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Use of Ce(IV) oxidizing agent for the derivatization of polycyclic aromatic hydrocarbons for liquid chromatography–electrochemical detection

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) were oxidized with Ce(IV), and the resulting quinones were determined by reductive-mode liquid chromatography–electrochemical detection. This oxidation is a rapid, automatable step, involving addition of Ce(IV) reagent to the PAH sample and cleaning up the derivative with C_{18} solid-phase extraction. Using a C_{18} analytical column and a 2-propanol–phosphate buffer as the mobile phase, detection limits were in the ppb range for naphthalene, phenanthrene and anthracene, with linearity over 3–5 orders of magnitude. Method validation was performed by addition of the PAHs to tap water and determining the levels by reference to a calibration curve. The three PAHs can be simultaneously derivatized and determined under the same chromatographic conditions. Analysis of a motor-oil sample is also shown.

INTRODUCTION

Determination of polycyclic aromatic hydrocarbons (PAHs) is an area of great importance in environmental analysis. Both gas chromatography and liquid chromatography (LC) are methods of choice. In LC, reversed-phase separation followed by UV or fluorescence (FL) detection is most commonly used. PAHs exhibit high UV and FL sensitivity, and FL detection limits are in the sub-ppb[a] range. Multi-component analysis is easily achieved by reversed-phase chromatography. However, there exists a need for developing simple, rapid methods for individual PAHs which show carcinogenicity and mutagenicity [1].

One possibility for such analyses would be LC, combined with electrochemical detection (ED), a method which offers high selectivity and sensitivity. However, in aqueous media, most PAHs do not undergo any oxidation or reduction reactions at

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[a] Throughout this article, the American billion (10^9) is meant.

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typical working electrode materials such as glassy carbon. The electrochemistry of PAHs has received a great deal of attention, particularly with regard to fundamental radical-ion chemistry. Electrochemical analysis of PAHs is usually performed in aprotic media. Single-electron additions and abstractions have been demonstrated in numerous papers, where electrochemistry was coupled to electron-spin resonance spectrometers [2–6]. This method has generally not been used for analytical purposes. There has been only one report describing the analysis of anthracene in non-aqueous LC–ED at a potential of +0.80 V *vs.* saturated calomel electrode (SCE) with a glassy carbon electrode [1]. Low-pmol detection limits were achievable for anthracene and aminoanthracene.

Here, we report the LC–ED analysis of Ce(IV)-oxidized PAHs. The final products from this oxidation step are quinones, which can be detected by reductive-mode LC–ED at mild potentials (*ca.* −0.40 V *vs.* Ag/AgCl) [7]. Ce(IV) is a powerful oxidizing reagent which has been used in organic synthesis to convert PAHs to quinones [8,12]. The mechanism of oxidation involves electron abstraction from the PAH by Ce(IV) to generate Ce(III) and a cation free radical, the latter being attacked by water molecules, to yield the final quinone product.

EXPERIMENTAL

Reagents

PAHs (anthracene, naphthalene, phenanthrene and pyrene), quinones (anthraquinone, 1,4-naphthoquinone and phenanthrenequinone), sodium phosphate (monobasic), sodium hydroxide, ceric ammonium nitrate (CAN) and nitric acid were purchased from a variety of sources, including Aldrich (Milwaukee, WI, USA), J. T. Baker (Phillipsburg, NJ, USA.), Pfaltz & Bauer (Flushing, NY, USA), Sigma (St. Louis, MO, USA) and Chem Service (West Chester, PA, USA). PAHs and quinones were purified by recrystallization from ethanol or tetrahydrofuran (THF). 2-Propanol and THF were OmniSolv grade from EM Science (Cherry Hill, NJ, USA). Water was deionized with a Nanopure system from Barnstead (Boston, MA, USA.). PAH standards were prepared in THF–water (50:50). The Ce(IV) reagent used for all derivatizations was 0.1 *M* CAN in 1.0 *M* HNO_3.

Apparatus

Cyclic voltammetry (CV) was performed using a Model CV-1B cyclic voltammograph from Bioanalytical Systems (BAS, West Lafayette, IN, USA). The working electrode was glassy carbon, with a Ag/AgCl reference and a Pt-wire auxiliary electrode. Cyclic voltammograms were recorded on a Hewlett-Packard (Palo Alto, CA, USA) XY recorder.

