Submitted on Aug. 11, 2020: revised Oct. 19, 2020

Arsenic Analysis in Japan

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1. Introduction

"Arsenic in Drinking Water" [1], a report of the National Research Council in the United States, compared arsenic concentrations of urine among Taiwan [2], Japan [3], Italy [4], and Argentina [5] residents as listed in Table 1. The NRC report stated that the similarity between the results of the Taiwanese [2] and European studies [4] was strong. The report guessed that the high values in the Japanese study were almost certainly associated with seafood consumption, and arsenosugars were a likely source of the DMA; however, the inorganic concentrations were noteworthy, and demethylation during storage or analyses could be a cause. The urinary arsenic concentrations are much higher in native Andean females exposed to high concentrations of arsenic in drinking water [5].

Table 1. Comparison of arsenic species in urine (mean μg/L ±standard deviation) in four studies, modified from Table 3-14 of Ref.[1].

Location	Inorganic	MMA	DMA	OF	AsT	Ref.
Taiwan	1.7±1.1	2.0±1.0	3.3±2.5	13.7	20.7±7.0	[2]
Japan	11.4±5.9	3.6±2.8	35.0±20.8	71.0	121±101	[3]
Europe	1.9±1.2	1.9±1.4	2.1±1.5	11.3	17.2±11.2	[4]
Argentina	66±41	7.1±12	185±110	13	274±98	[5]

Abbreviations: MMA and DMA, see Table 2; OF, other forms of arsenic; AsT, arsenic total.

The 102 Japanese control values reported by Yamauchi et al. [3] in Table 1 were in fact originated from Yamato's paper [6]. Yamato was the same group as Yamauchi. Yamauchi et al. [3] modified the Yamato's values [6], e.g. inorganic 12.7±7.1, or urine-density-corrected 11.9±6.5, to 11.4±5.9 as in Table 1, without any description of the reason of difference. Yamauchi used the Yamato's values until 1999 in Yamauchi's various reports; sometimes after modified and sometimes the same values as Yamato's paper [6]. Yamauchi sometimes cited Yamato's paper [6], but sometimes without citation.

The focus of the present chapter is to clarify the reason why arsenic concentrations of Japanese controls were one order of magnitude higher than other countries, from the viewpoint of analytical chemistry. Goyer et al. updated the report in 2001 [7], however the above-mentioned reasons were not updated.

The inorganic arsenic analyses in biological samples in Japan had been

performed by one research group solely, i.e. Yamauchi's group, and thus the analytical results have never been checked. The present chapter is the first check of the report of Japanese arsenic analyses for 44 years since Yamamura and Yamauchi [8] began the arsenic analysis.

The arsenic analyses were performed between late 1976 to early 2000 by Yamauchi's group, using the HG-AAS (hydride generation atomic absorption spectrometry) and since 1998 by SR-XRF (synchrotron radiation X-ray fluorescence) with Nakai [9,10]. The published dates of Refs.[9,10] were 1999 but their experiments were in December 1998. The samples measured by Yamauchi's HG-AAS [9] were the same person's hair as those of Nakai's SR-XRF report [10], but different hair shafts. These two reports have no contradiction.

The error sources of arsenic analysis using HG-AAS and SR-XRF will be concluded as follows in the present chapter:

(i) Glass test tubes were used until 1984. Glass usually contains As(III).

(ii) Urine and hairs were digested by NaOH, and thus As(III) was oxidized during NaOH digestion.

(iii) pH values used during hydride generation were not appropriate, and thus the arsenic recovery was very low.

(iv) The analyzed hair was not enough, and sometimes the arsenic was less than the detection limit, but the quantitative values were reported even one-order of magnitude below the detection limit.

(v) Lead (Pb) Lα peak was erroneously assigned to As Kα.

(vi) Selective excitation using SR-XRF of As Kα without exciting Pb Lα was not satisfactorily achieved because of the stray light of the synchrotron beamline.

2. Arsenic species

Arsenic is classified into several kinds in its chemical states. Inorganic arsenic is classified into As(III), arsenite, arsenous acid, AsO_3^{3-}, AsO_2^-, or As_2O_3; and As(V), arsenate, arsenic acid, AsO_4^{3-} or As_2O_5. Some compounds referred to in the present chapter are listed in Table 2. Some other representative arsenic compounds are shown in the NRC report [1]. The structural formula of most of the important arsenic compounds are tabulated by Reid et al. [11].

Table 2. Typical arsenic chemical species taken from NAS report "Arsenic in Drinking Water" [1] after modified.

