JAAS FULL PAPER
www.rsc.org/jaas

Complexation effect of antimony compounds with citric acid and its application to the speciation of antimony(III) and antimony(V) using HPLC-ICP-MS

Jian Zheng, Akihiro Iijima and Naoki Furuta*

Environmental Chemistry Laboratory, Department of Applied Chemistry, Chuo University, 1-13-27 Kasuga, Bunkyo-ku, Tokyo 112-8551, Japan. E-mail: nfuruta@chem.chuo-u.ac.jp

Received 1st March 2001, Accepted 12th June 2001
First published as an Advance Article on the web 19th July 2001

In this work, a complexation effect of Sb compounds with citric acid was observed using electrospray mass spectrometry (ES-MS). It was found that both Sb(III) and Sb(V) could form complexes readily with citric acid in an aqueous solution at room temperature. These complexes were found to be very stable in various matrices (moat water and aqueous extracts of airborne particulate matter), therefore, a novel HPLC-ICP-MS analytical method for the speciation of Sb(III) and Sb(V) in environmental samples was developed by using the observed complexation effect. Sb(III)- and Sb(V)-citrate complexes were separated on a PRP-X100 anion-exchange column with 10 mmol l^{-1} EDTA–1 mmol l^{-1} phthalic acid (pH 4.5) as a mobile phase. All complexes were retained on the separation column, and none of them eluted in the solvent front. Low detection limits of 0.05 μg l^{-1} and 0.07 μg l^{-1} were achieved for Sb(III) and Sb(V), respectively. The calibration curves were linear over the range of 1.0–250 μg l^{-1} for the investigated Sb species. The precisions, evaluated by using the relative standard deviation (%RSD) with a 2 μg l^{-1} standard solution, were 1.8% and 3.3% for Sb(III) and Sb(V), respectively. Several advantages of the developed method, such as improving chromatographic separation, stabilizing Sb compounds in a water sample, and preventing Sb(III) from oxidizing to Sb(V) during the ultrasonic-assisted and microwave-assisted extraction of an airborne particulate matter (APM) sample using 26 mmol l^{-1} citric acid as an extraction solvent, and alleviating the adsorption of Sb compounds on the sample surface, were observed. The developed method enabled us to detect the most toxic Sb specie, Sb(III), in an APM sample for the first time.

Introduction

For elemental speciation analysis in environmental studies, the determination of redox species such as As(III)/As(V), Se(IV)/Se(VI), Cr(III)/Cr(VI), Fe(II)/Fe(III), Sb(III)/Sb(V), and V(IV)/V(V), is very important. This is due to the fact that the toxicity and the environmental cycling behavior of elements are strongly dependent upon their oxidation states. In terms of Sb compounds, trivalent antimony compounds are generally more toxic than pentavalent forms,[1,2] and the metabolism of antimony also depends on its oxidation state.

Many analytical methods have been developed in past years for the speciation of Sb(III) and Sb(V). Among them, a hydride generation (HG) technique was widely used for the selective determination of Sb(III) and Sb(V), *e.g.*, Sb(V) was not measured directly, but calculated from the difference between the total concentration and the concentration of Sb(III).[3–5] Since some unknown hydride-forming Sb compounds exist in the environment,[6] precautions have to be taken when a HG technique is employed. A hyphenated technique, such as HPLC-ICP-MS is more preferable because the high separation power provided by HPLC and the high sensitivity offered by ICP-MS make it possible to determine Sb(III) and Sb(V) simultaneously at ultra-trace levels.

Compared with the speciation of As(III) and As(V), the separation of Sb(III) and Sb(V) was found to be more difficult. Ion chromatography was selected by most researchers for the separation of Sb(III) and Sb(V).[7–11] The use of a strong anion-exchange column (Hamilton PRP-X100) with phthalic acid as a mobile phase was widely used for the simultaneous separation of Sb(III) and Sb(V) in the past decade.[7] Under these conditions, the elution of Sb(V) was readily achieved, while long retention times and severe peak-tailing were encountered for Sb(III).[7] To solve these problems, Zhang *et al.*[8] proposed the use of a shorter 2 cm guard column with tartrate as a mobile phase, however, a broad peak for Sb(III) was still observed. Another more effective technique is the use of a complexing mobile phase. Lintschinger *et al.*[9] realised the separation of Sb(III) and Sb(V) on a Hamilton PRP-X100 column with a mobile phase containing 20 mmol l^{-1} ethylenediaminetetraacetic acid (EDTA) and 2 mmol l^{-1} potassium hydrogenphthalate (pH 4.5). Krachler and Emons[10] separated Sb(III) and Sb(V) on a Dionex AS14 column with 1.25 mmol l^{-1} EDTA adjusted to pH 4.7 as a mobile phase. Zheng *et al.*[11] successfully resolved Sb(III) and Sb(V) on a silica-based anion-exchange column (Synchropak Q300) with a mobile phase of 2 mmol l^{-1} phthalic acid and 5 mmol l^{-1} EDTA at pH 4.5. Although a good separation of Sb(III) and Sb(V) can be achieved with the use of a complexing mobile phase, one drawback of this technique was that Sb(V) normally eluted in or very close to the solvent front, which is generally not desirable for the identification and determination of species.

It is well recognized that speciation analysis consists of at least three steps: sample preparation, separation of different species and, finally, determination of the separated species. In the past, as described above, many efforts have been made either for the development of powerful separation systems to separate more species,[12] or for the improvement of the detection system to enhance the sensitivity and selectivity using various spectroscopic methods, such as AAS,[8] ICP-AES[13] and ICP-MS.[7,9–11] Little attention has been paid to the sample preparation, the most important and the weakest link in the sequence of analytical procedures for elemental speciation. For the redox speciation of Sb, the main difficulty encountered is the oxidation of Sb(III) to Sb(V). This oxidation reaction may take place during the sample storage, extraction of Sb

 DOI: 10.1039/b101943k

compounds from a solid matrix, and determination. In our previous work[11] on the speciation of Sb compounds in aqueous extracts of airborne particulate matter (APM), Sb(v) was found to be the major specie among several unknown Sb compounds. However, no Sb(III) could be detected. Analysis of real soil and natural water samples, which contain high concentrations of Sb, also showed that just one antimony specie, Sb(v), could be detected.[14] It is considered that oxidation of Sb(III) to Sb(v) might have occurred during sample preparation. Besides the potential oxidation of Sb(III) to Sb(v), Lintschinger *et al.*[14] also observed the adsorption of added Sb(III) and Sb(v) on the soil particles. In this work, we found that both Sb(III) and Sb(v) can form stable complexes readily with citric acid. Based on the observed complexation effect, we developed a novel analytical method for the speciation of Sb(III) and Sb(v) in environmental samples.

