



Application of the O₂-doped ECD to isomer differentiation
by James Allen Campbell

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemistry

Montana State University

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Abstract:

The addition of oxygen to the nitrogen carrier gas of a constant current electron capture detector (BCD) is shown to provide increased sensitivity and isomer distinction for environmentally important compounds such as polychlorinated biphenyls, polycyclic aromatic amines and hydroxides with appropriate EC-enhancing tags, chloroanthracenes and chlorophenanthrenes, methylanthracenes and methylphenanthrenes, and 2,3,7-trichlorodibenzo-p-dioxin. In most cases, the isomers of these particular compounds can be distinguished from the other isomers based solely on the measured response enhancements.

The oxygen doped BCD has been applied to compound identification where only partial resolution with capillary column gas chromatography is obtained. In addition, in instances where several compounds coelute and have the exact same retention times, the mole fraction of each component in the unresolved peak can be determined.

Atmospheric pressure ionization mass spectrometry (APIMS) gives an indication of the ions formed with and without the presence of oxygen in the source. For the chloroanthracenes, methylanthracenes, and methylphenanthrenes, actual oxygen incorporation is observed when oxygen is present. In contrast, for the polycyclic aromatic amines derivatized with trifluoroacetic anhydride (TFAA), the reaction with oxygen to produce an induced response involves a charge transfer between the oxygen anion and the analyte molecule.

Several TFAA derivatives of the aminoanthracenes and aminophenanthrenes were examined using electron impact mass spectrometry and chemical ionization mass spectrometry. Negative chemical ionization mass spectrometry with methane and isobutane as the reagent gases was evaluated as a method of isomer differentiation of these compounds and shows considerable promise.

In order to improve the precision involved in the measurement of response enhancements, two methods with parallel and series arrangement of the ECDs were utilized. The parallel arrangement with dual columns represents a significant improvement in the reproducibility of response enhancement measurements. The series detector arrangement seems to be confusing in view of possible additional reactions in the detectors.

The addition of ethyl chloride to an electron capture detector increased the response of anthracene and similar molecules with a low normal BCD response and those that react with the gaseous electron through a resonance type of reaction mechanism. The addition of ethyl chloride to the detector does not significantly increase the baseline frequency, in contrast to the addition of oxygen. The negative ions formed in this reaction have been identified.

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TO ISOMER DIFFERENTIATION

by

James Allen Campbell

A thesis submitted in partial fulfillment
of the requirements for the degree

of

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in

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APPROVAL

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James Allen Campbell

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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Date July 27, 1984

DEDICATION

I would like to dedicate this thesis to the memory of my father who recently passed away. He had his own particular definition of love and will always be remembered in my thoughts and actions. In addition, this is dedicated to the living, my mother, my wife, and son whose constant encouragement made this all possible.

VITA

James Allen Campbell was born December 14, 1948 in Wolf Point, Montana, the younger of two sons of Donald W. and Harriet V. Campbell. He attended public schools in Montana and graduated from Stanford High School in May, 1966.

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ABSTRACT

The addition of oxygen to the nitrogen carrier gas of a constant current electron capture detector (ECD) is shown to provide increased sensitivity and isomer distinction for environmentally important compounds such as polychlorinated biphenyls, polycyclic aromatic amines and hydroxides with appropriate EC-enhancing tags, chloroanthracenes and chlorophenanthrenes, methylanthracenes and methylphenanthrenes, and 2,3,7-trichlorodibenzo-p-dioxin. In most cases, the isomers of these particular compounds can be distinguished from the other isomers based solely on the measured response enhancements.

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Several TFAA derivatives of the aminoanthracenes and aminophenanthrenes were examined using electron impact mass spectrometry and chemical ionization mass spectrometry. Negative chemical ionization mass spectrometry with methane and isobutane as the reagent gases was evaluated as a method of isomer differentiation of these compounds and shows considerable promise.

