μ-SPE of Bisphenol A in Beverage and Water using Multi-walled Carbon Nanotubes-Reinforced Agarose Film

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Abstract

Bisphenol A (BPA) is a synthetic chemical widely applied as the monomer in the manufacture of polycarbonate plastics and epoxy resins. In this work, a micro-solid phase extraction (μ-SPE) technique using multi-walled carbon nanotubes (MWCNTs)-reinforced agarose film function as an adsorbent coupled with high performance liquid chromatography-fluorescence detection was developed for the analysis of BPA in beverage and environmental water. Under the optimal extraction conditions, the method offered excellent linearity with correlation coefficient, r = 0.9977, in the range of 0.1 to 100 ppb. The limit of detection (LOD) and limit of quantification (LOQ) were 0.02 and 0.1 ppb respectively.

The technique demonstrated acceptable relative recoveries average ranging 81.0 to 98.7% using carbonated beverage, river and sea water samples spiked at 1 and 10 ppb. The proposed μ-SPE was proven simple, cost-effective and consuming trace amounts of organic solvents; thus, contributing towards green chemistry.

Keywords: Agarose, beverage, bisphenol A, film, microextraction, water.

Introduction

Bisphenol A (BPA) is a synthetic chemical which has been used frequently in the fabrication of plentiful products. It acts as the monomer base when producing polycarbonate plastics and epoxy resins. BPA is also known as an endocrine disrupting compound that causes negative health effects to human. It also causes genotoxicity, neurotoxicity and cytotoxicity in aquatic organisms[1]. Trace of BPA can be detected in environment especially in surface water like river, lake and sea as it is discharged into aquatic environment by different pathways: manufacturing process of BPA based products, effluents from municipal and industrial wastewater treatment plant and direct discharge through landfill leachate[2]. Besides, trace of BPA ranging from 3.5 to 59.8 ng/L was also reported in tap water[3].

BPA has increasingly grabbed the attention of society in the concern of health safety. Therefore, there are number of methods demonstrated in order to determine the BPA residues in food and water samples to ensure the safety of the products. Initially, researchers utilized liquid-liquid extraction (LLE) and solid phase extraction (SPE) techniques to extract BPA from various types of sample matrices[4-8]. These two techniques are popular due to the ability of combining clean-up, pre-concentration and signal enhancement in just a single step. However, both LLE and SPE show drawbacks. LLE demands a big volume of organic solvents, forms emulsions, laborious and time consuming whereas SPE is expensive and has poor reproducibility.

Driven by raising awareness on environmental protection, liquid-phase microextraction (LPME) and solid-phase microextraction (SPME) were developed. SPME was first proposed by Arthur and Pawliszyn[9] in 1990 with the use of polymer-coated fiber. LPME is a solvent-minimized LLE. Both of these sample pretreatment techniques are inexpensive, simple and consuming minimal solvent as compared to both LLE and SPE. The LPME offered high sensitivity in ultra-trace detection levels in milk[10] and water[11] mainly because of the high analyte's enrichment with minute amounts of extractant.

Rastkari et al[12] applied SPME to determine the BPA in canned food12. In this research, the fiber was coated with single-walled carbon nanotubes (SWCNTs) adsorbent rather than common PDMS adsorbent. SWCNTs fiber showed an improved thermal stability, higher extraction capacity and longer life span when comparing to PDMS fiber. Apart from that, Chang et al[13] also demonstrated headspace-SPME to extract BPA from water and plastic containers and succeeded in the extraction of BPA with ultra-trace detection level.

This study provides an alternative green method to determine the BPA in beverage and water samples. Moreover, the method applied in this study is simple, inexpensive, applying minimal solvent and environment friendly compared to LLE and SPE.

Material and Methods

Reagent, standard solution and material: HPLC grade methanol (MeOH), acetonitrile (ACN), isopropanol (IPA) and tetrahydrofuran (THF) were purchased from Merck (Darmstadt, Germany). Analytical grade agarose, multi-walled carbon nanotubes (MWCNTs) and bisphenol A (BPA) reference standard were attained from Sigma-Aldrich (St. Louis, Missouri, United States). 500 ppm of BPA standard stock solution was prepared by dissolving 0.005 g of BPA in a 10 mL volumetric flask and diluting to volume with methanol. Series of working standard solutions was prepared by diluting the standard stock solution with methanol. All the solutions were stored at 0^0C in darkness when they were not in use. The sample solution was stirred using a hot plate stirrer purchased from Favorit (Semenyih, Malaysia) and a stirring bar (12 × 4 mm) during extraction.

