Development of electrodeposited nanostructural poly (o-aminophenol) coating as a solid phase microextraction fiber for determination of bisphenol A

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ABSTRACT

In this research nanostructural poly (o-aminophenol) was synthesized by electropolymerization and used for solid phase microextraction fiber procedure (SPME). Thin film of Poly (o-aminophenol) (4 µm thickness) was shaped by sweep potential for 45 min on the surface of stainless steel wire. Polymer was synthesized by potentiostat procedure too. Prepared polymer by sweep potential procedure showed nanostructures on the surface. Acetic anhydride was employed for derivatization of bisphenol A (BPh-A) and analysis of acetylated BPh-A was utilized by gas chromatography-flame ionization detector (GC-FID). Affecting parameters on derivatization and extraction such as amount of acetic anhydride, stirring rate, temperature, ionic strength and extraction time were optimized. The limit of detection (LOD) and relative standard divisions (RSDs%) were achieved 0.6 µg L<sup>-1</sup> and less than 6.8%, respectively under optimized conditions. Finally proposed method was used for extraction of bisphenol A from leaching of baby and drinking water bottles. Relative recovery was achieved 98% for leaching from drinking bottle. In leaching from plastic baby bottle, bisphenol A (BPh-A) was detected in the range 5–15 µg L<sup>-1</sup>.

Keywords: Poly (o-aminophenol), Nanostructure, Bisphenol A, Solid phase microextraction, Gas chromatography-flame ionization detector

1. Introduction

Solid phase microextraction (SPME) is a simple, solvent free and green sample preparation technique which includes the employing of a small amount of polymeric sorbent coated on a thin fiber for extraction [1]. Nowadays, commercial SPME fibers are available. The important disadvantages of commercial SPME fibers are high cost, fragility of the fibers and weakness of sorbents for extraction of polar compounds. For domination of these drawbacks, conductive polymers (CPs) such as polyaniline (PANI) and polypyrrole (PPy), coated on the surface of metallic fiber, are good choices [2] but thermal instability of these CPs is a problem when gas chromatography is applied for followed analysis. Many solutions such as changing of counter ion [3] or doping carbon nanotube [4] and nanosilica [5] in the matrix of polymer, have been suggested for modifying of thermal stability and other properties of PANI and PPy. Application of other new CPs with multi-functional groups can be an alternative way for modifying of thermal stability, selectivity and extraction efficiency of SPME fibers.
Poly (o-aminophenol) (POAP) has been an interesting polymer during three past decades. Many of researches have been performed about its synthesis, structure and application in sensor, biosensor and corrosion protection [6]. The POAP is formed by electrochemical oxidation of o-aminophenol (o-AP) on the surface of different electrode in aqueous solution. o-AP can be polymerized electrochemically in alkaline, neutral and acidic media. However, while a conducting polymer film is only produced in acidic media, POAP prepared in neutral and alkaline media leads to a nonconducting polymer film so, the polymer thickness of POAP synthesized in basic and natural media is limited within 10–100 nm due to a self-limiting growth [7].

The o-AP is containing –OH and –NH$_2$ groups in ortho position on a phenyl ring. Polymerization of the o-AP can be performed by both groups therefore a ladder structure could be formed which has better thermal stability rather than PANI [8]. POAP has been employed to build biosensors, because it has been showed permselective properties so the interference from different electroactive species can be significantly reduced during the analysis of biological samples by utilizing a biosensor based on POAP. Moreover, as both hydroxyl and amino groups are involved in the electropolymerization process of o-aminophenol, large amounts of biological macromolecules such as glucose oxidase [9,10] or horseradish peroxidase [11] could be immobilized in poly(o-aminophenol), which results in higher sensitivity of the sensor as compared with sensors based on other polymers. On the other hand the presence of POAP as a sensing material can decrease the oxidation overpotential of some molecules, so it has shown the electrocatalytic behavior [12].

