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Preconcentration of toxic elements in artificial saliva extract of different smokeless tobacco products by dual-cloud point extraction

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ABSTRACT

Smokeless tobacco (SLT) has been associated with oral cancer. In present study, the total and artificial saliva extracted toxic elements (TEs), arsenic (As), cadmium (Cd), nickel (Ni) and lead (Pb) were estimated in SLT products (gutkha, green and brown snuff). Dual-cloud point extraction has been used for the preconcentration of As, Cd, Ni and Pb in artificial saliva extract, using complexing reagent, ammonium pyrrolidinedithiocarbamate. The dual-cloud point extraction procedure was based on forming complexes of elemental ions with complexing reagent and subsequent entrapping of the complexes in nonionic surfactant. Then the surfactant rich phase containing the complexes was treated with nitric acid, and detected ions were back extracted again into aqueous phase at the second cloud point extraction stage, and finally determined by electrothermal atomic absorption spectrometry. The validity of methodology was tested by simultaneously analyzing certified reference material and spike recovery test. The experimental enhancement factors of As, Cd, Ni and Pb were found to be 52, 58, 48 and 44, respectively. The concentration of As, Cd, Ni and Pb in artificial saliva extracts corresponds to 18–24, 45–75, 30–55, and 20–30%, respectively, of the total contents of TE in different types of SLT products (gutkha, brown and green snuff). It was estimated that 10 g intake of SLT products (gutkha, brown and green snuff) could contribute 2.2–13, 21–69, 3.4–48 and 10–75% of the provisional maximum tolerable daily intake of As, Cd, Ni and Pb, respectively for adults ~60 kg.

1. Introduction

The use of SLT is limited to some areas in all continents, and it can be used orally or nasally. Nowadays the interest is growing in the patterns, distribution, consumption, and compositions of SLT products and their use in various parts of the world [1]. The use of SLT is integral to the culture of South Asia and its users have been estimated to be above 100 million [2].

The SLT has been established to cause one of the ten leading cancers i.e. oral cancer, irreversible gingival recession, oral submucous fibrosis, other oral pathologies, worsening of asthma, nicotine addiction, cardiovascular diseases, accidental inhalation and its consequent complications in children [3]. Time trend analysis of Pakistan and India has shown an increase in oral cancer among the peoples due to increased consumption of the alternative chewing products [2,4]. The chewing tobacco product is basically a flavored and sweetened dry mixture of areca nut, catechu, and slaked lime with tobacco [5]. The chewing SLT product, gutkha, has been classified as carcinogenic to humans and may be associated with oral disease [4,6].

Gutkha is commercially available in foil packets/sachets and tins, made of sweetened dry mixture of areca nut, catechu and slaked lime with tobacco [7]. It was reported that the gutkha has stimulant and relaxation effects [8]. Moist snuff consists of fire- and air-cured dark tobaccos that are finely cut. It is now the most popular form of SLT in the US; where the sales of this product have increased by 77% over the past 15 years [9]. Moist snuff is also very popular in Sweden, where it is called 'snus'. One reason for the popularity of moist snuff in both countries is that it has become more user-friendly. Traditional moist snuff users place a 'pinch' of the finely ground tobacco between the gingiva and buccal mucosa. Modern moist snuff products are sold in small, pre-portioned pouches similar to teabags. The brown moist snuff (BM) contains roasted tobacco leaves, gum, calcium oxide and water, while green moist snuff (GM) is prepared from fresh tobacco leaves, oil, menthol and water.

In general, tobacco plants accumulate heavy metals like Pb, Cd, and Ni preferentially. The As, Cd, Ni and Pb are known to be carcinogenic for humans [10]. Tobacco is known to contain numerous classes of carcinogenic substance such as tobacco-specific nitrosamines, which are often regarded as a major factor in smokeless tobacco-related

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carcinogenesis [11]. The combined exposure of nitrosamines and other classes of organic and inorganic substances, including toxic metals and metalloids enhances the carcinogenetic effects [10,11]. Human exposure to As via different routes such as water and foods can lead to diverse disease processes. However, intake of As from non-food sources is often overlooked although they may be a contributory factor in the development of disease and this requires further investigation. The effect of As exposure on human health was observed in population of south and southeastern Asia, particularly in Bangladesh, Taiwan, India and Pakistan [12–14]. Lindberg et al. reported that As content of tobacco and zarda, which are often included in betel quid, highlighted that tobacco chewing can further increase the risk of As induced skin lesions among people of As endemic areas [15].

