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Application Note 265

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Quantification of Polyaromatic Hydrocarbons with a Boron-Doped Diamond Electrode

INTRODUCTION

Boron-doped diamond (BDD) is a unique working electrode material. Commercially available electrochemical cells made using boron-doped diamond are robust and can operate at higher applied potentials than other working electrode materials such as carbon, gold, or platinum, which tend to form oxide layers when elevated potentials are employed. When operating at higher electrode potentials, certain organic compounds that typically do not react at lower potentials can be forced to react at the BDD electrode surface. The oxidation of organic compounds at BDD can be divided into two mechanism types,[1] depending on the applied potential:

- Direct electrochemical oxidation, in the region of water stability before oxygen evolution, where only reactions involving simple electron transfer can occur. One of the more important aspects of the BDD electrode is that higher potentials can be used to force electrochemically unreactive analytes to oxidize at potentials that are impractical with conventional carbon electrodes due to the adverse effects of mobile phase (water) oxidation.
- Indirect electrochemical oxidation caused by a sequence of reactions that are initiated by HO radicals formed at the BDD surface in the potential region of oxygen evolution (water electrolysis). Due to the high reactivity of HO radicals, these reactions are confined to an adsorbed thin film adjacent to the electrode surface. The hydroxylated adduct that is formed becomes electrochemically active, and it is its oxidation that is being measured.

Some aromatic compounds can be electrochemically active either due to aromatic rings (e.g., indole) or substituents on the aromatic ring (e.g., phenol, aniline, etc.). However, polyaromatic hydrocarbon (PAH) compounds, like many other aromatic compounds and impurities, do not respond to electrochemical detection using typical carbon-based electrodes. To extend the usefulness of electrochemical detection (ECD), the authors investigated a process termed electrotagging that renders inert compounds electrochemically active.

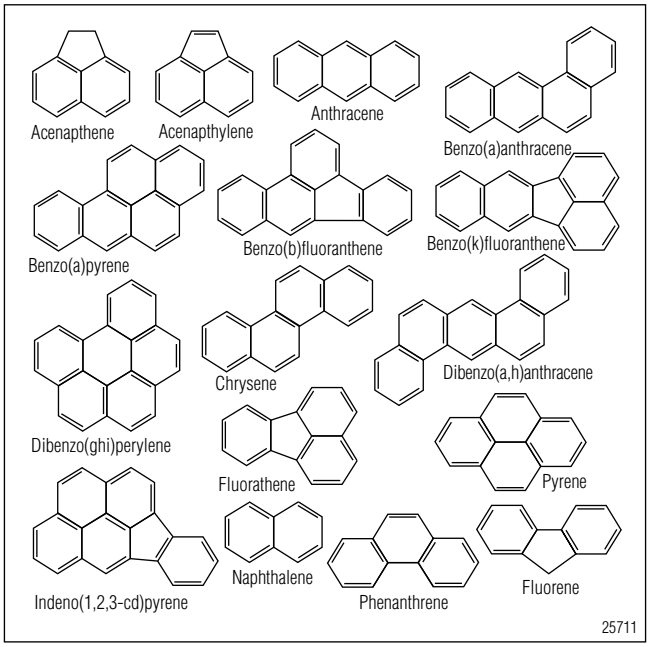

Figure 1. Structures of sixteen PAH compounds analyzed by HPLC-ECD.

To demonstrate the power of this approach, gradient HPLC with BDD was used to detect and quantify to low pg levels sixteen PAH compounds: naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, dibenzo(ghi) perylene, and indeno(1,2,3-cd)pyrene. Structures for these compounds are shown in Figure 1. These electrochemically unreactive PAHs are only being used as model compounds to illustrate the applicability of this approach to the analysis of other electrochemically inert compounds (e.g., drugs, impurities and degradants).

Interestingly, ECD with BDD has been used to measure electrochemically inert genotoxic aminopyridines,[2] tosylates,[3] and besylates,[4] as well as DNA adducts formed from the interaction of nucleic acids and a genotoxic material.[5,6,7] Once a compound has been determined to have the capacity to cause DNA damage, it must be quantified. Draft policies are being written to specify threshold of toxicological concern (TTC), no observable effect limits (NOEL), lowest observable effect limits (LOEL), and permitted daily exposure (PDE) values. Generally, a TTC amount of 1.5 µg/day is considered appropriate for most genotoxic materials that may be associated with a pharmaceutical product.

