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A new fluorescence reagent: Synthesis, characterization and application for speciation of arsenic (III)/(VI) species in tea samples

Nur Öksüz^a, Şerife Saçmacı^{b,*}, Mustafa Saçmacı^a, Ahmet Ülgen^b

^a Bozok University, Department of Chemistry, Faculty of Arts and Sciences, TR-66200 Yozgat, Turkey

^b Erciyes University, Department of Chemistry, Faculty of Sciences, TR-38039 Kayseri, Turkey

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ABSTRACT

A sensitive and selective fluorimetric method for the rapid determination of trace amounts of As(III)/As(V) species in certain tea samples forms the basis of the study. 3',6'-bis(diethylamino)-2-[(1E)-(4,5-dimethyl-2-furyl)methylene]amino}spiro[isindole-1,9'-xanthen]-3(2H)-one (DMBD), was synthesized and characterized as a new fluorescence reagent for the speciation of As(III)/As(V) species. As(III) was quantitatively recovered with fluorescence reagent at concentrations between 0.5 and 1.5 mol L⁻¹ of HNO₃, while As(V) was not quantitatively recovered at any pH. The optimum conditions for the speciation of As(III)/As(V) species were investigated on certain commonly tested experimental parameters such as acidity and pH of the sample, amount of reagent, effects of temperature, and interfering ions etc. In order to determine trace amounts of total arsenic, KI and L(+)-ascorbic acid were used to reduce As(V) to the trivalent state in the sample solution prior to determination. The concentration of As(V) in the sample solution were calculated by the difference in concentration between As(III) and the total arsenic in the sample solution. The study yielded a linear function of the concentration of As(III) in the 0–5 mg L⁻¹ range with a detection limit of 0.22 µg L⁻¹. The relative standard deviation was found to be 0.3% for the 0.5 mg L⁻¹ As(III) level. The proposed method was successfully applied for the determination of trace amounts of As(III) and As(V) in certain tea samples. The results of analysis of the certified reference material (INCT-TL-1 tea sample) was found to be in good agreement with the certified value.

1. Introduction

Heavy metals are defined as metallic elements that have a relatively high density compared to water (Fergusson, 1990). Besides the fact that heaviness and toxicity are inter-related, heavy metals also include metalloids, such as arsenic, which are able to induce toxicity at a low level of exposure (Duffus, 2002). Recent years have witnessed, an increasing ecological and global public health concern associated with environmental contamination via these metals (Brad, 2002).

Arsenic is a ubiquitous element which is detected at low concentrations in virtually all environmental matrices. The major inorganic forms of arsenic include As(III) and As(V) while the major the organic forms are the methylated metabolites namely monomethylarsonic acid, dimethylarsinic acid and trimethylarsine oxide (Agency for Toxic Substances and Disease Registry (ATSDR)). As the degrees of toxicity of inorganic As(III) and As(V) are considerably different, it is not sufficient to determine the total content of arsenic in a given sample in order to estimate its physiological or environmental risks. Instead, alternative ways and means should be designed to identify their individual

quantification (Nielsen & Hansen, 1997).

Environmental pollution by arsenic occurs as a result of natural phenomena such as volcanic eruptions and soil erosion, and anthropogenic activities (Agency for Toxic Substances and Disease Registry (ATSDR)). Several arsenic-containing compounds are produced industrially, and they are used to manufacture products with agricultural applications, such as insecticides etc. They are also been used in veterinary medicine for the eradication of tapeworms in sheep and cattle (Tchounwou & Wilson, 1999). Arsenic compounds have also been used in the medical field for at least a century in the treatment of syphilis, yaws, amoebic dysentery, and trypanosomiasis (Tchounwou & Wilson, 1999; Centeno, Gray, Mullick, Tchounwou, & Tseng, 2005).

Analyzing the toxic effects of arsenic is complicated since the toxicity is highly influenced by its oxidation state and solubility, as well as by many other intrinsic and extrinsic factors (Centeno et al., 2005; Centeno, Tchounwou, et al., 2005). Several studies have indicated that the toxicity of arsenic depends on the exposure dose, frequency and duration, the biological species, age, and gender, individual susceptibilities, genetic and nutritional factors as well (Abernathy et al., 1999).

* Corresponding author.

E-mail address: sacmaci@erciyes.edu.tr (Ş. Saçmacı).

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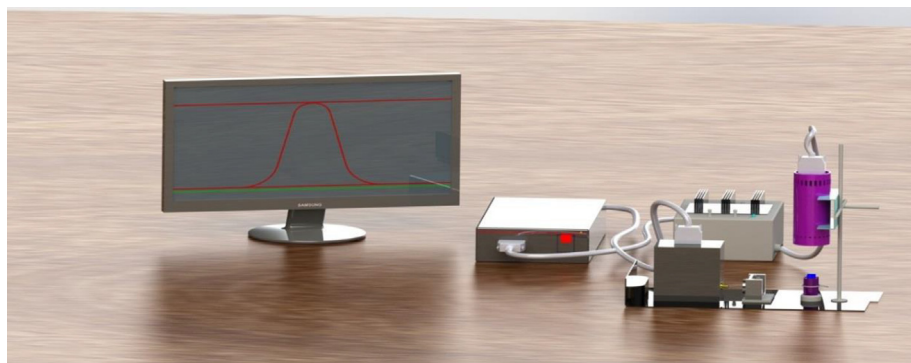


Fig. 1. Fluorimetry system.

