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Nonhydrolytic sol-gel approach to facile creation of surface-bonded zirconia organic-inorganic hybrid coatings for sample preparation. I. Capillary microextraction of catecholamine neurotransmitters

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ABSTRACT

Nonhydrolytic sol-gel (NHSG) route was used for the creation of novel zirconia-polypropylene oxide (ZrO_2-PPO) sol-gel hybrid sorbents in the form of surface coatings for the extraction and preconcentration of catecholamine neurotransmitters and molecules structurally related to their deaminated metabolites. In comparison to other sorbents made of inorganic transition metal oxides, the presented hybrid organic-inorganic sorbents facilitated reversible sorption properties that allowed for efficient desorption of the extracted analytes by LC–MS compatible mobile phases. The presented sol-gel hybrid sorbents effectively overcame the major drawbacks of traditional silica- or polymer-based sorbents by providing superior pH stability (pH range: 0–14), and a variety of intermolecular interactions. Nonaqueous sol-gel treatment of PPO with $ZrCl_4$ was employed for the derivatization of the terminal hydroxyl groups on PPO, providing zirconium trichloride-containing end groups characterized by enhanced sol-gel reactivity. NHSG ZrO_2-PPO sorbent provided excellent microextraction performance for catecholamines, low detection limits (5.6–9.6 pM), high run-to-run reproducibility (RSD 0.6–5.1%), high desorption efficiency (95.0–99.5%) and high enrichment factors (~1480–2650) for dopamine and epinephrine, respectively, extracted from synthetic urine samples. The presented sol-gel sorbents provided effective alternative to conventional extraction media providing unique physicochemical characteristics and excellent extraction capability.

1. Introduction

Analytical tools for efficient extraction, preconcentration and detection of catecholamines (dopamine, epinephrine and norepinephrine) in biological matrices (such as urine) are important from a clinical point of view. Catecholamines and their metabolites have been investigated as potential biomarkers for the diagnosis and monitoring of tumors associated with different types of cancers and neural disorders [1]. Excess production of catecholamines by these tumors can cause "hypercatecholaminemia" which may cause health complications such as cerebrovascular accident, heart failure, cardiomyopathy and other potent impacts on the cardiovascular system [2]. Catecholamines are excreted in urine mainly in the following forms: unchanged, deaminated metabolites, and *o*-methylated amines (metanephrines). Analyzing catecholamines in urine, plasma or blood samples require sample preparation, preconcentration and cleanup steps essential for the minimization of any interfering components that might be present in biological matrices. In current practices, catecholamine sample pretreatments are predominantly performed by solid phase extraction (SPE) utilizing two types of sorbents: (a) polymeric reversed-phase resins and (b) phenylboronic acid-functionalized silica particles. Polymeric sorbents are typically made of *N*-vinylpyrrolidone and divinylbenzene monomers [3], and they possess excellent pH stability as well as balanced hydrophilic-hydrophobic characteristics, but low specific affinity toward the polar catecholamines which can be improved through chemical modification (derivatization) of catecholamines prior to their extraction. This is typically accomplished via formation of diphenylboronate-catecholamine complex [4] to facilitate their analysis by HPLC [5] or capillary electrophoresis [6]. A notable shortcoming is that extraction beds prepared from organic polymers possess slow mass transfer characteristics analogous to the chromatographic stationary phases prepared from polymeric materials [7]. This may result in delayed or incomplete desorption of the extracted analytes from the sorbent bed causing sample loss and/or carryover problems.

Silica particles with phenylboronic acid (PBA) ligand have also been widely used in solid-phase extraction (SPE) for cleanup and

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enrichment of catecholamine samples. PBA ligand has high affinity toward *cis*-diol groups present in the catecholamines [8]. The activation of the complexation ligand (phenylboronate, pKa ~9.5 [9]) requires conditioning of the SPE cartridge with high-pH buffer (pH 10–12) [10] giving rise to the main drawback of PBA-SPE cartridges due to inadequate pH stability of silica-based particles which are known to have a narrow operational pH window (pH 2–8) [11–14]. Alumina has been used for the extraction of catecholamines providing a pH-stable sorbent in the form of SPE sorbent [15–21]. Extreme strong adsorptive characteristics of alumina requires pH manipulation and the use of phosphate buffers for the desorption and elution of the extracted catecholamines.

Using different metal/metalloid alkoxide precursors, Malik and coworkers [22–25] have developed a number of sol-gel CME extraction phases providing excellent pH stability (0.0–14.0) in CME-HPLC as well as enhanced thermal stability in CME-GC operations. They included titania- [22,26], zirconia- [23] and germania-based [14,24,25] hybrid organic-inorganic CME coatings. The sol-gel coating route provides a simple, convenient and effective approach to synthesizing organic-inorganic hybrid materials [27]. The key to the success of the sol-gel coating (in addition to the unique physical and chemical properties of the created hybrid materials) is the chemical bonding of the sol-gel coating to the substrate (e.g., fused silica fiber or capillary).