Name	Abbreviation	Chemical formula
arsenous acid	As(III)	AsO_3^{3-}, AsO_2^-
arsenic acid	As(V)	AsO_4^{3-}
monomethylarsonic acid	MMA(V)	$CH_3AsO(OH)_2$
dimethylarsinic acid	DMA(V)	$(CH_3)_2AsO(OH)$
trimethylarsine oxide	TMAO(V)	$(CH_3)_3AsO$
arsenobetaine	AB	$(CH_3)_3As^+CH_2COO^-$
arsine		AsH_3
monomethylarsine		CH_3AsH_2
dimethylarsine		$(CH_3)_2AsH$

trimethylarsine		$(CH_3)_3As$

Arsenite refers to As_2O_3 powder, and its water solution is called arsenous acid. When As_2O_3 solid is dissolved into water, it dissociates electrolytically as $3H^+ + AsO_3^{3-}$ or $H^+ + AsO_2^-$, and we express H_3AsO_3 (ortho) or $HAsO_2$ (meta). The arsenous acid is a mixture of ortho and meta species. Ortho is the major component in the arsenous acid. These are classified as oxo acids. In Japan, a Chinese character which means sour is used for expressing the acids, and the same Chinese character is also used for oxygen, as "the sour origin". Japanese sometimes confuse between acids and oxides. This confusion is probably originated from the fact that a non-metallic oxide, which is dissolved into water, becomes acid. However, dissolving metallic oxide such as Na_2O yields a base solution.

3. Gutzeit method

The Gutzeit method is used for detecting the arsenic element in the analyte by changing wet $AgNO_3$ paper into black. The first report of H. W. Gutzeit was dated up to 1879 [12]. The Gutzeit method was explained in Reckleben et al. [13] as;

$$H_3As+6AgNO_3+3H_2O=6Ag+H_3AsO_3+6HNO_3, \quad (1)$$

$$H_3As+3AgNO_3=Ag_3As+3HNO_3. \quad (2)$$

Based on these expressions, AsH_3 changed the $AgNO_3$ to black color Ag_3As or Ag_3AsO_3. The Gutzeit method has been widely used to detect the arsenic [14,15].

4. Principles of HG-AAS arsenic analysis

The sensitivity of Ge, As, Se, Sn, Sb, Te, Pb and Bi are not enough for AAS. In order to achieve high sensitivity, hydride has been used. The Gutzeit method is a hydride generation method, where arsenic becomes arsine molecular gas and detected by the blackening of $AgNO_3$(aq) paper by the arsine. Holak [16] applied hydride generation method in 1969 to AAS, where Nakahara [17] explained the reaction by Zn-$SnCl_2$-KI as:

$$Zn + 2HCl \xrightarrow{} ZnCl_2 + 2H^{\cdot} \xrightarrow{As^{3+}/As^{5+}} AsH_3 + H_2(\text{excess}). \quad (3)$$

Hydride generation by sodium borohydride $NaBH_4$ was first reported by Braman et al. [18] in 1972 for atomic emission spectrometry, and a little bit behind Braman et al. but almost simultaneously reported by Schmidt and Royer [19] for AAS. Nakahara [17] showed the hydride generation by sodium borohydride as follows:

$$NaBH_4 + 3H_2O + HCl \xrightarrow{} H_3BO_3 + NaCl + 8H^{\cdot} \xrightarrow{As^{3+}} AsH_3 + H_2(\text{excess}). \quad (4)$$

Braman and Foreback [20] reported the use of $NaBH_4$ for separately quantifying As(III) at pH4-5, and then again adding $NaBH_4$ at pH1-2 to the same solution but after the removal of As(III), in order to quantify the residual As(V). The pH value was later revised as pH3.5-4.0 [21] from the first value of pH4-5 [20].

The breakdown of the chemical reaction process from As(V) to AsH_3 was explained by a two-step process by Aggett and Aspell [22] as follows:

$$As(V) \xrightarrow{HCl} As(III), \quad (5)$$

$$As(III) \xrightarrow{NaBH_4} AsH_3. \quad (6)$$

The reaction (5) is faster than reaction (6) at an acidic condition of pH<1. The redox electric potential E of As(V)-As(III) couple is a function of pH as shown in Fig.1 [22,23] and thus differentiation between As(III) and As(V) is possible by changing the pH.

Yamamoto et al. [24, 25] experimentally determined the pH values for the speciation of As(III) and As(V) as shown in Fig. 2. When pH>5, we can detect As(III) only. When pH<0 or in hydrochroric acidity, we can detect the sum of As(III) and As(V). We must note here that the $[H^+]$ concentration determined by Yamamoto et al. [24,25] was higher than that of Braman et al. [21] by one or two orders of magnitude, i.e. the pH value is smaller by 1 or 2. The recovery rates of As(III) and As(III)+As(V) were 100 % for 5<pH<6 and pH<0, respectively, as are found from Fig. 2.

Thermodynamically favorable directions of the reaction between As(III) and As(V) are electrochemically predictable using the redox potential, because the Gibbs free energy is expressed as $\Delta G = -nFE$, where n is the charge and F the Faraday constant. When pH>10, As(III) --> As(V), and when pH<0, As(V) --> As(III).

