

Full Length Research Paper

Determination of bisphenol A in exposed bottled water samples to direct sun light using multi walled carbon nanotubes as solid phase extraction sorbent

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Polycarbonate plastics (PC) containing bisphenol A (BPA) are used for the production of bottles for storage drinking water. Trace amounts of BPA have been detected in ten different brands of bottled water samples from Khartoum supermarkets. Residues of BPA in bottled water were pre-concentrated using solid-phase extraction and determined by high performance liquid chromatography with fluorescence detector. Recently, multiwalled carbon nanotubes (MWCNTs) have great attention because of their application in many fields. MWCNTs were used as adsorptive material for solid phase extraction which it was packed in mini column prior to pre-concentration step. The results indicate that the mean concentration of BPA in ten bottled water brands was 4.28 ng/ml for those stored at room temperature (25°C) and 11.81 ng/ml for those exposed to direct sunlight (40°C). The concentration of BPA in bottled water exposed to direct sun light was significantly higher than those stored at room temperature. Recovery of BPA from water was 92.5%. Detection limit for BPA were 0.30 ng/ml. Good correlation coefficients (>0.9996) were obtained. The method has been successfully applied for the determination of trace BPA in bottled water samples.

Key words: Bisphenol A, plastic bottled drinking water, solid phase extraction, multiwalled carbon nanotube, high performance liquid chromatography.

INTRODUCTION

Bisphenol A (2,2-di(p-hydroxyphenyl) propane, BPA) has been used for production of polycarbonates, epoxy resins, polysulphones and polycrylate resins (Letcher et al., 2005; Joskow et al., 2006). BPA is obtained by condensation of phenol with acetone in the presence of an ion-exchange resin as a catalyst. Because BPA and its derivatives can be harmful to living organisms, their presence and concentration in food products must be

monitored.

Polycarbonate plastics (PC) are characterized by great strength, stability, elasticity, and low density. For these reason they have been widely used for production of food packaging, bottles of water, kitchen utensils, medical equipment (Hao-Chang et al., 2010; Rivas et al., 2009; Schecter et al., 2010). Substances capable of releasing BPA are used to coat the interior surfaces of bottles

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such as epoxy resins, plastics and fibers (Sung-Hyun et al., 2010; Joaquim et al., 2010; Munguia-Lopez and Soto-Valdez, 2001; Aldea et al., 2008; Wu et al., 2010). BPA residues have been detected in water stored in packages made of PC. BPA can be released from PC and migrate to the water inside the bottle. This migration is promoted by acidity of the water stored, elevated temperature, mechanical cleaning, and use of detergents for cleaning this packaging (Coulier et al., 2010; Carvalho et al., 2015; Lane et al., 2015).

Since the BPA level reflects the amount of the analyte that has been recently adsorbed, the determination of BPA is useful for assessing occupational and environmental exposure. However, monitoring BPA in bottled water is difficult task due to the low concentration of the analyte. Therefore, the determination of BPA in bottled water samples need a pretreatment step as well as sensitive instrumental technique such as chromatographic technique high performance liquid chromatography (HPLC) equipped with fluorescence detection (John and Irving, 2013). Various methods such as liquid-liquid extraction (LLE) (Jiang et al., 2015; Larki et al., 2015) and solid phase extraction (SPE) (Yang et al., 2013; Gallart-Ayala et al., 2010) have been employed as sample pretreatment procedure for the determination of BPA. Among the aforementioned sample pretreatment procedures, solid-phase extraction based on the use of different types of adsorbents such as C_{18} (Liu et al., 2014), C_8 (Hadjmohammadi et al., 2010) and multi-walled carbon nanotubes (MWCNTs) (Ma et al., 2015).

In order to achieve valuable and effective adsorbents for the SPE of the target analytes BPA, new kind of adsorbent for improving the enrichment performance are needed. In recent years, a novel carbonaceous material multi-walled carbon nanotubes (MWCNTs) was successfully observed to adsorb increasing attentions. On the basis of their peculiar electronic, metallic and structural characteristics, they have been exploited in analytical and other fields.

