Research Article

Determination of Polycyclic Aromatic Hydrocarbons in Water Using Nanoporous Material Prepared from Waste Avian Egg Shell

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For the first time a biocompatible calcium carbonate vateritic polymorph was recrystallized from eggshell waste and its application for the extraction of polycyclic aromatic hydrocarbons in water samples was demonstrated. This nanoporous calcium carbonate was used as sorbent in dispersive micro-solid-phase extraction method. In this approach 50 mg of the calcium carbonate material having about 25 nm pores was placed in a 5 mL of water sample and ultrasonicated for 30 min. The cloudy sample was centrifuged at 13500 rpm for 2 min. The aqueous layer was then discarded and the $CaCO_3$ material was dabbed dry with a lint-free tissue. The analytes were then desorbed with 100 μL of dichloromethane by ultrasonication for 5 min. Finally, the extract was analyzed by gas chromatography flame ionization detector. Experimental parameters affecting the extraction recoveries were optimized. Using optimum extraction conditions, calibration curves were linear with correlation coefficients of 0.9853 to 0.9973 over the concentration range of 0.05 to 30 ng/mL. This method showed a detection limit as low as 0.004 ng/mL (at signal-to-noise ratio of 3). Performance of the dispersive micro-solid-phase extraction was compared with a previously optimized solid-phase extraction technique. The developed method displayed good extraction recoveries (85 ± 8–110 ± 4%) with high enhancement factors (388–1433-fold) and good repeatability (% RSD < 13) and involved the use of minimal solvents. Analysis of seawater from Dammam Port revealed the presence of the analytes at concentrations between 0.15 ± 0.01 and 13.43 ± 1.54 ng/mL.

1. Introduction

In recent times, the search for new advanced materials has been the focus of numerous research interests. Due to their potential applicability, researchers are now dedicating much effort to the study of these materials, with continuous inspiration derived from nature through Biomimicry—a recent research area that seeks to understand and take inspiration from natural phenomena in order to solve human problems [1]. Of particular interest are the biomineralization processes by which living organisms produce minerals often to harden existing tissues. An example is the eggshell which is predominantly composed of calcium carbonate ($CaCO_3$) [1, 2]. In the field of analytical chemistry, great attention has been paid to nanoporous materials from different sources including

$CaCO_3$ from waste avian eggshells. The electrostatic nature of $CaCO_3$ particle [2] and its porous architecture make it a promising candidate as a solid sorbent for the extraction of polycyclic aromatic hydrocarbons (PAHs).

PAHs are ubiquitous pollutants that are found in different environmental matrices at different concentrations [3, 4]. Significant accumulation of PAHs in the aquatic ecosystem had been caused by anthropogenic inputs like oil spills, sea navigation, urban runoff, water, and industrial wastes [5–7]. High concentrations of PAHs are found in marine coastal environment near cities and industrial plants [8]. Initially, concern about PAHs was only focused on their carcinogenic property [9, 10]. Recently, however, searchlight has been beamed on their antagonism of hormonal functions and their potential effect on reproduction in humans, as well
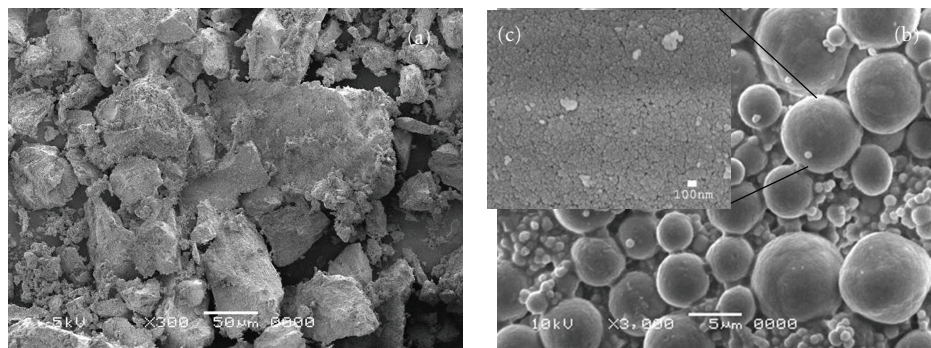
FIGURE 1: Scanning electron micrographs of (a) grinded powder of goose eggshell (b) recrystallized CaCO₃ material and (c) nanoporous surface of a single-calcium carbonate vateritic ball.