A hybrid modified electrode was also prepared by electropolymerization of o–AP in the presence of sulfonated nickel phthalocyanine. The modified electrode could electrocatalyze NO oxidation and has been employed as NO sensor [13]. The copolymer poly(aniline-co-o-aminophenol) has been exhibited favorable properties to its application in sensors, electrocatalysis, analytic determinations and rechargeable batteries [14,15]. The pH dependence of the electroactivity of the copolymer is much better than that of PANI. Poly(aniline-co-o-aminophenol) has been employed as sensor of catechol [16] and ascorbic acid [17]. POAP has been applied as a molecular imprinting polymer for sensor preparation. In this regard, an electrochemical sensor for nicotine based on the electropolymerization of o-PA as monomer and nicotine as template was proposed by Zhaoyang et al. [18]. Compared with nicotine imprinting membranes, the POAP film, was homogeneous, had nanometric thickness and its synthesis was easy. Other application of POAP film was reviewed by Tucerri [6].

Bisphenol A, (BPh-A), 2,2-bis(4-hydroxyphenyl) propane, is one of the highest volume chemicals in the world. It was applied for production of polycarbonate with the second largest outlet being epoxy resins [19]. A broad variety of food contact materials stand out among their uses, mainly derived from polycarbonates (infant feeding bottles, storage containers, tableware, returnable water, milk bottles and water pipes) and epoxy resins (internal protective lining for food and beverage cans, coating on metal lids for glass jars and bottles and drinking water storage tanks) [19]. The BPh-A has shown estrogenic activity so it acts as an endocrine disruptor. Furthermore, researches also has been indicated the potential of BPh-A to disrupt thyroid hormone action [20], to cause proliferation of human prostate cancer cells [21] and to block testosterone synthesis [22] at very low part-per-trillion doses So, there are needs for introducing of new analytical procedures particularly application of modern sample preparation methods (such as microextraction techniques) in order to have reliable tools for control of human exposure to BPh-A. SPME with commercial fibers was utilized for extraction of BPh-A frequently [23–27]. Molecularly imprinted polymeric fiber was employed for selective extraction of BPh-A from complex matrix which was shown by Tan et al. [28].
The POAP, as a multi-functional group compound, can be a remarkable sorbent for extraction of different analytes so, in this paper, nanostructural POAP coated on the stainless steel wire has been prepared by electropolymrization under cyclic voltammetry and finally the polymeric coating was used as a SPME fiber for extraction of BPh-A from aqueous matrix. Different effective parameters on derivatization and extraction of BPh-A have been evaluated and optimized and finally the method has been applied for determination of BPh-A in leaching from drinking and plastic baby bottle that are made from polycarbonate.

2. Experimental

2.1. Materials

Bisphenol A was obtained from Sigma & Aldrich (St. Louis, USA) and dissolved in methanol to make stock solution at the concentration of 500 mg L\(^{-1}\). Intermediate standard solution was prepared at concentration 10 mg L\(^{-1}\). More diluted working solutions used in optimization studies were prepared daily by diluting different amounts of the intermediate standard solution with pure water. All solutions were stored at 4 °C prior to use. HPLC grade methanol was purchased from Merck (Darmstadt, Germany). Sodium dodecyl sulfate (SDS), sulfuric acid, sodium chloride, sodium sulfate and o-aminophenol were purchased from Merck (Darmstadt, Germany). Sodium carbonate and acetic anhydride for derivatization of BPH-A was obtained from Merck too. Pure water was prepared by OES (Overseas Equipment & Services) water purification system (OK, USA). The surgical grade stainless steel plunger of a disposable spinal needle (27G, Bartar Co., Tehran, Iran) was used as the substrate of the SPME fibers.

