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A hybrid material as a sorbent phase for the disposable pipette extraction technique enhances efficiency in the determination of phenolic endocrine-disrupting compounds

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ABSTRACT

In this study, the hybrid material 3-*n*-propyl(3-methylpyridinium) silsesquioxane chloride (Si3Py$^+$Cl$^-$) was synthesized and investigated as a novel sorbent phase for the disposable pipette extraction (DPX) technique coupled to high-performance liquid chromatography-florescence detection. This sorbent phase was characterized by scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR). Aqueous samples containing the phenolic endocrine-disrupting compounds bisphenol A (BPA), 17α-ethynylestradiol (EE2), 4-*tert*-octylphenol (4-t-OP), 4-octylphenol (4-OP) and 4-nonylphenol (4-NP) were subjected to DPX procedures and a series of optimizations was performed to determine the ideal extraction conditions using this approach. The proposed sorbent phase exhibited higher extraction efficiency than DPX-RP (reversed phase tips containing styrene-divinylbenzene), commonly used for the determination of the phenolic endocrine- disrupting-compounds under study. Satisfactory analytical performance was achieved with linear ranges from 2 to 100 $\mu g\,L^{-1}$ for 4-t-OP and 1–100 $\mu g\,L^{-1}$ for the other analytes. Limits of detection of 0.60 $\mu g\,L^{-1}$ for 4-t-OP and 0.30 $\mu g\,L^{-1}$ for other analytes, RSDs ranging from 1 to 20% and relative recoveries of 83–116% were obtained. Based on these satisfactory results, this sorbent phase represents a valuable alternative for the extraction of compounds with polar moieties in their structure.

1. Introduction

Phenolic compounds are widely used in a number of different applications and exhibit a broad range of applicability in the chemical industry. Subsequently, due to the fact that they are often not totally degraded in effluent treatment plants [1], these compounds have been found in different environmental matrices [2–5]. The main concern in this regard is associated with the fact that they are endocrine-disrupting, which can interfere in the human endocrine system causing dysfunction of reproduction systems, a decrease in immune function and enhanced incidence of cancer [6–9]. Therefore, the presence of these pollutants in different environmental matrices needs to be monitored.

The determination of phenolic compounds is usually carried out by chromatographic techniques, including gas chromatography (GC) and liquid chromatography (LC), coupled to different detectors. However, due to the complexity of some environmental matrices and the analytes often being present in low concentrations, a suitable sample preparation technique needs to be applied prior to the chromatographic analysis. An appropriate sample preparation technique allows the preconcentration of the analytes, a decrease in the matrix effect through the removal/separation of the analytes from interferents in the matrix, and the transfer of the analytes to a medium compatible with the analytical instrumentation [10]. Several extraction and microextraction techniques have been developed and applied as sample preparation tools for the determination of a number of compounds from different chemical classes.

Solid-phase extraction (SPE) has been widely employed for the extraction of phenolic compounds from water. However, this technique is associated with long extraction times and the use of relatively large volumes of solvents [11–14]. Other reported techniques are solid-phase microextraction (SPME) [15,16], hollow-fiber liquid phase microextraction (HF-LPME) [17], magnetic solid-phase extraction (MSPE) [18], dispersive

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liquid–liquid microextraction (DLLME) [19,20] and single-drop microextraction (SDME) [20].

One well-known sample preparation technique is disposable pipette extraction (DPX), which is an extraction technique based on SPE [21]. The standard procedure consists of the following steps: (*i*) conditioning to activate the sorbent sites; (*ii*) sample aspiration followed by air aspiration to allow the dynamic mixing between sorbent and sample; (*iii*) cleaning using solvents to remove matrix interferences; and (*iv*) desorption of the analytes using a solvent or a mixture of solvents [22]. The dynamic mixing of the solid sorbent with the sample using air aspiration provides fast equilibrium between the analyte and sorbent and thus the extraction time is reduced. Another advantage is the lower consumption of solvent and sorbent phase compared to SPE.

The choice of the sorbent to be used for the DPX procedure is dependent on the analytes and constituents of the sample matrix. A variety of DPX devices with different extraction phases is commercially available: DPX-RP – reversed phase tips that contain styrene-divinylbenzene as a sorbent, ideal for non-polar and slightly polar compounds. DPX-WAX – weak anion exchange tips that contain a sorbent with poly-amino groups for adsorption interactions with acidic compounds as well as a strong reversed phase for many drugs and metabolites. DPX-CX – cation exchange tips that contain a sorbent with sulfonic acid groups for strong adsorption interactions with basic compounds. DPX-WCX – weak anion exchange tips that contain a sorbent with poly-carboxylate groups for the adsorption of basic compounds. DPX-SI – tips that contain silica gel for the rapid cleanup of environmental samples. DPX-C18 – tips that contain C18 sorbent for rapid sample cleanup to remove matrix interferences [22–24]. The main applications of these commercially available DPX devices are in the extraction of non-polar compounds and acid and basic drugs and also for cleanup procedures. However, based on the available literature, there appears to be a need for further studies involving the determination of phenolic compounds in water samples using commercial-DPX. This sample preparation technique enables the use of alternative materials as sorbent phases. In this study, the polymer 3-*n*-propyl(3-methylpyridinium) silsesquioxane chloride ($Si3Py^+Cl^-$) was used for the first time as the extraction phase for DPX. This material possesses functional groups that can interact selectively with the more polar moieties of the analytes. Thus, the matrix interference can be reduced and enhanced sensitivity can be achieved.