Preparation of sample: Two types of soft drink samples packed in epoxy tin bottle were obtained from local department store in Kuala Terengganu, Malaysia. The soft drink samples were degassed for 20 min in an ultrasonic bath prior to extraction. The environmental water samples were collected from river and sea around Kuala Terengganu, Malaysia and kept in freezer prior to extraction.

Preparation of multi-walled carbon nanotubes-reinforced agarose film: Agarose solution (1.0%) was prepared by dispersing agarose (0.30 g) with deionized water (30 mL) in a 100 mL beaker. The solution was boiled to ensure that all agarose powder was dissolved completely. Then, MWCNTs (90 mg) were added into the boiled agarose solution and stirred to mix well. An aliquot of the warm solution (4 mL) was pipetted into a glass Petri dish (50 mm in internal diameter). The solution was allowed to cool and gel for at least 30 min at room temperature. The Petri dish was dried for 24 h at room temperature. The adsorbent film preparation was adopted from Loh et al.[14] The multi-walled carbon nanotubes-reinforced agarose film (MWCNTs-AF) formed was cut into a square piece (1 cm x 1 cm) with a scissor to function as an adsorbent film.

Micro-solid phase extraction procedure: The sample was adjusted to pH 6 using either 0.1 M hydrochloric acid (HCl) or 0.1 M sodium hydroxide (NaOH). Then, sample solution (30 mL) was pipetted into a 40-mL sample vial and a stirring bar was put into it. A piece of MWCNTs-AF was conditioned by dipping in IPA for 2 min followed by deionized water for 1 min to activate the sorbent surface. The film was then placed into the sample solution for extraction. After stirring at a speed of 650 rpm for 30 min, the MWCNTs-AF was removed and put into a 500-µL safe-lock-tube. The film was sonicated with ACN (200 µL) for 15 min. The ACN was then filtered through 0.2 µm nylon syringe filter prior to quantification using high performance liquid chromatography-fluorescence detection (HPLC-FD). Figure 1 shows the schematic diagram of micro-solid phase extraction (µ-SPE) system using MWCNTs-AF as adsorbent film.

High performance liquid chromatography conditions: All analyses were performed using high performance liquid chromatography (HPLC) (Shimazu, Kyoto, Japan) coupled with fluorescence detection (FD) (Shimazu, Kyoto, Japan). The HPLC conditions were modified from Loh et al[15]. The chromatographic determination of BPA was carried out on a reversed phase C18 column (4.6 × 150 mm, 5 µm) that was purchased from Sigma-Aldrich (St. Louis, Missouri, United States). The chromatographic determination was carried out using isocratic mobile phase which consisted of acetonitrile-deionized water (50:50) (v/v) at the column temperature of 30^0C. The flow rate and volume of injection were set at 1.0 mL/min and 10 µL respectively. The excitation and emission wavelengths were set as 275 nm and 305 nm respectively.

Optimization and validation of µ-SPE-MWCNTs-AF-HPLC-FD: The µ-SPE-MWCNTs-AF technique was optimized for sample volume, concentration of MWCNTs, stirring speed, extraction time, desorption solvent, desorption time and salt addition. The method was then validated in terms of linearity, relative recovery, limit of detection (LOD) and limit of quantification (LOQ). The LOD was defined as the concentration that produced a peak height of three times the background noise (ratio of signal to noise = 3:1). The LOQ was calculated based on the sample concentration with a peak height of ten times the background noise (ratio of signal to noise = 10:1).

Results and Discussion

Optimization of µ-SPE-MWCNTs-AF technique: Seven extraction parameters were examined comprehensively to increase the analyte enrichment in this study. Sample volume, amounts of multi-walled carbon nanotubes (MWCNTs), stirring speed, extraction time, type of desorption solvent, desorption time and salt addition were optimized thoroughly before applying this technique in the analysis of beverages and water samples. The optimization was performed with the use of deionized water samples spiked with BPA to give a concentration of 20 ppb. Triplicate extraction was performed for each parameter to ensure the consistency of the results.

The sample volume determined the adsorption capacity of the MWCNTs that were sandwiched in the agarose film. The effect of sample volume ranging from 20 to 40 mL was examined. Based on figure 2a, the increment of BPA peak area was observed with increasing of sample volume. However, the statistical evidence was sufficient to prove that there was no significant difference ($p>0.05$) between 30 and 40 mL although BPA peak area was slightly higher when 40 mL of sample volume was applied. The difference was mainly due to the random error. Therefore, 30 mL of sample volume was deemed to be the most optimal sample volume and was applied for further experiments.