2.2. Instrumentation

The SPME device was purchased from Supelco (Bellefonte, PA, USA) and used for SPME experiments with commercial fibers (85 µm PA and 65 µm PDMS/DVB). A homemade SPME holder was assembled and used to perform extraction with the fibers produced in the present work. A piece of stainless steel wire (3 cm) was mounted into the SPME device and used as a working electrode to make the SPME fiber. Electrochemical polymerization was carried out with a potentiostat/galvanostat AutoLab (Echo Chemie, Netherlands). A SP-3420A gas chromatograph equipped with a split/ splitless injector and a flame ionization detector (BFRL, Beijing, China) was employed for all experiments. The injector was equipped with a low-volume insert designed for the analysis by SPME (Restek, Bellefonte, PA, USA). Nitrogen (99.999%) was used as carrier and make-up gas. The carrier and make-up gas flow rate was set at 1.7 and 30 mL min\(^{-1}\), respectively. The chromatographic separation was performed using a DB-35ms, 10 m×0.25 mm, fused silica capillary column with a 0.15 µm stationary phase thickness (Supelco, Bellefonte, PA, USA). The injector and detector temperatures were set at 260 and 280 °C, respectively. The column temperature was initially maintained at 100 °C for 1 min; subsequently, the temperature was increased to 250 °C (at a rate of 30 °C min\(^{-1}\)) and held for 10 min. Surface characteristic studies of the poly(o-aminophenol) coating was performed using field emission-scanning electron microscopy (FE-SEM) (Hitachi, S-4160, Japan). Chemical bonding characterization of the coating was investigated using Fourier transform infrared spectroscopy (FT-IR-350, Jasco Co., Tokyo, Japan). Thermogravimetric analysis (TGA) was performed using a Rheometric Scientific TGA 1500 instrument.

2.3. Preparation of SPME coating

Poly(o-aminophenol) film was prepared according to work of Kunimura et al [29] with some modification. Polymerization was performed with a potential-sweep electrolysis by using a standard three-electrode cell. Stainless steel wire (o.d. 0.2 mm) and platinum electrode were used as working and counter electrodes. A length of 1 cm from the end part of stainless steel wire was
immersed into the polymerization solution. The electrode potential was cycled between 0 and 1.3 V versus an Ag/AgCl electrode at 50 mV s\(^{-1}\) in a 0.5 M \(\text{Na}_2\text{SO}_4\) solution (pH 1.0) containing 100 mM o-aminophenol and 0.05 mM SDS (as the counter ion and catalyst). Solution was stirred magnetically by a 1 cm stir bar at 800 rpm. For the comparison purpose, polymer was synthesis at a constant potential of 1.3 V for 45 min too. To make the coating adhere firmly to the surface of the wire, the wire surface was first roughened by a smooth sand paper and then washed in methanol while sonicating. After polymerization, prepared fiber was thoroughly rinsed with distilled water and thermally conditioned before use. Thermal conditioning of the fiber was carried out by heating at 150 °C for 20 min, then at 200 °C for 20 min, and finally at 290 °C for 20 h in a GC injector port under a nitrogen atmosphere.

2.4. Derivatization and SPME procedure

A 3.0 mL standard solution of BPH-A containing 0.05 mol L\(^{-1}\) of sodium carbonate was transferred into a 7-mL glass vial from Supelco (14 mm i.d.). A 13 mm×3 mm Teflon coated stir bar was used in the vial for stirring the solution. For derivatization step, amount of 25 µL acetic anhydride was added to the vial. The vial was capped and reaction was performed for 5 min at 300 rpm and room temperature. After the reaction, 0.6 g of sodium chloride was added to the solution and magnetically stirred until dissolving. For extraction step, the SPME fiber was immersed into the sample solution under optimum stirring rate (600 rpm) at room temperature. After 30 min, the fiber was retracted into the needle and immediately introduced into the GC injection port (260 °C). Injection was made in splitless mode and desorption time obtained at 3 min.