MWCNTs used as adsorbents for the preconcentration of BPA from bottled water samples for chromatographic analysis (Yaqi et al., 2003), because of high surface area and inner volume, stability, high enrichment factor and mechanical strength. MWCNTs packed in mini-column pre-treated with methanol to concentrate BPA. The strong interaction may be attributed to the unique structure of carbon nanotubes, the hexagonal arrays of carbon atoms in graphene sheets of carbon nanotubes surface have strong interaction with the two benzene rings of BPA. The facts mentioned earlier show that MWCNTs may have great analytical potential as effective solid-phase extraction adsorbent for BPA.

In the present paper, the aim of the present study was to investigate the presence of BPA in ten brands of bottled drinking water under different storage situations in the province of Khartoum, Sudan. The applicability of MWCNTs as packing adsorbents for solid phase

extraction was investigated by using BPA as model compound. The analyte were extracted by a MWCNTs packed solution, and the analyte attracted on MWCNTs were eluted with suitable amount of methanol. Finally, the elute was analyzed by high performance liquid chromatography with fluorescence detector.

MATERIALS AND METHODS

Apparatus, reagents, and water samples

Bisphenol A was obtained from Acros Organics (NJ). Standard stock solutions (1000 $\mu\text{g/ml}$) containing this compound were prepared by dissolving an appropriate amount of bisphenol A in methanol. Working standard solutions 1, 10, 20, 40 and 50 ng/ml were prepared by an appropriate dilution of stock solutions with water. Methanol was purchased from ScharalaceChemi SA, Barcelona, Spain. Sodium hydroxide and hydrochloric acid were guarantee grade reagents (Beijing Chemicals Corporation, Beijing, China). MWCNTs with an average external diameter of 30 to 60 nm were kindly provided by Tsinghua-Nafine Nano-Powder Commercialization Engineering Center.

The Mini column (6.0 \times 1.0 cm i.d.), employed for packing the MWCNTs was made of polyethylene containing glass wool placed at both ends of mini column aiming to prevent sorbent losses during the system operation.

A MWCNTs-packed mini column was prepared by modifying 0.5 g of MWCNTs was packed into the mini column. The 20- μg polypropylene upper and lower frits remained at each end of the column to hold the MWCNTs packing in place. Prior to use, the entire solid-phase extraction assembly was carefully washed with sufficient methanol.

To reduce the organic and inorganic contaminants, all beakers, calibrated flasks, and other glassware used in the experiments were cleaned sequentially with tap water, neutral detergent, and tap water, then soaked in nitric acid for 48 h and cleaned with Mill Q purified water.

Ten brands (labeled 1-10) of commonly consumed bottled water were randomly purchased from local supermarkets in Khartoum, Sudan. A set of ten different brands of bottled water was purchased from among those stored at room temperature (25°C) while the second set (the same brands as those in the first set) was set purchased from among those exposed to direct sun light (39 to 42°C). In each case, water in the bottles was immediately extracted and analyzed. Two water bottles per brand were used in the analysis. The two sets of bottles were stored for 1 week prior to extraction. A sample of water (25 ml) was transferred from each bottle to a separating glass funnel. Then, the solid-phase extraction method was used.

Solid phase extraction procedure

As a pre-concentration step, the MWCNTs packed mini-column was washed by 5 ml of methanol and activated with 5 ml of water. Then, a 25 ml of bottled water sample spiked with BPA was passed through the mini-column at a flow rate of 2 ml/min (Pei et al., 2008). After the sample solution had passed through, the mini-column, it was washed with 5 ml of 10% methanol aqueous solution to remove matrix materials from the column. Then, the BPA retained on the SPE packing was eluted with 2.5 ml of methanol. Finally, 20 μl of methanol elute was injected into the HPLC system for the HPLC determination. By using the standard solutions of BPA, the external calibration curve can be acquired, and then the

Table 1. HPLC Conditions for the Analysis of BPA.

HPLC system	PerkinElmer Flexar FX-10 UHPLC
Injection volume	50 μ l
Column	PerkinElmer C8 (150 mm \times 4.6 mm, 5 μ m)
Mobil phase	Acetonitrile/Water (60/40)
Flow rate	2 ml/min
Detector wavelength	Excitation: 275 nm/Emission: 313 nm
Detector response time	0.1 s
PMT, Em BDW	Super high, wide
Run time	7 min

Table 2. Physical characteristics and pH of bottled water samples.

