Microchemical Journal 124 (2016) 290–295

Contents lists available at ScienceDirect

Microchemical Journal

journal homepage: www.elsevier.com/locate/microc

Arsenic speciation in artificial saliva extract of smokeless tobacco products by extraction methodologies coupled with electrothermal atomic absorption spectrometry

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ARTICLE INFO

Article history:
Received 6 June 2015
Received in revised form 3 September 2015
Accepted 3 September 2015
Available online 11 September 2015

Editor: J. Sneddon

Keywords:
Arsenic speciation
Artificial saliva
Smokeless tobacco products
Cloud point and solid phase extractions

ABSTRACT

Simple and rapid preconcentration methods viz. cloud point extraction (CPE) and solid phase extraction (SPE) were applied for the determination of As^{3+} and total inorganic arsenic (iAs) in artificial saliva extract (ASE) of smokeless tobacco (SLT) product (*mawa*). The iAs species were separated from organic As by adsorbing on titanium dioxide (TiO_2), whereas the organic As was eluted out. The retained iAs species was eluted by 1.0 mL of 0.5 M NaOH solution. The CPE method has been used for the preconcentration of As^{3+}, using ammonium pyrrolidinedithiocarbamate (APDC) as complexing reagent. The resulting solutions of each method were determined by electrothermal atomic absorption spectrometry with a mixture of palladium and magnesium nitrates as modifier. The % recovery of As^{3+} and iAs were found to be >98%. The adsorption capacity of TiO_2 for iAs was calculated to be 11.5 mg g^{-1}. The accuracy of methods was tested by simultaneously analyzing certified reference material, Virginia tobacco leaves and spike recovery test. Under the optimized experimental conditions, the detection limits for As^{3+} and iAs were 28 and 20 ng mL^{-1} for CPE and SPE, respectively. The enrichment factor for SPE and CPE were obtained as 25 and 20, respectively. The concentration of As^{+3} and As^{5+} were found in the range of 26–45 and 50–98 ng g^{-1}, respectively in studied SLT product. The artificial saliva extractable arsenic (As) corresponds to 19%–25%, of total As in SLT product.

1. Introduction

The use of smokeless tobacco (SLT) is limited to some areas in the world, and it can be used orally or nasally. The main types of SLT in Western and Asian countries are chewing tobacco, with different gradients, and snuff. Although interest is growing in the patterns, distribution, consumption and compositions of SLT products and its use in various parts of the world [1,2]. The chewing SLT products are composed of a mixture of tobacco, catechu, areca nut, slaked lime, flavors and sweetened by different spices, except snuff, which is made by different processing of tobacco leaves [3]. The *mawa* product, for example, is made up of small pieces of sun-cured areca nut mixed with tobacco flakes and slaked lime.

Agricultural soils and products may become contaminated by As due to natural processes and human activities. Pesticides and herbicides have been major sources of As in agriculture, while other agronomic practices and pollution arising from nearby industries also represents potential sources of this metalloid in agricultural soils [4]. Tobacco (*Nicotiana tabacum*), like other crops, can absorb As from soil and concentrates in the leaves [5]. In the last decades, As received much attention as humans may be exposed to this toxic element through occupational and environmental exposure [6]. The International Agency for Research on Cancer has classified it as carcinogenic to humans [4].

The exposure of As may cause gastrointestinal irritation, decreased production of red and white blood cells, abnormal heart rhythm, damage of blood vessels, "pins and needles" sensation in hands and feet as well as damage to the internal organs [7,8]. The effect of As exposure on human health was observed in populations in south and southeastern Asia, particularly in Bangladesh, Taiwan, India and Pakistan [9–11]. Different forms of As species (either by arsenic valence or by associated anions) are known to have different toxicities [7]. The most toxic As species are in inorganic forms, such as, arsenite (As (III) O_3^{3-}) and arsenate (As(V)O_3^{4-}). Arsenite is 60 times more toxic than arsenate [12]. In contrast, organic arsenicals in food and plants (arsenobetaine, monomethyl

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http://dx.doi.org/10.1016/j.microc.2015.09.002
0026-265X/

arsenic acids, dimethylarsonic acids, arsenochline and arsenosugars, etc.) have less toxicity [7]. Inorganic arsenicals belong to group I carcinogens, it causes cancers of the skin, lungs and bladder [13–15].