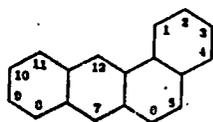
In order to improve the precision involved in the measurement of response enhancements, two methods with parallel and series arrangement of the ECDs were utilized. The parallel arrangement with dual columns represents a significant improvement in the reproducibility of response enhancement measurements. The series detector arrangement seems to be confusing in view of possible additional reactions in the detectors.

The addition of ethyl chloride to an electron capture detector increased the response of anthracene and similar molecules with a low normal ECD response and those that react with the gaseous electron through a resonance type of reaction mechanism. The addition of ethyl chloride to the detector does not significantly increase the baseline frequency, in contrast to the addition of oxygen. The negative ions formed in this reaction have been identified.

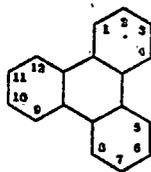
INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) and substituted polycyclic aromatic hydrocarbons (SPAHS) are present as environmental pollutants formed from both natural and anthropogenic sources. Natural sources include forest and prairie fires and in situ synthesis from degraded biological material. Anthropogenic sources of PAHs and SPAHS include the burning of coal refuse banks, residential fireplaces, and commercial incinerators.

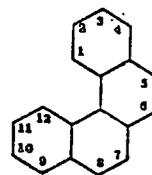
PAH mixtures can be extremely complex, since they can contain numerous isomeric compounds. A case in point is a simple sample containing the isomeric compounds with four-fused rings. These would include benz[*a*]anthracene (A), triphenylene (B), benzo[*c*]phenanthrene (C), chrysene (D), and naphthacene (E). The structure and numbering scheme for these compounds are shown below.



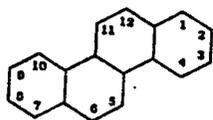
A



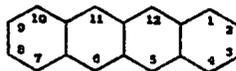
B



C



D



E

It follows that for example, a monosubstituted (amine, chloride, methyl, etc.) naphthacene has three isomers. Accounting for all of the isomeric possibilities for the five compounds for monosubstitution, the total number is 29. For a disubstituted naphthacene, the number of isomers is 20, and a disubstituted benz[*a*]anthracene has 66 isomers. The total number of disubstituted isomers for the five compounds then probably exceeds 100. As the number of substituted positions increases, the number of possible isomer combinations becomes enormous. This can pose quite a challenging problem for the analytical chemist to identify possible isomers in a sample. Even if the sample contained a single component, one isomer of the sixty-six possible disubstituted isomers of benz[*a*]anthracene, the problem of identification is still complex. Mass spectrometry, which is normally used for this type of analysis, alone is frequently unable to provide positive identification because of the large number of possible isomers for a given molecular mass. In addition, for some substituted PAHs, a strong dependence of carcinogenicity and mutagenicity on the position of substitution has been found. In view of these observations, identification of specific isomers is extremely important. Therefore, a technique which provides assistance in the identification of substituted PAHs will be a welcomed addition to the arsenal of analysis techniques presently available to the analytical chemist.

In the next section the background and history of some of the more environmentally important substituted polycyclic aromatic

hydrocarbons which include the polychlorobiphenyls, polycyclic amines and hydroxides and dioxins will be discussed followed by a short review of the typical analytical techniques for the identification of PAHs and SPAHs. The history and development of the electron capture detector (ECD) and the use of oxygen as a dopant will be reviewed. A discussion of the model for instrumental response with oxygen doping and the application of other dopants will follow.

Polychlorobiphenyls (PCBs)

Chlorinated biphenyls have been available as commercial products with a variety of applications for over fifty years. They were marketed under the trade name of Aroclors by Monsanto in North America and in other parts of the world under various names, such as Clophen (G.F.R.), Phenoclor (France), and Kennechlor (Japan). These PCB formulations, since their development in 1929, have been utilized chiefly as electrical coolant and insulating fluids in transformers and capacitors, but also in heat exchange and hydraulic systems, as plasticizers in paints, adhesives, caulking compounds and dye-carriers in carbonless copy paper. Their escape from these systems or their disposal in wastes has led to worldwide environmental contamination with PCBs. The presence of PCBs in the environment became apparent only after the ECD had been extensively applied to the monitoring of organochlorine pesticide residues, during the early 1960s. Chromatograms of wildlife sample extracts were found to exhibit many electron-capturing responses which could

not be attributed to organochlorine pesticide residues. These were first identified as PCBs by Jensen (1) who confirmed their presence with mass spectrometry.

