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Cloud point assisted dispersive ionic liquid -liquid microextraction for chromium speciation in human blood samples based on isopropyl 2-[(isopropoxycarbothiolyl)disulfanyl] ethane thioate

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ABSTRACT

An efficient and fast method based on isopropyl 2-[(isopropoxycarbothiolyl)disulfanyl] ethane thioate (IICDET) were used for the speciation and determination of trace amount of Cr(III and VI) in human biological samples by cloud point assisted dispersive ionic liquid –liquid microextraction (CP-DILLME). Cr(VI) has carcinogenic effects, so, speciation of chromium in human body such as blood cells is very important. The cloudy solution was achieved by the mixture of acetone and IL ($[C_8MIM][PF_6]$) in human blood samples containing Cr(III) ions that were already complexed by IICDET at pH 4.5. After reduction Cr(VI) to Cr(III) by ascorbic acid, chromium speciation was obtained based on total chromium determination by electro thermal atomic absorption spectrometry (ET-AAS) and difference between total Cr and Cr(III) content. In addition, Cr speciation in human blood cells was calculated based on IICDET/CP-DILLME and hematocrit blood test (HCT). After optimized conditions, the enrichment factor (EF), Linear range and limit of detection (LOD) was obtained 25.2, 0.02–1.75 μg L^{-1} and 5.4 ng L^{-1} in human biological samples respectively. The validation of methodology was achieved by certified reference material (CRM) and ICP-MS technique.

1. Introduction

Recently, heavy metals get distinguished from other toxic pollutants which, due to their non-biodegradability can accumulate in living tissues, thus becoming concentrated throughout the food chain and can be readily absorbed by the human body. Even a very small amount of them can cause severe physiological or neurological damage to the human body. Chromium is a major pollutant for the environment, usually as a result of some industrial pollution including tanning factories, steelworks, industrial electroplating and wood preservation. Among the elements that currently need to be determined in environmental and pharmaceutical products, chromium is important in view of some health risks such as chromosomal aberration, mutations, carcinogenicity, and transformation in cultured cells and a variety of DNA lesions [1–3].

Chromium species exist mainly in two different oxidation states in the environment, Cr(III) and Cr(VI), which have contrasting physiological effects. Cr(III) compounds play an important role in the metabolism of glucose and certain lipids; in addition, it is considered an essential trace element for the maintenance of an effective protein metabolism in humans [4–6]. On the contrary, the Cr(VI) toxic and carcinogenic effects present are due to their strong oxidation potential and their relatively small size, which enables them to penetrate through biological cell membranes, providing damage to macromolecules such as proteins and DNA. Cr(VI) inhibits the enzymatic sulfur uptake of the cell and is also harmful to lungs, liver and kidneys [7–9].

Chromium can enter the human body through breathing or drinking water, and the level of it in the air, water and biological samples is very low. Chromium concentration in drinking water is generally less than 2 μg L^{-1} [10]. The World Health Organization (WHO) states that the guideline values of 50 μg L^{-1} Cr(VI) are considered to be too high as compared to its genotoxicity, and the

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national health and nutrition examination survey (NHANES) claims normal chromium levels found in blood are 0.1–1.7 μg L^{-1} and 0.24–1.8 μg L^{-1} in urine [11–13]. Cr(VI) compounds, once inside the bloodstream, are actively transported into red blood cells (RBC) via nonspecific anionic channels and then rapidly reduced to Cr(III) which becomes bound to hemoglobin in RBCs ($Cr^{6+} \rightarrow Cr^{5+} \rightarrow Cr^{4+} \rightarrow Cr^{3+}$). Therefore, chromium levels in the red blood cells indicate exposure to Cr(VI) and can be changed to Cr(III) within these cells [14]. The reactive species produced by the reduction of Cr(VI), particularly Cr(III), can form both ionic and coordinate covalent complexes with DNA as inter strand cross-links (DNA-Cr-DNA). In order to provide a timely warning of chromium exposure, it is highly desirable to develop suitable procedures for chromium speciation.

Sensitive techniques for determination of chromium species include ion chromatography, inductively coupled plasma mass spectrometry [15], luminescence quenching [16], stripping voltammetry [17], co-precipitation [18,19], flame atomic absorption spectrometry (F-AAS) [20], neutron activation analysis (NAA) [21], inductively coupled plasma optical emission spectrometry (ICP-OES) [22,23], inductively coupled plasma-mass spectrometry (ICP-MS) [24], ion chromatography inductively coupled plasma-mass spectrometry (IC- ICP-MS) [15] and electrothermal atomic absorption spectrometry (ETAAS) [25,26] analysis techniques that were frequently coupled with a prior preconcentration and/or separation steps. However, the high instrumental and operational costs and the high detection limits are common disadvantages of many of these methods. Sample preparation procedures such as liquid-liquid extraction (LLE) [27,28], homogeneous liquid-liquid extraction [29,30], solid phase extraction (SPE) [4,31], liquid-phase microextraction (LPME) [32] and cloud point extraction (CPE) [33] are developed to simplify analytical approaches as it reduces costs.