Experimental

Chemicals and reagents

All of the chemicals and reagents used in this study were of analytical grade. The water used was Milli-Q purified water (18.3 MΩ cm) that was prepared by further purification of de-ionized water by a Milli-Q system (Milli-Q SP ICP-MS, Millipore, Tokyo, Japan). Phthalic acid, HNO_3 (70%), methanol (99.9%) and EDTA were obtained from Kanto Chemical Co., Inc. (Tokyo, Japan). Citric acid was purchased from Aldrich (Milwaukee, WI, USA). A stock solution of Sb(III) was made up from a commercially available concentrate, Spex plasma standard (Metuchen, NJ, USA). A stock solution of Sb(v) was prepared from potassium hexahydroxoantimonate(v) (Kanto Chemical Co., Inc., Tokyo, Japan). Working standard solutions of Sb(III) and Sb(v) were prepared by appropriate dilution from 1000 mg l^{-1} stock solutions with Milli-Q water or citric acid. All working standard solutions of Sb(v) and Sb(III) were prepared daily to prevent any possible species change. The used mobile phases for chromatography were freshly prepared by dissolving an appropriate amount of phthalic acid and EDTA to 1 l with Milli-Q water to obtain the required concentration. The pH of the mobile phase was adjusted by the dropwise addition of 20% NH_4OH and 1 mol l^{-1} HNO_3. Rubidium with a concentration of 20 μg l^{-1} was added to the mobile phase as an internal standard. The resulting solutions were filtered through a 0.45 μm membrane filter and degassed before use.

Instrumentation

The ICP-MS instrument used was an HP 4500 (Yokogawa Analytical Systems, Tokyo, Japan). The sample introduction system used included a Scott-type spray chamber fitted with a concentric nebulizer. The chromatographic system consisted of a JASCO PU-1580I (metal-free) intelligent HPLC pump (Japan Spectroscopic Cooperation), a syringe-loading injector (Model 9725i, Rheodyne six-port injection valve) with a 100 μl loop and an inert PRP-X100 anion-exchange column (Hamilton Company, Reno, Nevada, USA, 250 × 4.6 mm id, 10 μm particle size; stable between pH 1 and 13), which was packed with a styrenedivinylbenzene copolymer with trimethylammonium exchange sites. The chromatographic system was interfaced with an ICP-MS instrument using 200 mm of PEEK (polyether ether ketone) capillary tubing (0.25 mm id) to connect the column outlet to the inlet hole of the nebulizer. The chromatographic results were processed using Chromatosoftware (Yokogawa Analytical Systems, Tokyo, Japan). Quantifications were performed in the peak area mode. The operating conditions for HPLC-ICP-MS are summarized in Table 1. The ICP-MS measurement conditions, given in Table 1, were optimized daily using a standard built-in software procedure for the injection of a 10 μg l^{-1} solution containing Li, Y, Ce and Tl. To achieve the best sensitivity, specifically, the nebulizer gas flow rate was optimized with a 2 μg l^{-1} Sb standard solution. Two antimony isotopes of ^{121}Sb and ^{123}Sb (with natural abundances of 57.21 and 42.79%) were monitored.

Table 1 Operating conditions for HPLC and ICP-MS instruments

HPLC	
Column	Hamilton PRP-X100 (250 × 4.6 mm id)
Mobile phase	10 mmol l^{-1} EDTA–1 mmol l^{-1} phthalic acid at pH 4.5
Flow rate	1.5 ml min^{-1}
Injection volume	100 μl
Column temperature	Ambient
ICP-MS	
Forward rf power	1300 W
Plasma Ar flow	15.0 l min^{-1}
Auxiliary Ar flow	1.0 l min^{-1}
Nebulizer Ar flow	1.2 l min^{-1}
Data acquisition mode	Time resolved analysis
Integration time	100 ms
Isotopes monitored	^{87}Rb, ^{121}Sb and ^{123}Sb
Total analysis time	600 s

A Micromass LCT (Altringham, UK) electrospray time-of-flight (TOF) mass spectrometer equipped with an atmospheric pressure ionization (API) source operated in a nebulizer-assisted electrospray mode was used for the investigation of complexation between Sb compounds and citric acid in this study. The nebulizer gas was N_2 gas. The electrospray potential was set to 3.2 kV in a negative ion mode and the sample cone voltage was set at 15 V. The extraction cone voltage was set at 3 V. Samples were prepared in MeOH/H_2O (v/v, 50/50) and introduced into the ion source using an infusion pump at a constant flow rate of 10 μl min^{-1} for 2 min. Mass spectral acquisition was usually performed from *m/z* 100 to *m/z* 1000. Data were acquired by operating the data system in a total ion current (TIC) acquisition mode and several scans were summed to obtain the final spectrum. Mass calibration was performed using a solution of NaI (2000 μg ml^{-1}) for the negative ion mode. More details about the experimental conditions are described elsewhere.[15]

Preparation of Sb-citrate complexes

One ml standard solutions of 1000 μg ml^{-1} Sb(v) and Sb(III) were mixed with 9 ml solutions of 29 mmol l^{-1} citric acid in a 15 ml polyethylene tube. These solutions were kept for 15 min at room temperature to complete the complexation, after which the complexes were present in the matrix with a Sb concentration of 100 μg ml^{-1}. These solutions were diluted with 26 mmol l^{-1} citric acid to obtain the required concentration.