The Cd has no known functions in the metabolism of eukaryotes. It was reported that Cd is an inhibitor of the enzymes with sulphydryl groups and disrupts the pathways for the oxidative metabolism [16]. It was studied that administration of Pb to experimental animals, results in the production of lipid peroxidation in brain and hepatic tissues [17]. As is the case with Cd, tobacco plants absorb Ni from the soil and concentrate it in the leaves. The Ni is currently classified as Group 1 "carcinogenic to humans" by the International Agency for Research on Cancer [18]. The Ni has been long known to produce nasal and lung cancers [19].

The habit of chewing gutkha and other SLT products is widespread across all socio-economic groups in Pakistan, particularly in lower socio-economic strata who constitute the major population. Although the majority of the peoples believed that the consumption of SLT creates adverse effects on health, they still continued the use of these substances for the taste, followed closely by the low cost and convenient availability. It is evident that costs and consequences of tobacco use impose a heavy social and economic burden on a nation.

Several analytical techniques, electrothermal atomic absorption spectrometry (ETAAS) [20], inductively coupled plasma-atomic emission spectroscopy [21] and inductively coupled plasma mass spectrometry [22] are used for the determination of trace elements in tobacco products with sufficient sensitivity. The analysis of TE 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 [23]. The preconcentration method, cloud point extraction (CPE) has been discussed by several authors, comprising this method as green chemistry [20,24]. 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 [25,26]. As the CPE is primarily based on the hydrophobic interaction between the solutes and surfactant, other hydrophobic species can be extracted into the surfactant-rich phase and may interfere with the analysis of interested analytes.

Here with a dual-cloud point extraction (d-CPE) technique, the drawbacks of traditional CPE problems are overcome. The d-CPE procedure is carried out twice during a single sample pretreatment process. The first part of d-CPE procedure is done just as traditional CPE. The surfactant is added into the solution containing the analytes that are hydrophobic or can form hydrophobic complexes with suitable ligands. After heating in thermostatic bath and centrifugation, the interested analytes and other hydrophobic interfering species are extracted into the surfactant-rich phase. But, instead of the direct analysis, another round of CPE procedure, in which, surfactant-rich phase is treated with another aqueous solution is done. After heating at control temperature in thermostatic bath and centrifugation, the interested analytes are back-extracted into aqueous phase [27,28]. The obtained aqueous extract can be analyzed by different techniques. Due to the introduction of the second CPE procedure, the effects of the surfactant on the analysis and separation are eliminated. The removal of interfering species through the d-CPE procedure improves the analysis method selectivity extensively, and besides the aqueous sample solution is naturally compatible with the conditions of atomic absorption spectrometry.

Very little research has been carried out on the intake of toxic elements (TE) from the different SLT products, gutkha, green and brown moist snuff 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 TE (As, Cd, Ni and Pb) concentrations in SLT product (gutkha and moist snuff) consumed and available in Pakistan. To estimate the TEs contents by extracting in artificial saliva, may help to better understand the levels of TEs to which users of SLT products are immediately exposed. The proposed d-CPE method was used to eliminate the effects of surfactant on the analysis and separation of trace quantity of toxic elements. Nitric acid was chosen as back extraction agent to extract elemental ions (As, Cd, Ni, Pb). The APDC was employed as a chelating agent in d-CPE. The effect of different variables, concentration of complexing reagent, Triton X-114, pH, equilibrium time and temperature was investigated and discussed in detail. The intake of total and extractable TE contents was also calculated.

2. Materials and methods

2.1. Study population

A survey was carried out about the chewing habit of gutkha and moist snuff, from people (both genders), with age range of 12–60 years, of different cities of Pakistan. Before the start of this study, all users were informed about the aim of the study, and all agreed to participate 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. From the analysis of 642 questionnaires, we found that more than 58% people consumed both moist snuff and gutkha, and 32% people consumed only gutkha (mostly laborers and drivers). About 10% of these participants were also smokers.

2.2. Sampling

Samples of different brands of gutkha ($n = 11$) and moist snuff ($n = 14$) 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 of each product collected at different time intervals (Jan to Dec 2012). 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 was obtained from ELGA LabWater system (Bucks, UK). The CRM Virginia tobacco leaves (ICHTJ-cta-VTL-2) were purchased from International Atomic Energy Agency, Vienna (Austria). 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 (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}) were obtained from Fluka Kamica (Bush, Switzerland). The artificial saliva was prepared according to McKnight-Hanes and Chou formula [29,30]. The artificial