Because of their resistance to breakdown and their other physical properties which are similar to those of the organochlorine pesticides, particularly DDT and its metabolites, the PCBs tend to bioaccumulate towards the top of the food web, namely in the fatty tissues of predatory fish, mammals and birds. PCBs have been found in all parts of the environment. Their presence and distribution have been reviewed by Risebrough *et al.* (2), Peakall (3), Jensen (4) and Nisbet and Sarofin (5). Pal *et al.* (6) have reviewed the fate of PCBs in soil-plant systems as well as in other environmental substrates. Fishbein (7) has reviewed some of the chromatographic and biological properties of the PCBs.

Polycyclic Aromatic Amines and Polycyclic Aromatic Hydroxides

The occurrence of polycyclic aromatic amines (PAA) in coal-derived liquids and residues was reported as early as 1958 by Karr *et al.* (8). Other workers have implied or demonstrated the presence of PAAs having one or two aromatic rings in synfuel products (9,10). More recently, however, even more highly mutagenic polycyclic aromatic amines (PAAs) have been detected in some synfuels materials (11-14) and have been found to be the determinant mutagens in some coal-derived materials (13,15-17). These species include recognized or suspected carcinogens such as 1-aminonaphthalene, 2-aminonaphthalene, 4-aminobiphenyl, 1-

anthracene, and 2-aminoanthracene (18-20). Lee, Later, and Wilson have found that mutagenic activity within an isomer group is structure dependent (21).

Many hydroxy type compounds have been shown to be present in synthetic fuel products and several methods have been developed for their isolation (22-25). Compounds such as hydroxyfluorenes, hydroxynaphthalenes, and hydroxybiphenyls have been identified in these synfuels (23). These compounds like the polycyclic aromatic amines may possess mutagenic and carcinogenic characteristics (25).

Chlorinated Dioxin

Chlorinated dioxins were first synthesized as early as 1872 by German scientists, but only recently has an enormous interest been focused on the polychlorodibenzo-p-dioxins (PDDs) and in particular one of the 22 isomers of the tetrachlorodibenzo-p-dioxin (TCDD) group, 2,3,7,8-TCDD, as a result of an explosion of a safety valve in an industrial plant producing trichlorophenol at Sevesco in northern Italy in July 1976. As the explosion was the result of overheating of the plant reactor, 2,3,7,8-TCDD, the most toxic (26) compound of the series, was produced in much larger amounts than in the normal process, where its concentration is restricted to low parts per million levels (27).

Times Beach, Missouri, has a most unenviable reputation as the town too poisoned to live in. Just a few miles west of St. Louis, its fate has been sealed as the result of some poor waste disposal practices, insufficient environmental laws in the early 1970s, and

political pressures for action. Because of the contamination and the federal government's decision to buy the town, Times Beach is expected to virtually disappear.

The story traces back to the 1960s and begins with agent orange. Hoffman-Taft, one of the original defendants in the agent orange trial, made 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) for the Department of Defense for a while, but ceased production in 1969, about the time ecological concerns led the military to halt spraying. In November of 1969, the plant in Verona, Missouri, was leased to North Eastern Pharmaceutical and Chemical company, and then later sold to Syntex Agribusiness, which let the pharmaceutical company stay to produce hexachlorophene.

