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Restricted access carbon nanotubes for direct extraction of cadmium from human serum samples followed by atomic absorption spectrometry analysis

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ABSTRACT

In this paper, we propose a new sorbent that is able to extract metal ions directly from untreated biological fluids, simultaneously excluding all proteins from these samples. The sorbent was obtained through the modification of carbon nanotubes (CNTs) with an external bovine serum albumin (BSA) layer, resulting in restricted access carbon nanotubes (RACNTs). The BSA layer was fixed through the interconnection between the amine groups of the BSA using glutaraldehyde as cross-linker. When a protein sample is percolated through a cartridge containing RACNTs and the sample pH is higher than the isoelectric point of the proteins, both proteins from the sample and the BSA layer are negatively ionized. Thus, an electrostatic repulsion prevents the interaction between the proteins from the sample on the RACNTs surface. At the same time, metal ions are adsorbed in the CNTs (core) after their passage through the chains of proteins. The Cd^{2+} ion was selected for a proof-of-principle case to test the suitability of the RACNTs due to its toxicological relevance. RACNTs were able to extract Cd^{2+} and exclude almost 100% of the proteins from the human serum samples in an online solid-phase extraction system coupled with thermospray flame furnace atomic absorption spectrometry. The limits of detection and quantification were 0.24 and 0.80 μg L^{-1}, respectively. The sampling frequency was 8.6 h^{-1}, and the intra- and inter-day precisions at the 0.80, 15.0, and 30.0 μg L^{-1} Cd^{2+} levels were all lower than 10.1% (RSD). The recoveries obtained for human blood serum samples fortified with Cd^{2+} ranged from 85.0% to 112.0%. The method was successfully applied to analyze Cd^{2+} directly from six human blood serum samples without any pretreatment, and the observed concentrations ranged from < LOQ to 2.52 μg L^{-1}.

1. Introduction

An adequate sample preparation is indispensable for metal analyses in complex samples using spectrometry techniques. The matrix effect can modify analytical responses and damage equipment, mainly when the samples are slimy and rich in proteins, for example, biological fluids (e.g., total blood, plasma, serum, milk). Thus, strategies based on organic matrix decomposition have been frequently employed as the combustion [1] and microwave-assisted techniques [2]. In both cases, mineralization processes are carried out in drastic conditions with high temperatures, pressures in acidic and oxidant media, or both. To the best of our knowledge, matrix decomposition is the main sample preparation procedure for metal analyses in biological fluids, despite its slowness and potential danger when used.

Unlike metals, organic compounds are extracted from different samples employing soft techniques such as solvent extraction [3,4], solid phase extraction [5], and cloud point [6,7], among others. These techniques are able to extract molecules without damaging them because drastic conditions in terms of temperature and acidity are not employed. However, when these extractions are carried out in biological fluids, the proteins from the sample are not totally excluded, and several problems can occur during the analyses, such as the matrix effect and blockage of tubes. For such samples, an additional protein precipitation step is frequently employed as a cleanup process.

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An alternative for facilitating a gentle extraction of analytes from the biological fluids and total protein elimination is to do so through restricted access materials (RAMs) [8]. RAMs are sorbents that are able to retain organic compounds (with low molecular weight) from biological fluids, eliminating macromolecules as proteins. These sorbents limit the accessibility into sites within the pores only to small molecules (physical barrier), whereas macromolecules are excluded due to the presence of hydrophilic groups covering the sorbent surface (chemical barrier) [8]. Recently, some studies have been devoted to chemically modifying the surface of common sorbents as silica or organic polymers, converting them to sorbents that are able to exclude macromolecules. A very successful strategy is based on the formation of a bovine serum albumin (BSA) layer around silica [9–13] or molecularly imprinted polymers [14]. In these cases, when the sample is percolated through the sorbent in a pH larger than the isoelectric point of the proteins, both proteins from the sample and proteins from the sorbent layer (BSA) are negatively ionized, causing an electrostatic repulsion between both. Then, the proteins from the sample are excluded, and the analytes of low molecular weight pass through the BSA layer and are retained in the internal sorbent.

As far as we know, the RAMs have never been employed to extract metals from biological fluids, and their use has been exclusively devoted to the organic compound extractions. However, we believe that if a sorbent that is able to adsorb metals is covered with a BSA layer, the metals can be retained and the proteins excluded. This possibility is extremely promising for metal analyses from biological fluids because the slow and dangerous procedures of sample preparation based on matrix decomposition could be replaced by a simple solid phase extraction with RAM.

Carbon nanotubes (CNTs) are excellent sorbents for the preconcentration of metals due to their high surface area and inner volume, stability, and mechanical strength, as well as the possibility of establishing π–π interactions [5]. Several applications have been described in the literature using CNTs to concentrate metals from water [15–17], the environment [18–20], and food [21], among others. However, the use of CNTs in direct extractions of metals from protein fluids has not yet been successful because these sorbents can be blocked by proteins retained from the sample [22].