Extraction of Sb from airborne particulate matter (APM)

The extraction of Sb from APM with water was described in our previous work.[6,11] Briefly, 0.3 g of APM was weighed into a screw-top polypropylene bottle (15 ml), and then 6 ml of Milli-Q water were added. The extraction was performed using a horizontal shaker (Iuchi WCS-150, Osaka, Japan) for four hours or one day at room temperature. The solution was then centrifuged (Iuchi Pasolina, Osaka, Japan) for 25 min at 1500 *g*. The supernatant was removed and filtered through a 0.45 μm membrane filter (Millipore, USA). Sub-samples of each extract were taken for total element analysis by ICP-MS and for speciation analysis by HPLC-ICP-MS. In this work, other extraction procedures were also employed. (1) Ultrasonic-assisted extraction: 0.1 g of APM was weighed into a screw-top polypropylene bottle (20 ml), and then 10 ml of Milli-Q water or citric acid (5 or 26 mmol l^{-1}) were added. The extraction was performed in an ultrasonic water bath, and sonicated for 30 min at room temperature. When necessary, N_2

gas was introduced into the extraction bottles continuously during the extraction process. (2) Microwave-assisted extraction: 0.1 g of APM was weighed into a PTFE digestion vessel (100 ml), and then 10 ml of Milli-Q water or citric acid (26 mmol l^{-1}) were added. The extraction was performed in an MLS 1200 MEGA microwave digestion system from Milestone (Sorisole, Italy) equipped with a rotor for six PTFE digestion vessels (max. 11 MPa) at 100 W for 20 min. The extract was filtered through a 0.45 μm membrane prior to any measurements.

Results and discussion

Complexation effect between Sb compounds and citric acid

For the speciation analysis of Sb compounds, the oxidation of Sb(III) to Sb(V) was observed by many researchers.[11,16,17] Efforts to stabilise Sb species have been made in the past. It was reported that Sb(III) solutions could be stabilised even at 40 °C for a period of 12 months in 50 mmol l^{-1} citric acid solutions.[17] Mohammad *et al.*[18] also used citric acid (12% w/v) to suppress hydride formation from Sb(V) during the determination of Sb(III). However, the chemistry of the interaction of citric acid with Sb compounds was not elucidated in either case, although complexation was assumed to be involved. Recently, Ulrich *et al.*[19] reported the complexation of Sb(III) with citric acid based on the observation of changes in chromatographic retention times; no Sb(V) complex was observed through this approach. In contrast, Guy *et al.*[13] demonstrated the complexation between Sb(V) and citric acid, while no complexation between Sb(III) and citric acid was observed.

In this work, we examined the complexation of Sb compounds with citric acid using electrospray mass spectrometry (ES-MS). Stock solutions of Sb(III) and Sb(V) were mixed with citric acid according to the procedure described in the experimental section, to give a concentration of 100 μg ml^{-1} Sb(III) and Sb(V) in solutions of 26 mmol l^{-1} citric acid. These solutions were diluted with 99.9% MeOH (1 + 1) prior to the ES-MS measurement. Fig. 1 shows the ES-MS spectra obtained in a negative mode. In both cases, the obtained ES-MS spectrum was characterised by the base peaks at *m/z* 191 and 383, which could be attributed to the deprotonated citrate and citrate dimer, respectively, due to the excess of citric acid in the test solutions. Peaks resulting from Sb species were observed at *m/z* 361 and 363 for a Sb(V)-citric acid solution, and at *m/z* 501 and 503 for a Sb(III)-citric acid solution, respectively.

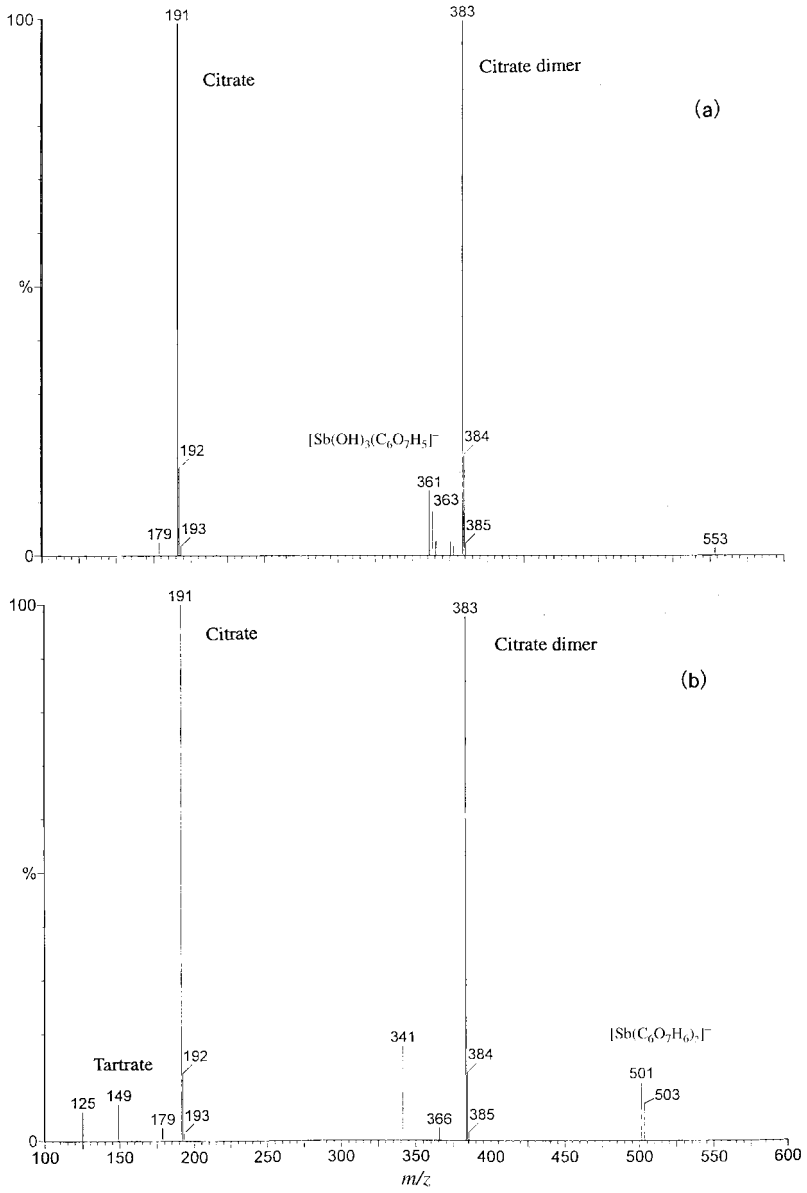

Fig. 1 ES-TOF mass spectra of Sb-citrate complexes in a negative mode: (a) Sb(V)-citrate and (b) Sb(III)-citrate.