According to EPA's records, wastes from the plant were being disposed of properly by shipping them to a waste facility owned by what is now Rollins Environmental Service near Baton Rouge, Louisiana. But in early 1971, allegedly to save money, North Eastern contracted with a firm called Independent Petrochemical to haul away its sludge bottoms. Independent, in turn, subcontracted the job to Russell Bliss, a waste oil hauler in Missouri. The records show that Bliss hauled away 18,500 gal of waste bottoms containing dioxin from the Verona plant, which he apparently stored in waste oil tanks near Fronenac, Missouri, between February and October 1971. But Bliss used some of this contaminated waste oil to spray horse arenas in May of 1971. Three stables apparently were sprayed, and the consequences were severe. Over the next few days and weeks, hundreds of animals got sick and died, including at

least 65 horses. One six-year old child, the daughter of one of the stable owners, developed an inflamed and bleeding bladder after playing in the soil of the arena, and three other children and one adult complained of skin lesions after exposure to the stables. All of the symptoms disappeared after exposure was halted and have not recurred.

State of Missouri investigators, reasoning that something must have been in the oil that was sprayed, sent samples for analysis. The Center for Disease Control, with few clues to go on, took until 1974 to identify dioxin as the toxic compound in the oil. They eventually determined that the oil was contaminated at about 33 ppm, a level far higher than any that occurred in Vietnam from agent orange. Crystals of trichlorophenol found during the soil analysis led the investigators to the Hoffman-Taft plant in Verona, and the thinking was that 2,4,5-T production was the culprit. But it was then discovered that the hexachlorophene wastes made by North Eastern had been disposed of improperly.

Spraying began in Vietnam in January 1962, using a variety of herbicide mixtures. Small amounts were used at first, but the amounts jumped at the end of 1965 and heavy use continued until 1969. Mounting concerns about damage to Vietnam's ecology led to a tapering off and finally a halt to the spraying of 2,4,5-T in 1970. Of the seven or so herbicide formulations used, the most significant was called agent orange, an oily liquid that was a 50-

50 mixture of the n-butyl esters of 2,4,5-T and 2,4-dichlorophenoxyacetic acid (2,4-D).

The major source of PCDDs is the massive production and use of pentachlorophenol (PCP) and other chlorophenols that are widely used as herbicides, insecticides, and wood preservatives. These compounds contain a variety of chlorine-containing contaminants and by-products that have different structures but a behavior that is similar to that of the PCDDs, and also their molecular weights lie in the same range. In addition, chlorinated dioxins have been found on fly ash particulate matter generated by municipal incinerators (28) and chimney particulate matter from wood combustion (29).

The overall picture indicates dioxins are extremely toxic and carcinogenic based on animal studies, but the picture is much less clear about human health effects. Dioxin's lethality is compared to other poisons in Table 1. It is clear that an analytical technique that would distinguish isomers of the dioxins, as well as the previously described compounds, would be of tremendous importance.

Analytical Techniques for PAH and SPAH Identification

As previously indicated, PAH mixtures can be extremely complex, since they contain numerous isomeric compounds. The success of the chemical analysis, whether it be quantitative or qualitative (of even a single component), hinges on the resolving power and sensitivity of the analytical method employed.

Table 1. Dioxin's lethality compared to other poisons

Substance	Animal	Minimum lethal dose (moles per kg body weight)
Botulinum toxic a	Mouse	3.3×10^{-17}
Tetanus toxin	Mouse	1.0×10^{-15}
Diphtheria toxin	Mouse	4.2×10^{-12}
2,3,7,8-TCDD	Guinea Pig	3.1×10^{-9}
Bufotoxin	Cat	5.2×10^{-7}
Curare	Mouse	7.2×10^{-7}
Strychnine	Mouse	1.5×10^{-6}
Muscarin	Cat	5.2×10^{-6}
Diisopropylfluoro-phosphate	Mouse	1.6×10^{-5}
Sodium cyanide	Mouse	2.0×10^{-4}

Source: EPA

In the following sections, the most important analytical techniques for the analysis of PAHs and substituted PAHs will be discussed. The emphasis will be placed on chromatographic methods, mass spectrometry, and spectroscopic methods.