5. Problems in Yamauchi's method

5.1. Glass test tube

Yamamura and Yamauchi [8] first published a paper on arsenic analysis from their research group, where urine, blood, and hair of arsenic factory employees were analyzed. Urine of 25-50 mL, blood 5-10 g, hair 0.5-1.0 g were digested by acids and analyzed by HG-AAS. The range of urinary arsenic levels of the factory employees was 44-501 μg/L, and that of 125 control was 6-389 μg/L. Though the details of the sample preparation procedures were not described in their paper, the arsenic concentrations of 125 controls seems to be too high.

Yamauchi and Yamamura [26] began the speciation analysis of As(III) and As(V) similar to the method of Braman's papers [20, 21].

Yamamura and Yamauchi [27] reported on urine, blood, and hair of arsenic factory employees similar to Yamamura and Yamauchi [8] (1976), but this time they quantified As(III), As(V), MMA(V), and DMA(V) separately. Hair arsenic of 15 controls were 0.03-0.07 As(III), 0.08-0.20 As(V), and 0.11-0.23 μgAs/g total As. MMA and DMA were not detected. The details of the sample preparation procedures were again not described in their paper, but the inorganic arsenic concentrations seemed to be too high

as the controls.

Yamauchi and Yamamura [28] published a paper on inorganic arsenic, MMA, DMA, and TMAO quantification of brain, hair, kidney, liver, lung, muscle, skin, and spleen of hamsters after orally administrated DMA. The experimental details were as follows: the hair sample was put into a 50-mL glass-stoppered test tube, and after adding 5 mL of 2 N NaOH, heated in a water bath at 85°C for 3 hours, finally HG-AAS analysis was performed. The inorganic As(III) and As(V) were not separately quantified from this paper but the total inorganic arsenic was quantified.

Yamauchi et al. [29] published a review paper, where they stated, "Arsenic was speciated in biological samples by the method of arsine generation and atomic absorption spectrometry [28]. To prepare samples for speciation of arsenic, 0.5 g of hair or 0.5 mL of urine were transferred into polypropylene tubes containing 3 mL of 2 N NaOH and heated in a heating block at 95°C for 3 hours."

It is therefore very strange that although the glass test tube was used in the cited paper [28], but it was stated as a polypropylene tube. It has been well known already in early 1970s that glass vessels should be avoided for the arsenic analysis [30] because it contains As_2O_3 as an antifoaming reagent. Recently, antimony (Sb) is added to the glass for the same purpose. Antimony is also used as a catalyst of synthesizing polyethylene terephthalate (PET). Therefore we always need the blank test for arsenic or antimony analysis. High purity arsenic reagents, especially high purity organic chemicals such as DMA, should be stored in a non-glass bottles in order to avoid the arsenic contamination.

All the Yamauchi's papers have been checked with respect to the glass test tubes. The papers which Yamauchi published in 1985 were two kinds; some papers used glass tubes, and some used non-glass tubes. After 1986, all the Yamauchi's papers used non-glass tubes, while all the papers before 1984 used glass tubes, as long as the paper refers to the test tube material, e.g. glass or polypropylene. It is now concluded that the higher arsenic concentrations of controls before 1985 were due to the use of glass test tubes. Yamauchi has never referred to this error in his papers. Yamauchi sometimes used old data before 1985 in the papers published after 1986, in such a way as the paper of Yamauchi et al. [29], which was published in 1997. Thus the data in his group are not reliable.

5.2. NaOH decomposition

Yamauchi and his co-authors digested biological samples in 2 N NaOH solution, and kept it at 85-95 °C for 3 hours. It is well known that As(III) is easily oxidized to As(V) if pH>10 at room temperature [31]. The test tube was stoppered or corked tightly and heated, and sometimes the temperature became over 100 °C. The basicity of 2 N NaOH was strong enough that the As(III) had easily oxidized all into As(V). The speciation analysis of As(III) and As(V) became therefore meanngless as long as NaOH digestion is used. Yamauchi performed speciation analysis of inorganic As(III) and As (V) between 1979 and 1984 using this NaOH digestion. Yamauchi stopped the speciation analysis of As(III) and As(V) in 1984, and in place of speciation, they quantified the total inorganic arsenic As(III)+As(V) since 1984 but for one exception. This exception was the forensic analysis of suspect person's hair of an arsenic murder case done by Yamauchi [9] with Nakai [10]. This forensic analysis was reviewed with

respect to the SR-XRF analysis by Kawai [32]. Yamauchi detected 90 ppb As(III), i.e., 0.090 μgAs(III) per one gram of hair, of the suspected person after the hair was digested by 2 N NaOH for 3 hours at 100°C [9]. This evidence was one of the main reasons of death penalty in the arsenic murder incident in Japan.

Yamauchi et al. [33] published a paper in 1984 on arsenic in land water in various area of Japan, including water in Toroku Mine, which was notorious for heavy arsenic pollution since 1920, and several lawsuits were in progress during 1980s [34]. Yamauchi et al. [33] compared two sample preparation methods as follows:

The first method was the direct method. The land water was directly analyzed by the HG-AAS. Yamauchi et al. [33] concluded that the As(III), As(V), MMA, DMA, and TMAO can be separately analyzed, but the accuracy was not enough. The concentration was sometimes one order of magnitude lower than the second method.