If a genotoxic material is used or created in the making of a pharmaceutical product, the general guideline is to control the amount down to as low as reasonably practical (ALARP). The use of ECD, combined with the BDD analytical cell, enables the detection of these aromatic, genotoxic compounds down to tens of pg on column.

EQUIPMENT

Coulochem® III detector equipped with a Model 5020 Guard Cell

Amperometric cell (Model 5040) equipped with a BDD working electrode and a 25 µm gasket

CONDITIONS

Column:	C18 PAH, 2.1 × 100 mm, 4 µm
Column Temp.:	35 °C
Sample Temp.:	10 °C
Mobile Phase A:	Buffer/Water/Acetonitrile (225:675:100), 25 µL/L H_2O_2
Mobile Phase B:	Buffer/Acetonitrile (200:800), 25 µL/L H_2O_2
Buffer:	200 mM Sodium perchlorate, 100 mM perchloric acid in polished, deionized water
Flow Rate:	0.60 mL/min
Injection Vol.:	10 µL
Rinse Vol.:	10 µL, Water/Acetonitrile (10:90)
Flush Vol.:	30 µL
Run Time:	30 min
Guard Cell Potential:	+500 mV (vs Pd reference)
BDD Cell Potential:	+1750 mV (vs Pd reference)
Filter:	5 s

Gradient:

Time (Min)	%A	%B
0.0	70.0	30.0
7.0	42.0	58.0
16.0	0.0	100.0
25.0	0.0	100.0
25.1	70.0	30.0
30.0	70.0	30.0

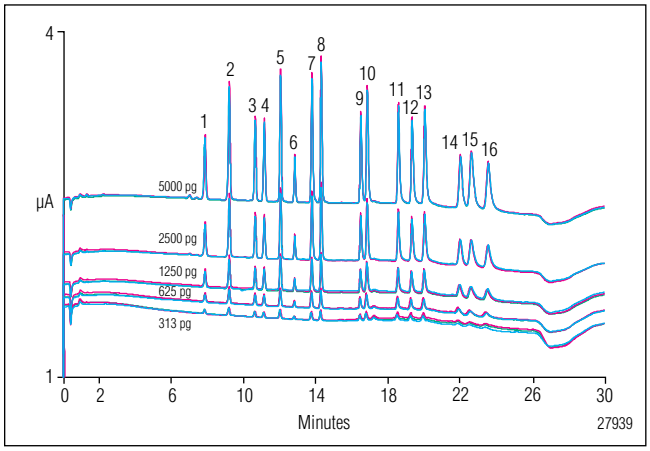

Figure 2. HPLC-ECD chromatogram overlays of 16 PAHs, from 313 to 5000 pg on column, in triplicate.

REAGENTS AND STANDARDS

In order to minimize background currents, the highest quality EC-compatible reagents (e.g., sodium perchlorate [Fluka, P/N 71853], acetonitrile [EMD, P/N AX0142-1], perchloric acid [GFS Chemicals, P/N 67]) were used. Hydrogen peroxide, 30%. Further polish deionized water with a C18 cartridge as outlined in ESA Technical Note 70-1668.[8]

Standards were dissolved in acetonitrile and diluted serially across the amounts (39 to 5000 pg on column) used in this application.

RESULTS AND DISCUSSION

The overlaid chromatograms, in triplicate, for these analytes are shown in Figure 2. All peaks were resolved with acceptable reproducibility. The use of gradient elution, unusual in HPLC-ECD methods but accommodated by the BDD electrode, provides flexibility in the range of analytes that can be quantified in a single run.

The method showed good linearity for all 16 PAHs from amounts of 39 to 5000 pg on column for the first 13 analytes, and 313 to 5000 pg for the last 3 eluting analytes (Figures 3a and 3b). The linear correlation coefficients for all 16 PAH calibration curves were found to be greater than 0.996.