Hydrolytic sol-gel (HSG) route [11] was used to create those microextraction media. Non-hydrolytic (nonaqueous) sol-gel (NHSG) route has been investigated extensively in the field of catalysis for the creation of metal/metalloid oxides [28,29]. In a nonaqueous environment, transition metal halide (e.g., $ZrCl_4$) concurrently undergoes alcoholysis and condensation reactions leading to the formation of transition metal oxides. [30] NHSG-generated transition metal oxides possess better water-tolerance, enhanced homogeneity, and more Lewis acid sites than Bronsted acid-base sites [28,29,31,32]. NHSG route can provide uniformly dispersed transition metal oxide particles in organic solvents and allows for facile surface modification with organic moieties [33–35]. The later property is important for the use of nonaqueous sol-gel route for the creation of hybrid organic-inorganic material with covalently bonded organic ligands. Here we present a systematic investigation on the synthesis and analytical evaluation of a novel zirconia-based sol-gel hybrid organic-inorganic sorbent to provide a biocompatible extraction medium integrating amphiphilic properties with enhanced thermal-, mechanical- and pH stability characteristics that are important for the analysis of aqueous samples of free catecholamines and molecules structurally related to their deaminated metabolites.

2. Experimental section

2.1. Materials and instruments

Zirconium (IV) butoxide, zirconium (IV) chloride, ethanol, 1-butanol, toluene, hydroxy-terminated polypropylene oxide (M_{avg} 3500), glacial acetic acid, catechol, quinol, resorcinol, 4-hydroxybenzoic acid, benzoic acid, vanillin, acetaminophen, dopamine hydrochloride, epinephrine hydrochloride, and serotonin hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO). HPLC grade solvents (methanol. dichloromethane, tetrahydrofuran), polypropylene microcentrifuge tubes and micropipette tips were purchased from Fischer Scientific (Waltham, MA). Fused silica capillary (250 μm i.d.) with polyimide external protective coating was purchased from Polymicro Technologies (Phoenix, AZ). The following chromatographic equipment was used in this study: (a) an Agilent 1100 series HPLC system equipped with a Diode Array Detector (Agilent Inc., Santa Clara, CA), (b) a Varian 3800 model gas chromatograph with a flame ionization detector (currently Varian is a part of Agilent), (c) Rhyeodyne 6-ports valve (IDEX Health & Sciences, Oak Harbor. WA). (d) an in-laboratory built purging/filling system [36,37].

2.2. Hydrothermal pretreatment of fused silica capillary

A one-meter segment of fused silica capillary (250 μm i.d.) was rinsed with 2 mL each of dichloromethane, methanol, and water using a gas pressure-operated purging/filling system [36] at 10 psi. Both ends of the capillary were then sealed using an oxy-acetylene torch. The sealed capillary was placed in the GC oven and conditioned by raising the temperature from 40 °C to 350 °C at a rate of 1 °C/min, holding the capillary at 350 °C for 200 min. After thermal conditioning, the capillary was cooled to room temperature and cut open on both ends using an alumina wafer. It was then placed in the GC oven with one end connected to the GC injection port allowing nitrogen gas to flow through the capillary, and the other end was left open and secured in the GC oven. Thermal conditioning of the capillary was performed under nitrogen purge (1 mL/min) as follows: (40 °C–350 °C at rate of 10 °C/min, 120 min hold time at 350 °C). The capillary was then cooled down to room temperature and its inner surface was ready for coating.

2.3. Preparation of sol-gel zirconia-PPO coated capillary via non-hydrolytic sol-gel (NHSG) route

2.3.1. Solvents drying

In the NHSG process, the solvents must be free from water. For this, the solvents (butanol, toluene) were dried over molecular sieve (type 4A) by placing 15 mL of each solvent in a separate vial. A 10-g amount of the molecular sieve was added to each solvent and vortexed for 2 min and then left airtight in the hood overnight. 2-mL aliquot of each treated solvent was transferred to a micro-centrifuge vial and centrifugation was performed (10,000 rpm for 2 min) to eliminate any possible contamination from the molecular sieve particles. To test if the dried solvents still contained water, 0.5 g of anhydrous copper sulfate (white) was mixed with 1 mL of each dried solvent, then the mixture was thoroughly vortexed. The mixture was centrifuged to precipitate the copper sulfate powder, which would turn blue in the presence of water. The drying procedure was repeated until no color change of $CuSO_4$ was observed.

2.3.2. Modification of organic polymer with zirconium tetrachloride

Prior to the preparation of sol-gel sorbents, the terminal hydroxyl groups of polypropylene oxide (PPO) were modified with zirconium tetrachloride. For this, PPO and $ZrCl_4$ were taken in a 25 mL round-bottom flask in molar ratio of 1:2 (PPO: 0.6 mmol, $ZrCl_4$: 1.2 mmol) and dissolved in anhydrous toluene (300 μL). The solution was stirred for 12 h at 60 °C and then allowed to reach room temperature before using it for the preparation of sol solution.

2.3.3. Preparation of sol solution for the NHSG route

The sol solution was prepared as follows: in a polypropylene centrifuge vial, 46 mg of zirconium tetrachloride was dissolved in 74 μL of dried 1-butanol. In a second vial, 80 mg of modified PPO was mixed with 180 μL of dried toluene and vortexed thoroughly for 1 min and it was left in the hood for 6 h. Thereafter, polymer solution was vortexed for 1 min and then it was transferred to the first vial containing butanolic solution of zirconium tetrachloride. The mixture was vortexed thoroughly to ensure homogeneity. The gelation time of this mixture was ~2 h. Based on this gelation time, the coating of the capillary was performed after the solution was