3. Results and discussion

3.1. Characterization of POAP coating

The morphological structures of POAP coating were investigated with FE-SEM and have been shown in Figure 1. POAP coating prepared by sweep potential showed granular structures which were contained the large number of nanoparticles (o.d. 50 nm or smaller) attached to each other (Fig. 1a and b). However, in some places, nanoparticles were placed beside each other without any granular structure (Fig. 1d). The thickness of the coating was obtained about 4 µm (Fig. 1c). It seems low conductivity of polymer causes the thickness does not increase in the period of electropolymerization [29]. Figure 1e and f demonstrate surface morphology of POAP prepared by constant potential. As can be seen, polymer film is uniform and flat and there is no granular structure. Application of the fiber prepared by constant potential shows 4 times weaker result than the one prepared by sweep potential. It seems the higher surface area of the polymer film prepared by sweep potential plays important role in extraction of analyte. For gas chromatography applications, SPME fibers must be have efficient thermal stability. The TGA curve of the POAP coating under argon atmosphere at a heating rate of 10 °C min\(^{-1}\) has been shown in Figure 2a. This film was found to start a slow loss of weight around 300 °C. The weight loss was at most 4% at this temperature which could be attributed to the evaporation of water moisture trapped in the pores of the film. So the present SPME fiber coating is stable at temperatures below 300 °C due to ladder chemical structure [29]. Then the fiber is suitable for gas chromatographic analysis. Investigation of infrared spectroscopy of POAP was carried out by many researchers and more chemical structures have been proposed. o-AP has two functional groups which contains –OH and –NH\(_2\) sites. Polymerization of o-AP can be performed by both sites. Due to spectroscopic measurements, different structures have been proposed for POAP. Besides a completely ring-closed or ladder structure with phenoxazine units [8,30–32], other two structures, a partially ring opened and another partially hydrolyzed, have been considered for POAP. In-situ Raman spectroscopy measurements propose
that the POAP medium contains alternating oxidized (quinonoid) and reduced (N-phenyl-
-p-phenylenediamine) repeating units [33,34]. Zhang et al. introduced 1,4-substituted molecular structure for POAP [33,34] allows explaining the interaction of the polymer with metal ions. The
cation capturing process by POAP was certified to simultaneous presence of -OH and –NH 2
groups of the polymeric backbone, in which the lone-pair electrons are available to form metal complex. IR studies have been indicated that the POAP film-growing process in alkaline media involves the deprotonation of the aminophenol molecule, which is probably chemisorbed at the metal surface, followed by oxidation and electropolymerization reactions. In this whole process, the polymerization affects the –OH group by the formation of C–O–C bond while the
–NH 2 groups are preserved [35].

In this study Fourier transform infrared spectroscopy (FT-IR) was used to investigate the functional groups of the polymer. Spectrum has been revealed in Figure 2b. Broad peak between 3000 and 4000 cm⁻¹ assigned to the symmetric stretching of NH and OH in aromatic system. Strong peaks at 1582 and 1379 cm⁻¹ are belonging to C-N stretching vibrations for quinoide structure or combination band for protonated aromatic amine [36]. Strong peak at 1460 cm⁻¹ can be assigned for NH scissoring vibrations. Weak peak at 1037 cm⁻¹ is belonging to C-C stretching vibrations in benzene ring [36]. Consequently, a blend of structures could be considered for POAP film. A weak peak at 2926 cm⁻¹ assigned to stretching of CH in aliphatic system which can be considered for dodecyl sulfate counter ion. SDS plays two roles for preparation of POAP. At first it acts as a catalyst and oxidation potential of o-AP was shifted to less positive potentials (almost 0.075V) and the oxidation current increased, as compared with the process in the absence of SDS. The rate of polymerization also increased considerably in the presence of SDS [37] on the other hands dodecyl sulfate has been used as a frequent counter ion in conductive polymer preparation that used for microextraction process because it increases thermal stability of polymeric film [38].
3.2. Optimization of conditions
For evaluation of POAP film as a solid phase microextraction fiber, effects of various parameters that can probably influence the performance of the derivatization and extraction of BPH-A, including amounts of acetic anhydride and sodium carbonate, salt concentration, stirring rate, extraction time and temperature were investigated. All experiments were performed three times.