The LC system consisted of an LDC Constametric III pump (Riviera Beach, FL, USA), a Model 7010 injector with a 20-μl loop (Rheodyne, Cotati, CA, USA), a LiChrospher C_{18} 5 μm, 125 mm × 4.6 mm I.D. column (E. Merck, Darmstadt, Germany) and a BAS Model LC-4B amperometric detector with a glassy carbon working electrode, stainless-steel auxiliary and Ag/AgCl reference. The mobile phase was continuously kept under a He blanket. Data were recorded on a Göerz-Metrawatt (Vienna, Austria) Model SE120 strip-chart recorder. Peak heights were measured manually.

The mobile phase for oxidized PAH analysis was 2-propanol–0.05 *M* NaH_2PO_4 (30:70) (pH 6.5) [11]. The electrode was maintained at either −0.40 V for oxidized phenanthrene, naphthalene and pyrene analysis or at −0.60 V for oxidized anthracene.

PROCEDURE

Cyclic voltammetry

A 0.1% (w/v) solution of the PAHs (naphthalene, phenanthrene, pyrene or anthracene) was prepared in THF–water (50:50), and 5 ml of this were added to 5 ml of 0.1 *M* CAN in 1.0 *M* HNO_3. After 1 min reaction time, a cyclic voltammogram was recorded at a scan rate of 100 mV/s. Solutions (0.1%, w/v) of the quinones in THF–water (50:50) were prepared and 5 ml were added to 5 ml 1.0 *M* HNO_3. A cyclic voltammogram was recorded 1 min after addition.

Derivative clean-up for LC–ED work

A 1-ml volume of the analyte–Ce(IV) reaction mixture was applied to a preconditioned 1-ml C_{18} solid-phase extraction tube (Supelco, Bellefonte, PA, USA). Excess reagent was washed off with two 1-ml washings of deionized water. The derivative was eluted with 0.5 ml 2-propanol, and this solution was diluted with 0.5 ml deionized water prior to analysis.

Optimization and percent derivatization

A 0.001% (w/v) solution of naphthalene in THF–water (50:50) was used in the optimization study, and the peak height of the derivative was measured, under the chromatographic conditions stated above, as a function of various derivatization parameters. Volume ratio of analyte to Ce(IV) solution and reaction time were optimized in this way. Under optimized conditions, percent derivatizations were calculated for the PAHs by comparing the derivative peak with the peak of the quinone standard and taking into account the dilution of the analyte and the increased weight of the derivative compared to the analyte. The percent derivatizations are listed in Table I.

Detection limits and linearity

A stock solution of the PAHs (0.1%) was diluted and derivatized until no derivative peak could be seen by LC–ED. Detection limits and linearity are shown in Table I for each PAH.

TABLE I

PERCENT DERIVATIZATIONS, DETECTION LIMITS AND LINEARITY

Analyte	Percent derivatization, average ± S.D. ($n = 3$)	Limit of detection (ppb)	Linear range (ppb)	r^2
Naphthalene	76.6 ± 3.6	5	10–10 000	0.9993
Phenanthrene	37.5 ± 1.1	50	50–100 000	0.9998
Anthracene	42.1 ± 1.2	50	50–100 000	1.0000

TABLE II

DETERMINATION OF PAHs ADDED TO TAP WATER

Sample	Concentration found (μg/l), average ± S.D. ($n=3$)	Actual	Relative error (%)
Naphthalene 1	30.4 ± 1.4	32.0	5.00
Naphthalene 2	16.7 ± 0.5	18.0	7.22
Naphthalene 3	75.1 ± 3.1	71.0	5.77
Phenanthrene 1	520 ± 4	520	0
Phenanthrene 2	342 ± 12	360	5.00
Phenanthrene 3	974 ± 5	920	5.87
Anthracene 1	522 ± 3	544	4.04
Anthracene 2	750 ± 2	732	2.46
Anthracene 3	257 ± 5	276	6.88

Single-blind spiking studies and application

Known amounts of the PAHs (naphthalene, phenanthrene or anthracene) were added to tap water. These solutions were diluted with an equal volume of THF and derivatized and analyzed using an external calibration plot, for which the standards were prepared in THF–water (50:50). The results are listed in Table II. Naphthalene, phenanthrene and anthracene were added at the 1-ppm level to tap water and also analyzed.