In our previous work,[15] we investigated the ES-MS spectra of Sb(v) and Sb(iii) in aqueous solutions, and found that $Sb(OH)_6^-$ (*m/z* 223 and 225) was the predominant species of potassium hexahydroxyantimonate, while $[Sb_2(C_4O_6H_2)_2]^{2-}$ (*m/z* 267, 268 and 269) was the major species of potassium antimonyl tartrate. Therefore, the ES-MS spectra shown in Fig. 1 clearly indicated the complexation of Sb(v) and Sb(iii) with citric acid. The peaks at *m/z* 361 and 363 [Fig. 1(a)] could be assigned to $[Sb(OH)_3(C_6O_7H_5)]^-$, and peaks at *m/z* 501 and 503 [Fig. 1(b)] could be assigned to $[Sb(C_6O_7H_6)_2]^-$, *i.e.*, the initial Sb(iii)-tartrate complex was replaced by Sb(iii)-citrate complex.

Development of analytical procedures for the speciation of Sb(iii) and Sb(v): figures of merit

Considering the fact that the formed Sb(iii)- and Sb(v)-citrate complexes are negatively charged ions in solution, anion-exchange chromatography was selected for the separation of Sb-citrate complexes. After a series of optimizations in terms of the selection of HPLC columns and the optimization of the ionic strength and the pH of a mobile phase, it was found that good separation between Sb(iii)- and Sb(v)-citrates could be obtained on a Hamilton PRP-X 100 column with 10 mmol l^{-1} EDTA–1 mmol l^{-1} phthalic acid (pH 4.5) as a mobile phase. A representative chromatogram obtained with a standard solution of Sb-citrate (10 μg l^{-1} as Sb) is shown in Fig. 2. It is clearly shown that both Sb-citrate species have interaction with the column, and neither of them eluted in the solvent front, which often results in difficulties for the quantification of low concentrations of Sb species.

To hyphenate the developed chromatographic separation system with ICP-MS, the operation conditions of ICP-MS, especially the nebulizer Ar gas flow rate, were optimized to achieve the best sensitivity for the detection of Sb signals. At first, the flow rates of the nebulizer gas were varied between 0.8 and 1.45 l min^{-1} (Fig. 3, relative standard deviations of individual measurements are less than 5%) when a 2 μg l^{-1} Sb standard solution was introduced. These experiments revealed that maximum sensitivity for both ^{121}Sb and ^{123}Sb signals could be obtained at a gas flow rate of 1.20 l min^{-1}. Taking into account the minimum intensity of the blank Sb signals at the same flow rate, a nebulizer gas flow rate of 1.20 l min^{-1} was used throughout the rest of the experiments.

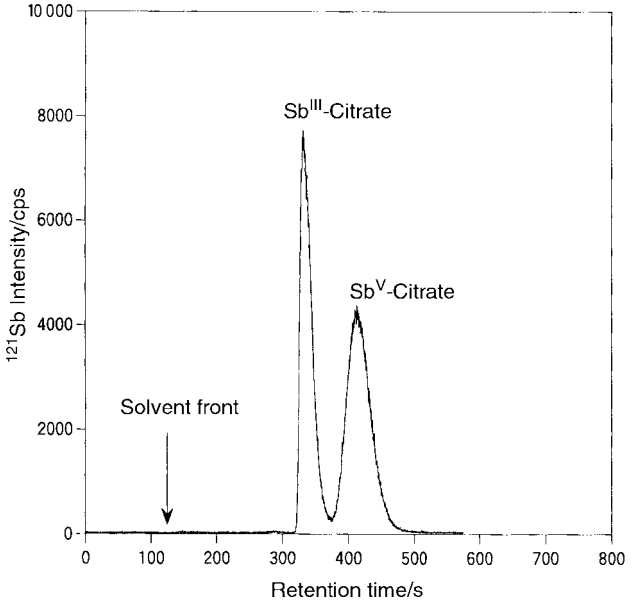

Fig. 2 Chromatographic separation of Sb(iii)-citrate and Sb(v)-citrate (each 10 g l^{-1} as Sb). Chromatographic conditions: PRP-X100 anion-exchange column with 10 mmol l^{-1} EDTA–1 mmol l^{-1} phthalic acid (pH 4.5) as a mobile phase at a flow rate of 1.5 ml min^{-1} under ambient temperature. A Sb isotope of *m/z* 121 was monitored by ICP-MS.

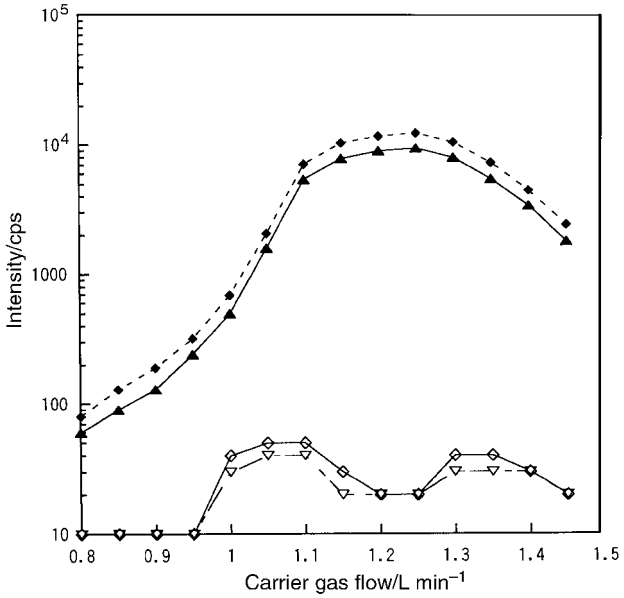

Fig. 3 Effect of nebulizer gas flow rate on the intensity of Sb ion counts: (◆) ^{121}Sb of 2 μg l^{-1} Sb standard solution; (▲) ^{123}Sb of 2 μg l^{-1} Sb standard solution; (◇) ^{121}Sb of blank solution; and (▽) ^{123}Sb of blank solution.