Based on the proven characteristics of CNTs to preconcentrate different metals, we propose, for the first time, the modification of these sorbents with an external BSA layer, resulting in restricted access carbon nanotubes (RACNTs) that are able to extract metals directly from untreated protein fluids, excluding all of the macromolecules. The RACNTs were synthesized, characterized, and used in an online solid-phase extraction of Cd^{2+} ions directly from human serum samples without any pretreatment. The analyses were carried out using the flow injection analysis (FIA) system with detection by thermospray flame furnace atomic absorption spectrometry (TS-FF-AAS). The Cd^{2+} ions were employed for a proof-of-principle case to appraise the suitability of the RACNTs due to its high toxicity in living organisms and its bioaccumulation potential because of the frequent occupational and environmental expositions of humans to this metal [23].

2. Materials and methods

2.1. Instrumentation

An atomic absorption spectrometer Shimadzu AA-6800 (Shimadzu®, Tokyo, Japan) equipped with a hollow cathode lamp for cadmium and a deuterium lamp for background correction was used in the analyses. Measurements were carried out at the peak height at 228.8 nm, and the lamp currents were set at 5 mA. The TS-FF-AAS was operated with acetylene and airflow rates of 3.0 L min^{-1} and 10.0 L min^{-1}, respectively. Moreover, the apparatus was composed of a nickel tube (Ni 200 Realum, UNS N 2200, São Paulo, Brazil) of 10-cm length and 2.5-cm i.d., containing six holes of 2.5-mm i.d. addressed to the burner, allowing for flame penetration inside the tube. A non-porous Al_2O_3 ceramic capillary (Friatec®, Mannhein, Germany) (10-cm length, 0.5-mm i.d., and 2.0-mm o.d.) was used to conduct the sample towards the nickel tube. A JEOL LV-JSM 6360 microscope (Tokyo, Japan) was used to obtain the images of scanning electron microscopy (SEM) and the spectra of energy-dispersive spectroscopy (EDS). Transmission electron microscopy (TEM) images were carried out using a JEOL JEM 2100 microscope. A chromatograph from Shimadzu® equipped with a 20AD pump (Shimadzu®), an electronic six-port switching-valve model 11R-0016H (Valco® Instruments, Houston, USA), and a UV-detector model SPD-20A (Shimadzu®) was used in the protein elimination tests.

The FIA system consisted of a peristaltic pump (Ismatec® IPC-08, Glattzbrugg, Switzerland), furnished with Tygon® tubes to propel all sample and reagent solutions, while a homemade injector commutator (in acrylic) was used to select the preconcentration-washing and elution/sampling steps.

2.2. Reagents and solutions

All solutions were prepared using ultra-high purity water (18.2 MΩ cm) from a Milli-Q system (Millipore®, Bedford, MA, USA). All chemicals were of analytical grade and used without further purification.

The multi-walled CNTs with outer wall diameters from 6 nm to 9 nm and lengths of 5 μm were purchased from Sigma-Aldrich® (St. Louis, MO, USA). BSA, glutaraldehyde, and sodium borohydride (all from Sigma-Aldrich®) were used for the CNTs coating.

A standard stock solution of Cd^{2+} (1000 mg L^{-1}) was purchased from Sigma-Aldrich®. Nitric acid and phosphate buffer (all from Sigma-Aldrich®) were used in the FIA system in the elution and cleaning steps.

2.3. RACNTs preparation

Initially, 500 mg of CNTs were oxidized with 30 mL of HNO_3 (65%) according to the method described by Zhijie et al. [24]. The material was then extensively washed with water and coated with a BSA layer, following the procedure of Moraes et al. [14] on coating a molecularly imprinted polymer. A 20 mL 1% (m/v) BSA prepared in 50 mmol L^{-1} phosphate buffer (pH 6.0) was poured through a cartridge containing 500 mg of oxidized CNT at 1.0 mL min^{-1}. Next, 5 mL of 25% (m/v) glutaraldehyde aqueous solution was slowly percolated through the cartridge, and the system was maintained in standby during 5 h. Finally, 10 mL of 1% (m/v) sodium borohydride aqueous solution was poured through the material at 1 mL min^{-1}. The cartridge was extensively washed with water (pH approximately 7.0) to eliminate all residues from the synthesis. The new covered material was defined as restricted access carbon nanotubes (RACNTs).

2.4. RACNTs characterization

The presence of the BSA layer on the RACNT was investigated by SEM, EDS and TEM. For the SEM and EDS, the samples were previously covered with a thin layer of platinum. The electron acceleration voltage was 15.0 kV. For TEM, the samples were dropped on the copper grid with holey carbon film and analyzed using an acceleration voltage of 200 kV.