Dispersive liquid-liquid micro-extraction (DLLME) is a miniaturization of the traditional LLE technique, where the extraction phase is a drop of a few microlitres of a water-immiscible solvent that can be directly immersed in the sample and dispersed by organic solvent. Although organic solvents (i.e., octanol, cyclohexane, and toluene) are useful as extraction phase, recently the use of ionic liquids (IL) has been proposed in LLE. They have various advantages over traditional organic solvents, such as low vapour pressure, high stability, large viscosity, adjustable miscibility and polarity, good extractability for different organic and inorganic compounds [34–37].

Cloud point extraction (CPE) utilizes the clouding behavior of a solution that containing a nonionic surfactant is heated before being allowed to settle. Because the surfactant is dehydrated during the settling process, the liquid separates into aqueous and surfactant-rich phases. The cloud point phenomenon has been used in separation science for extraction, purification and preconcentration [38,39].

The aim of this work is speciation of Cr(III) and Cr(VI) in human blood cells, whole blood and serum samples (35 exposed subjects and 35 unexposed controls) based on IICDET by CP-DLLME. Experimental parameters affecting the extraction process were optimized and the performance of the proposed method was evaluated.

2. Materials and methods

2.1. Apparatus

Determination of chromium was performed with a spectra GBC electro-thermal atomic absorption spectrometer (ET-AAS, Plus 932, Australia) using a graphite furnace module (GF3000, GBC). The operating parameters for the metal of interest were set as recommended by the manufacturer. A hollow cathode lamp (GBC) operating at a current of 6 mA and a wavelength of 357.9 nm with a spectral bandwidth of 0.2 nm was used. The working range, mode and volume injection have obtained 0.5–45 μg L^{-1}, Peak Area and 20 μL, respectively. The pH values of the solutions were measured by a digital pH meter (Metrohm 744). A micro-hematocrit instrument (Jouan, Italy, A13-HCT) was used for determination of the ratio of blood cells/serum (HCT %) in whole blood samples. Inductivity coupled plasma mass spectrometry (ICP-MS) was used for determination of ultra-trace chromium in standard and human blood samples (Perkin Elmer, QP, Elan6000 DRC, RIPI, Iran).

2.2. Reagents and materials

All reagents with ultra-trace analytical grade were purchased from Merck Germany. Iodine, dichloromethane (CH_2Cl_2), dithiocarbomate (CH_2NS_2), sodium thiosulfate ($Na_2S_2O_3$), magnesium sulfate ($MgSO_4$) and hexane (C_6H_{14}) were prepared from Merck. Cr(III) stock solution was prepared from an appropriate amount of the nitrate salt of this analyte as 1000 mg L^{-1} solution in 0.01 mol L^{-1} HNO_3 (Merck). Cr(VI) stock solution was prepared from an appropriate amount of the 1 g of Potassium salt (K_2CrO_4) of this analyte as 1000 mg L^{-1} solution in 1% HCl. Standard solutions were prepared daily by dilution of the stock solution. The pH adjustments were made using appropriate buffer solutions including sodium phosphate (H_3PO_4/NaH_2PO_4, 0.1 mol L^{-1}) for pH 2–3, ammonium acetate ($CH_3COOH/CHCOONH_4$, 0.1 mol L^{-1}) for pH 4–6, sodium borate ($NaBO_2$/HCl, 0.1 mol L^{-1}) for pH 7, and ammonium chloride (NH_3/NH_4Cl, 0.1 mol L^{-1}) for pH 8–10 (Merck). Polyoxyethylene octyl phenyl ether (TX-100, CAS N: 9036-19-5) as the anti-sticking agent was purchased from Merck. Ultra-pure lithium heparin (H0878, CAS N: 9045-22-1, 100KU) and sodium citrate (S4641, CAS N: 6132-04-3, 25 g) were purchased from Sigma-Aldrich in Iran. Isopropyl 2-[(isopropoxycarbothioyl) disulfanyl] ethane thioate was synthesized and purified in RIPI laboratories (IICDET; $(CH3)_4$ $(CO)_2$ S_4). Ultrapure water (18 MΩ cm) was obtained from Millipore continental water system (Bedford, USA), and 1-octyl-3-methylimidazolium hexafluorophosphate was prepared from Sigma Aldrich (Germany, $C_{12}H_{23}N_2 \cdot PF_6$, CAS N: 304680-36-2, 5 g). Graduated micro centrifuge conical tubes with cap (2–10 mL) were purchased from Sigma-Aldrich (Germany, Product N: SIAL311GZ2F).

2.3. Sampling

For sampling, all glass tubes were washed with a 0.5 mol L^{-1} HNO_3 solution for at least 24 h and thoroughly rinsed 6 times with ultrapure water before use. As chromium concentrations in whole blood and serum are very low, even minor contamination at any stage of sampling, sample storage and handling, or analysis has the potential to affect the accuracy of the results. Heparin is commonly used as anticoagulants in human blood samples. The blood collection tube with heparin was aliquoted into Eppendorf tubes (5 mL) and kept at −20°C for one week. For analysis in whole blood, 10 μL of pure heparin (free chromium) is added to a 10 mL blood sample of painting workers from Iranian car factory, Tehran, Iran. Hematocrit percentage (HCT %) was determined by centrifuging heparinized blood in a capillary tube (micro-hematocrit tube) at 10,000 rpm for 8 min. The human blood sample was maintained at −20 °C in a cleaned glass tube. Serum and blood samples were collected from exposed (35) and unexposed (35) subjects from Iranian car factory between 20 and 45 years of age.