Bottled water brands	pH	Color
1	7.5	Clear
2	7.2-8.2	Clear
3	7.4	Clear
4	7-8	Light blue
5	6.5-8.5	Light blue
6	8.0	Light blue
7	7,8	Light blue
8	7.5	Clear
9	7.5	Clear
10	7.4	Light blue

concentration of BPA in the eluate can be gotten. Finally, the recoveries of BPA at two situation storage at room temperature and exposed to direct sun light for one week obtained by comparing the concentration of BPA in the elute with the spiked concentration levels.

Instruments

Standards and bottled water samples were analyzed with a PerkinElmer Flexar FX-10 HPLC system including a PerkinElmer Series 200, a Fluorescence detector. The separation was performed on Mini Column.

A 744-pH Meter Metrohm (Switzerland) was used for pH measurement. Millipore SAS 67120 Molsheim system for deionized water (Table 1).

RESULTS AND DISCUSSION

Possible factors that may influence the enrichment include the eluent and its volume, sample volume, pH of the sample and flow rate of the sample. These parameters will place an important role on the concentration of the target analytes.

Effect of sample pH on adsorption

The adsorption of BPA on MWCNTs depends on pH

value for the extraction of BPA. Ten brands of bottled water samples were purchased at a Khartoum city markets, the amount of BPA was determined by HPLC after SPE on MWNTs. Table 2 obtained the physical characteristics and pH of BPA of the ten examined bottled water brands.

Effect of eluent concentration and volume

The eluent concentration is one of the most important parameters of desorption of BPA from the mini-column. It was found that methanol was an effective eluent for BPA analyte. And therefore, it was accepted as an eluent. To find the required volume of methanol to elute the analyte from the mini column, eluent volumes up to 10 ml were investigated. The experimental results indicated the quantitative recoveries (95%) could be achieved when the volume of methanol was in the range 2 to 10 ml. Because application of a smaller volume of eluent contributes to obtaining a higher preconcentration factor, 2.5 ml of methanol was adopted as the eluent throughout the experiments.

Effect of flow rate of sample solution

In solid-phase extraction system, the flow rate of sample

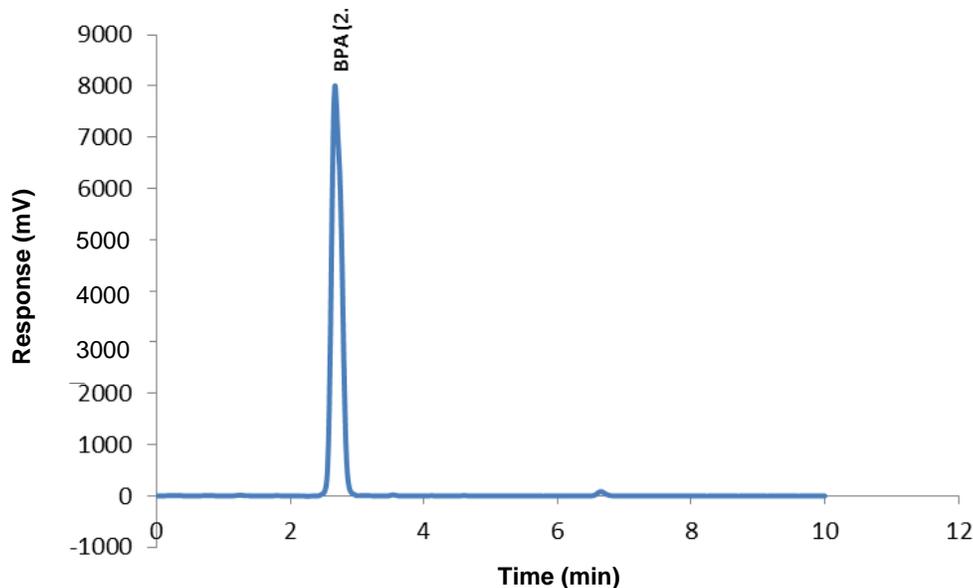


Figure 1. Chromatogram obtained the standard of BPA (1 ng/ml).

solution does not only affect the recovery of analyte, but also controls the analysis time. After optimization of HPLC instrument, determination of BPA was performed at flow rate of 2 ml/min, an example of chromatogram obtained the standard of BPA (1 ng/ml) as shown in Figure 1.

