.
The terms, retention, extraction and recovery, were used to explain the separation performance of the SPE systems. The retention ratio was calculated comparing the analyte concentration in the sample solution loaded in SPE columns with that in the solution passed through the columns providing only the information about the concentration of analyte sorbed in the SPE systems. On the other hand, the analyte concentrations in the column effluent, EPW wash solution and eluent were measured to understand the extraction and recovery behavior of the SPE columns. The extraction ratio of each column for the individual species was calculated by comparing the numbers of mol of analyte in the eluent with the cumulative number of mol of analyte present in the total effluents. Numbers of mol of analyte recovered in all fractions were compared with the numbers of mol of analyte in the solution loaded to the column to calculate the recovery ratio.

3.0 Results and discussion

3.1 Optimization of variables

3.1.1 Sample loading flow rate

The flow rate of the metal-rich sample solution has a reasonable impact on the metal retention rate in SPE columns (Bag et al., 1998). Effect of sample loading flow rates adjusted in the range of 0.2–4.0 mL min\(^{-1}\) (Table 2) was checked at the optimum conditions. Quantitative retention up to the flow rates of 0.25 mL min\(^{-1}\) was observed. The retention capacities decrease gradually with the increase of flow rates above 0.25 mL min\(^{-1}\). Such behavior indicates the constant retaining capability of the MRT gel at the initial loading period. Therefore, a flow rate of 0.2 mL min\(^{-1}\) was applied to ensure maximum retention of the analyte from MRT Gel SPE columns.
3.1.2 Selection of eluent and eluent concentration

The eluent should be able to extract the analyte without affecting the quantitative determination of analytes (Chen et al., 2009). Analytes retained in the TE-01 and AN-01 SPE columns were eluted with HNO₃ (4 mL) of varying concentrations (0.1–6.0 M). The recovery patterns were similar and the recovery rates became constant for the eluent concentration above 0.5 M (Figs. 1a and 1b). However, greater than or equal to 5.0 M acids were recommended for the elution of bound ions in TE-01 and AN-01 SPE columns (IBC Advanced Technologies, 2007, 2009). Hence, a combination of 1.0 M HNO₃ (2 mL) and 6.0 M HNO₃ (1 mL) was selected as eluent for the subsequent experiments to ensure the complete elution of the analyte when treated with TE-01 or AN-01. On the other hand, only 0.1–4.0 M NaOH (2 mL) or 0.1–6.0 M HNO₃ (2 mL) was found unsuitable for the elution of analytes from As-01. Combinations of 0.1–4.0 M NaOH (1 mL) followed by 2.0 M HNO₃ (1 mL) and vice-versa were used to check the elution of arsenic species from the As-01 column (Figs. 1c and 1d). The recovery was achieved at quantitative maximum for the following eluent combination: 0.1 M HNO₃ (1 mL) + 2.0 M NaOH (1 mL), and was applied for the next experiments with As-01 MRT gel column.

3.2 Retention behavior of the MRT gel SPE columns

The retention efficiency of the MRT gel SPE columns for different arsenic species at varying pH is illustrated in Fig. 2. The retention (%) of As(III) was negligible with TE-01 and AN-01 SPE columns. Average retention efficiency (%) of 92±3.7 was observed with As-01 column at the pH 4 to 10 while it was highest at pH 7 (96±1.2). As(III) mainly exists as a neutral species, As(OH)₃, at the entire range of the studied pH. Thus, the macrocyclic materials immobilized in the TE-01 and AN-01 columns were not capable of retaining the neutral form of As(III). Almost complete retention of As(V) and MMA was achieved at pH 4 to 7 with all the MRT gel SPE columns. As(V) and MMA remain in the anionic form within
that pH range, as evident from the corresponding $pK_a$ values. Therefore, all of the MRT gel columns investigated can retain the anionic form of As(V) and MMA. DMA, which exists as a cation in the acidic medium, was retained at an average efficiency (%) of 94±3.3 with As-01 column between pH 4 and 6 while the retention was not that notable with TE-01 and AN-01 columns.

Data evaluation showed that the most significant finding of our work was with As(III). Yu et al. (2003) checked 11 SPE systems at pH 5.6 and found that none of them were capable of retaining As(III) quantitatively. Yalcin and Le (2001) worked with 7 SPE systems and reported that Alumina-A, -B and -N (normal phase in acidic, basic, and neutral activity; from Millipore-Waters, Missisauga, ON, Canada) and silica-based LC-SCX (sulfonic acid-bonded; from Supelco, Bellefonte, PA, USA) columns can retain As(III) at the pH of 5.5. None of those SPE systems were recommended for As(III) separation considering the difficulty in elution. In our study, at pH 7, As(III) was completely retained at As-01 SPE column followed by quantitative recovery.