Under the optimized operating conditions for both HPLC and ICP-MS (shown in Table 1), a reliable analytical procedure for the speciation of Sb(iii)- and Sb(v)-citrates with high sensitivity was established. Table 2 summarizes the analytical characteristics of the developed method. All quantifications were performed based on the peak area. It was found that the calibration curves were linear over the range of 1.0–250 μg l^{-1} for the Sb species studied. Low detection limits of 0.05 and 0.07 μg l^{-1} were obtained for Sb(iii) and Sb(v), respectively. The precisions, evaluated by using the relative standard deviation (%RSD) with a 2 μg l^{-1} standard solution, were 1.8% and 3.3% ($n=3$) for Sb(iii) and Sb(v), respectively. In our previous work,[11] we obtained detection limits of 0.3 μg l^{-1} for Sb(iii) and 0.1 μg l^{-1} for Sb(v), with a conventional anion-exchange column (Synchropak Q300) and a conventional stainless-steel HPLC system. In this work, an inert PEEK anion-exchange column (PRP-X100) and a metal-free inert HPLC pump, as well as PEEK tubing for all connections were used. Thus, the background signals for both ^{121}Sb and ^{123}Sb could be decreased from initially 50–70 counts to 9–13 counts, resulting in a much better signal-to-background ratio.

Table 2 Analytical figures of merit of the developed method

Species	Retention time/s (%RSD)	DL[a]/μg l^{-1}	Repeatability[b] (%)	Dynamic range/μg l^{-1}	R^{2c}
Sb(iii)	325 (0.8)	0.05	1.8	1–250	0.9997
Sb(v)	406 (1.0)	0.07	3.3	1–250	0.9994

[a]Detection limits were determined as the elemental concentration which provides a signal three times the standard deviation ($n=7$) of the blank. [b]Repeatability was determined for peak area by calculating the relative standard deviation (%RSD of three successive measurements; concentration of each analyte was 2 μg l^{-1}). [c]Correlation coefficients (R^2) were evaluated based on peak area.

Table 3 Comparison of detection limits ($\mu g\ l^{-1}$) for Sb(III) and Sb(V) using HPLC-ICP-MS techniques as reported in the recent literature

Reference	Technique	Sb(III)	Sb(V)
Smichowski *et al.*[7]	HPLC-ICP-MS	7.5	0.9
Smichowski *et al.*[7]	HPLC-HG-ICP-MS	0.4	0.08
Lintschinger *et al.*[9]	HPLC-ICP-MS	0.8	0.5
Ulrich[20]	HPLC-ICP-MS	3.0	0.5
Ulrich *et al.*[19]	HPLC-ICP-MS	0.29	0.06
Lindemann *et al.*[21]	HPLC-ICP-MS	1.7	0.14
Zheng *et al.*[11]	HPLC-ICP-MS	0.3	0.1
Krachler and Emons[16]	HPLC-USN-ICP-MS	0.014	0.012
Krachler and Emons[22]	HPLC-HG-ICP-MS	0.008	0.020
Zheng *et al.*, this work	HPLC-ICP-MS	0.05	0.07

Therefore, detection limits of 0.05–0.07 $\mu g\ l^{-1}$ were obtained in this work. Previously, a large difference between detection limits of Sb(III) and Sb(V) was reported by many authors,[7,19–21] because Sb(V) eluted at the solvent front with a very sharp peak resulting in a lower detection limit, while Sb(III) was strongly retained on the anion-exchange column with a broad peak leading to a much higher detection limit. In our chromatographic separation system, both Sb species were retained on the column and eluted with sharp peaks, thus, similar detection limits for Sb(III)- and Sb(V)-citrate could be obtained. Also, it should be noted that the detection limits obtained in this work are among the lowest values reported in the literature with the HPLC-ICP-MS technique (Table 3). These detection limits are sufficiently low for the speciation of inorganic Sb species in environmental samples.

Analytical applications

Stability of Sb species in a moat water sample. A moat water sample was collected in the Palace in Tokyo. This water sample was first analysed for Sb species using a method reported by Lintschinger *et al.*,[9] and only Sb(V) was detected [Fig. 4(a)]. The spike of the Sb(III) standard in the moat water sample indicates that the spiked Sb(III) was oxidized to Sb(V) within 30 min [Fig. 4(b) and (c)] due to the presence of oxidizing substances in the moat water sample. The spiked Sb(III), however, could be stabilized by adding citric acid at a concentration of 26 $mmol\ l^{-1}$ due to the formation of a Sb(III)-citrate complex. After adding citric acid, the Sb(III) standard (5 $\mu g\ l^{-1}$) was spiked into the moat water sample, then, the water sample was stored in a refrigerator at 4 °C. It was found that the spiked Sb(III) was stable; there was no oxidation of spiked Sb(III) to Sb(V) during 10 days storage. This observation implies that the addition of citric acid is useful for preventing Sb(III) from oxidising to Sb(V) during sample storage for the speciation analysis of Sb compounds in aqueous solutions.

Determination of Sb(III) and Sb(V) in an APM sample. Since a high concentration of Sb was detected in APM samples collected in Tokyo, Japan, the impact of APM on humans has been of public health concern. Considering the fact that trivalent antimony compounds are generally more toxic than pentavalent forms,[1,2] it is desirable to have the chemical species information of Sb in APM in order to have a better risk assessment. In our previous work,[6,11] the speciation of Sb compounds in aqueous extracts of APM was investigated. Sb(V) was found to be the major specie among several unknown Sb compounds and trace trimethylantimony species (TMSb). However, no Sb(III) could be detected. It is considered that oxidation of Sb(III) to Sb(V) might have occurred during the extraction process. Therefore, the control of Sb(III) oxidation becomes the key to obtain the original chemical speciation information of Sb in an APM sample.