Several analytical techniques, electrothermal atomic absorption spectrometry (ETAAS) [16], inductively coupled plasma-atomic emission spectroscopy [17] and inductively coupled plasma mass spectrometry [18] are used for the determination of trace elements with sufficient sensitivity. The analysis of As concentrations in biological/environmental samples might be considered a difficult analytical task, mostly due to the complexity of the matrix and the low concentrations of analyte, which require sensitive instrumental techniques and often a preconcentration step [19]. Moreover, it is necessary to develop sensitive and precise methods to identify and quantify iAs species in real samples. For the speciation of As, the separation and preconcentration methods reported in the literature are solid phase extraction [19,20], gold nanoparticle loaded on activated carbon with bis(4-methoxysalicylaldehyde)-1,2-phenylenediamine as sorbent [21], cloud point extraction [16] and dual cloud point extraction methods [22]. The SPE is a simple and effective technique for As speciation. It can eliminate transition metal interferences and improve the selectivity and sensitivity of the measurement [23]. Numerous substances have been used as solid phase extraction sorbents for the separation and preconcentration of As species, such as anion exchange resin [24], PTFE turnings [25], mesoporous TiO_2 [26,27] and immobilized yeast [28]. The CPE method can be used for the extraction of As^{3+} species, based on the phase separation of nonionic surfactants in aqueous media [16]. These methodologies are simple, low cost, environmentally friendly and provides high enrichment factor.

Very little research has been carried out on the intake of As (in populations from all age groups in Pakistan and other Asian countries) with regards to different SLT products. Based on the above information, the present study was aimed at investigating the level of total As, iAs species (As^{3+}, As^{5+}) in *mawa* that is consumed and available in Pakistan. To estimate the As contents by extracting in artificial saliva to better understand its level to which users of *mawa* are immediately exposed to toxicants. The iAs was determined by SPE using TiO_2 as the adsorbent, while As^{3+} was determined by the CPE method using APDC as a complexing reagent. The optimal conditions for As speciation were also investigated in detail. The accuracy of these methodologies has been validated by spiked recovery test.

2. Materials and methods

2.1. Study population

A survey was carried out about the *mawa* product–consuming habits of people (both genders), at an age range of 30–60 years, from different cities of Pakistan. Before the start of this study, all users were informed about the aims of this study, and all agreed to provide information about chewing habits of SLT products and signed the form. A questionnaire was administered to them for collecting the details regarding physical data, ethnic origin, health, duration and frequency of SLT consumption, age and consent. This study was approved by the ethics committee of NCEAC, University of Sindh, Pakistan.

2.2. Sampling

Samples of different brands of *mawa* product ($n = 12$) were collected/bought from the local markets as per their availability and usage by the people of South Eastern Province of Pakistan. Samples of the same brand were mixed together to obtain a representative sample of that product. Brand names have not been disclosed in this paper due to legal requirements. Ten composite samples were made by homogenizing the mixture by removing the wrappers of samples collected at different time intervals (Jan 2014 to Dec 2014). All samples were dried at 80 °C. The dried samples were ground with agate mortar and pestle, sieved through nylon sieves with mesh sizes of 125 μm, and then stored in the labeled sample bottles.

