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The Application of Multiwalled Carbon Nanotubes as Solid Phase Extraction Sorbent for Pre-concentration of Bisphenol A in Canned Food

Abstract

A simple, reliable and suitable analytical method was developed for solid phase extraction of bisphenol A in several canned food products such as beverages, fruits, vegetables and foodstuffs bought in Sudan markets. The analysis done by using a packed Multi Walled Carbon Nanotubes (MWNTs) mini-column followed by high-performance liquid chromatography with fluorescence detection. In summary, we have preliminarily demonstrated that as promising sorbents, MWNTs have great potential for solid phase extraction of BPA. The recommended parameters of proposed method influencing the preconcentration of the analytes, such as pH of the sample, flow rate and sample volume have been used. Under the optimized conditions, the calibration graphs were linear with the correlation coefficient ranged 0.9906-0.9999. According to the results, the concentrations of BPA in samples exposed to direct sunlight were higher than samples that stored at room temperature. The range of BPA level of beverages were (0.3-0.8ng/ml) at room temperature where it were (1.5-2.1ng/mL) at beverages exposed to direct sunlight , in vegetables the levels were (20.0-38.0ng/g) at room temperature and (30.0-43.0ng/g) in vegetables exposed to direct sunlight , the levels in fruits were (6.0-24.0ng/g) at room temperature and in fruits exposed to direct sunlight were (8.0-38.0ng/g), in fat-containing products the levels of BPA at room temperature were (2.1-45.0ng/g) and (3.0-55.0ng/g) in products which exposed to direct sunlight. Limits of detection (S/N=3) from 0.1ng/mL in beverages to 5.5 ng/g in fruits. The accuracy of proposed method was tested by recovery measurements on standard bisphenol A spiked canned food samples, the recoveries ranged 90.2-102.0% were obtained.

Keywords: Bisphenol A; Canned food; Multiwalled carbon nanotubes; Solid phase extraction; High performance liquid chromatography; Florescence detector

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Abbreviations: BPA: Bisphenol A; GC: Gas Chromatography; HPLC: High-Performance Liquid Chromatography; SPE: Solid Phase Extraction; CNTs: Carbon Nanotubes; MWCNTs: Multi Walled Carbon Nanotubes; LODs: Limit of Detections

Introduction

The most effective method for the preservation of food from heat or chemical treatment is through sealing of food in tight containers [1,2]. Different types of food such as fish, meat, fruits and vegetable can be stored safely in airtight sealed containers [3-5]. In general the process of canned foods involve washing, cutting and filling in metal containers a long with a canning fluid such as water, salted water or fruit juice. Bisphenol A (2,2-bis(4-hydroxyphenyl)propane) (BPA) is a toxic chemical used essentially as a monomer or starting material in the production of cans, epoxy resin and polycarbonate bottles. BPA can leach into foods from the lacquer lining of cans and from other food containers and bottles made from certain plastic.BPA is an **endocrine disruptor** meaning that it's a chemical that can interfere with the endocrine (or hormonal system). Endocrine disruptors can cause cancerous tumors, birth defects and other developmental disorders. BPA was shown to be associated with breast and prostate cancers, genital defects in males [6-8]. Due to possible low dose effects of unknown toxicological relevance and weekly oestrogenic BPA is the subject of many investigating assessing, its potential toxicity and human exposure for a more conclusive risk assessment [9,10]. Nowadays the exposure of human to BPA is estimated to be mainly by food consumption and widely spread, while air, dust and water. BPA in food and beverages accounts of the majority of daily human exposure.

Chromatographic techniques such as GC or HPLC are among the most widely used methods for analysis of harmful organic contaminants such as BPA [11-13]. However their sensitivity and selectivity are usually insufficient for direct determination of these contaminants at very low concentration level in complex matrix food samples. Therefore, a sample extraction and clean up prior to chromatographic analysis is usually critical. Over the last two decades, solid phase extraction (SPE) has become the most powerful technique available for the sensitive and selective sample preparation prior to analytical chromatography. SPE extends the lifetime of chromatographic systems and improves qualitative and quantitative analysis. Different types of solid phase extraction (SPE) sorbents including C_{18} or C_8 silica [14], polystyrene-divinylbenzene polymers [15], sol–gel organic–inorganic hybrid sorbents [16] and various carbonaceous sorbents have been used, however it shows relatively low recovery for some relatively polar analytes. Carbon nanotubes (CNTs) it is a family type of nanomaterials made up entirely of carbon [17]. This family contains Multi Walled Carbon Nanotubes (MWCNTs) which it is a new material has attracted special interest due to their high mechanical strength, young's modulus, thermal and electrical conductivity, and chemical stability [18]. Several researches work has been reported on the use of MWCNTs as solid phase extraction (SPE) sorbents for the analysis of diverse types of pesticides [19-22].

