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Simultaneous preconcentration of toxic elements in artificial saliva extract of smokeless tobacco product, mainpuri by cloud point extraction method

Sadaf Sadia Arain [a,*], Tasneem Gul Kazi [a,1], Jamshed Bashir Arain [a,b], Hassan Imran Afridi [a,1], Kapil Dev Brahman [a,2], Faheem Shah [a,1], Naeemullah [a,1], Salma Arain [a,1], Abdul Haleem Panhwar [a,1]

[a] *National Centre of Excellence in Analytical Chemistry, Sindh University, Jamshoro, Sindh 76080, Pakistan*
[b] *Muhammad Medical College Mirpurkhas, Sindh, Pakistan*

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ABSTRACT

It has been extensively investigated that smokeless tobacco chewing can lead mainly to inflammation of oral cavity. In present study, the total and artificial saliva extracted toxic elements, arsenic, cadmium, nickel and lead were estimated in smokeless tobacco product, mainpuri. Cloud point extraction has been used for the preconcentration of arsenic, cadmium, nickel and lead in artificial saliva extract, using complexing reagent, ammonium pyrrolidinedithiocarbamate. Total and extractable toxic elements were measured by electrothermal atomic absorption spectrometry. The chemical variables of cloud point extraction were optimized. The validity of methodology was tested by simultaneously analyzing certified reference material (Virginia tobacco leaves) and spike recovery test. The artificial saliva extractable levels of arsenic, cadmium, nickel and lead ranged from 15–22, 45–70, 35–58, and 18–32%, respectively, of total elemental contents in mainpuri samples. It was estimated that intake of 10 g of different brands of mainpuri contributing the 5.88, 55.0, 45.0 and 40.3% of the provisional maximum tolerable daily intake for arsenic, cadmium, nickel and lead, respectively for adults of ~60 kg.

1. Introduction

The consumption of tobacco products and number of smokers have been increasing steadily world over. The use of smokeless tobacco (SLT) is limited to some areas in all continents, and it can be used orally or nasally. The main types of SLT in Western and Asian countries are chewing tobacco and oral snuff. Although interest is growing in the patterns, distribution, consumption and compositions of SLT products and its use in various parts of the world (Subramanian et al., 2004). Use of SLT is integral to the culture of South Asia. The SLT users in India and Pakistan together have been estimated to be above 100 million (IARC, 1996; Gupta and Ray, 2003).

The chewing SLT products are composed of mixture of tobacco, catechu, areca nut, slaked lime, flavors and sweetened by different spices, except snuff which are made by different processing of tobacco leaves (Richter and Spierto, 2003). The mainpuri one of the SLT product, made up of pieces of tobacco leaves, finely cut betel nut and other ingredients are mixed thoroughly with lime (Gupta and Ray, 2004). Mainpuri tobacco, taking its name from a mainpuri district in the northern state of Uttar Pradesh (Gupta and Ray, 2003). The mainpuri is chewed slowly, and in this process the aqueous extract due to saliva is not only absorbed locally but also ingested to enter the circulation system.

Generally, chewing of SLT products including mainpuri was found to be closely associated with the occurrence of oral cancer and other problems in children and adults (Boucher and Mannan, 2002; Shah et al., 2002; Gupta and Ray, 2003). The incidence of oral submucous fibrosis is common, especially in the youngsters who consume SLT products, even after a short period of use (Nair et al., 2004). In Pakistan, oral cancer ranks 2nd in all malignancies among both males and females, with the highest reported incidence in the world. In the absence of alcohol use, chewing of SLT products remain the main etiological risk factors (Khawaja et al., 2005; Mazahir et al., 2006).

Elemental level of tobacco is a function of many factors like soil characteristics, climatic conditions and plant variety (Angelova et al., 2003; Lugon-Moulin et al., 2006). Tobacco plant (*Nicotiana*

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[*] Corresponding author. Fax: +92 22 9213431.
E-mail addresses: ssadiashafi@gmail.com (S.S. Arain), tgkazi@yahoo.com (T.G. Kazi), surgeon_jamshed@yahoo.com (J.B. Arain), hassanimranafridi@yahoo.com (H.I. Afridi), kr_brahman@yahoo.com (K.D. Brahman), shah_ceac@yahoo.com (F. Shah), khannaeemullah@ymail.com (Naeemullah), salma_arain67@yahoo.com (S. Arain), haleem_analyst@yahoo.com (A.H. Panhwar).
[1] Fax: +92 221 771560.
[2] Fax: +92 22 9213431.

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tabacum) is well known for its capacity to concentrate toxic elements (TEs) from its growing environment (Golia et al., 2007).

Arsenic (As) is a toxic element and classified as a human carcinogen (WHO, 1981). 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 the internal organs (Abernathy et al., 2003; Hossain, 2006). The effect of As exposure on human health was observed in population of south and southeastern Asia, particularly in Bangladesh, Taiwan, India and Pakistan (Das et al., 2004; Rmalli et al., 2005; Hall et al., 2006; Lin and Liao, 2008; Baig et al., 2009). Cadmium (Cd) is a non-essential, potentially toxic, largely pollutant ion, which is accumulated by tobacco plants and is transferred in tobacco smoke to humans (Tsadilas and Soilp, 2000; Chen and Teo, 2001; Sravrides, 2006). The Cd is an inhibitor of the enzymes with sulphydryl groups and disrupts the pathways for the oxidative metabolism (Miccadei and Floridi, 1993; Verougstraete et al., 2003). The tobacco plant in addition to other TEs also absorbs lead (Pb) and nickel (Ni) from the soil and concentrates them in the leaves (Goyer, 1996). The Ni is currently classified as Group 1 "carcinogenic to humans" by IARC (2004) and the Pb exposure causes lipid peroxidation in brain and hepatic tissues (Stohs and Bagchi, 1995).