3.3 Extraction and recovery behavior of the MRT gel SPE columns

The extraction behavior of the MRT gel SPE columns with four arsenic species is illustrated in Fig. 3. A similar extraction pattern was observed with TE-01 and AN-01 SPE columns; As(III) was not captured, As(V) was captured at an average rate (%) of 99±0.5 until pH 8, MMA extracted at an average percent rate of 99±0.60 at pH 6 and 7, and the highest extraction (%) of 71±4.6 was observed at pH 7 for DMA. With As-01 SPE columns, the average extraction (%) was 96±3.2 at pH 4–6 for As(V), MMA and DMA, while it was 99±1.1 at pH 7–9 for As(III).

Recovery (%) of the arsenic species with the MRT gel SPE columns is shown in Fig. 4. TE-01 SPE columns showed quantitative recovery performance at the entire studied pH range for all the arsenic species. AN-01 SPE columns showed similar behavior with As(III), As(V)
and DMA while fluctuating recovery was achieved for MMA at different pH. A gradual increase in the recovery (%) was observed from pH 4 to pH 10 with As-01 SPE columns, and expected maximum recoveries were achieved for all the arsenic species at pH 7.

The extraction and recovery behavior of the MRT gel SPE columns leads us to the following assumptions: (i) TE-01 and AN-01 columns are not effective for As(III) separation but can be used to separate other target species (As(V), MMA and DMA) quantitatively at varying pH conditions; (ii) selective separation and complete elution of As(III) is possible with the As-01 column; (iii) the As-01 column can also be used to preconcentrate the targeted water-soluble arsenic species for the determination of total arsenic content in the samples, if selective separation is not desired; and (iv) column regeneration process is simple because the retained analytes are completely eluted.

3.4 Interference studies
Cations of alkaline and alkaline earth metals are always found in water samples and have the capability to compete with the target metal ions during the binding with the SPE material, and common anions have the ability to bind with the target metal ions. In their presence, the efficiency of the SPE material to bind the target ions may be reduced resulting in a reduction of the recovery. The effects of matrix ions in water samples on the recovery of the spiked sample solutions of 100 µM As(III), As(V), MMA and DMA were investigated. The recovery of different arsenic species in the presence of 10 mM of different ions (Na⁺, K⁺, Ca²⁺, Mg²⁺, Cl⁻, NO₃⁻, CH₃COO⁻, PO₄³⁻, SO₄²⁻, ClO₄⁻) in the water samples were observed in the range of 95±2.7–100±3.2%. Therefore, there is limited possibility of the interference from the matrix ions commonly found in sample waters, which is may be due to the selective separation capability of the MRT gel SPE materials.
3.5 Retention capacity and regeneration of the SPE columns

Retention capacity of the MRT gel SPE columns is important for determining the stability of the MRT gel SPE columns during the separation process. Analyte concentration and breakthrough volume (the volume of sample that causes the target analyte to be eluted from the SPE columns) were used to find out the retention capacity (Yu et al., 2003). After arsenic-spiked sample solutions were passed through the SPE columns, the retention capacity was expressed in terms of mmol of analyte captured in one gram of SPE material. The retention capacities of the MRT gel SPE columns at pH 7 were calculated as follows: 0.40±0.02 mmol g\(^{-1}\) TE-01, 0.39±0.02 mmol g\(^{-1}\) AN-01 and 0.31±0.01 mmol g\(^{-1}\) As-01 (sample solution– 10 mM of As(V), matrix– H\(_2\)O, flow rate– 0.2 mL min\(^{-1}\), elution– 2 mL of HNO\(_3\) + 1 mL of 6 M HNO\(_3\) + 1 mL of EPW, for TE-01 and AN-01 SPE columns and 1 mL of 0.1 M HNO\(_3\) + 1 mL of 2.0 M NaOH + 2 mL of EPW). The result was in good agreement with the certified values for the MRT gel SPE columns (IBC Advanced Technologies, 2006, 2007, 2009). The regeneration ability of the MRT gel SPE columns was investigated, and it was observed that more than 100 loading and elution cycles can be performed without the loss of analytical performance. SPE systems with macrocycles attached onto solid supports allow selective separation of analytes from matrix facilitating the repeated use of the macrocycles (Bradshaw et al., 1988; Horwitz et al., 1992; Izatt, 1997).