The second method was the NaOH digestion method. The water sample was put into the 2 N NaOH in the glass-stoppered test tube, and then decomposed for three hours at 85°C. Yamauchi et al. [33] stated that the accuracy of the total inorganic arsenic concentration was satisfactory, but that the speciation of As(III) and As(V) was not possible. They also checked that MMA, DMA, and TMAO were not decomposed to inorganic arsenic during the hot NaOH digestion. After this comparison, Yamauchi's group began to use the NaOH decomposition method hereafter, and they have never used the direct method since then.

However, the high concentration of inorganic arsenic in NaOH digestion method in Ref.[33] was due to the contamination from glass arsenic. They used many test tubes, and thus the contamination from glass was not always the same, thus large errors were introduced by this NaOH sample preparation. Even after Yamauchi found that the glass tubes were a source of arsenic contamination, they kept continuing the NaOH method. The only change was polypropylene tubes in place of glass tubes since 1985.

Yamauchi testified in courts as an arsenic medical expert for the defendant Toroku Mining company. His testimony was that the arsenic in human body was rapidly demethylated and oxidized to pentavalent to make it non-toxic, as well as it was quickly excreted through the metabolic process. He testified at least 1982 and 1988 at two different trials. He testified in 1982 at the First Team, First Trial [35], based on the As(III) and As(V) concentrations of Toroku Mine water referring the data to be published as Yamauchi et al. [33]. Yamauchi again testified in 1988 (Second Team, First Trial) by the total arsenic concentrations, based on the total inorganic arsenic [33]. Although Yamauchi had already recognized at the testimony in 1988, that the glass test tubes used in the paper published in 1984 [33] contaminated the quantitative results, he testified by using the contaminated data in 1988.

5.3. pH values for speciation

Yamauchi divided the NaOH solution of biological specimen into two volumes, and one was adjusted to pH1-2 or pH1.5, and the other to pH3.5-4.0 or pH3.5. These pH values were the same as those used by Braman and Foreback [20], but different from those of Yamamoto et al. [24,25]. After Yamauchi became to analyze only the total inorganic arsenic at 1984, Yamauchi et al. [28] still separated the NaOH-digested solution of specimen into two volumes: one solution was adjusted to pH1-2, and the other to pH3-4. Yamauchi et al. analyzed the two solutions in the same procedure as before,

but the results reported was only the total inorganic arsenic. The separation had thus no meaning at all. As can be found from Fig.2, the pH3-4 was not high enough to quantify the As(III) because 5-10 % of As(III) was originated from As(V). The pH should be pH5 in place of pH3-4.

It is also found from Fig.2, that the pH1.5 was not low enough in order to quantify the total inorganic arsenic, because the recovery rate was not 100 % for As(V); only 30% was recovered. Usually, 5 M hydrochroric acid solution (pH<0) was needed. It is true that the recovery rate depends on the temperature or co-existing ions, Yamauchi only followed the pH values of Braman and Foreback [20] every time, without checking the recovery rate.

5.4. Detection limit

Yamauchi usually digested 500 mg of hair into 2 mL and 2 N NaOH solution. It was then divided into two volumes, and 30 mL each the buffer solution was added in order to adjust the pH at 1.5 or 3.5. In this procedure the detection limit was 0.03 μgAs per 1 g of hair, which was equivalent to 0.5 ng/mL detection limit. However, at the forensic analysis in 1998, 50 mg hair of the suspected person was digested into the 2 mL and 2 N NaOH solution, and thus the detection limit was 10 times worse, i.e. 0.3 μgAs per 1 g hair, which was equivalent to 0.5 ng/mL. It was very strange that Yamauchi reported 0.090 μg As(III) per 1 g of hair, which was below the detection limit. Additionally, As(III) was not possible to discriminate from As(V) because of the NaOH digestion and of inadequate pH values.

6. Selective excitation of SR-XRF

The X-ray fluorescence peak of As Kα is at 10.5 keV, and Kβ at 11.7. The Kα X-ray is emitted by the electron transition by 2p --> 1s and Kβ by 3p --> 1s. Lead (Pb) is usually found in synchrotron radiation beamlines, because metallic Pb is frequently used for X-ray shielding. The Pb Lα line is situated also at 10.5 keV, the same energy as As Kα. The Pb Lβ is at 12.6 keV. The Lα is due to 3d --> $2p_{3/2}$ and the major intensity of Lβ to 3d --> $2p_{1/2}$ transitions [36].

The 10.5 keV peak cannot be assigned by itself to arsenic. We usually check the presence of arsenic by the Kαβ doublet. The intensity ratio is crudely 100 to 15 for Kα to Kβ. Lead is also checked by the Lαβγ triplet. The intensity ratio of Lα to Lβ is around 1 to 1, but it changes as the excitation mode, such as, for example, photon energy, exit angle of X-ray fluorescence, surface roughness, and spectrometer resolution [36-43]. If synchrotron beamline is available, we can use the selective excitation in order to excite arsenic without exciting the lead.