Effect of volume of the sample solution

To obtain reliable and reproducible analytical results and a high concentration factor, it is very important to get satisfactory recoveries for BPA in a large volume sample solutions as possible. So it is necessary to obtain the breakthrough volumes. Following the experimental procedure, the recoveries of the analyte at different volumes were obtained. The effects of sample volumes on the recoveries of the BPA are good recoveries (>92.5%) were obtained for BPA when the volumes of sample solutions were under 50 ml. Although a decline of recoveries was observed for the BPA in 100 ml of sample solutions, the recoveries for the analyte were still above 90%, which was acceptable in trace analysis. A sample volume of 25 ml was selected in the subsequent analysis of several environmental samples.

Comparison studies

For comparative studies, the results obtained in this research were compared with those of other researches. As mentioned in breakthrough volume experiment earlier, the recoveries (>92.5%) were obtained when the volumes of bottled water samples were under 50 ml. In some

cases, it appears that the recoveries obtained in this present work with bottled water samples are better than those obtained in some of other research work (Syed, 2011). In this research, C_{18} were used for the pre-concentration of several BPA aqueous solution and recoveries obtained were 80%. In research of Shan et al. (2014), another adsorbent C_8 was used for the pre-concentration of BPA in water samples at 50 ng/ml and recoveries were 89.2%.

In respect to the extraction recoveries, the aforementioned facts show that multi-walled carbon nanotubes were more effective than or as effective as some other commercially available solid phase extraction adsorbents such as C_{18} , C_8 for the solid phase extraction of BPA. Carbon nanotubes are hollow nanosize tubes that constitute a new structure of graphite carbon consisting of one or several concentric tubules with a helically wound hexagonal honeycomb lattice. The reason why the MWCNT's have stronger interaction with BPA perhaps is that the hexagonal arrays of carbon atoms in graphene sheets in their structures may have strong interaction with the benzene ring in their structures.

Linearity of calibration curves and correlation coefficients

Calibration was performed with mixed standard solutions of the BPA. To determine the range in which response was a linear function of amount injected, standard solutions containing 10, 20, 30, 40 and 50 ppm BPA in the mobile phase were injected. Each solution was injected in twice. Good correlation coefficients (>0.9996)

Table 3. Limits of detection and quantitation and Recovery of BPA from 25 ml Bottled water spiked at 1 ppm after SPE on MWNTs.

Parameter	LOD (ng/ml)	Recovery (%)
BPA	0.30	92.5

Table 4. BPA concentrations in bottled drinking water products.

Examined bottle	Country of origin	Content (ml)	¹ Mean concentration \pm ² SD of samples stored at room temperature (ng/ml)	³ %RSD	¹ Mean concentration \pm ² SD of samples exposed to sunlight (ng/ml)	³ %RSD
1	Sudan	500	3.40 \pm 0.15	4.3	8.32 \pm 0.33	4.0
2	Sudan	600	4.50 \pm 0.18	3.9	7.90 \pm 0.36	4.6
3	Sudan	400	4.32 \pm 0.14	3.2	10.76 \pm 0.30	2.8
4	Sudan	600	3.10 \pm 0.13	4.2	9.64 \pm 0.32	3.3
5	Sudan	500	6.24 \pm 0.17	2.8	10.10 \pm 0.31	3.1
6	Sudan	300	3.62 \pm 0.14	4.0	12.23 \pm 0.24	2.0
7	Sudan	400	3.21 \pm 0.12	3.6	16.85 \pm 0.20	1.2
8	Sudan	500	4.59 \pm 0.10	2.2	14.32 \pm 0.23	1.6
9	Sudan	600	5.65 \pm 0.11	1.9	12.80 \pm 0.27	2.1
10	Sudan	300	4.21 \pm 0.13	3.1	15.27 \pm 0.23	1.5

¹Mean concentration (three replicates). ²Standard deviation. ³Percentage relative standard deviation.

were obtained. Limits of detection (LOD) were defined as the concentrations giving peak heights five times the standard deviation of the baseline signal. The limits of detection and recovery of BPA from water are shown in Table 3. The compounds were added to the water at a concentration of 1 ppm. The method proposed is characterized by high recovery.