Most cases of human toxicity from arsenic have been associated with exposure to inorganic arsenic. Inorganic trivalent arsenite is 2–10 times more toxic than pentavalent arsenate (Fergusson, 1990). Due to that, the U.S. Environmental Protection Agency (USEPA) lowered the standard of As in drinking water from 50 to 10 $\mu\text{g As L}^{-1}$, although at present, there is no known safe limit for As (Peralta-Videa et al., 2009). Another main route of arsenic exposure occurs through the ingestion of food containing arsenic, although arsenic levels in food are less strictly regulated compared to drinking water (World Health Organization, 2008). Exposure to inorganic As by food can exceed exposure by drinking water, and total arsenic concentrations consumed through food can rise up to $\sim 40 \mu\text{g day}^{-1}$, with the majority consisting of organo arsenicals (Kurzius-Spencer et al., 2014). In this context, the development of methods for separation/preconcentration of arsenic species are necessary.

The determination of elements with different oxidation stages in aqueous solutions is referred to as “speciation” (Macedo et al., 2009; Monasterio, Altamirano, Martinez, & Wuilloud, 2009). For the determination and speciation of As(III)/As(V) in various real samples, where the concentrations of arsenic are usually found at trace levels, highly sensitive and selective preconcentration techniques are required. Several analytical techniques have been used for arsenic determination at trace levels including hydride generation (HG) (Nielsen, Sloth, & Hansen, 1996), zetapotential analyzer (Saçmacı, Saçmacı, & Kök, 2018), electrothermal atomic absorption spectrometry (ETAAS) (Pozebon, Dressler, Gomes Neto, & Curtius, 1998) and inductively coupled plasma atomic emission spectrometry (ICP–AES) (Jitmanee, Oshima, & Motomizu, 2005) or mass spectrometry (ICP–MS) (Packer & Ciminelli, 2005).

Tea is one of the most common drinks all over the world, which is produced from the leaves of a shrub called *Camellia sinensis* (Dufresne & Farnworth, 2001). About 98% of people prefer tea to all other beverages available (Al-Saleh et al., 2008). Seventy five percent of the estimated 2.5 million metric tons of desiccated tea is processed as black tea, which is used by people in many countries (Lasheen, Awwad, El-Khalafawy, & Abdel-Rassoul, 2008). Around 18–20 billion cups of tea are consumed daily in the world (Ashraf & Mian, 2008).

Many studies have proved that tea has certain beneficial effects on human health such as the prevention of Parkinson’s disease, cardiovascular disease (Qin & Chen, 2007), cancer (Siddiqui, Raisuddin, & Shukla, 2005), immune disorders (Mark, 2007) and decrease in blood cholesterol levels (Fujita & Yamagami, 2008). Rapid urbanization and industrialization in recent decades have increased the levels of these metals in tea and other foods (Han, Shi, Ma, & Ruan, 2005). Plants adsorb these trace elements from growth media (Herrador & González, 2001); while other sources include the application of pesticides and fertilizers (Atafar et al., 2010).

As part of the current research (Saçmacı, Saçmacı, & Ülgen, 2017; Yıldız, Saçmacı, Saçmacı, & Ülgen, 2017), for a sensitive and selective monitoring of arsenic ions in some tea samples, we report herein a

novel method for the determination and speciation of arsenic, using the new fluorogenic ligand 3',6'-bis(diethylamino)-2-[(1*E*)-(4,5-dimethyl-2-furyl)methylene]amino}spiro[isoindeole-1,9'-xanthen]-3(2*H*)-one (DMBD). Factors affecting complexation and the effects of foreign species were investigated. Optimum conditions were used for the determination of As(III) in tea samples in the presence of other ions. The method was further applied for the determination of arsenic in commercial bottled water, tap water and natural water samples spiked with arsenic. The method was verified by analyzing samples of a certified reference material. The results showed good agreement with the certified value and the recoveries were sufficiently high.

2. Experimental

2.1. Apparatus

Fluorescence measurements were carried out through a home-made fluorimetry system equipped with a violet laser lamp. Instrumental parameters were controlled via a software program that we made (Fig. 1) (Saçmacı et al., 2017; Yıldız et al., 2017). Absorption spectra were recorded on a Hitachi (150-20) model UV–Vis spectrophotometer using a 1-cm quartz cell. IR spectra were recorded on a Perkin Elmer Spectrum Two Model FT-IR spectrophotometer using the ATR method in the range of 4000–400 cm^{-1} . The ^1H and ^{13}C NMR spectra were measured with a Bruker Avance III 400 MHz spectrometer. The chemical shifts (δ ppm) were recorded in parts per million (ppm) down field from TMS (assigned as zero ppm). The purity of the tested compound was determined by combustion elemental analyses with a Leco-932 CHNS-O elemental analyzer.

2.2. Reagents and the standard solutions

All the reagents were of the highest available purity and were used without purification (Merck, Darmstadt, Germany). Laboratory glassware was kept overnight in a 10% (v/v) HNO_3 solution and then rinsed with ultrapure water. A 1000 mg L^{-1} stock solution of As(III) was prepared by As_2O_3 (Merck). Similarly, a 1000 mg L^{-1} stock solution of As(V) was prepared by dissolving KH_2AsO_4 (Sigma). Working solutions were prepared from these stock solutions on a daily basis at 1.0 mol L^{-1} of HNO_3 . Hydrochloric acid, sulfuric acid, methanol, ethanol, sodium thiosulfate and potassium iodide were obtained from Merck. DMBD was firstly synthesized and characterized and used as a fluorescence reagent for the analysis of arsenic species. A solution of DMBD was prepared by dissolving 0.1 mg of the reagent in a 100-mL volumetric flask with ethanol.

2.3. Synthesis of the fluorescence reagent

The synthetic route of DMBD is shown in Fig. 2, in which rhodamine 6G hydrazide (RGH) was synthesized firstly. RGH was synthesized from

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