The absorption threshold of As Kα is 11.9 keV (As K edge), while the threshold of exciting the Pb Lα is 13.0 (Pb L_3 edge). Therefore, when the synchrotron beam energy is tuned between 12.0 and 12.9 keV, arsenic can be solely excited without exciting the lead. This is the selective excitation method utilizing the tunability of the synchrotron radiation. The monochromator is easily adjustable at any desired energy of the beamline within a few minutes. Conseqently arsenic is theoretically measurable without the interference of lead.

7. Stray light

Figure 3 is a typical SR-XRF spectrum of a hair shaft, measured and reported by Nakai [10] and Yamauchi [9]. The incident beam width was 4 mm. Yamauchi detected the 0.090 μgAs(III) per one gram of the suspected person's hair by HG-AAS as mentioned above, just a couple of days before the synchrotron radiation experiment. One shaft of hair was selected to measure the As Kα peak from the hairs of the suspected person. The 10.5 keV peak in Fig. 3 was assigned to "As" by Nakai [10]. However, the right end of the spectrum of Fig.3 was 13 keV, and thus Fig.3 was not a selective excitation. The incident elastic scattering peak was not shown in Fig.3, and Nakai was requested by the court to open the whole energy range of the spectrum. Then Nakai's testimony was changed to 15 keV excitation on the BL-4A beamline at KEK-PF synchrotron facility, when he opened the whole energy spectrum. Because of the presence of 10.5 and 12.6 keV peaks in Fig. 3, which clearly correspond to the Pb Lα and Lβ peaks, Kawai had pointed out that the peak assignment "As" in Fig. 3 is not the arsenic but it was lead. However when the whole spectrum of Fig. 3 up to 20 keV was opened to the court, Nakai stated that the 12.6 keV peak was not the Pb Lβ, but an escape peak of the Compton scattering of the incident 15 keV X-rays. Nakai also testified that the "As" peak at 10.5 keV in Fig. 3 was not the Pb Lα but As Kα. It is found from Fig. 3 that the width of the 12.6 keV peak is too narrow as an escape peak [44] of the Compton scattering, and thus 12.6 keV peak is not an escape but is natural to conclud the Pb Lβ. If Nakai wants to prove it the escape peak, he must only show a different excitation energy spectrum so that the escape peak shifts. He must have measured several different excitation energies.

Nakai opened the whole energy X-ray spectrum to the court. But the lawyers requested Nakai its numerical data. He opened the numerical data by pages of paper. Figure 4 is the logarithmic plot by Kawai, based on the Nakai's numerical data sheet submitted to the court. From the log plot spectrum, the intensity between 17-20 keV are non-zero intensity as is found from Fig. 4, although Nakai pleaded that Fig. 4 was 15 keV selective excitation. The integral intensity between 16 and 20 keV is 28 % of the elastic scattering intensity at 15 keV. Thus Pb Lβ, the threshold energy to excite the Lβ is 15.2 keV, could be excited by the 16-20 keV stray light, even the "15 keV" selective excitation was used. The selective excitation of 15 keV was not a genuine selective excitation to exclude the Pb Lβ. The 15 keV incident energy has no meaning at all because it does not exclude the Pb Lα.

This kind of strange forensic report of Nakai [10] was due to the HG-AAS forensic report of Yamauchi [9], which was performed a couple of days before the Nakai's SR-XRF measurement. Yamauchi and Nakai collaborated for the forensic analysis, where the conclusion of Nakai [10] should agree with that of Yamauchi [9]: As(III) was detected in the hair of the suspected person both by HG-AAS and also by SR-XRF. However arsenic concentration of the hair was below the detection limit of HG-AAS, As(III) was not detectable due to wrong pH and due to the NaOH digestion; and As Kα in SR-XRF was not the arsenic but the Pb Lα peak.

In order to detect the arsenic Kα buried in the strong Pb Lα signal from the X-ray shield of the beamline, the stray light intensity, including the higher order harmonics of the monochromator, should be less than ppm if we want to detect the ppm arsenic. It should be eliminated less than ppb if we want to detect the ppb level arsenic. Such a weak stray light intensity is usually not possible.

Nakai [45] that he could quantify impurity elements of Sn 7.4 pg and Sb 10.5 pg in a 100 μm diameter arsenic trioxide single particle using the BL08W beamline at SPring-8. The three-digit precision "10.5" is never obtained from a single particle analysis, because the X-ray fluorescence intensity strongly depends on the thickness of the particle, especially the thickness between 0 and 100 μm changes the intensity drastically. Three-digit concentration is only obtainable by a powder of bulk amount using e.g. ICP-AES (inductively coupled plasma atomic emission spectrometry). Nakai recently opened the truth by the prosecutor's document [46], that the quantitative values 10.5 and 7.4 were not the results of SR-XRF analysis, but they were obtained by an ICP-AES, though Nakai [45] described that 7.4 and 10.5 were obtained by SR-XRF at SPring-8. The too good precision of the Nakai's three-digit 10.5 pg absolute value was pointed out by a researcher who spent a large amount of money for the experiment on the same beamline at SPring-8 after the Nakai's paper [45], but could not obtain such a precise concentration.