3.6 Scheme for selective separation of arsenic species

The differences in extraction and recovery pattern of MRT gel SPE columns for different arsenic species enabled us to propose a selective separation method. The method is based on the selective retention of the arsenic species followed by quantitative selective recovery at the elution step. Retention, extraction and recovery behavior of three MRT gel SPE columns: TE-01, AN-01 and As-01 were studied and combined to design a multi-step separation technique for quantitative measurement of As(III), As(V), MMA and DMA. Another MRT
gel SPE column, AnaLig AN-02, was also checked. The retention, extraction and recovery behaviors of the AN-02 column were somewhat similar with those of AN-01 column. Therefore, AN-02 column can be considered as an alternative of AN-01 column in the separation process. The scheme for selective separation with subsequent quantitative measurement of the arsenic species by GF-AAS technique is shown in Fig. 5.

At pH 5, As(V) and MMA were quantitatively retained in the TE-01 SPE column while As(III) and DMA remained in the column effluent. The captured species was eluted with HNO₃. The eluted solution was separated into two equal portions, and pH was adjusted to 5 and 8 respectively. When each of the pH-adjusted portions independently treated with AN-01 SPE columns, As(V) and MMA were quantitatively extracted and recovered from the eluted solution, respectively, at pH 5 and pH 8. The column effluent containing As(III) and DMA were adjusted to pH 9, and treated with As-01 SPE column. DMA remained in the solution that passed through the SPE material while As(III) was selectively captured. Captured As(III) was eluted with the eluent combination of 0.1 M HNO₃ followed by 2.0 M NaOH. GF-AAS were used to determine the concentration of the individual arsenic species.

3.7 Analytical characteristics

The concentrations of As(III), As(V), MMA and DMA in the treated solutions from MRT gel SPE columns were measured using GF-AAS. At optimum conditions, the linear range was found to be 0.01–0.32 µg mL⁻¹ As(III), 0.01–0.78 µg mL⁻¹ As(V), 0.01–0.35 µg mL⁻¹ MMA and 0.01–0.54 µg mL⁻¹ DMA. The method detection limits were calculated by three times the standard deviation (n = 15) of the blank. The values were 0.06 µg L⁻¹ for As(III) and As(V), and 0.05 µg L⁻¹ for MMA and DMA. The precision of the method was also studied. The repeatability, as relative standard deviation, was 0.65, 2.93, 2.25 and 1.20%, calculated from 10 replicate measurements at the 1.0 µM of As(III), As(V), MMA and DMA respectively.
3.8 Accuracy and applications

The accuracy of the proposed separation scheme was evaluated by analyzing two EC-JRC-IRMM CRMs, namely BCR-713 (effluent wastewater) and BCR-610 (groundwater) (Table 3). None of the arsenic species measured in this work has either certified or literature values. Our values for the total of all arsenic species determined for both BCR-713 and BCR-610 were in good agreement with the certified value, the calculated recoveries, 97% for BCR-713 and 94% for BCR-610, were satisfactory. The proposed separation scheme was also applied to the analysis of local natural water samples (tap water and river water) and was validated by spiking the samples with known amounts of As(III), As(V), MMA and DMA (Table 4). The recoveries from spiked solutions were varied in the range 98±1.6–102±1.7%.

4.0 Conclusions

The application of three MRT gel SPE columns (TE-01, AN-01 and As-01) for selective separation of four different arsenic species (As(III), As(V), MMA and DMA) was demonstrated. Retention behaviors of the arsenic species were varied with the change of pH at the range of 4 to 10. TE-01 and AN-01 SPE columns were unable to retain As(III) while As-01 showed the ability to retain all the species at a certain pH quantitatively. Either HNO₃ or a combination of HNO₃ and NaOH were used as eluent to recover the ‘captured’ species from the MRT gel structure. However, the recovery ratio was also found to depend on the pH. pH-dependent retention and recovery behavior of the MRT gel SPE columns were used to design a selective separation scheme for quantitative determination of a particular arsenic species in the sample solution. It is possible to overcome the tedious preconcentration process by following the proposed selective separation technique. To the best of our knowledge, it is the first ever report dealing with SPE columns equipped with immobilized macrocyclic material as sorbent material for selective determination of arsenic in water. In addition, quantitative retention followed by recovery of As(III) was achieved with As-01 column
which was previously not achieved with any other reported SPE systems. Easy operation, virtually unlimited loading and elution capability of the sorbent material without losing the analytical performance and high-sensitive separation ability can make the proposed technique as a useful one for selective separation of arsenic species from natural waters.