3.2.1. Optimization of derivatization conditions
Derivatization can reduce the polarity of some analytes and can improve the extraction efficiency and also it leads to better peak shape, and higher sensitivity. Acetic anhydride is a common derivatization reagent that has been applied for blocking of hydroxyl group of phenolic compounds frequently [23,39,40]. The derivatization with acetic anhydride was performed in situ. Consequently, the experimental variables affecting to both the extraction and derivatization processes were studied together. Derivatization of bisphenol A with acetic anhydride performs in basic condition. Sodium carbonate usually has been used for adjustment of sample pH hence in this research effect of different concentration of sodium carbonate was investigated. Figure 3a show the consequences. The concentrations of 0.05 and 0.1

![Fig. 2. Thermal gravimetric analysis (a) and FT-IR spectra of POAP coating prepared by sweep potential.](image)
mol L\(^{-1}\) reveal maximum derivatization efficiency. Therefore, 0.05 mol L\(^{-1}\) of sodium carbonate was applied for adjustment of pH. Under optimized pH, the amount of acetic anhydride was evaluated for the best derivatization efficiency. Effect of different amount of acetic anhydride on extraction efficiency was summarized in Figure 3b. As can be seen, concentration more than 0.75% (V/V) has not significant effect on derivatization efficiency therefore this concentration was selected as an optimum point. Acetylation of phenolic compounds usually is completed at a short time nonetheless in this research the reaction time was studied too. The results satisfy that the times more than 5 min have not considerable effect on the reaction recovery (the curve has not shown).

### 3.2.2. Optimization of extraction conditions

Effective parameters such as ion strength, stirring rate, extraction temperature and time were evaluated and optimized. The ion strength of the sample solution was studied by spiking a series of NaCl concentrations of 0–0.3 g L\(^{-1}\). The response increases with the increase of ion strength; however, the extraction efficiency slightly decreases under the high salt content (Fig. 4a). A salt level of 0.2 g mL\(^{-1}\) of NaCl was used in the following experiments. The fiber is directly immersed in the liquid samples, and partitions between the sample matrix and the stationary phase were happened. Agitation of the sample is often carried out with a small stirring bar to decrease the time necessary for equilibrium and to decrease the tension of the static aqueous film. The stirring bar is of dimension 10 mm × 3 mm. The effect of the stirring rate on the responses was tested from 250 to 1000 rpm. At a higher stirring rate of 600 rpm, a significant decrease in the area response was observed (Fig. 4b). Moreover, better result was obtained at a relatively medium stirring rate than at the lower and higher ones. Thus, a stirring rate of 600 rpm was chosen for further experiments. Temperature has kinetic and thermodynamic effects on extraction recovery. On the other hand, solubility of analyte in water increases at high temperature, so, effect of temperature in the range of 10 to 45 \(^{\circ}\)C (Fig. 5a). Best results were achieved at 15 \(^{\circ}\)C but for simplicity room temperature was applied as an optimal temperature. The extraction time was studied from 10 min to 60 min (Fig. 5b). The result shows that the equilibrium time is reached until 40 min when a further increase of the extraction time does not result in a significant increase in the detector response but for shortening the analysis time, an extraction time of 30 min was established in all the experiments.