The analysis of TEs concentrations in biological/environmental samples might be considered a difficult analytical task, mostly due to the complexity of the matrix and the low concentration of these elements, which requires sensitive instrumental techniques and often a preconcentration step (Soylak et al., 2011; Gopikrishna et al., 2004; Yunes et al., 2003; Suvardhan et al., 2004). The analytical potential of cloud point extraction (CPE) has been discussed by several authors, comprising this method as green chemistry (Manzoori and Tabrizi, 2002; Ghaedi et al., 2008). The CPE is based on the phase behavior of non-ionic surfactants in aqueous solutions, which exhibit phase separation after an increase in temperature or the addition of a chelating agent (Baig et al., 2010; Espergham et al., 2011; Shah et al., 2011).

Several analytical techniques, electrothermal atomic absorption spectrometry (ETAAS) (Kazi et al., 2009), inductively coupled plasma-atomic emission spectroscopy (Ryan and Clark, 2010) and inductively coupled plasma mass spectrometry (Musharraf et al., 2012) are used for the determination of trace elements in tobacco products with sufficient sensitivity. Very little research has been carried out on the intake of TEs from mainpuri by the population of all age group in Pakistan and other Asian countries.

Based on the above information, present study was aimed to investigate the total TEs (As, Cd, Ni and Pb) concentrations in SLT product (mainpuri) consumed and available in Pakistan. To estimate the TEs contents by extracting in artificial saliva to better understand the levels of TEs to which users of mainpuri are immediately exposed. The CPE method was applied for the determination of TEs in artificial saliva extract. For proposed CPE method, ammonium pyrrolidinedithiocarbamate (APDC) was used as a chelating agent and Triton X-114 as non-ionic extractant. The effect of different variables, concentration of complexing reagent, Triton X-114, pH, equilibrium time and temperature were investigated and discussed in detail. The accuracy of CPE method was evaluated by spiked recovery test. The intake of total and extractable TEs contents was also calculated. According to authors knowledge the analysis of mainpuri for TEs was not done previously.

2. Materials and methods

2.1. Study population

A survey was carried out about the chewing habit of mainpuri, from people (both genders), age ranged 15–60 years, of two cities of Pakistan. A questionnaire was administered to them for collecting the details regarding physical data, ethnic origin, health, duration and frequency of mainpuri 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 mainpuri ($n=12$) 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 composites samples of each product were made by homogenized the mixture by removing the wrappers of samples of each product collected at different time interval (January to December 2011). 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, then stored in the labeled sample bottles.

2.3. Reagents and glassware

Ultra-pure water obtained from ELGA labwater system (Bucks, UK). Certified reference material Virginia tobacco leaves (ICHTJ-cta-VTL-2) was purchased from International Atomic Energy Agency, Vienna (Austria). Concentrated nitric acid (65%), hydrogen peroxide (30%), α-amylase was obtained from Merck (Darmstadt, Germany). The nonionic surfactant (Triton X-114), was obtained from Sigma (St. Louis, MO, USA), and was 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 (3–8) by the addition of 0.1 M HNO_3/NaOH solution in acetate buffer. The certified standard solutions of As, Cd, Ni, Pb (1000 mg L^{-1}) and APDC were obtained from Fluka Kamica (Bush, Switzerland). The artificial saliva was prepared according to McKnight-Hanes and Whitford (1992), Chou and Hee (1994) formula. The artificial saliva was made from different salts (Sigma) in g L^{-1}, KCl (0.625), $CaCl_2 \cdot 2H_2O$ (0.166), $MgCl_2 \cdot 6H_2O$ (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 (Chou and Hee, 1994).

Glasswares and polyethylene containers were soaked in 10% (v/v) HNO_3 for 24 h; washed with distilled water, finally with de-ionized water and dried in such a manner to ensure that no any contamination from glasswares occur.

2.4. Instrumentation

The determination of As, Cd, Ni and Pb were 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, pyrocoated graphite tubes with integrated platform and an autosampler AS-800 and deuterium lamp as background correction system. Hollow cathode lamps were used as radiation sources. Hollow cathode lamps (PerkinElmer) operating at recommended current were used for all cases. The instrumental parameters are shown in Table 1. 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 mainpuri 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 to 80 °C at intensification frequency of 35 kHz.

2.5. Determination of pH

Weighed 1 g sample of each brand of mainpuri product, added 10 mL of ultrapure water in flask (100 mL capacity), and placed in an electrical shaker at 30 rpm for 30 min, then filtered the solution through Whatman no. 42 filter paper and extracts were taken to determine the pH.

2.6. Microwave assisted acid digestion

A microwave assisted acid digestion procedure was carried out, in order to achieve a shorter digestion time. Weighed 0.2 g replicates six samples of CRM (Virginia tobacco leaves), 0.2 g triplicate (dry weight) of different brands of mainpuri directly into PTFE flasks (25 mL in capacity). For further validation, spike recovery study of each element in a real mainpuri sample, at three concentration levels were also carried out. Added to each flask was 2 mL of a freshly prepared mixture of concentrated $HNO_3–H_2O_2$ (2:1, v/v). The flasks were kept for 10 min at room temperature and then placed in a covered PTFE container, then heated at 80% of the total power (900 W) for time intervals 3–5 min. After digestion, the flasks were left to cool and the resulting solution was evaporated to semidried mass to