as their ability to depress immune function [11]. These concerns have prompted both the World Health Organization (WHO) [12] and the United States Environmental Protection Agency (USEPA) [13] to formulate regulations for the protection of drinking and source water systems in order to safeguard the populace from such harmful pollutants, many of which are considered as potential carcinogens. For many superficial water systems, the European Union (EU) has set maximum admissible concentrations of 100 ng/L for both anthracene and fluoranthene, and 1200 ng/L for naphthalene [14].

Development of methodologies for the analysis of PAHs is, therefore, one of the important aspects of environmental analytical chemistry [15]. In this regard, on-line and off-line solid-phase extraction (SPE) has been widely studied for trace analysis of PAHs [15, 16]. It can be combined with gas chromatography-mass spectrometry (GC-MS) or gas chromatography-flame ionization detector (GC-FID). Unfortunately, due to their mainly nonpolar nature and adsorption onto walls of extraction vessels, PAHs are not very amenable for multistep extraction procedures such as SPE and liquid-liquid extraction [17–21]. These techniques may also involve large solvent volumes which may not be in consonance with good environment-conscious practices. A greener approach should involve fewer steps and minimization of solvent waste. Solvent minimization through the use of commercial polymeric materials for sorption in solid-phase microextraction (SPME) [22–24] and stir bar sorptive extraction (SBSE) [25, 26] have been attempted for the analysis of PAHs in water. However, these materials are often expensive. Alternative sorption material that can carry out this function at a lower cost and offer more convenience in handling would be a welcome development. The objectives of this study were, therefore, set to synthesize nanocrystal sorbent materials from waste egg shells and to apply these in the solvent minimization method of dispersive micro-solid-phase extraction (D-μ-SPE) with GC-FID for the analysis of selected PAHs in water.

2. Experimental

2.1. Preparation of Nanoporous CaCO₃ Vateritic Polymorph.
To prepare the nanoporous CaCO₃ sorption material, waste avian egg shells from quails were dried and broken into small pieces. These pieces were then grinded and the coarse lump was treated with ammoniacal hypochlorite bleach solution (5%) for about 15 min to remove the egg shell membrane. 10 g of clear, bleached egg shells were powdered and decalcified with 100 mL hydrochloric acid (2 M) for 12 hrs. Vateritic calcium carbonate crystals were then grown from this solution using Weiner's vapor diffusion method [27, 28]. Previously, vateritic balls of CaCO₃ were observed during precipitation reaction in the presence of organic additives [29]. These balls were of high porosity and proved to be efficient in drug delivery [30]. The presence of inorganic and organic substances, such as magnesium ions and aspartic acid, may influence the growth of calcium carbonate and control its morphology [31, 32]. Hence, these substances can be used to adjust the porosity of the produced nanoporous material. Figure 1 shows the eggshell scanning electron micrographs before (a) and after crystallization (b). About 25 nm pores can be seen in the figure (c).

2.2. Reagents and Chemicals.
Individual PAH standards (99.5% purity) were purchased from Sigma (St. Louis, MO, USA). HPLC-grade solvents were from J. T. Baker, (NJ, USA). Ultrapure water was purified in a Milli-Q-water purification system (Millipore, Bedford, MA, USA). Standard working solution was prepared in methanol everyday using stock solution. All reagents and solvents used were of analytical or HPLC-grade unless otherwise mentioned. Seawater samples were collected from Dammam (Eastern province of Saudi Arabia) in Teflon jars in accordance with USEPA surface water sampling SOP (EPA, 1991) and analyzed on the same day. Methanol (10%, v/v) was added to these samples and mixed thoroughly in order to avoid adsorption of PAHs onto glassware.