It has been recently reported that BPA may leach out into food and beverages samples from cans and plastic bottles which they were stored at two different temperatures at room temperature and direct sun light temperature. In the present work we use MWCNTs as mini-column packing adsorbents for S solid phase extraction (SPE) of BPA from can. The BPA in the samples after extraction using solid phase extraction (SPE) was analyzed using HPLC with fluorescence detector.

Materials and Methods

Reagents

BPA (purity 99.7%) was obtained from Acros Organics (NJ). Standard stock solutions (1000µg/mL) containing this compound were prepared by dissolving an appropriate amount of BPA in methanol. Working solution was prepared by an appropriate dilution of stock solutions with water. Methanol (chemical purity 100%) was purchased from Scharalace Chemi SA, Barcelona, Spain. Sodium hydroxide (≥98%) and hydrochloric acid (35%) were guarantee grade reagents (Beijing Chemicals Corporation, Beijing, China). MWNTs with an average external diameter of 30-60nm were kindly provided by Tsinghua-Nafine Nano-Powder Commercialization Engineering Center.

Apparatus

The mini column (6.0×1.0cm 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.

Solid-phase extraction mini column

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

Instrumentation

HPLC consisted of a high-pressure gradient pump (modelL-7100, Merck), a column thermostat, a six-port injection valve equipped with a 100µL injection loop was used, eluent used is methanol/water (60/40), was used as eluent, column size was C8 (150mmx4.6mm, 5µm) , and the extract volume was 50µl. A fluorescence detector (model 1080, Merck) set at 275/305nm. Peaks were integrated using the McDacq software (Leonberg, Germany). Data given in this work were obtained in a time period of 2 months by applying different HPLC methods. A 744-pH Meter Metrohm (made in Switzerland) was used for pH measurement. Millipore SAS 67120 Molsheim system for deionized water.

Evaluation of MWCNTs as SPE sorbents

To evaluate the analytical potential of MWCNTs as solid phase extraction (SPE), BPA were adopted as model compound. The effects of different parameters, such as the sample volume, the eluent volume, and the pH of sample solution, on the recoveries were carefully investigated.

Analysis of samples

From April to June 2014 soft drinks bottles and canned food samples were purchased from Khartoum local supermarkets which they covered at least 80% of markets share of canned food products sold in Sudan. Some of the samples were stored at room temperature and the other exposed to direct sunlight for <1 month before analysis at June 2014. The samples distributes as follow: 6 samples of beverages bottles (cola drink, orange soft drink, lemon soft drink) 3 samples stored at room temperature and 3 samples exposed to direct sun light, 4 samples of canned vegetables (corn, crushed tomatoes) ,4 samples of canned fruits (pineapple, mango), 4 samples of canned fat containing-products (tuna, sardine), 2 samples of each kind of canned food were stored at room temperature and the other 2 samples were exposed to direct sun light.

Sample preparation

Beverages After the beverages had been degassed in an ultrasonic bath, an aliquot of either 15.0 or 20.0mL was diluted 1:1 with buffer (K_2HPO_4/NaOH). If necessary, pH values were adjusted to 7.0 by adding a standardization buffer which it was prepared by adding 29.1mL of 0.1 molar NaOH to 50mL of 0.1 M of dipotassium phosphate. The sample was applied to the mini column after the column had been washed with 5mL of methanol/water (10/90, v/v), BPA was eluted with 2.5mL of methanol/water (70:30, v/v). The eluate was collected in a 5mL measuring flask. This was filled to the ring mark with methanol/water (70:30, v/v). An aliquot of 100 µL was injected into HLPC.