A sample of used motor oil from an automobile was also analyzed. A 1-ml volume of the motor oil was extracted with 10 ml of THF. The THF was diluted with 10 ml water and derivatized and analyzed by the described method. Naphthalene (naphthoquinone) and phenanthrene (phenanthrenequinone) were determined by using standard addition. The THF used for extraction of the motor oil contained 0, 50 and 100 ppm each of naphthalene and phenanthrene and these were analyzed. Also, known amounts of each of the two PAHs were added to the THF used for extraction and this was analyzed with the other samples. This procedure is based on a similar report in the literature [13]. Results are given in Table III.

TABLE III

ANALYSIS OF MOTOR OIL EXTRACT

Analyte	Native level (μg/ml), average ± S.D. ($n=3$)	Added level (μg/ml), average ± S.D. ($n=3$)	Actual	Relative error (%)
Naphthalene	77 ± 4	10 ± 0.1	11	9.1
Phenanthrene	25 ± 1	35 ± 0.4	33	6.1

RESULTS AND DISCUSSION

Cyclic voltammetry

For initial studies, cyclic voltammetry was used to determine if an electroactive derivative was formed with a Ce(IV)-oxidized PAH. The organic literature reports that Ce(IV) oxidation of PAHs leads to the formation of quinones [8–12], which are electrochemically reducible. In performing these initial studies, it became clear that the derivatization solvent greatly affected the rate of oxidation. In this regard, methanol and acetonitrile proved to be poor choices, as they complex with the Ce(IV), rendering it incapable of oxidizing the PAH. THF proved to work best as derivatization solvent. Thus, all studies were performed using THF–water (50:50) as the derivatization solvent.

Fig. 1 shows the cyclic voltammogram recorded for Ce(IV)-oxidized phenanthrene, as well as phenanthrenequinone at the same pH. The two are superimposable, lending evidence that the derivative of Ce(IV)-oxidized phenanthrene was phenanthrenequinone. A reversible reduction wave was seen at +0.35 V *vs.* Ag/AgCl, which phenanthrenequinone also showed. This was also the case for Ce(IV)-oxidized naphthalene, the cyclic voltammogram of which paralleled that for 1,4-naphthoquinone, and Ce(IV)-oxidized anthracene, whose cyclic voltammogram exactly

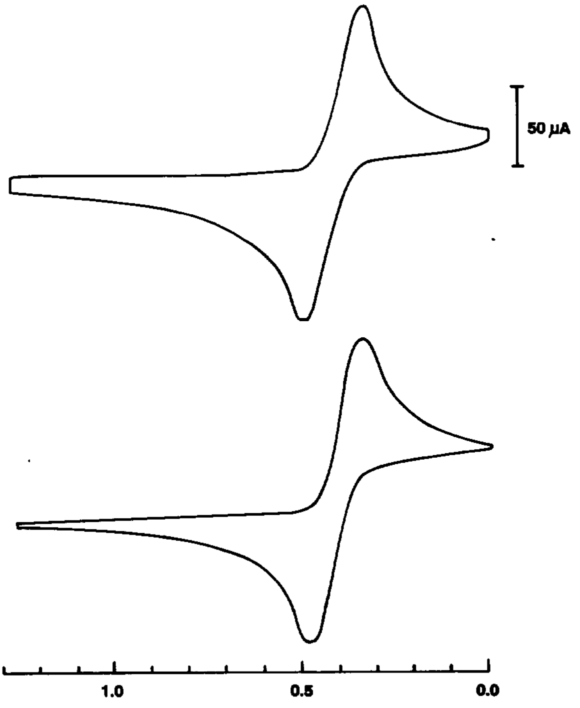

Fig. 1. CV of Ce(IV)-oxidized phenanthrene (top) and phenanthrenequinone (bottom), both in 1 *M* HNO_3. Glassy carbon working electrode, Pt auxiliary and Ag/AgCl reference electrode. 0.0 V to +1.3 V, 0.100 V/s scan rate, 50 μA sensitivity.

matched that for anthraquinone. These results were consistent with what the organic literature reported for oxidation products of PAHs [8–12]. There have been no literature reports of the oxidation products of pyrene. The cyclic voltammogram for Ce(IV)-oxidized pyrene showed the presence of more than one redox couple, suggestive of multiple derivatives. This was confirmed by LC–ED results.

Derivative clean-up for LC–ED work

In order to use the Ce(IV) oxidation procedure for LC–ED analysis of PAHs, it was necessary to clean up the derivative prior to injection. Injection of unreacted Ce(IV) ion to a high-performance liquid chromatographic column would lead to decreased column life, as well as a large solvent–matrix peak due to the Ce(IV) reducing at the electrode. Moreover, the reaction must be quenched so that further oxidation of the derivative does not occur. This was accomplished using a C_{18} solid-phase extraction step, which eliminated Ce(IV) and Ce(III) as well as HNO_3 from the final analysis solution.