2.3. Dispersive Micro-Solid-Phase Extraction and Solid-Phase Extraction.
The D-μ-SPE was carried out as follows: the nanoporous vateritic CaCO₃ (50-mg) was first conditioned with dichloromethane (DCM) (for 5 min in ultrasonicator) and then placed in 5 mL water sample spiked with 10 μL of the mixed PAHs solution (10 μg/mL of each compound) in a 10 mL glass centrifuge vial. This was then ultrasonicated

for 30 min and the resultant cloudy sample centrifuged at 13500 rpm for 2 min. The aqueous layer was discarded and the material was dabbed dry by placing it on a lint-free tissue to remove excess moisture. A 100 μL of DCM was then added to the nanoporous calcium carbonate crystals and ultrasonicated for 15 min for analyte desorption. Finally, 2 μL of DCM containing the desorbed analytes was injected into the GC-FID machine using autosampler. In order to avoid any carryover effects, each extraction was performed using a freshly prepared nanoporous vateritic CaCO$_3$ material.

SPE experiment was performed based on previously optimized extraction conditions [33].

2.4. GC-FID Analysis. Determination of PAHs was carried out using Agilent GC 7890A. Chromatographic separation of the seven PAHs was accomplished with a DB-1 fused-silica capillary column (30 m × 0.32 mm I.D, 1 μm film thickness) from J&W Scientific. Helium was used as carrier gas at a flow rate of 0.45 mL/min. Sample injection was in the splitless mode with an injection volume of 2 μL. The GC oven temperature program was optimized: initial temperature of 70°C was held for 2 min and then ramped at 10°C/min to 220°C. It was maintained at this temperature till the end of the run (33 min). The injection port temperature was set at 250°C and that of the FID detector was set at 300°C. The hydrogen and air flow rates were set at 40 mL/min and 400 mL/min, respectively.

3. Results and Discussion

The extraction mechanism for D-μ-SPE is similar to SPME and SBSE. Preliminary studies were conducted to investigate the most appropriate solvents (DCM, toluene, methanol, and hexane) for conditioning nanoporous vateritic balls of CaCO$_3$. Conditioning with organic solvent resulted in significant improvement in the extraction performance of the nanoporous CaCO$_3$, probably due to improved wettability of the sorbent in the presence of the solvent. Other parameters that might influence the extraction efficacy of the procedure were investigated prior to using it for the determination of PAHs in real water samples. The objective of the optimization procedure was to obtain maximum analyte recovery and sensitive determination. The parameters investigated were extraction time, sample pH, extraction solvent, and desorption time. All experiments were performed in replicates of three.

To optimize the effect of different extraction time on the efficacy of the method, experiment was performed by keeping all other conditions constant and varying the extraction time from 10 to 70 min. Results are given in Figure 2; 30 min appeared to be the sufficient time that would allow optimal interaction between the PAHs and the nanoporous crystals. Increasing the extraction time beyond the 30 min resulted in negative to no apparent effect on most of the analytes. At longer extraction time (>30 min), heat produced by the ultrasonication would negatively affect the D-μ-SPE process. Hence 30 min was adopted during subsequent trials.
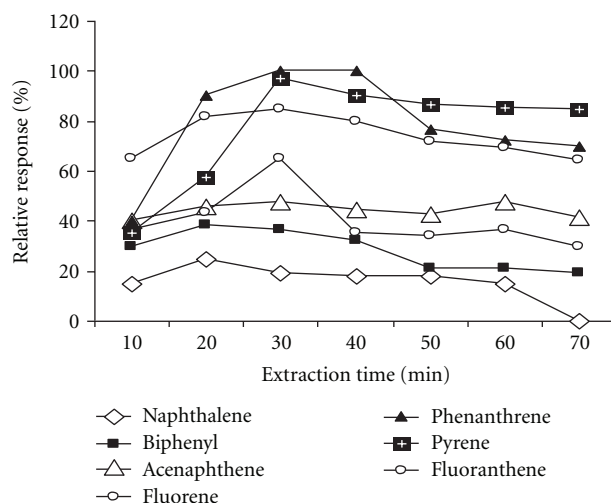