Fruits and vegetables In the case of fruits and vegetables the whole content of the can only the solid portion was homogenized with Ultra-Turrax mixer. One gram of the homogenized sample was mixed with 2mL of methanol in a 15mL polypropylene centrifuge tube. After 1min of shaking; centrifuging was carried out at 2800g for 5min. The supernatant liquid was removed with a Pasteur pipet, and the solid residue was extracted for a second time with 1mL of methanol. The combined supernatants were filtered through a glass microfiber filter GF/F. Then, the sample had been loaded to the mini column, the mini column had been washed with 5mL of methanol/water (10:90 v/v), after that BPA was eluted with 4ml of methanol/water (70:30 v/v). The eluate was collected in a 5 mL measuring flask, which was then filled to the ring mark with methanol/water (70:30 v/v). An aliquot of 100 µL was injected into HPLC.

Fat- Containing Food stuffs. For the analysis of tuna and sardines the liquid part was removed by sieving, and only the solid portion was homogenized. A 1g aliquot of the homogenized sample (fish, 10g) was filled in a centrifuging tube. One milliliter of methanol and 1mL of hexane (fish, 10mL of methanol and 10mL of hexane) were added, and the mixture was shaken for 1min. After centrifuging at 2800g for 20min, the methanol phase was transferred into a centrifuging tube. The solid residue and hexane phase were extracted for a second time by adding a fresh 1mL (fish, 10mL) portion of methanol. The combined methanol extracts were filtered through a glass microfiber filter GF/F. The filtrate was evaporated under a constant steam of nitrogen to approximately 2mL, diluted 1:10 with water, and purified as described above. An aliquot of 100µL was injected into HPLC.

Standard Addition For each food matrix the samples were analyzed as described above. The amounts of BPA in samples were determined in preliminary experiments. The addition of 10, 20 and 30ng of BPA (100, 200 AND 300µL of a 100ng/mL BPA standard solution in methanol) to the sample aliquot used proved to be appropriate. Peak areas were plotted against the amount of analyte added. The recovery was determined by dividing the slope of the linear regression line for the standard addition by the slope of the linear regression line of BPA standard solutions.

Results and Discussion

Effect of sample pH on adsorption

The adsorption of BPA on MWCNTs depends on pH value. For the extraction of BPA, however, the pH of the sample solutions had no effect on the recoveries of bisphenol A in range of pH 2-4, although a dramatic decrease in recoveries of BPA was observed due to deprotonation of hydroxyl of the phenol. The highest recovery (%) was obtained at pH 7 as shown in Figure 1, therefore was chosen as the optimum pH to prepare sample solutions.

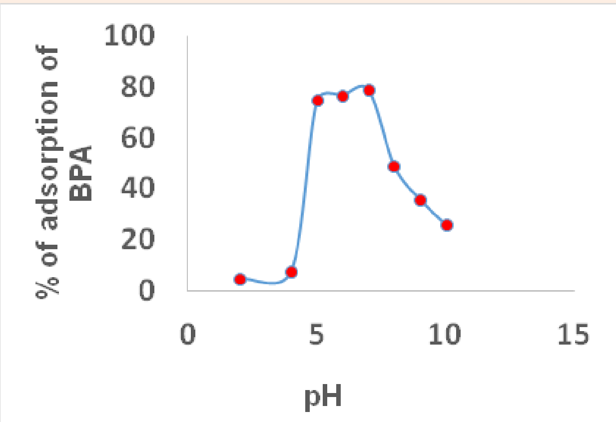

Figure 1: Effect of pH on adsorption of BPA .

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 [23,24] 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 10mL were investigated. The experimental results indicated the quantitative recoveries (95%) could be achieved when the volume of methanol was in the range 2-10mL. Because application of a smaller volume of eluent contributes to obtaining a higher pre-concentration factor, 2.5mL of methanol was adopted as the eluent throughout the experiments.

Effect of flow rate of sample solution

The flow rate of sample solutions affects the retention of BPA on the adsorbent an duration of analysis. Therefore, the effect of flow rate of sample solutions on the desorption of BPA was examined under the optimum pH condition according to recommended procedure. It was found that flow rates up to 8mL/min (the maximum flow rate of our vacuum pump) for sample solution loading on the cartridge had no effect on the recoveries of the analyte. Therefore, 8mL/min was chosen as the flow rate of sample solutions.

Effect of volume of the sample solution on the recoveries of BPA

In order to explore the possibility of enriching low concentrations of analytes from large volumes, the effect of sample volume on the retention of BPA was also investigated. For this purpose, 700, 750, 800, 850, and 1000mL of sample solutions containing 1.0µg of BPA were passed through the mini column with optimum flow rate. It was found that quantitative recovery (>95%) of BPA was obtained for the sample volume up to 750mL. above 750mL the recovery for BPA decreased slightly. In this experiment, 750ml of sample solution was adopted for the pre-concentration of BPA from food samples.