The aim of this study was to propose a novel sorbent phase for the analysis of BPA, EE2, 4-t-OP, 4-OP and 4-NP using DPX with $Si3Py^+Cl^-$. The extraction conditions were optimized using both univariate and multivariate strategies and the analytical parameters of merit were determined in environmental water samples with separation/detection by high-performance liquid chromatography coupled to fluorescence detection. In addition, the chemical interactions between the new sorbent employed in this study and the analytes were evaluated and discussed.

2. Experimental

2.1. Reagents and materials

The phenolic compounds BPA (99%), EE2 (98%), 4-t-OP (97%), 4-OP (99%) and 4-NP (99.8%) were obtained from Sigma-Aldrich (Milwaukee, WI, USA). Individual stock solutions at a concentration of 1000 mg L^{-1} were prepared in methanol (MeOH). Working solutions containing a mix of the analytes at a concentration of 100 mg L^{-1} in MeOH were prepared by appropriate dilution of the stock solution. Plastic pipette tips (1 mL, without sorbent material) were acquired from DPX Labs (Columbia, SC, USA). Acetonitrile (ACN) and MeOH were supplied by JT Baker (Mallinckrodt, NJ, USA). Water was purified in an ultrapure water system (Mega Purity, Billerica, USA). Tetraethylorthosilicate (TEOS), ethanol, chloropropyltrimethoxysilane (CPTS), toluene and 3-methylpyridine were also obtained from Sigma-Aldrich (Milwaukee, WI, USA). Phosphate dibasic, citric acid, hydrochloric acid and sodium chloride were obtained from Vetec (Rio de Janeiro, Brazil). EPA 525 PAH mix B was obtained from Sigma-Aldrich (Milwaukee, WI, USA) with 500 μg mL^{-1} of each compound in acetone.

2.2. Instrumentation and chromatographic conditions

Chromatographic analysis was performed on a Shimadzu Prominence LC 20AT series HPLC system (Shimadzu, Kyoto, Japan) equipped with a fluorescence detector (RF 20A series) with a 20 μL loop and Rheodyne 7725i manual injector (Rohnert Park, CA, USA). Chromatographic separations were performed in reversed phase mode using a C18 column (ZORBAX Eclipse XDB®, 250 mm × 4.6 mm i.d., 5 mm film thickness, Agilent, CA, USA). The injection volume was 20 μL at a flow rate of 1 mL min^{-1} of mobile phase in gradient mode. The mobile phase consisted of a mixture of ACN (A) and water (B) in the following gradient compositions: 0–5.5 min A 60% and B 40%; 5.5–7.5 min A 80% and B 20% and isocratic mode held up to 25 min; 25–26 min return to the initial conditions which were held up to 30 min. The fluorescence detector was set to analyze the excitation wavelength of 277 nm and the emission wavelength of 307 nm. The chromatographic data were evaluated with LCsolution software (Shimadzu, Kyoto, Japan).

2.3. Synthesis of the sorbent phase

The polymer $Si3Py^+Cl^-$ was prepared by the sol-gel processing method. Tetraethylorthosilicate (TEOS), ethanol and an aqueous HCl solution were mixed in a round-bottomed flask, and the resulting solution was stirred for 2.5 h at room temperature (298 K). A solution of 3-*n*-chloropropyltrimethoxysilane (CPTS) was then added and the solution was stirred for 2 h at room temperature. The temperature of the solution was increased to 328 K and the mixture was allowed to stand for 60 h open to the ambient atmosphere until the gelation process occurred. The resulting gel was powdered, washed with ethanol and then dried under vacuum (133×10^{-3} Pa) at room temperature. The dry gel was immersed in a round-bottomed flask containing a solution prepared by mixing pure 3-methylpyridine and dry toluene. Each mixture was heated at the reflux temperature of the solvent for approximately 3 h. The solids were filtered, washed with ethanol and dried for 2 h under vacuum (133×10^{-3} Pa) at room temperature [25]. The chemical structure of this synthesized compound is represented in Fig. 1.

2.4. Characterization of the sorbent phase

Structural information was obtained by Fourier transform infrared spectroscopy (FTIR), using a Varian 3100 spectrometer (Santa Clara, CA, USA). FTIR spectra were generated from KBr pellets. The morphology of the polymer $Si3Py^+Cl^-$ was evaluated by scanning electron microscopy (SEM), using a Hitachi TM 3030 microscope (Tokyo, Japan).

2.4.1. Optimization of DPX procedure

A series of experiments were carried out to optimize the DPX procedure. Extractions were performed using 700 μL of ultrapure water spiked with the analytes at a concentration of 30 μg L^{-1} for each compound. The desorption step was performed using 200 μL of organic solvent. The sample volume was kept constant at 700 μL to allow a satisfactory dynamic mixture between the sorbent phase and the aqueous sample inside the pipette (1 mL