Figure 2: Effect of extraction time on the relative response of seven PAHs at experimental conditions of 5 mL sample volume (20 ng/mL), 5 min desorption in DCM (100 μL) and 50 mg CaCO$_3$ nanocrystals as sorbent.
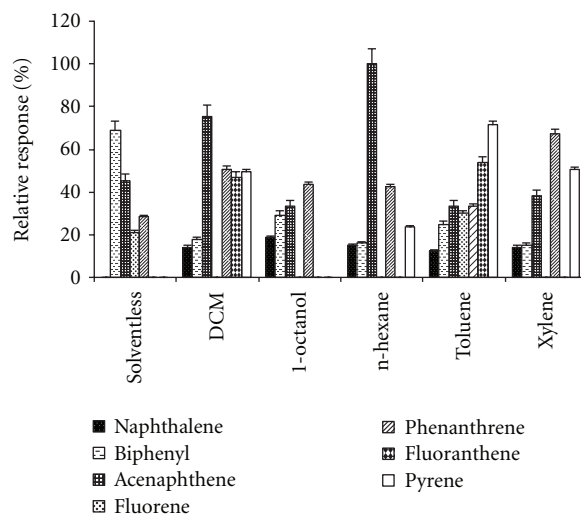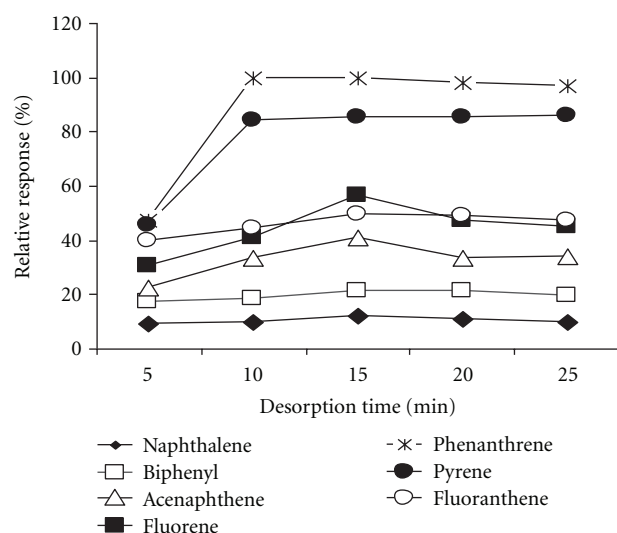

Figure 3: Effect of various conditioning solvents on the relative response of seven PAHs at experimental conditions of 5 mL sample volume (20 ng/mL), 10 min ultrasonic extraction, 5 min desorption time, and 50 mg CaCO$_3$ nanocrystals as sorbent.

Extraction was performed in the absence of any solvent and compared to the results obtained in the presence of five different conditioning solvents. The presence of solvent has significantly enhanced the extraction procedure. Although some compounds performed relatively better in other solvents, only toluene was able to extract all the seven analytes simultaneously as shown in Figure 3. Hydrophobic interaction [37] seems to govern the sorption of PAHs to the porous nanocrystals. The better performance of the extraction procedure with toluene might be due to its better facilitation of the interaction of these nonpolar analytes with the hydrophobic hollow spaces within the nanocrystals. Although both xylene and toluene contain an aromatic ring,

TABLE 1: Method parameters using D-μ-SPE and GC-FID for PAHs determination in water.

PAHs	Slope ± SD	Intercept ± SD	$R^{2\text{a}}$	LOD[b] (ng/mL)	LOQ[c] (ng/mL)	EF[d]	RSD (%), $n = 3$
Naphthalene	1.02 ± 0.03	4.79 ± 0.13	0.9953	0.022	0.073	1433	2.71
Biphenyl	0.46 ± 0.02	6.23 ± 0.31	0.9857	0.012	0.04	567	4.88
Acenaphthene	1.04 ± 0.10	11.02 ± 1.10	0.9853	0.008	0.027	968	9.96
Fluorene	0.95 ± 0.05	10.48 ± 0.62	0.9939	0.01	0.033	682	5.65
Phenanthrene	1.33 ± 0.14	6.64 ± 0.72	0.9939	0.008	0.027	839	11.21
Fluoranthene	0.72 ± 0.09	97.57 ± 10.70	0.9950	0.006	0.02	1376	12.5
Pyrene	0.64 ± 0.64	10.23 ± 1.02	0.9973	0.004	0.013	388	10.51

[a] Coefficient of determination for 0.05–30 ng/mL.
[b] LOD: limit of detection at S/N = 3.
[c] LOQ: limit of quantitation at S/N = 10.
[d] EF: enhancement factor (ratio of peak area for extracted sample relative to unextracted one).