Linearity of calibration curves and correlation coefficients

For HPLC method the 18 calibration curve obtained by injecting BPA standard solutions range from 0.2 to 50.0ng/mL, good correlation coefficients ranged from 0.9906-0.9999 were obtained and Limit of detections (LODs) depending on the sample amount and HPLC method as well as the food matrix were in range from 0.1ng/Ml to 5.5ng/g all these results summarized in Table 1.

Analytical performance of the method

The analytical performance of the whole analysis method was assessed by applying the standard addition method. The precision of the method applied, evaluated as the RSD, obtained in Table 2. Recovery tests were carried out with standard BPA, spiked canned food samples and the results are also presented in Table 2.

BPA levels in canned foodstuffs.

BPA levels found in canned foodstuffs are given in Table 3. Traces of BPA were detected in all samples, which the canned food products stored at room temperature and the same products exposed to direct sunlight for <1 month. Very low BPA levels of the beverages at room temperature obtained which they were ranging from 0.3ng/mL to 0.8ng/mL. On the other hand, the soft drinks which they were exposed to direct sun light which the temperature was <40^{0}C the levels of BPA was higher ranging from 1.5ng/mL to 2.1ng/mL .

Table 1: Analytical Data.

Product	Sample Amount	Standard Addition Correlation Coefficient R^2	LOD (S/N=3)
Beverages			
Cola drink	20.0 mL	0.9956	0.3 ng/mL
Orange soft drink	15.0 mL	0.9959	0.1 ng/mL
Lemon soft drink	15.0 mL	0.9999	0.1 ng/mL
Vegetables			
Corn	1.0 g	0.9949	2.9ng/g
Crushed tomatoes	1.0 g	0.9906	1.9ng/g
Fruits			
Pineapple light	1.0 g	0.9958	1.3 ng/g
Mango	1.0 g	0.9942	5.5 ng/g
Fat-Containing Products			
Tuna	10.0 g	0.9989	0.4 ng/g
Sardines	10.0g	0.9939	0.2 ng/g

Table 2: Determination and recoveries of BPA spiked canned food samples.

product	Added (ng/ ml)	Found (ng/ml)	Recovery (%)
Beverages			
Cola drink	10	9.35	93.5±4.9
Orange soft drink	10	9.02	90.2±2.0
Lemon soft drink	10	9.45	94.5±3.5
Vegetables			
Corn	10	9.23	92.3±3.6
Crushed tomatoes	10	9.66	96.6±8.2
Fruits			
Pineapple light	10	10.1	101.0±10.4
Mango	10	9.92	99.2±9.9
Fat-Containing Products			
Tuna	10	9.64	96.4±8.1
sardines	10	10.2	102.0±10.6

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Table 3: BPA Concentrations in Canned Beverages, Vegetables, Fruits and Fat containing products Obtained by rounding standard deviation date calculated from four determination.

Product	Country of Origin	Content	Amount of Solid (g)	Date of Starge	Date of Analysis	Conc. of Samples Stored at Room Temp. BPA±SD (ng/g)	Conc. of Samples Exposed to Sunlight BPA±SD (ng/g)
Beverages							
Cola drink	Sudan	300 mL	-	Apr-14	Jun-14	0.8±0.1	2.1±0.1
Orange soft drink	Sudan	300 mL	-	Apr-14	Jun-14	0.4±0.1	1.8±0.1
Lemon soft drink	Sudan	300 mL	-	Apr-14	Jun-14	0.3±0.0	1.5±0.0
Vegetables							
Corn	Egypt	300g	280	Apr-14	Jun-14	38±3.1	43±3.5
Crushed tomatoes	Sudan	400g	-	Apr-14	Jun-14	20±2.9	30±2.0
Fruits							
Pineapple light	Sudan	500g	350	Apr-14	Jun-14	6.0±2.1	8.0±3.0
Mango	Sudan	400g	250	Apr-14	Jun-14	24±4.0	38±3.5
Fat-Containing Products							
Tuna	Sudan	200g	150	Apr-14	Jun-14	45±6.5	55±7.1
Sardines	Sudan	200g	100	Apr-14	Jun-14	2.1±0.3	3.0±0.4

Table 4: Comparison of the published methods with the proposed method in this work.