In this work, we observed the complexation effect of Sb compounds with citric acid, and found that this effect could be used for the stabilization of Sb(III) species. This observation gives us inspiration to explore the applicability for the determination of Sb(III) and Sb(V) in APM by using citric acid as an extraction solvent. It is expected that the extracted Sb(III) and Sb(V) will form their citrate complexes during the extraction process to avoid the change of oxidation state and, thus, the original Sb(III), if it exists in APM, will be detected.

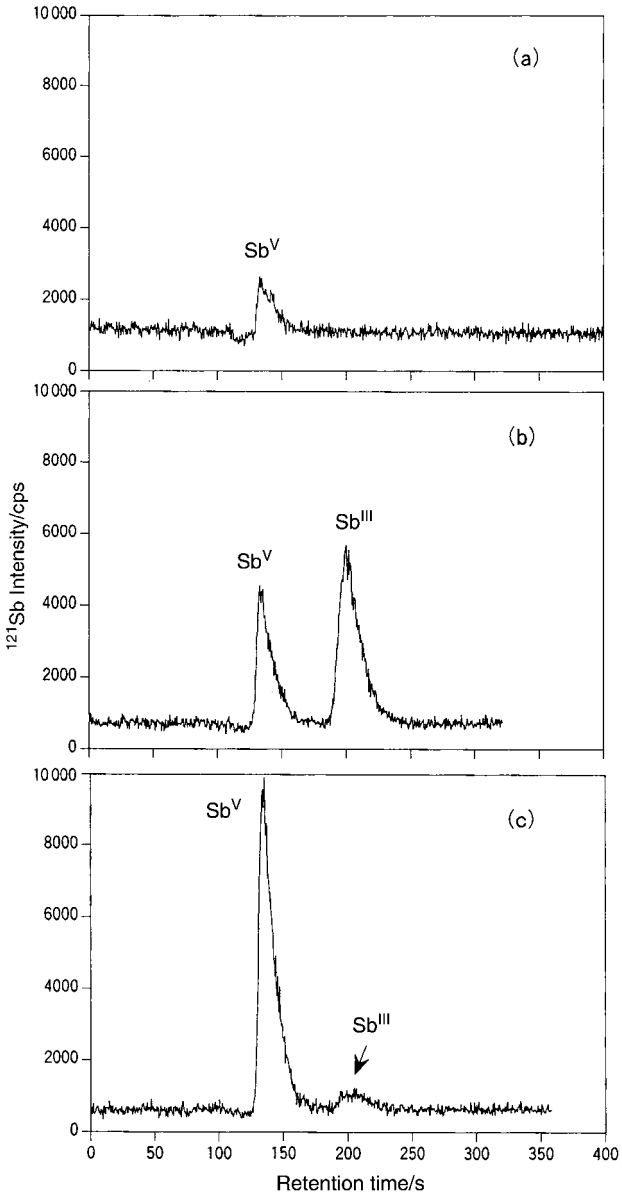

Fig. 4 Rapid oxidation of Sb(III) to Sb(V) in a moat water matrix. Chromatographic conditions: PRP-X 100 anion-exchange column with 20 $mmol\ l^{-1}$ EDTA–2 $mmol\ l^{-1}$ phthalic acid (pH 4.5) as a mobile phase at a flow rate of 1.5 $ml\ min^{-1}$ under ambient temperature. (a) Original moat water sample; (b) moat water spiked with Sb(III) (5 $\mu g\ l^{-1}$), immediately after spiking; and (c) moat water spiked with Sb(III) (5 $\mu g\ l^{-1}$), after 30 min.

For this purpose, an APM sample ($104 \pm 3\ \mu g\ g^{-1}$ Sb) was collected in Tokyo, Japan in July, 2000, according to the method described before.[6,11] The total concentration in the collected APM sample was determined by ICP-MS after acid digestion. To check the accuracy of measurements of total Sb, a Japanese quality control sample for APM (AS-1, Japan Environment Agency) and a standard reference material of urban particulate matter, NIST SRM 1648 (NIST, Gaithersburg, USA) were analyzed. The certified Sb concentration in AS-1 is 92 $\mu g\ g^{-1}$, while a Sb concentration of 45 $\mu g\ g^{-1}$ in NIST 1648 is given as an information value. The detected total

Sb concentrations in these two reference materials were $92.4 \pm 4.1\ \mu g\ g^{-1}$ for AS-1 and $46.9 \pm 0.9\ \mu g\ g^{-1}$ for NIST 1648, respectively, which is in good agreement with the certified or information value. The extraction of Sb species was performed using water and/or citric acid (5 or 26 mmol l^{-1}) in a sonicated water bath for 30 min. Due to the limited amount of the APM sample, all the experiments were performed in duplicate, and the average value was presented unless otherwise stated. The obtained extraction yields were: 13, 11 and 15% with water, 5 and 26 mmol l^{-1} citric acid using ultrasonic-assisted extraction, respectively. To investigate the possible species change during the extraction process, 50 $\mu g\ l^{-1}$ Sb(III) and 50 $\mu g\ l^{-1}$ Sb(V) were spiked into the APM sample separately, prior to the extraction operation. After the extraction, the obtained extracts were analysed in terms of Sb species using the HPLC-ICP-MS analytical method described above. The obtained recovery data of Sb(III) and Sb(V) are summarized in Fig. 5. It was found that a very low recovery ($<60\%$) for both Sb(III) and Sb(V) was obtained with water extraction due to the oxidation of Sb(III) and the re-adsorption of added Sb standards on the APM particles. With 5 mmol l^{-1} citric acid, 90% of added Sb(V) could be recovered because of the formation of a Sb(V)-citrate complex, which resulted in an alleviation of re-adsorption of Sb(V) on APM particles, while the recovery for Sb(III) could not be improved under these conditions. With an increase in citric acid concentration up to 26 mmol l^{-1}, the recovery of Sb(III) was improved significantly, up to 74%, due to the suppression of Sb(III) oxidation by forming a Sb(III)-citrate complex. In addition, when N_2 gas was introduced continuously into the extraction bottle during the extraction, the recovery for Sb(III) could be further improved to 84%, and the recovery for Sb(V) also increased up to 97%. Since no oxidation or reduction for the spiked Sb(III) and Sb(V) could be observed by comparison with chromatograms obtained from the original APM sample [Fig. 6(a)–(c)] when 26 mmol l^{-1} citric acid was used, it is considered that the further recovery improvement for both Sb(III) and Sb(V) was due to the alleviation of re-adsorption effect when N_2 was introduced. It should be noted that Sb(III) was detected in the citrate extract of the APM sample [Fig. 6(a)]. This is the first evidence for the existence of the most toxic Sb(III) in an APM sample, and the ratio of Sb(V) to Sb(III) was found to be *ca.* 4.6 : 1.