FIGURE 4: Effect of desorption time on the relative response of seven PAHs at experimental conditions of 5 mL sample volume (20 ng/mL), 30 min extraction time, 50 mg CaCO$_3$ nanocrystals as sorbent.

the latter solvent has lower viscosity which enabled it to have a better interaction with both the analytes and the hydrophobic pores of the sorbent. Toluene was then selected as conditioning solvent in subsequent experiments.

After the extraction, desorption was performed in 100 μL of DCM at five different times: 5, 10, 15, 20 and 25 min. Figure 4 indicates no significant difference in the relative responses of biphenyl and naphthalene between 5 and 25 min. However, both pyrene and phenanthrene were desorbed at the optimum time of 10 min, while 15 min was necessary for acenaphthene, fluorine, and fluoranthene desorption. Consequently, 15 min was taken as desorption time in the remaining parts of this study.

Five pH values (2, 4, 7, 9 and 12) were examined in order to study their effect on the D-μ-SPE method. Results obtained show generally no significant difference in the peak areas of the analytes when the extraction procedure was performed at the acidic and basic pH values as compared to the neutral pH. This indicates the absence of significant impact of pH on the analytes which may be explained from the lack of ionizable groups on the PAHs. As a result, all subsequent trials in this study were performed at neutral pH. Nanoporous CaCO$_3$ crystals are easily soluble at very low sample pH (<2).

3.1. Method Performance and Quantitative Analysis.

Various performance criteria including enhancement factor (EF) of the extraction procedure (the ratio of peak areas for extracted sample relative to unextracted one), relative standard deviation (RSD, $n = 3$); linearity and limit of detection (LOD) (at signal-to-noise ratio, S/N = 3) were used to assess the performance of this method. Table 1 shows linearity of the detector response between the concentrations of 0.05 and 30 ng/mL, with coefficient of determination ranging from 0.9853 to 0.9953. The instrument responsivity was greatly enhanced by the high EF of the extraction method. In addition, the repeatability of this method is good as signified by RSD of <13%. LOD (ng/mL) values of the different analytes were obtained at S/N of 3, with pyrene having the lowest value of 0.004. The LODs are low enough for the successful determination of the PAHs in water. For quantitative determination of the analytes, the limits of quantitation (LOQ) were estimated from S/N equaled 10.

Characteristics of the CaCO$_3$ nanocrystal-based D-μ-SPE-GC-FID method can be seen to compare favorably with previously reported techniques in the literature (Table 2). Extraction performance of D-μ-SPE is comparable with previously established microextraction techniques [34–36]. As shown in Figure 5, both D-μ-SPE and SPE techniques showed comparable extractions performance. However, SPE required longer extraction time and huge amount of organic solvents. Table 3 shows the percentage recoveries of D-μ-SPE and SPE techniques spiked at the same concentrations (5 ng/mL). The percentage relative recoveries (ratio of the peak area for spiked seawater sample to that of spiked ultrapure water) indicate the absence of negative matrix effect. The optimized D-μ-SPE-GC-FID method was successfully applied to the analysis of real water samples

TABLE 2: CaCO$_3$ nanoporous crystal-based D-μ-SPE-GC-FID method characteristics as compared with literature.

Method	SV[a]	ET[b]	LOD (ng/L)	%RSD	RR[c]	N[d]	Reference
IL-SPME-GC-MS[e]	10	40	4-5	6.1–11.4	80–110	5	[34]
SBSE-LC-FLD[f]	10	60	0.2–2	4.7–13.5	58–100	15	[35]
SPE-HPLC-UV[g]	20	20	5–40	1–6	94–106	5	[36]
D-μ-SPE -GC-FID	5	30	4–22	2.7–12.5	84–110	7	Present

[a]Sample volume (mL).
[b]Extraction time (min).
[c]Relative recovery (%).
[d]Number of PAHs determined.
[e]Nafion membrane-supported ionic liquid-solid phase microextraction (SPME) with GC-MS.
[f]Stir bar sorptive extraction (SBSE) and liquid chromatography (LC) with fluorescence detection.
[g]Online SPME with LC and ultraviolet detection.