	Detection Method	Extraction Method		Beverages BPA Levels	Canned Vegetables BPA Levels	
Food additive,2011	G C-MS	DLLME	5.0ng/l	0.03-4.7µg/l	-	
Food control,2013	GC-MS	DLLME	0.3µg/kg	-	3.0µg/kg	3.4µg/kg
This work	HPLC-FL	SPE	5.50.1ng/ml ng/g	0.3-2.1ng/ml	20-43ng/g	6.0-38ng/g

In the case of canned vegetables BPA concentrations were determined in solid portions, which are the parts of the can content actually consumed. In corn, BPA concentration was found to be 43ng/g of solid portion when it was exposed to direct sun light but at the room temperature it was found to be 38 ng/g,which is in agreement with previously reported data ranging from 18.4 to 95.3ng/g [25]. In crushed tomatoes BPA concentration, determined by analyzing the whole content of the can, was 20ng/g at room temperature and more concentrate when it was exposed to direct sun light which it was 30ng/g, which is consistent with the amount of BPA reported in the literature [26,27] (<10-21ng/g).

Most canned fruits exhibited BPA levels at room temperature in the range from 6.0ng/g for the light pineapple to 24.0ng/g for mango, we obtained that the levels of BPA higher when they were exposed to direct sun light the found to be 8.0ng/g for pineapple and 38.0ng/g, and this is corresponds to the data published by Thomson et al. [28]. In fat-containing ready to eat products BPA levels at room temperature were 45ng/g for tuna and 2.1ng/g for sardine, The BPA concentration in the solid portion of tuna can when it was exposoed to direct sun light 55ng/g and 3.0ng/g for sardine which obviously higher than that stored at room temperature. The above results showed that the concentration of BPA in all samples exposed to direct sunlight is higher than samples that stored at room temperature. Also, MWCNTs were at least as effective as C_{18} for the solid phase extraction of BPA, but they were more effective. The reason that the MWNTs have a stronger interaction with BPA perhaps is that the hexagonal arrays of carbon atoms in graphene sheets in their structures may have a strong interaction with the benzene rings of BPA.

Comparison study

Table 4, compares the characteristic data of present method with those reported in literatures) [29,30]. Generally, The BPA levels obtained in the two reported work and this work was approximate to each other. We obtained that in beverages the levels of BPA is higher in the reported than our work but in the canned vegetables and fruits is much higher than in this work and this exactly due to higher temperture that canned exposed to when they were stored in direct sun light.

Conclusion

A simple and reliable method was developed for solid phase extraction (SPE) of BPA in several canned food products by using a MWCNTs packed mini column. The results obtained canned food products. For the more polar analytes, BPA, MWCNTs as solid phase extraction (SPE) adsorbents are more powerful than C_{18}, because they possess a higher capability to extract BPA from larger volume of sample solutions. That the analytes retained on MWCNTs can be easily desorbed and no carryover is observed in the next analysis is another advantage of present method. In summary, we have preliminarily demonstrated that as promising sorbents, MWNTs have great potential for solid phase extraction of BPA.

It should mention that the samples of canned food products were collected in April 2014 one is stored at room temperature and the other same products exposed to direct sunlight before analysis in June 2014. We found that the concentration of BPA in canned food products is low in general, the average BPA level in all products in two situations was 0.8μg/g. The fact that the canned food products stored at room temperature had lower levels of BPA demonstrate that migration of BPA from can coatings into the food is extremely slow and is negligible. But, the canned food products which exposed to direct sunlight for >1 month had relatively high levels of BPA due to the fast migration from their can coatings into food during the can sterilization process.

Variation of BPA levels in different canned food products (0.14-4.71μg/g) 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 can sterilization condition (temperature - duration) used by different canned food products companies. We found in our analysis that if someone eating in just one meal with at least one canned food product, their levels of BPA are as much as those that have been shown to cause health effects So, we have some recommendations to avoid the dangerous effects of BPA ,Cook your food fresh, **Buy in glass bottles**. But even they may have trace amounts of BPA we have ways to beat BPA from canned food. Don't use canned baby formula, don't **eat canned food if you are pregnant, Also,** Buy in bottles, not cans**.** Many products, like crushed tomato, are available in bottles as well as cans, Start cooking instead of just heating. The fact that 17% of the Sudanese dieses comes out of cans is just a scandal when we are surrounded by fresh food. Cook it from scratch and avoid the problem altogether.

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