Chromatographic Methods

High Performance Liquid Chromatography

A major advantage of HPLC for the determination of PAHs is the general applicability to even completely involatile compounds of interest. Ultraviolet (UV) absorption and fluorescence detectors are generally used in series; the UV detector is universal for PAHs, whereas, the fluorescence detector provides high specificity.

Several workers (30-32) have described the use of variable wavelength UV detection to achieve some degree of selectivity. Thoms and Zander (33) used complete UV absorption spectra of PAHs for the identification in HPLC eluents. Other researchers (30,34) have employed absorbance ratios at several wavelengths for qualitative analyses.

Selective fluorescence quenching of certain PAHs in the presence of nitromethane has been investigated as a selective detection system for HPLC. Sawicki *et al.* (35) and later Dreeskamp *et al.* (36) reported that in the presence of nitromethane, the fluorescence emission of six-membered ring PAHs were quenched to a much greater degree than those containing a fluoranthenic structure. Recently, Konansh *et al.* (37) investigated further the potential of this phenomenon for the selective detection of the

benzofluoranthene isomers in the presence of the benzopyrene isomers and perylene.

A recent development in spectroscopic detection of PAHs in chromatographic effluents is the use of multichannel rapid scanning spectrometers. These detectors permit the recording of fluorescence spectra "on-the-fly", thereby eliminating stop flow or valving to trap the chromatographic peak in the flow cell. Jadamec *et al.* (38) described the use of such a system for the characterization of petroleum fractions for the determination of the source of oil spills.

The potential of the combination of liquid chromatography-mass spectrometry (LC/MS) for the separation and identification of organic compounds has generated considerable research interest in the past few years. At present, two LC/MS approaches are available, i.e., direct liquid injection and depositing the effluent onto a moving belt from which the mobile phase is evaporated prior to introduction into the mass spectrometer. Both of these approaches transfer only a small portion of the LC solute into the MS. Christensen *et al.* (39) recently described a new LC/MS interface that combines several of the advantages of both the direct liquid injection and moving belt techniques. The LC effluent is concentrated by evaporation as it flows down an electrically heated wire. The concentrated effluent then flows through a small needle valve and is sprayed into the MS ion source.

Dark *et al.* (40) using the moving belt method, demonstrated the LC fraction of coal liquid samples with structural

characterization by LC/MS. Using LC retention data on two different columns and the molecular weight data, they identified a number of aromatic compounds with molecular weights up to 250 in the coal liquid. Other applications of LC/MS involving PAH separation and identification have been reported (41,42). A review of the LC/MS techniques by McFadden (43) emphasized the current applications of this technique. Many other applications of LC/MS have been reported involving PAHs (43-45). For isomer distinction, this suffers from the same problems associated with the use of the mass spectrometer which will be discussed in more detail later. In the future, LC/MS probably will find greater application in the determination of higher molecular weight PAHs that are not particularly amenable to GC analysis.

Gas Chromatography

The extreme complexity of the PAH mixtures demands the greatest resolution possible in their analysis, and in this respect, as chromatography with packed columns has fallen far short of the capabilities of glass capillary columns. Capillary column gas chromatography was refined by a number of workers and the present standard was reached in 1975 when Lee (46) showed that acid-leaching of Lewis acids from the glass from which capillaries were made greatly improved the deactivation and efficiency of columns. Figure 1 shows a chromatogram of coal tar PAH obtained with an acid-leached column, subsequently coated with SE-52. The