TABLE 3: Relative recoveries (%RR) of D-μ-SPE, SPE and the mean concentration of PAHs detected using D-μ-SPE of real water analysis.

PAHs	%RR of SPE 5 ng/mL spiked sample	%RR of D-μ-SPE 5 ng/mL spiked sample	Mean PAHs concentration detected from Dammam real water samples (ng/mL, $n = 10$)
Naphthalene	90 ± 3	92 ± 3	1.06 ± 0.02
Biphenyl	107 ± 5	110 ± 4	0.15 ± 0.01
Acenaphthene	84 ± 8	85 ± 8	1.90 ± 0.18
Fluorene	100 ± 6	104 ± 5	0.36 ± 0.02
Phenanthrene	90 ± 10	92 ± 10	3.22 ± 0.33
Fluoranthene	86 ± 10	87 ± 10	13.43 ± 1.54
Pyrene	94 ± 10	96 ± 10	0.43 ± 0.04

(with salinity between 38.35 and 41.45 part per thousand) from Dammam Port. Ten selected stations were sampled around the Dammam Port. Four stations were located inside the port operational area and two each one in either side of the port. Two stations were sampled inside the shipping lane. This port is the main gateway through which cargoes from all over the world enter the Eastern and Central Provinces of the Kingdom. The port is fully self-sufficient with mechanical and marine workshops, water treatment plants, and about 39 berths. It is one of the busiest ports in Saudi Arabia. Around the port, artisan and small fishing boats are operated for both commercial and recreational purposes. The nearby Dammam Corniche is considered to be highly polluted with sewage discharges and other inputs from agricultural and rain waters. PAHs concentrations between 0.15 ± 0.01 and 13.43 ± 1.54 ng/mL were found (Table 3).

4. Conclusion

Nanoporous polymorphic crystals of CaCO$_3$ were used as sorbent and were applied in the dispersive micro-solid-phase extraction of selected PAHs as target analytes. After separation of the analytes on gas chromatography, they were successfully quantified with external calibration using flame ionization detection. Different factors controlling the extraction procedures were investigated. Results have shown that in addition to solvent type, extraction and desorption time could have significant effect on the method performance. Comparison results have shown that this method could serve as a less costly and more viable alternative to other techniques
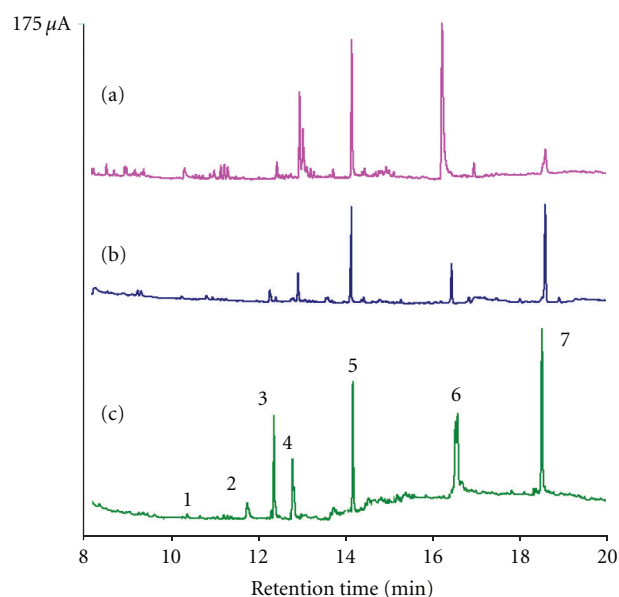

FIGURE 5: PAHs extraction chromatograms of (a) SPE extract of 20 ng/mL spiked ultrapure water sample (b) 2 μg/mL standard chromatogram (without extraction) and (c) D-μ-SPE-GC-FID of 20 ng/mL spiked water sample. Peak identification: (1) Naphthalene, (2) Biphenyl, (3) Acenaphthene, (4) Fluorene, (5) Phenanthrene, (6) Fluoranthene, (7) Pyrene.

that use commercial sorption materials. The method was successfully applied to the determination of PAHs in real water samples with promising results.

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