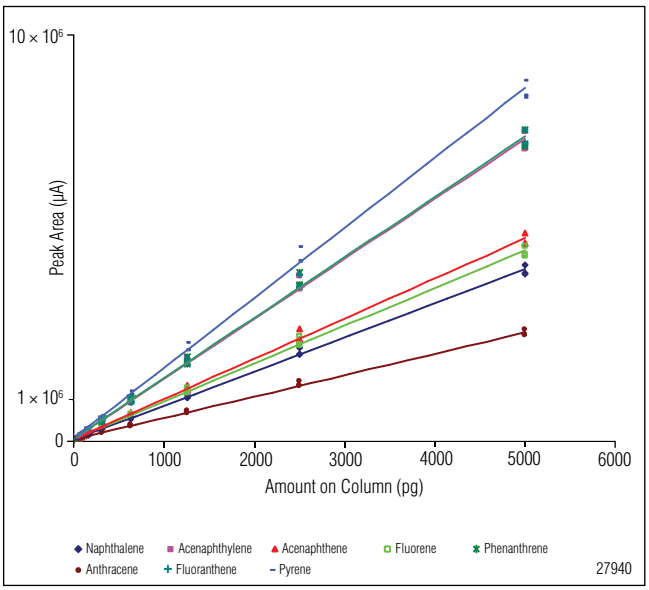

Figure 3a. Calibration plots of eight PAH analytes, naphthalene to pyrene.

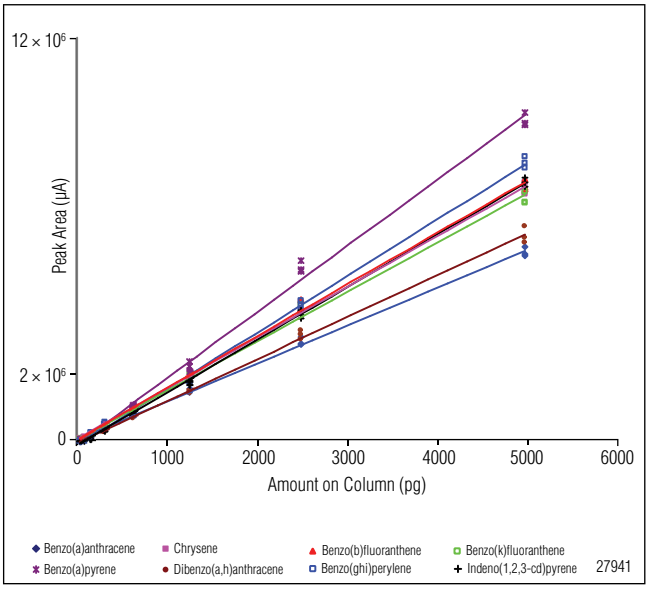

Figure 3b. Calibration plot of eight PAH analytes, benzo[a]anthracene to indeno(1,2,3-cd)pyrene.

Table 1. Estimates of LOD and LOQ Values for PAH Analytes by HPLC-ECD		
Analyte	**LOD (pg on column)**	**LOQ (pg on column)**
Naphthalene	20	60
Acenaphthylene	20	60
Acenaphthene	20	60
Fluorene	20	60
Phenanthrene	20	60
Anthracene	20	60
Fluoranthene	20	60
Pyrene	20	60
Benzo(a)anthracene	20	60
Chrysene	20	60
Benzo(b)fluoranthene	80	160
Benzo(k)fluoranthene	80	160
Benzo(a)pyrene	80	160
Dibenzo(a,h)anthracene	300	500
Dibenzo(ghi)perylene	300	500
Indeno(1,2,3-cd)pyrene	300	500

The use of ECD, combined with the BDD electrode, enabled the detection of these aromatic, genotoxic compounds down to tens of pg on column. The estimated limits of detection (LOD) with a S/N of 3:1 and limits of quantitation (LOQ) with a S/N of 10:1 are presented in Table 1. The LOD values for the first 10 peaks was ~20 pg on column. The peaks for the larger PAH analytes were broad, resulting in LOD values of 80–300 pg on column. In order to improve their sensitivity, a simple isocratic elution method specific to this region of the chromatogram can be used.

Although this work demonstrates use of a method for low-level detection of PAHs, it is feasible to use HPLC-ECD to detect other aromatic-based, analytes to low levels (typically <100 pg on column). As many of the identified genotoxins also contain an aromatic ring, this method can be used in their determination along several stages of a product's development.

CONCLUSION

The BDD working electrode, with its ability to be used at high potentials, extends the range of compounds that can be measured electrochemically. By generating reactive oxygen species, and with gradient elution conditions, compounds like PAHs can be modified *in situ* by hydroxylation reactions, thereby rendering the compounds electrochemically active.

This method shows good linearity, reproducibility, and sensitivity (~20 pg on column). Use of the Coulochem III detector, the boron-doped diamond electrode, and the chemistry demonstrated in this method allows for quantitative results for aromatic compounds that cannot be analyzed using conventional HPLC-ECD approaches.

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