8. Conclusions

Shraim and Hirano co-authored a paper with Yamauchi [47] using HPLC/ICP-MS. In their paper, the authors said that they have tested Yamauchi's NaOH digestion method and found that most of the arsenite(III) was oxidized to arsenate(V) as a results of alkaline digestion. Therefore they say the development of a method that keeps the integrity of the arsenic species intact. They reported on the development of a method for extraction of the hair-arsenic without altering the arsenic species oxidation state and analyzing the extracted species by HPLC-ICPMS. This paper has revealed that all the As(III) and As(V) speciation papers from Yamauchi's group had faults. This kind of check is quite important. This is an important paper cited by many other papers, for example, by Yáñez et al. [48].

We must discuss which analysis method is the ideal speciation method of arsenic in biological samples. HPLC/ICP-MS and IC (ion chromatography)/ICP-MS [11,47-49] are superior now compared with HG-AAS or even the SR-XRF. If the sample preparation is specially designed [50] as using cysteine, HG-AAS is still a good method. If the arsenic concentration is ~10 ppm level, HH(hand held)-XRF is an easy and good method for total arsenic analysis. If ppb level, TXRF (total reflection XRF) is useful for liquid samples [51,52].

Hijiki or *hijikia fusiformis* is a kind of seaweed, a popular food in Japan. Hijiki was once analyzed by Yamauchi and Yamamura [26], and concluded that high concentration of As(III) was contained, and thus not safe for human. However the As(III) detected in hijiki in 1979 was As_2O_3 in glass test tubes. We have in Japan forty-year long and wrong history of arsenic analysis. Frisch and Schwartz [53] pointed out the lack of validation of analytic techniques. The present chapter exemplifying that the hiding the truth in experiments performed by a major research group has distorted the arsenic chemical analysis of Japan for over 40 years.

The Arsenic Curry Murder Case is that Pb Lα was erroneously assigned to As Kα, and based on this erroneous assignment, an innocent person was sentenced to death penalty. The development of a reliable method of arsenic analyses in biological samples is very much needed to avoid such wrong decisions and XRF based techniques may play an important role in such development.

Acknowledgements
Thanks are due to Manor Kumar Sharma (Bhabha Atomic Research Centre), Nand Lal Mishra, and Susumu Imashuku (Tohoku University) for their advices on the redox reaction, due to Ursula E. A. Fittschen (Clausthal University of Technology) for the Gutzeit method, and Ramon M. Barnes for teaching the author the details of Braman's experiment.

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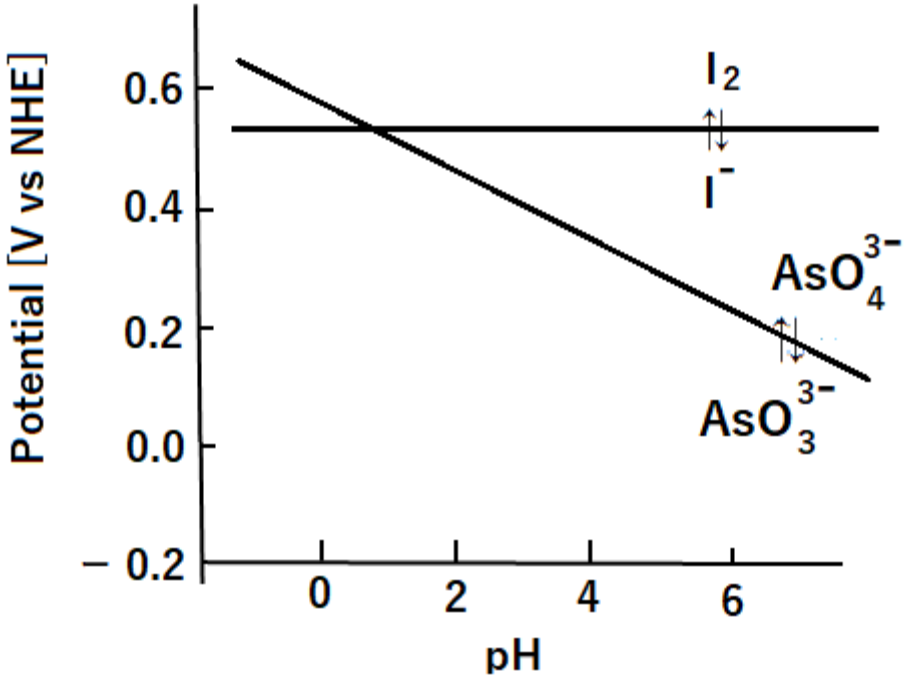

Fig.1. A schematic illustration of redox potential of As(V)-As(III) and I_2-I^- couples, taken from Aggett and Aspell [22] and Fujinaga [23] after modification. While As(III) + I_2 --> As(V) +2I^- at pH>4, As(V)+2I^- --> As(III) + I_2 in hydrochroric acid solution (pH<1).