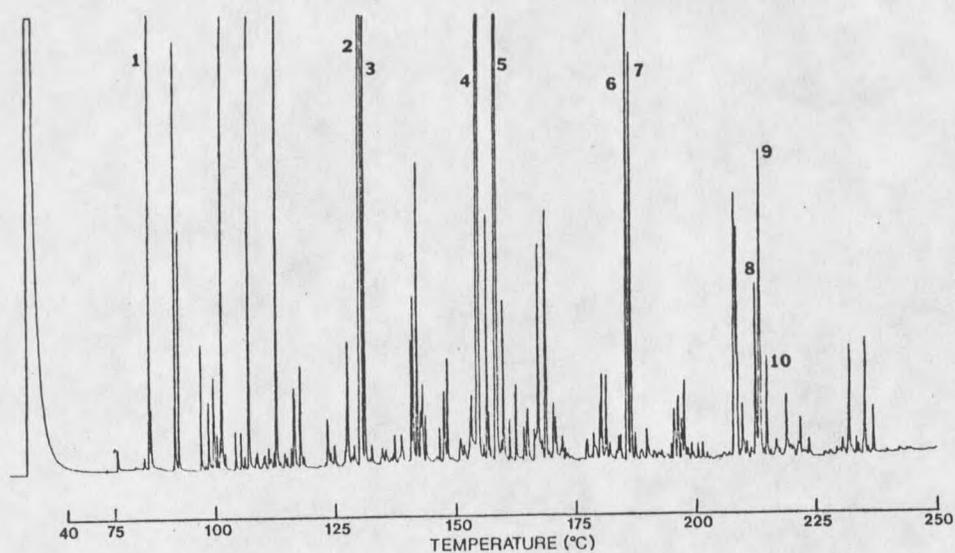


Figure 1. Capillary Column Gas Chromatogram of Coal Tar. Peak (1) Naphthalene, (2) Phenanthrene, (3) Anthracene, (4) Fluoranthene, (5) Pyrene, (6) Benz(a)anthracene, (7) Chrysene, (8) Benzo(a)pyrene, (9) Benzo(e)pyrene, (10) Perylene. (Reference 46).

performance of this column, as measured by the resolution between the isomer pairs phenanthrene-anthracene, benz[a]anthracene-chrysene, and benzo[e]pyrene-benzo[a]pyrene, represents the best resolution currently available. Fused silica columns are now used almost universally. Liquid crystal phases show pronounced selectivity for PAHs in column chromatography and allow the resolution of a variety of isomer pairs such as anthracene and phenanthrene.

The most widely used gas chromatographic detector for PAHs is the flame ionization detector (FID). This is a result of its universally accepted characteristics of excellent response linearity, sensitivity, and reliability. Response increases with molecular weight and response factors are similar for most structural isomers.

As early as 1965, Cantuti *et al.* (47,48) showed that the response of the electron capture detector (ECD) for PAHs was dependent on the structure of the compound, and that the detector could be selective for PAHs in hydrocarbon mixtures. Bjorseth and Ekland (49) measured the ECD/FID response ratios for 29 PAHs, and found that many isomers could be differentiated by measurements of these ratios.

Mass Spectrometry

In the last decade, mass spectrometry (MS) has gained wide acceptance for the analysis of PAHs. New rapid scan techniques coupled with high resolution chromatography and improved data

systems have greatly surpassed any other method or combination of methods used for PAH analysis.

Electron Impact

The electron impact mass spectra of PAHs are generally quite simple, mainly consisting of an intense molecular ion and lower intensity ions due to the loss of one to four hydrogen atoms. Doubly charged molecular ions are quite common and are usually near twenty percent of the abundance of the molecular ion. In most cases, differentiation of PAH isomers by electron impact mass spectra alone cannot be achieved. Even in the cases of isomers with very different structures, such as fluoranthene and pyrene, the mass spectra are most often indistinguishable. Figure 2 compares the EI mass spectra of the four-ring isomers, triphenylene, chrysene, benz[a]anthracene, naphthacene, and benzo[c]phenanthrene. All the mass spectra are essentially identical except for the benzo[c]phenanthrene. In this case, steric interaction between the protons on and 1 and 12 carbons facilitates the loss of these two protons with the subsequent formation of the benzo[ghi]fluoranthene ion. A large number of electron impact mass spectra for PAHs have been compiled and may be found in several reference books (50,51).

Mass spectrometry coupled with gas chromatography has been used to confirm the presence of PCB residues and characterizing the molecular composition of PCB formulations. Coupling of the gas chromatograph and mass spectrometer is normally carried out in such