2.3. Reagents and glassware

Ultra-pure water obtained from ELGA labwater system (Bucks, UK). Concentrated nitric acid (65%), hydrogen peroxide (30%), hydrochloric acid (37%) and α-amylase were obtained from Merck (Darmstadt, Germany). The nonionic surfactant (Triton X-114) was obtained from Sigma (St. Louis, MO, USA), and used without further purification. The sodium carboxymethyl cellulose and methyl-*p*-hydroxybenzoate were obtained from Daejung reagents chemicals (Korea), and Scharlau (Spain), respectively. The 0.1 M of acetate buffer was used to control the pH of the solutions. The pH of the samples was adjusted to the desired pH (1–8) by the addition of 0.5 M HNO_3/NaOH solution in acetate buffer. The certified standard solutions of As (1000 mg L^{-1}) were obtained from Fluka Kamica (Bush, Switzerland). The artificial saliva was prepared according to the Macknight-Hanes and Chou formula [29,30]. The artificial saliva was made from different salts (Sigma) in g L^{-1}, KCl (0.625), $CaCl_2.2H_2O$ (0.166), $MgCl_2.6H_2O$ (0.059), K_2HPO_4 (0.804), KH_2PO_4 (0.326), methyl-*p*-hydroxybenzoate (2.0), sodium carboxymethyl cellulose (10.0) and 2.5 units mL^{-1} (2500 units L^{-1}) of α-amylase. Titanium (IV) dioxide (TiO_2) (Merck, 99%, 0.5 mm) was utilized as a sorbent. The certified reference material (CRM) Virginia tobacco leaves (ICHTJ-cta-VTL-2) was purchased from the International Atomic Energy Agency, Vienna (Austria). Glassware and polyethylene containers were soaked in 10% (v/v) HNO_3 for 24 h; washed with distilled water then deionized water and dried in such a manner to ensure that no any contamination from glassware occur.

2.4. Instrumentation

The determination of As was carried out by means of a double beam Perkins-Elmer atomic absorption spectrometer model 700 (Norwalk, CT, USA) equipped with the graphite furnace HGA-400, autosampler AS-800 and deuterium lamp as background correction system. Hollow cathode lamps were used as radiation sources. The pyrocoated graphite tube with integrated platform was used. The analytical wavelength was set at 193.7 nm, operated at 7.5 mA with a spectral bandwidth of 0.7 nm. The graphite furnace heating program was set for different steps: drying, ashing, atomization and cleaning as temperature range °C/ramp time in seconds/holding time in seconds; 80–120/1/30, 300–600/10/20, 2000–2100/0/5, and 2100–2400/0/2, respectively. Portions of both standard or sample and modifier were transferred into auto-sampler cups, and 20 μL of standard or sample volume (10 μL) and 10 μL of modifier (3 μg Pd + 5 μg Mg $(NO_3)_2$) were injected into the electrothermal graphite atomizer. A horizontal flask electrical shaker (220/60 Hz, Gallenkamp, England) was used for shaking the samples. A pH meter (Ecoscan Ion 6, Malaysia) was employed for pH adjustments. A PEL domestic microwave oven (Osaka, Japan), programmable for time and microwave power from 100 to 900 W, was used for digestion of different SLT samples. The phase separation was assisted with a centrifuge ROWKA Laboratoryjna type WE-1, nr-6933 (Mechanika Phecyzyjna, Poland). A programmable ultrasonic water bath, model no. SC-121TH (Sonicor, Deep Park, NY, USA) was used for incubation with temperature ranging from 0 °C to 80 °C at an intensification frequency of 35 kHz.

2.5. Determination of total As in SLT

Weighed 0.2-g replicates from six samples of CRM (Virginia tobacco leaves), and in triplicate (dry weight) of all composite samples of *mawa* (0.2 g), were placed directly into PTFE flasks (25 mL in capacity). A total of 2 mL of a freshly prepared mixture of concentrated HNO3–H2O2 (2:1, v/v) was added to each flask. The flasks were kept for 10 min at room temperature and placed in a covered PTFE container, then heated at 80% of the total power (900 W) at time intervals of 3–5 min. After