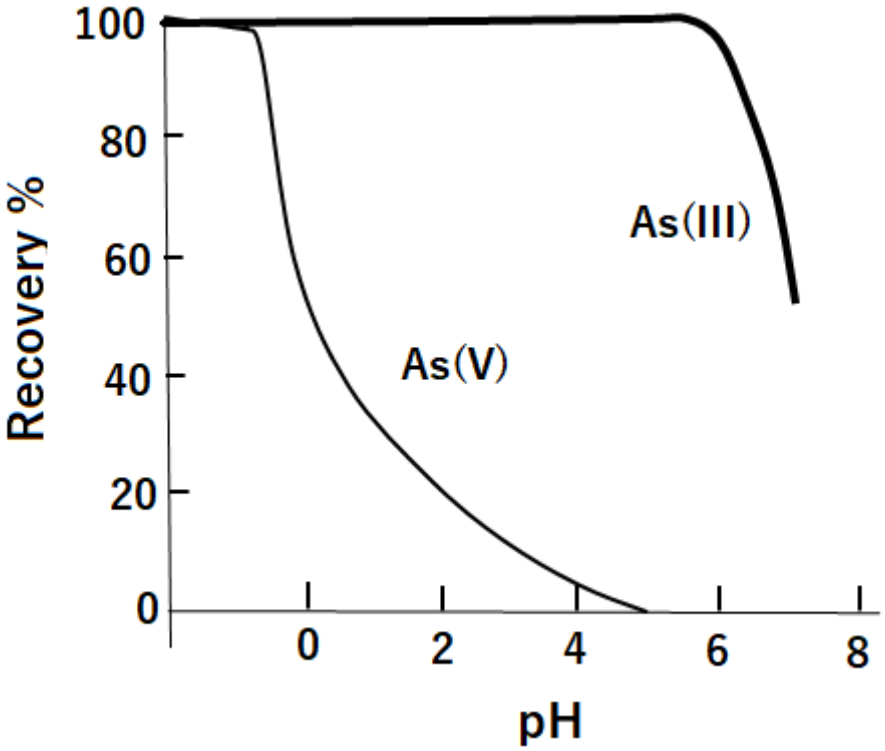

Fig.2. The recovery rate of As(III) and As(V) depending on pH, taken from Yamamoto et al. [24,25] after simplification.