BPA levels in plastic bottled water

Table 4 shows that the mean concentration of BPA in ten bottled water brands was 4.28 ng/ml for those stored at room temperature (25°C) and 11.81 ng/ml for those exposed to sunlight (40°C). The concentration of BPA in bottled water exposed to sun light was significantly higher than those stored at room temperature. Among the bottled water brands stored at room temperature, the difference between their BPA concentrations was not significant, e.g., 1 vs. 6, 2 vs. 8, 3 vs. 10, 4 vs. 7, 5 vs. 9. The same observation applied to those exposed to sun light and stored outdoor.

Because of the trace concentration of BPA and to facilitate the desired sensitivity and selectivity of HPLC measurements, we used SPE as an efficient pre-concentration step and it has several advantages over other separation techniques in view of trace and major concentrations of trace ions present in hazardous samples can be removed with equal ease, higher

enrichment factors, reusability of the adsorbent, absence of emulsion, minimal costs due to low consumption of reagents, ease of automation, environmental friendly and safety with respect to hazardous samples. Accordingly, several solid phase extractants have been employed for enrichment of traces of elements from dilute solutions.

In this respect, SPE has commonly been used as a selective technique for pre-concentration/separation of various inorganic species and enhanced selectivity is achieved because of the discriminatory binding of the analyte to a solid support and accumulated analyte is subsequently eluted with a small volume of solvent. The adsorbents with high surface area are preferred in solid phase extraction of traces of BPA. MWCNTs are adsorbent materials in the pre-concentration step for solid phase extraction method. According to our literature review, until now, the MWNTs had much better extraction capacity for the target, BPA in bottled water samples and more suitable to extract BPA in samples. In a word, MWCNTs would be a valuable and alternative adsorbent for the trace level analysis of BPA, and will be widely used in analytical and environmental fields.

Polycarbonate plastic has been the material of choice for food and beverage product containers for nearly 50 years, because it is lightweight, highly shatter-resistant, and transparent. It is widely used in various food containers and many other everyday items. Many plastic bottled water companies in Sudan use polycarbonate plastic for water bottles. Toyo'oka et al. (2008) found

the concentration of BPA in drinking water bottles made from polycarbonate to be between 3 and 10 ng/ml. While another study did not detect BPA in different plastic containers for beverages, including drinking water (Shao et al., 2005). Our results are in agreement with other studies which reported the presence of plastic residues in water stored in bottles (Tokuanaga et al., 2008; Amiridou and Voutsas, 2011). This could be attributed to the migration of BPA from the bottle material to the water since bottle quality may vary depending on the raw material and technology used in bottled water.

The fact that the bottled water products stored at room temperature had lower levels of BPA demonstrate that migration of BPA from bottle coatings into the water is extremely slow and is negligible. But, the bottled water products which exposed to direct sunlight for one week had relatively high levels of BPA due to the fast migration from their bottle coatings into water during the bottle sterilization process.

High concentrations of BPA levels in different bottled water products in this analysis is due to careless exposure of products to heat (sunlight) during storage and transportation. Also, it could be due to differences in can coatings (type, amount, etc.) and sterilization condition (temperature - duration) used by different canned food products companies.

CONCLUSION AND RECOMMENDATIONS

The proposed method involves concentration of BPA residues in drinking water samples by solid-phase extraction, then analysis by high performance liquid chromatography with fluorescence detection. Based on the experimental results, a simple and appropriate method was developed for the solid phase extraction and HPLC determination of BPA in bottled water samples. Under the optimized conditions, detection of limits of 0.30 ng/ml was achieved for BPA. The accuracy of the proposed method was tested by recovery measurement on spiked sample, the recovery of 92.5% were obtained. The experiment proved that BPA is present in the water samples stored at room temperature at concentrations in range 3.10 to 6.24 ng/ml which is lower than the concentrations of water samples exposed to direct sunlight in range from 7.90 to 16.85 ng/ml.

Here are some recommendations of what need to be done to avoid the risky effects of BPA, drink tap water, rely on BPA-free stainless steel water bottles instead of plastic bottles, and always store the bottles in dry, clear and cool place.

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