Acknowledgement

This research was partly supported by Grants-in-Aid for Scientific Research (K22042) from Ministry of the Environment, Japan.
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Table 1. Separation process of As(III), As(V), MMA and DMA using MRT gel SPE columns

<table>
<thead>
<tr>
<th>Step</th>
<th>Function</th>
<th>Solution</th>
<th>Volume (mL)</th>
<th>Flow rate (mL min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rinsing 1</td>
<td>0.1 M HNO₃</td>
<td>8</td>
<td>0.5</td>
</tr>
<tr>
<td>2</td>
<td>Rinsing 2</td>
<td>EPW</td>
<td>6</td>
<td>0.5</td>
</tr>
<tr>
<td>3</td>
<td>Conditioning</td>
<td>200 mM NaNO₃ + 0.1 M buffer solution*</td>
<td>32–40</td>
<td>0.2–0.5</td>
</tr>
<tr>
<td>4</td>
<td>Collection</td>
<td>100 µM As-species spiked sample solution</td>
<td>4</td>
<td>0.2</td>
</tr>
<tr>
<td>5</td>
<td>Washing</td>
<td>EPW</td>
<td>4</td>
<td>0.2</td>
</tr>
<tr>
<td>6</td>
<td>Elution 1</td>
<td>For TE-01 or AN-01 SPE columns</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 M HNO₃</td>
<td>2</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>For As-01 SPE column</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.1 M HNO₃</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>7</td>
<td>Elution 2</td>
<td>For TE-01 or AN-01 SPE columns</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>6 M HNO₃</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>For As-01 SPE column</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.0 M NaOH</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>8</td>
<td>Elution 3</td>
<td>For TE-01 or AN-01 SPE columns</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>EPW</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>For As-01 SPE column</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>EPW</td>
<td>2</td>
<td>0.2</td>
</tr>
</tbody>
</table>

*MES Buffer (pH 4–6), HEPES Buffer (pH 7–8), TAPS Buffer (pH 9–10)
Table 2. Effect of the sample loading flow-rates on the retention capacities (%) of the MRT gel SPE columns

<table>
<thead>
<tr>
<th>Flow rate (mL min⁻¹)</th>
<th>TE-01</th>
<th>AN-01</th>
<th>As-01</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.20</td>
<td>101±3.8</td>
<td>100±3.7</td>
<td>101±4.6</td>
</tr>
<tr>
<td>0.25</td>
<td>99±3.0</td>
<td>100±3.4</td>
<td>100±4.3</td>
</tr>
<tr>
<td>0.30</td>
<td>88±2.8</td>
<td>82±2.6</td>
<td>92±3.8</td>
</tr>
<tr>
<td>0.50</td>
<td>75±2.4</td>
<td>71±2.7</td>
<td>87±2.9</td>
</tr>
<tr>
<td>1.00</td>
<td>74±1.8</td>
<td>68±3.2</td>
<td>82±2.7</td>
</tr>
<tr>
<td>2.00</td>
<td>65±2.6</td>
<td>62±1.6</td>
<td>71±3.4</td>
</tr>
<tr>
<td>4.00</td>
<td>62±3.2</td>
<td>59±1.8</td>
<td>68±2.2</td>
</tr>
</tbody>
</table>
Table 3. Analysis of EC-JRC-IRMM CRMs for arsenic species

<table>
<thead>
<tr>
<th>Arsenic species</th>
<th>Effluent Wastewater CRM BCR-713 (µg L(^{-1}))</th>
<th>Groundwater CRM BCR-610 (µg L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>This work</td>
<td>Certified value</td>
</tr>
<tr>
<td>As(III)</td>
<td>1.9±0.3</td>
<td>NR</td>
</tr>
<tr>
<td>As(V)</td>
<td>7.1±1.2</td>
<td>NR</td>
</tr>
<tr>
<td>MMA</td>
<td>BDL</td>
<td>NR</td>
</tr>
<tr>
<td>DMA</td>
<td>0.4 ±0.1</td>
<td>NR</td>
</tr>
<tr>
<td>(\sum) (As-species)</td>
<td>9.4±1.4</td>
<td>9.7±1.1</td>
</tr>
</tbody>
</table>

*‘BDL’ – Below Detectable Limit; ‘NR’ – Not reported*
Table 4. Determination of arsenic species in the fortified samples of ‘real’ waters