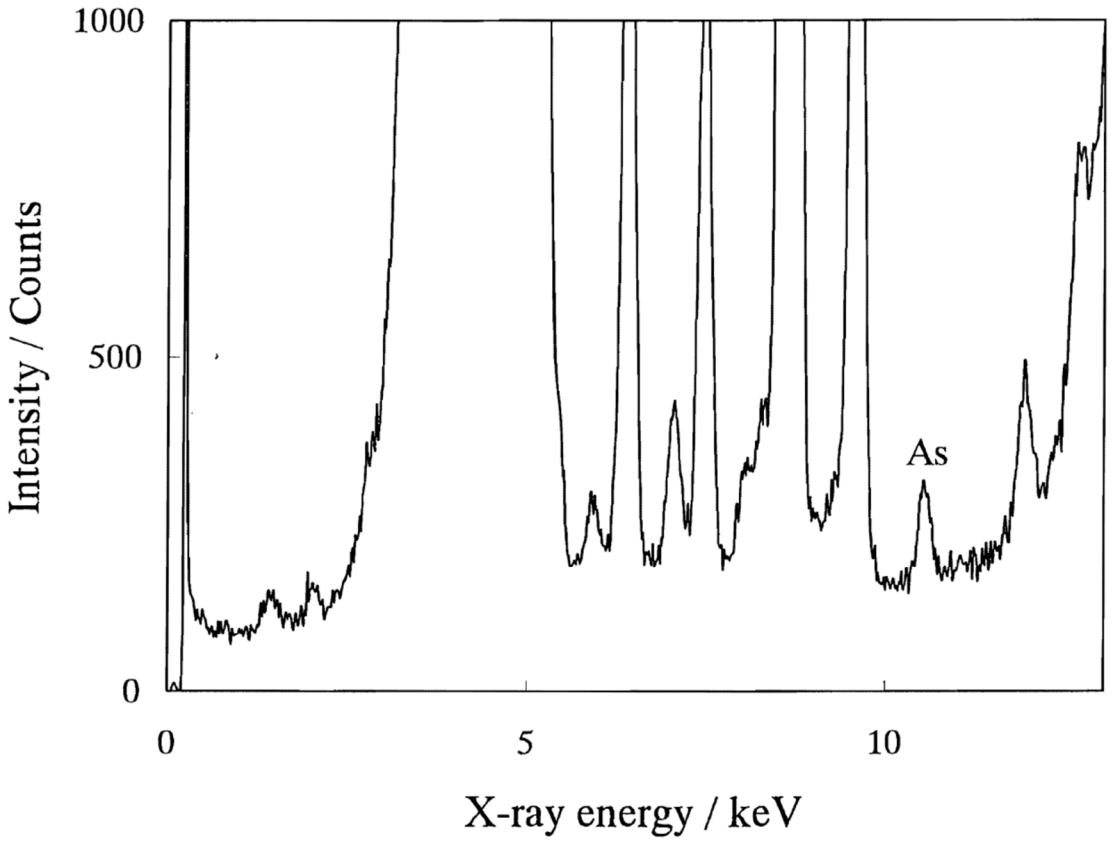

Fig.3. SR-XRF spectrum of suspect person's hair shaft measured on the BL-4A beamline of KEK-PF (High Energy Accelerator Research Organization, Photon Factory) by Nakai [10]. The Nakai's document [10] is a public document, and is not subjected to the copyright.

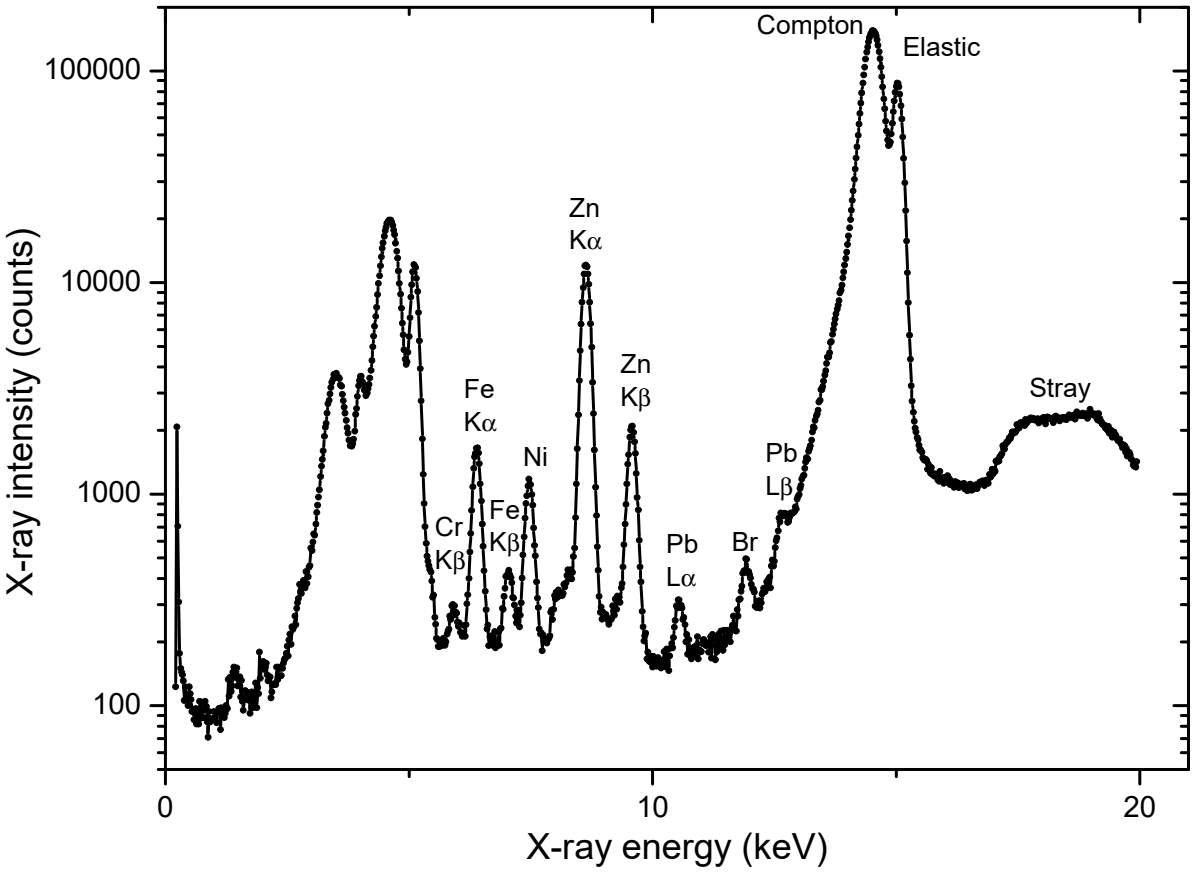

Fig.4. Plot of the whole spectrum of Fig.3, with logarithmic scale. Plot and peak assignments were done by Kawai. Sulfur Kα is not observed, even this spectrum is hair; hair usually contains 5-10% of sulfur. Fe, Cr, and Ni are stainless steel elements. Br is probably due to the flame retardant of electric circuits. These assignments indicate that the X-ray beam did not hit the hair shaft. Incident monochromatic beam energy was 15 keV, but strong stray light was also contaminated between 16-20 keV.