Analysis of oxidized PAHs was performed using a mobile phase reported for the LC–ED determination of naphthoquinone, anthraquinone and phenanthrene quinone on a C_{18} column [7]. The same applied potentials used for analysis in the cited paper [7] were also used. Reduction of anthraquinone requires a lower applied potential (−0.60 V) than phenanthrenequinone and naphthoquinone (−0.40 V). The optimum potential for oxidized pyrene was determined to be −0.40 V. Unfortunately, this four-ring PAH gave multiple derivatization products, leading to poor sensitivity and non-linearity (Fig. 2). However, the ratio of the two main derivative peaks was constant over the range 1–100 ppm, which implies that this ratio could be used to confirm the presence of pyrene in a sample.

OPTIMIZATION AND PERCENT DERIVATIZATION

The derivatization was optimized for naphthalene, and the optimized conditions were used for all PAHs. The optimum volume ratio of analyte solution to Ce(IV) solution was determined to be 1.00 ml analyte to 0.75 ml 0.1 *M* CAN in 1.0 *M* HNO_3. The reaction time was seen to plateau at 1 min, with no change in percent conversion up to 1 h. For convenience, 1 min was chosen as the optimum reaction time. A temperature study was not performed. All reactions were performed at ambient temperature.

Percent derivatization was calculated as outlined under Experimental. The retention time was the same for the derivative as the quinone standard for all PAHs, further solidifying the belief that the derivative was the corresponding quinone. Table I summarizes percent derivatizations for all three PAHs. As expected, the highest value was for naphthalene, 80%, for which the derivatization was optimized. Phenanthrene (37%) and anthracene (43%) showed about half the percent derivatization as naphthalene. These values could be improved by separately optimizing the derivatization for each, athough not done here. Nevertheless, these percent derivatizations were analytically useful, as well as quite practical, given the fact that the reaction time was 1 min and only required a C_{18} clean-up step prior to analysis. Possible reasons for incompete conversion of naphthalene include further oxidation and losses during the C_{18} step.

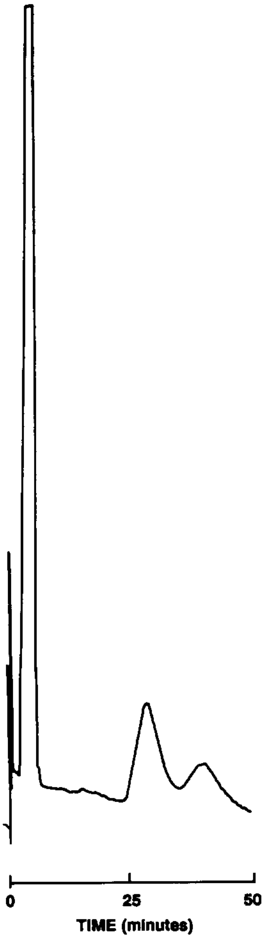

Fig. 2. Chromatogram of 10 ppm derivatized pyrene with electrochemical detection at potential −0.40 V *vs.* Ag/AgCl, 10 nA full scale (20 μl injection). LiChrospher C_{18} column, 5 μm, 125 × 4.6 mm I.D.; mobile phase, 2-propanol in 0.05 *M* NaH_2PO_4 (30:70) (pH 6.5); flow-rate, (pH 6.5); flow-rate, 1.0 ml/min.

Detection limits and linearity

Table I summarizes the detection limits and linearity for the oxidized PAHs. In all cases, the detection limits were in the ppb range, with the best, 5 ppb, for oxidized naphthalene. Linearity was at least three orders of magnitude in all cases. The detection limits were a bit high for typical LC–ED analysis. However, this work was performed in the reductive mode, where dissolved oxygen plays a major role in determining at what sensitivity level the detector could be effectively operated. It should be noted that if it were necessary to determine lower levels of analyte, pre-concentration during the C_{18} solid-phase extraction step could be employed.

Single-blind spiking study and application

In areas where an oil spill has occurred, high levels of PAHs can be found in the surrounding area's water supply. Thus analysis of PAHs in tap water was chosen as a means to validate the method. Individual PAHs were added to tap water and analyzed using the described method. Table II summarizes the results. In all cases, percent relative errors (REs) were within 8%.