<table>
<thead>
<tr>
<th>Arsenic species</th>
<th>Tap water</th>
<th></th>
<th></th>
<th>River water</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Added (µg L⁻¹)</td>
<td>Found (µg L⁻¹)</td>
<td>Recovery (%)</td>
<td></td>
<td>Added (µg L⁻¹)</td>
<td>Found (µg L⁻¹)</td>
</tr>
<tr>
<td>As(III)</td>
<td>0</td>
<td>BDL</td>
<td>–</td>
<td>0</td>
<td>0.7±0.12</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>19.5</td>
<td>19.3±0.10</td>
<td>99±0.5</td>
<td>20</td>
<td>19.4±0.41</td>
<td>99±2.1</td>
</tr>
<tr>
<td>As(V)</td>
<td>0</td>
<td>BDL</td>
<td>–</td>
<td>0</td>
<td>1.3±0.15</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>31.2</td>
<td>31.3±0.48</td>
<td>100±1.5</td>
<td>31.2</td>
<td>30.5±0.51</td>
<td>98±1.6</td>
</tr>
<tr>
<td>MMA</td>
<td>0</td>
<td>BDL</td>
<td>–</td>
<td>0</td>
<td>BDL</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>21.0</td>
<td>21.3±0.34</td>
<td>102±1.7</td>
<td>21.0</td>
<td>20.7±0.43</td>
<td>99±2.0</td>
</tr>
<tr>
<td>DMA</td>
<td>0</td>
<td>BDL</td>
<td>–</td>
<td>0</td>
<td>0.1±0.01</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>32.1</td>
<td>32.3±0.27</td>
<td>101±0.8</td>
<td>32.1</td>
<td>31.9±0.60</td>
<td>99±1.9</td>
</tr>
</tbody>
</table>

*‘BDL’ – Below Detectable Limit*
Figure 1: Selection of eluent and eluent concentration: (a) AnaLig TE-01 (HNO$_3$– 0.1/0.2/0.3/0.5/1.0/6.0 M) (b) AnaLig AN-01 Si (HNO$_3$– 0.1/0.2/0.3/0.5/1.0/6.0 M) (c) AnaLig As-01 PA (HNO$_3$– 0.1/1.0/2.0/4.0/6.0 M + NaOH– 2.0 M) (d) AnaLig As-01 PA (NaOH– 0.1/1.0/2.0/3.0/4.0 M + HNO$_3$– 2.0 M). Sample solution– As(V) (100 µM), matrix– H$_2$O, pH– 5, sample volume– 4 mL, flow rate– 0.2 mL min$^{-1}$ ($n$ =3).
Figure 2: Retention behavior of the MRT gel SPE columns. Sample solution– As(III), As(V), MMA and DMA (100 µM), matrix– H2O, pH– 4 to 10, sample volume– 4 mL, flow rate– 0.2 mL min⁻¹, elution– 1.0 M HNO₃ (2 mL) + 6.0 M HNO₃ (1 mL) + EPW (1 mL), for TE-01 and AN-01 SPE columns and 0.1 M HNO₃ (1 mL) + 2.0 M NaOH (1 mL) + EPW (2 mL), for As-01 SPE column (n = 3).
Figure 3: Extraction behavior of the MRT gel SPE columns: (a) AnaLig TE-01, (b) AnaLig AN-01 Si and (c) AnaLig As-01 PA. Sample solution—As(III), As(V), MMA and DMA (100 µM), matrix—H₂O, pH—4 to 10, sample volume—4 mL, flow rate—0.2 mL min⁻¹, elution—1.0 M HNO₃ (2 mL) + 6.0 M HNO₃ (1 mL) + EPW (1 mL), for TE-01 and AN-01 SPE columns and 0.1 M HNO₃ (1 mL) + 2.0 M NaOH (1 mL) + EPW (2 mL), for As-01 SPE column (n =3).
Figure 4: Recovery behavior of the MRT gel SPE columns: (a) AnaLig TE-01, (b) AnaLig AN-01 Si and (c) AnaLig As-01 PA. Sample solution—As(III), As(V), MMA and DMA (100 µM), matrix—H₂O, pH—4 to 10, sample volume—4 mL, flow rate—0.2 mL min⁻¹, elution—1.0 M HNO₃ (2 mL) + 6.0 M HNO₃ (1 mL) + EPW (1 mL), for TE-01 and AN-01 SPE columns and 0.1 M HNO₃ (1 mL) + 2.0 M NaOH (1 mL) + EPW (2 mL), for As-01 SPE column (n = 3).
Sample solution
As(III), As(V), MMA and DMA

pH 5
MES Buffer (0.1 M)

TE-01

‘Captured’
As(V) and MMA

Column effluent
As(III) and DMA

pH 9
TAPS Buffer (0.1 M)

As-01

‘Captured’
As(III)

Column effluent
DMA

Elution

pH 5
MES Buffer (0.1 M)

AN-01

As(III)

Elution

pH 8
HEPES Buffer (0.1 M)

AN-01

As(V)

Elution

MMA

DMA

Figure 5: Scheme for selective separation of the arsenic species by MRT gel SPE columns