In this study, MWCNTs acted as adsorbents to extract the BPA from sample solutions. The BPA cannot be extracted without MWCNTs in the agarose film as the agarose is the inert material. This assertive sentence was proven as there was no BPA peak shown in the chromatogram when the extraction was performed using spiked deionized water samples. Therefore, the concentration of MWCNTs reinforced in the agarose film was studied in the range of 0.1 to 0.4%(w/v). Figure 2b shows that the highest analyte enrichment was observed when the concentration of MWCNTs was increased to 0.3%(w/v) and the extraction efficiency dropped beyond this point.

According to Kamaruzaman et al[16], a high loading of adsorbents will lead to a higher extraction efficiency as the increase of active sites for adsorption. However, film with a higher percentage of MWCNTs [0.4% (w/v)] showed poorer extraction efficiency. This might be due to the reason that a greater amount of MWCNTs was forced to be sandwiched within an invariable concentration of agarose and thus, leading to the reduction in surface area for adsorption of BPA[14]. Therefore, 0.3% (w/v) of MWCNTs was chosen for further experiments.

Stirring speed shows an influential role in μ-SPE techniques as it enhances the kinetics of analytes by increasing the mass transfer[17]. In this study, the effect of stirring speed on extraction efficiency was examined by monitoring the chromatographic peak area response over 350 to 950 rpm. Figure 2c shows that the peak area of BPA increased gradually when the stirring speed became faster. The peak area reached maximum at a speed of 650 rpm. The kinetic of the BPA increased simultaneously with the increasing of stirring speed. Elevation of migration kinetic of the BPA enhanced the interaction between the BPA and film and thus improved the BPA mass transfer onto the film. However, at a stirring speed higher than 650 rpm, the vortex flow was observed and it reduced the contact between the BPA and film and thus causing a lower peak area[14]. Therefore, 650 rpm was adopted as the optimal stirring speed for the subsequent studies.

The μ-SPE-MWCNTs-AF is not an exhaustive extraction but a time-dependent process. Only a remarkable amount of targeted analytes is extracted from the sample solution and the amounts achieve maximum at equilibrium stage[18]. With the intention of determining the required equilibrium time for the analytes to be adsorbed on the film, the extraction time ranging from 15 to 35 min was examined. Figure 2d shows that when the extraction time increased from 15 to 30 min, the peak area also increased steadily. The peak area of BPA was highest at 30 min and it proposed that the extraction process reached equilibrium.

However, the peak area dropped significantly when the extraction time was above 30 min. Back-extraction may occur and the analytes were extracted back to the sample solution from the adsorbent film[19]. Therefore, 30 min was applied as the optimal extraction time in the subsequent studies.

BPA was desorbed from the adsorbent film using ultrasonication method. Desorption solvent should be selected wisely based on the solubility of analyte in the solvent to enhance desorption process. Several organic solvents that were compatible with the HPLC mobile phase including acetonitrile (ACN), methanol (MeOH), isopropanol (IPA) and tetrahydrofuran (THF) were investigated. The results found that the polar solvents (ACN and MeOH) exhibited better analyte desorption from the film than less polar solvents (IPA and THF). As BPA is a polar compound, therefore polar solvent possessed stronger capability to disrupt the interaction between the BPA and the MWCNTs and then desorbed the BPA. From the results, ACN showed greatest desorption efficiency. This might be due to its polar characteristic which exhibited a better affinity to BPA than the other solvents. Therefore, ACN was applied as the optimal desorption solvent for subsequent studies.

The μ-SPE-MWCNTs-AF requires desorption step to desorb analytes from the adsorbent film into desorption solvent prior to HPLC analysis. The desorption time range from 5 to 20 min was determined. As indicated in figure 3a, the desorption of BPA from the adsorbent film was enhanced with increasing of desorption time. The desorption efficiency reached optimum at 15 min. Beyond 15 min, there was a decrease of desorption efficiency. This decline may be caused by the re-adsorption of the targeted analytes onto the film because adsorption and desorption were both depending on equilibrium[20]. Consequently, 15 min was deemed to be the optimal desorption time and was chosen for further experiments.