Fig. 3 shows a chromatogram of naphthalene, anthracene and phenanthrene added to tap water at the 1-ppm level and determined using the described method.

Fig. 4 shows chromatograms of underivatized and derivatized motor-oil extract. The two peaks present in the derivatized sample were determined to be naphthoquinone and phenanthrenequinone by retention time comparison. These presumably arose from naphthalene and phenanthrene in the motor oil extract. However, this would be difficult to prove conclusively, as Ce(IV) oxidation of naphthol or phenanthrol also leads to the quinone products. Thus, these peaks may be due to a combination of both the parent PAH and the PAH alcohol. Using standard addition, the levels of naphthalene and phenanthrene in the motor oil were determined to be 77

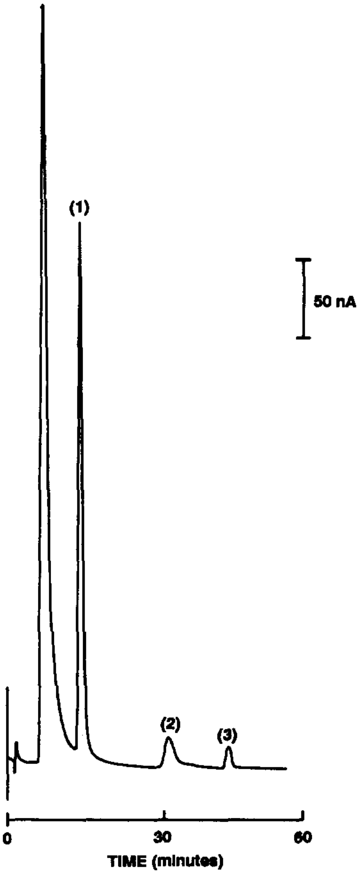

Fig. 3. Chromatogram of 1 ppm naphthalene (1), phenanthrene (2) and anthracene (3) in tap water. For conditions, see Fig. 2, except applied potential −0.60 V, 50 mA full scale.

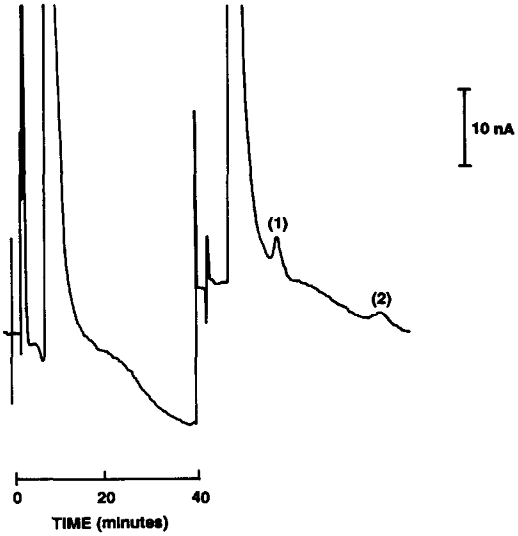

Fig. 4. Chromatogram of underivatized (left) and derivatized motor-oil extract. Peaks: 1 = naphthoquinone; 2 = phenanthrenequinone. Conditions as in Fig. 2.

μg/ml and 24 μg/ml, respectively. Analysis of fortified motor oil was also performed and subtracting out the values determined from the native oil, these added amounts were determined with 9% accuracy (Table III).

CONCLUSIONS

A rapid, simple procedure is described for the analysis of PAHs by LC–ED. Detection limits in the ppb range were attainable for all analytes, with linearity over several orders of magnitude. The approach has been shown valid for analysis of PAHs in tap water. High selectivity has been demonstrated through analysis of a motor-oil extract. The method has the potential for automation, and possesses the selectivity inherent in LC–ED.

The detection limits obtained with the described method (low-ppb) are orders of magnitude higher than what has been reported for LC with FL detection for these compounds (sub-ppb) [14]. It is clear that the described method offers no advantage over LC–FL in terms of detection limits. The advantage of the described method is the high selectivity which is obtainable, allowing an analyst to determine one or two PAHs present in a complex mixture. This was demonstrated with the analysis of a sample of used motor oil. It has been reported that LC–FL is not useful for determining one PAH present in a complex sample–matrix [14]. We believe that the described method may find utility for certain specific applications where one PAH must be determined in the presence of several. This selectivity can be gained by adjusting the applied potential or by adjusting derivatization conditions.

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