Since the extraction yield obtained with citric acid using ultrasonic-assisted extraction was still low (around 10%), the use of microwave-assisted extraction was also considered in this work. Preliminary results indicated that the extraction efficiency could be improved up to 35% by using microwave-assisted extraction under the conditions of 26 mmol l^{-1} citric acid with a microwave power of 100 W for 20 min. The obtained chromatogram is shown in Fig. 7. It can be seen that Sb(III) could be still detected although Sb(V) was the major species. The ratio of Sb(V) to Sb(III) was found to be 15 : 1 under these conditions. This result may be attributed to either the oxidation of Sb(III) or simply the more easy extraction of Sb(V)-citrate. To shed light on this observation, 50 $\mu g\ l^{-1}$ Sb(III) and Sb(V) were spiked into APM samples separately, prior to the microwave-assisted extraction. The obtained results showed that almost 100% of added Sb(V) could be recovered, while the recovery for Sb(III) was only 40%. Since an increase of the Sb(V) peak area was observed when Sb(III) was spiked, the oxidation of Sb(III) was confirmed to be responsible for the high ratio of Sb(V) to Sb(III) compared with that obtained with ultrasonic-assisted extraction. Assuming the oxidation of Sb(III) is 60%, based on the obtained recovery of Sb(III), the

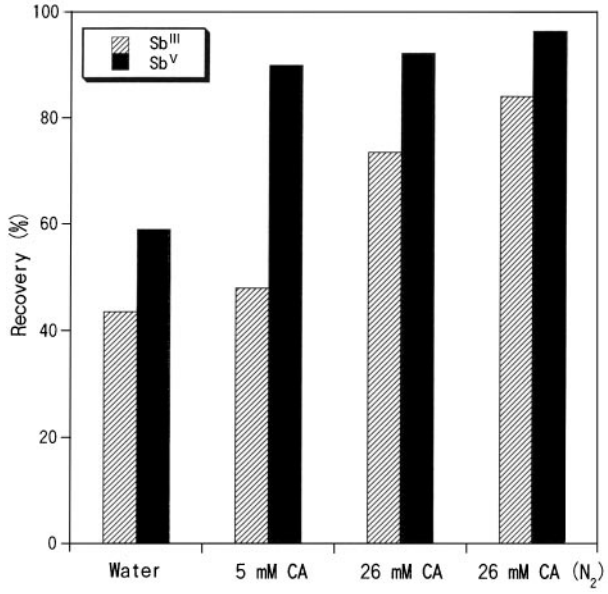

Fig. 5 Recovery of spiked Sb(III) and Sb(V) prior to APM extraction with different extraction procedures using ultrasonic-assisted extraction. CA stands for citric acid.

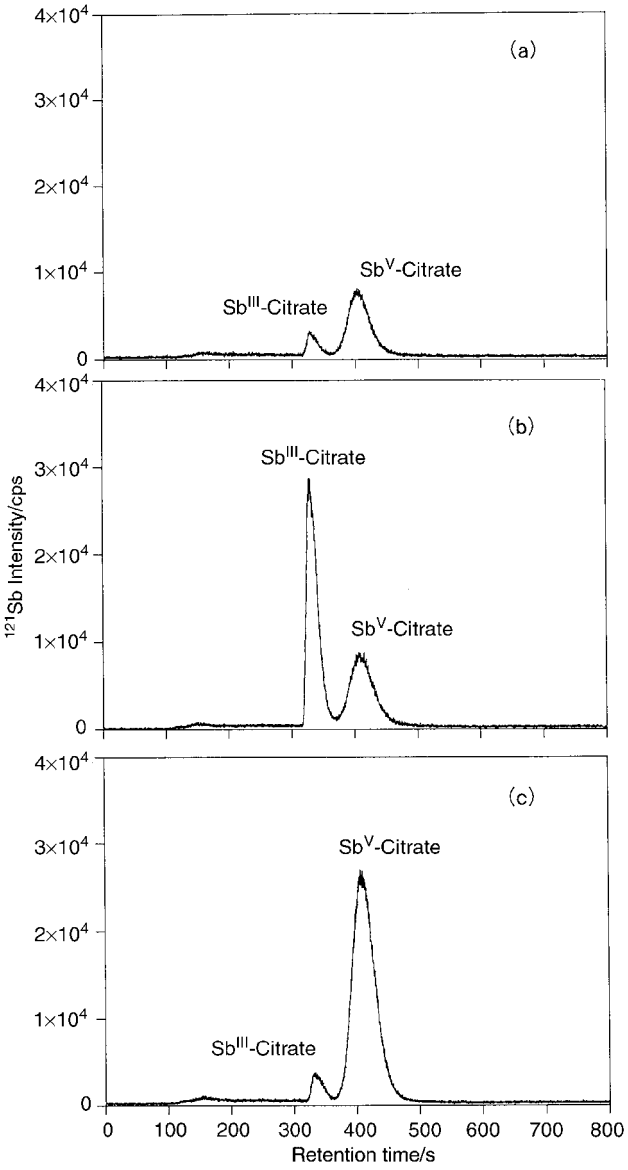

Fig. 6 Chromatograms of an APM sample using citric acid as an extraction solvent with ultrasonic-assisted extraction: (a) original APM sample; (b) APM sample spiked with Sb(III)-citrate prior to extraction; and (c) APM sample spiked with Sb(V)-citrate prior to extraction. See Fig. 2 caption for chromatographic conditions.