When adding salt into the sample solution, it will lead to an increase in ionic strength of the solution which in turn decreases the solubility of the targeted polar analyte and increases the salting out effect. The salting out effect was examined by adding sodium chloride (NaCl) into the sample solution to achieve final ionic strength ranging from 0 to 12.5%(w/v). Figure 3b shows that the extraction efficiency was increased slightly with the increase of ionic strength from 0 to 7.5%(w/v). However, as the peak area of each condition was very close to each other, the Post-hoc Tukey's test indicated that the results obtained were of no significant difference among the sample solutions that were added with NaCl to give the final ionic strength from 0 to 10.0%(w/v).

The slightly difference in peak area was mainly due to the random error that can be accepted by monitoring the relative standard deviation no more than 5%. Further adding of salt (12.5%(w/v)) into the solution showed a decline in extraction efficiency. According to Mousa et al[21], this might be due to the increase in viscosity of solution which impeded the mass transfer of analytes from solution to adsorbent film. As a conclusion, the extraction was performed without salt addition to simplify the extraction procedure and minimize the chemical usage.

Validation of μ-SPE-MWCNTs-AF: Under the optimum extraction conditions, the μ-SPE-MWCNTs-AF technique was validated for linearity, limit of detection (LOD), limit of quantification (LOQ) and relative recovery. The calibration was performed by spiking BPA into the deionized water samples. The results revealed that a good linearity in the range of 0.1 to 100 ppb with correlation coefficient, r, of 0.9977 was obtained. Thus, it can be concluded that the proposed μ-SPE that utilized MWCNTs-AF as adsorbent film was capable to extract consistent amounts of BPA with the increase of BPA concentrations.

The LOD and LOQ for BPA were calculated at signal-to-noise (S/N) ratio of 3 and 10 respectively. The LOD and LOQ were 0.02 and 0.1 ppb respectively. The LOQ calculated was lower than the maximum residue limit (MRL) of 0.25 ppb established by European Union. This revealed that the μ-SPE technique was sensitive to perform ultra-trace detection of BPA in beverage and environmental water samples.

Relative recovery and repeatability of the proposed method were examined by spiking the BPA in the carbonated beverages and environmental water samples to give the final concentrations of 1 and 10 ppb. Sample blank determinations were conducted and the samples were free from BPA. The results presented in table 1 show that the average of relative recoveries was acquired ranging from 81.0 to 98.7% with good repeatability indicated by relative standard deviations (RSDs) of < 3.1%. These results indicated that the matrix effect was negligible and the proposed method is applicable to analyze BPA in real samples.