![Fig. 3.](image-url)

**Fig. 3.** (a) Effect of sodium carbonate concentration and (b) effect of acetic anhydride amount on the derivatization efficiency of bisphenol A (sample volume, 3 mL; concentration of analyte, 200 µg L\(^{-1}\); amount of acetic anhydride (for (a)), 10 µL; amount of sodium carbonat (for (b)), 0.05 mol L\(^{-1}\); reaction time, 5 min; salt addition, 0.1 g mL\(^{-1}\); stirring rate, 400 rpm; extraction time, 30 min).
3.3. Method validation

The linearity, the repeatability and the detection limits of the proposed method were investigated. The correlation coefficient (0.9981) indicated a good linearity between 2 - 500 µg L\(^{-1}\). Under optimal conditions, LOD was achieved 0.6 µg L\(^{-1}\). Relative standard deviation for intra- and inter day were 4.0 and 6.1%, respectively. The amount of 6.8% was attained for fiber-to-fiber relative standard deviation too. On the other hands, extraction capability of the POAP coated fiber was compared with commercial SPME fibers. Poly acrylate (PA) and poly dimethylsiloxane/divinylbenzen (PDMS/DVB) commercial fiber were chosen for this comparison. Figure 6 shows the results. POAP coated fiber revealed better capacity for extraction of bisphenol A. It seems the chemical composition and surface configurations of coating are two effective factors for this investigation. POAP has more chemical functional groups compared to PA and PDMS/DVB coating. On the other side, POAP nanostructure morphology can help for more and fast extraction.
3.4. Real sample analysis

To examine the feasibility of the method, new SPME fiber was applied for analysis of bisphenol A released from milk and drinking water bottle. All the leachate samples were collected from the containers that had been filled with 50 mL of boiling hot water. Bisphenol A was below the LOD for drinking water bottle but was detected in the range 5–15 µg L$^{-1}$ in leaching from plastic baby bottle. Relative recovery was attained 98±3% for leaching from drinking bottle. Relative recoveries were reported in Table 1. Figure 7 shows the chromatograms from the leaching of baby bottle with and without spiking of bisphenol A. Releasing of bisphenol A from plastic baby bottle was investigated in four time reusing and the results were summarized in Figure 8. As can be seen, bisphenol A exists in the consecutive leaching but the amount of it reduces.

![Fig. 6. Comparison of POAP coated SPME fiber with PA & PDMS/DVB commercial fiber (sample volume, 3 mL; analyte concentration, 50 µg L$^{-1}$; 0.05 mol L$^{-1}$ sodium carbonate; amount of acetic anhydride, 25 µL; reaction time, 5 min; stirring rate, 600 rpm; salt addition, 0.3 g/mL; extraction time, 30 min at room temperature).](image)

| Added (µg L$^{-1}$) | Drinking water bottle | Plastic baby bottle (1$\text{st}$ leachate) |
|---------------------|----------------------|------------------------------------------|
|                     | Found | Relative Recovery (%) | Found | Relative Recovery (%) |
| 0.0                 | ND$^*$ | - | 15.1 | - |
| 10.0                | 9.7   | 97 | 25.4 | 103 |
| 20.0                | 20.6  | 103 | 35.3 | 101 |
| 50.0                | 51.0  | 102 | 64.1 | 98 |
| 100.0               | 94.1  | 94 | 109.1 | 94 |

* Not detected.
Fig. 7. GC-FID chromatograms after SPME of non-spiked (a) and spiked (b) leaching from plastic baby bottle with 10 µg L\(^{-1}\) of bisphenol A.

Fig. 8. Determined concentration of bisphenol A (BPH-A) in leaching from plastic baby bottle after four time reusing.
Conclusions
This study shows application of nanostructural poly (o-aminophenol) as a new SPME fiber combined with GC–FID is a precise method for reproducibly analyzing trace bisphenol A from aqueous samples. Better chromatographic shape and sensitivity were obtained by derivatization of bisphenol A using acetic anhydride. Different effective parameters were studied and optimized. The figures of merit belonged to the method were favorable. The dynamic range was achieved in the ranges of 2 - 500 µg L\(^{-1}\). The limits of detection and RSD were 0.6 µg L\(^{-1}\) and <6.8% respectively. The feasibility of using the SPME–GC–FID system to measure the amount of bisphenol A in leaching from plastic baby and drinking water bottle was tested. Bisphenol A was detected in the range 5–15 µg L\(^{-1}\).

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