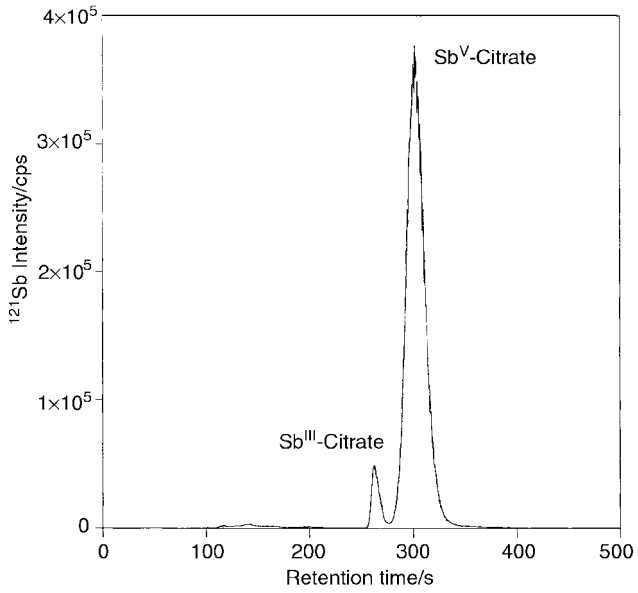

Fig. 7 A representative chromatogram of APM using citric acid as an extraction solvent with microwave-assisted extraction (100 W, 20 min). See Fig. 2 caption for chromatographic conditions.

original Sb(v)/Sb(iii) ratio should be 5.4 : 1, which is very close to the ratio obtained with ultrasonic-assisted extraction described above. It should also be pointed out that the sum of the Sb species determined with HPLC-ICP-MS method was 21% of the total concentration of Sb in the APM sample, which is less than the obtained extraction yield (35%). This result indicated that there were hidden Sb species in the citrate extract, in other words, only inorganic Sb compounds could be detected by the described method. Further investigation is needed to completely elucidate the Sb species present in APM samples.

Conclusion

A novel analytical method for the determination of inorganic Sb(iii) and Sb(v) in environmental studies was described. This method is based on the formation of Sb(iii)- and Sb(v)-citrate complexes. Evidence for this complexation effect was obtained with ES-MS analysis. Experimental results indicated that the formed Sb-citrate complexes were stable in an environmental matrix and a HPLC separation system was developed for the separation of Sb(iii)- and Sb(v)-citrates. After coupling this HPLC system to ICP-MS, very low detection limits were achieved, which made the developed method useful for the determination of Sb(iii) and Sb(v) in environmental samples.

When the developed method was applied to the Sb speciation in an APM sample, the two main factors responsible for the low recovery of spiked Sb(iii) during aqueous extraction were found to be the oxidation of Sb(iii) to Sb(v) and the adsorption of Sb compounds on the sample particles. The reason for the incomplete recovery of spiked Sb(v) seems to be due to the adsorption effect only. The use of citric acid as an extraction solvent was demonstrated to be useful in suppressing the oxidation of Sb(iii) to a great extent and also in alleviating the adsorption of Sb compounds on the sample particles, which made it possible to obtain the original Sb species information in the APM. Sb(iii) was detected in an APM sample for the first time. Taking into account the high toxicity of Sb(iii) species, long-term regular monitoring of Sb species in APM should be considered for a better risk assessment.

Acknowledgements

This research has been conducted under the joint research project "Studies on Speciation Analysis of Pollutant Elements released to the Environment by Human Activities" at the Institute of Sciences and Engineering of Chuo University.

References

1 D. G. Elinder and L. Friberg, in *Handbook on the Toxicology of Metals*, ed. L. Friberg, G. F. Nordberg and V. B. Vouk, 1979, Elsevier, Amsterdam, p. 283.
2 M. Krachler, J. Zheng and H. Emons, *Trends Anal. Chem.*, 2001, **20**, 79.
3 S. C. Apte and A. G. Howard, *J. Anal. At. Spectrom.*, 1986, **1**, 221.
4 T. Nakahara and N. Kikui, *Anal. Chim. Acta*, 1985, **172**, 127.
5 L. S. Cutter, G. A. Cutter and M. L. C. San Diego-McGlone, *Anal. Chem.*, 1991, **63**, 1138.
6 J. Zheng, M. Ohata and N. Furuta, *Analyst*, 2000, **125**, 1025.
7 P. Smichowski, Y. Madrid, M. B. de la Calle Guntinas and C. Camara, *J. Anal. At. Spectrom.*, 1995, **10**, 815.
8 X. Zhang, R. Cornelis and L. Mees, *J. Anal. At. Spectrom.*, 1998, **13**, 205.
9 J. Lintschinger, I. Koch, S. Serves, J. Feldmann and W. R. Cullen, *Fresenius' J. Anal. Chem.*, 1997, **359**, 484.
10 M. Krachler and H. Emons, *J. Anal. At. Spectrom.*, 2000, **15**, 281.
11 J. Zheng, M. Ohata and N. Furuta, *Anal. Sci.*, 2000, **16**, 75.
12 B. Michalke and P. Schramel, *J. Chromatogr. A*, 1999, **834**, 341.
13 A. Guy, P. Jones and S. J. Hill, *Analyst*, 1998, **123**, 1513.
14 J. Lintschinger, B. Michalke, S. Schulte-Hostede and P. Schramel, *Int. J. Environ. Anal. Chem.*, 1998, **67**, 1.
15 J. Zheng, A. Takeda and N. Furuta, *J. Anal. At. Spectrom.*, 2001, **16**, 62.
16 M. Krachler and H. Emons, *Anal. Chim. Acta*, 2001, **429**, 127.
17 M. B. de la Calle-Guntinas, Y. Madrid and C. Camara, *Fresenius' J. Anal. Chem.*, 1992, **344**, 27.
18 B. Mohammad, A. M. Ure, J. Reglinski and D. Littlejohn, *Chem. Speciation Bioavailablity*, 1990, **3**, 117.
19 N. Ulrich, P. Schaked and D. Zilberstein, *Fresenius' J. Anal. Chem.*, 2000, **368**, 62.
20 N. Ulrich, *Anal. Chim. Acta*, 1998, **359**, 245.
21 T. Lindemann, A. Prange, W. Dannecker and B. Neidhart, *Fresenius' J. Anal. Chem.*, 1999, **364**, 462.
22 M. Krachler and H. Emons, *J. Anal. At. Spectrom.*, 2001, **16**, 20.