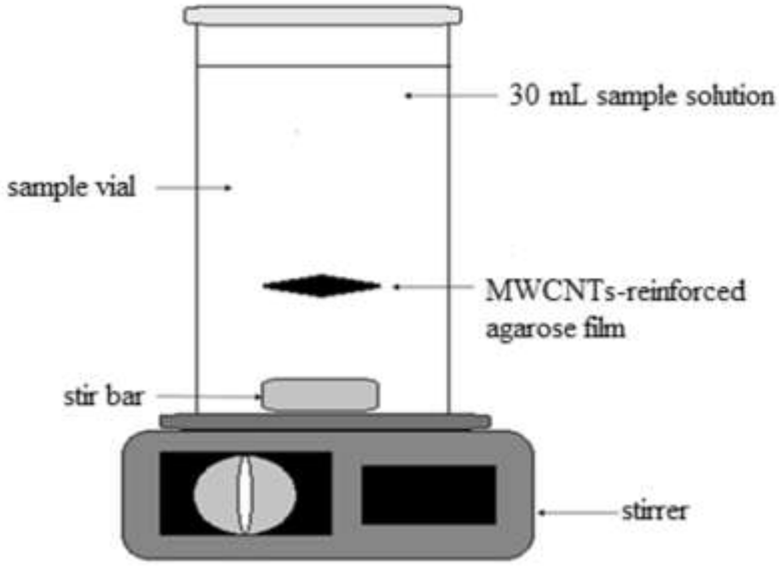

Figure 1: Schematic of μ-SPE-MWCNTs-AF system

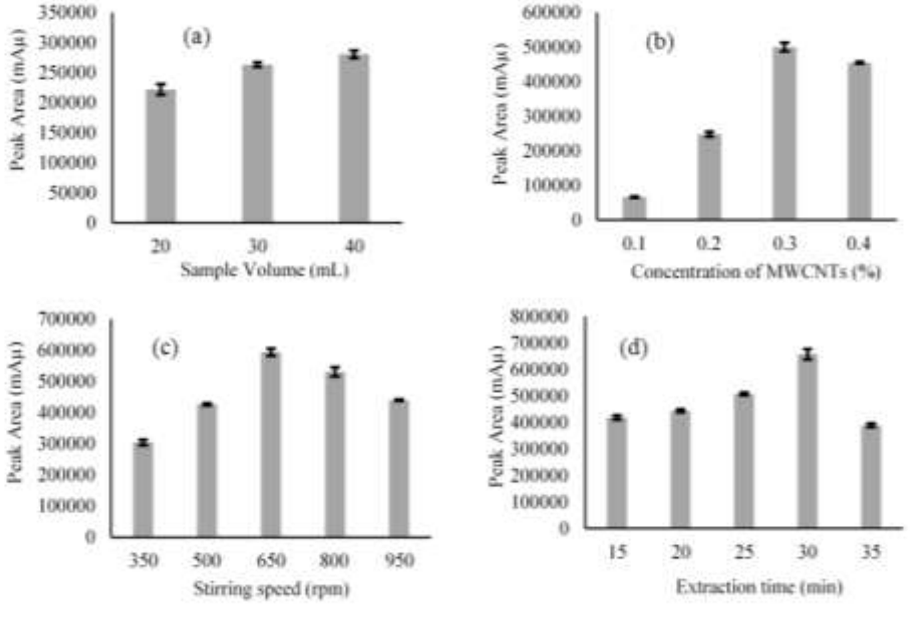

Figure 2: Effect of (a) sample volume, (b) concentration of MWCNTs, (c) stirring speed and (d) extraction time on the extraction of BPA from spiked deionized water samples using μ-SPE technique. Each error bar denotes the standard deviation of triplicate extractions

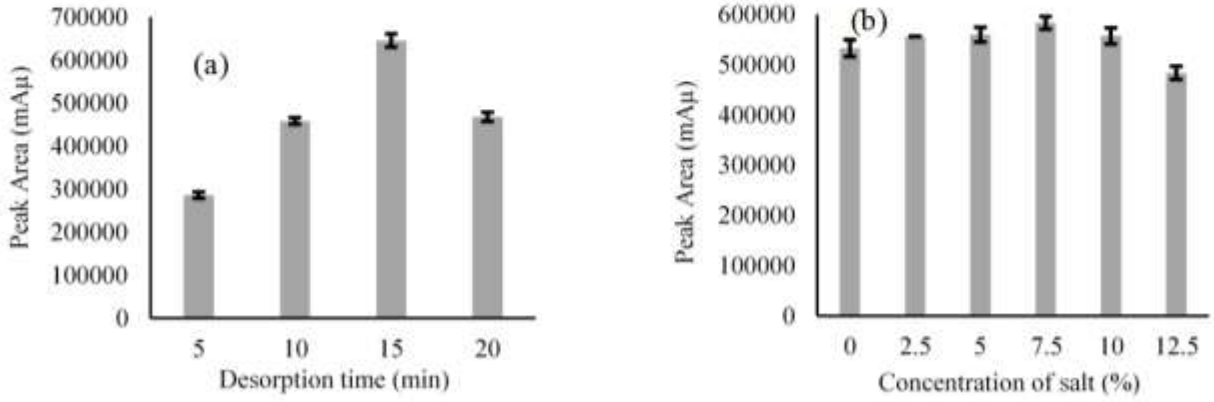

Figure 3: Effect of (a) desorption time and (b) concentration of salt on the extraction of BPA from spiked deionized water samples using µ-SPE technique. Each error bar denotes the standard deviation of triplicate extractions.

Table 1
Relative recovery study using µ-SPE for the extraction of BPA in beverage and water samples

Sample	**Average of relative recovery ± relative standard deviation, % (n=3)**	
	Spiked at 1 ppb	**Spiked at 10 ppb**
Carbonated beverage 1	98.7 ± 2.8	86.2 ± 1.8
Carbonated beverage 2	94.2 ± 2.0	84.1 ± 1.2
River water	81.0 ± 3.1	86.7 ± 0.9
Sea water	89.0 ± 3.1	95.2 ± 1.2

Conclusion

The proposed µ-SPE technique utilizes agarose as the medium to hold MWCNTs acting as the adsorbent to extract BPA from the samples. It possesses a number of strengths such as inexpensive, simplicity of operation, good recovery and excellent enrichment. The technique also offers a sensitive extraction as indicated by the ultra-trace LOD. Besides, this technique is environment friendly. This is because agarose is biodegradable material and the organic solvent required is in micro-scale format. Less hazardous chemical is utilized in this method. Therefore, this technique fulfils the concept of green chemistry which is widely pursued in these days.

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