(70) Haas, J. R.; Friesen, M. D.; Harwan, D. J.; Parker, C. E. *Anal. Chem.* **1978,** *50*, 1474.
(71) Poland, A.; Glover, E.; Kende, A. S. *J. Biol. Chem.* **1976,** *251*, 4926. Greenlee, W. F.; Poland, A. *J. Biol. Chem.* **1979,** *254*, 9814. Carl-stedt-Duke, J.; Kurl, R.; Poellinger, L.; Gillner, M.; Hannson, L. A.; Toftgard, R.; Hoberg, B.; Gustafsson, J. A. In ref 2, pp 355–365.
(72) Kooke, R. M. M.; Lustenhouwer, J. W. A.; Hutzinger, O. *Anal. Chem.* **1981,** *53*, 461.

(73) Petty, J. D.; Smith, L. M.; Bergqvist, P.; Johnson, J. L.; Stalling, D. L.; Rappe, C. In "Chlorinated Dioxins and Dibenzofurans in the Total Environment"; Choudhary, G., Keith, L. H., Rappe, C., Eds.; Ann Arbor Science Publishers: Ann Arbor, MI, 1983; pp 203–207.

Selectivity of Negative Ion Chemical Ionization Mass Spectrometry for Benzo[a]pyrene

L. R. Hilpert,* G. D. Byrd, and C. R. Vogt[1]

Center for Analytical Chemistry, National Bureau of Standards, Washington, D.C. 20234

Gas chromatography/negative ion chemical ionization mass spectrometry (GC/NICIMS) was used as a selective and sensitive technique for the detection of benzo[a]pyrene (Ba-P). Under optimized conditions, the molecular anion, M⁻·, of BaP was more than 3 orders of magnitude more abundant than that of its isomer benzo[e]pyrene (BeP) using methane as the reagent gas. Quantities of BaP as low as 1 pg can easily be detected in the selected ion monitoring mode and the response vs. concentration was linear over a range of 3 orders of magnitude. The absolute sensitivity and the selectivity for detection were found to depend on the pressure and temperature in the ion source of the mass spectrometer. NICIMS was used for the quantitative determination of BaP, indeno[1,2,3-cd]pyrene, and benzo[ghi]perylene in a sample of petroleum crude oil as part of the process of certifying the oil as a Standard Reference Material.

Negative ion chemical ionization (NICI) mass spectra can be obtained from certain organic compounds by resonance capture of thermal electrons if the molecules have positive electron affinities, and if the internal energy of the molecular anion is less than the electron affinity of the neutral species. Usually the major species formed is the molecular anion, M⁻·, which often yields relatively large ion currents and little fragmentation. The selectivity of NICI over electron impact (EI) has been well established and this feature has permitted NICI to have wide applications over the past few years in the analysis of compounds such as polychlorinated biphenyls (*1*), dioxins (*1–3*), pesticides (*1, 4, 5*), and nitrated polycyclic aromatic hydrocarbons (*6*). Ilda and Dashima (*7*) recently reported the methane negative ion chemical ionization mass spectra of 21 polycyclic aromatic hydrocarbons (PAH). Oehme (*8*) determined PAH in air particulate matter using NICI. He used a mixture of methane and nitrous oxide as the reagent gas to promote ionization by electron capture and ion/molecule reactions and was able to differentiate isomeric PAH based on the relative abundances of various species formed. Zackett, Ciupek, and Cooks (*9*) used negative ion chemical ionization charge inversion mass spectrometry as a highly selective means for determining polycyclic aromatic hydrocarbons in a solvent refined coal.

We have used NICI mass spectrometry as a sensitive and selective technique for the quantitative determination of

[1] Present address: Environmental Trace Substances Research Center, University of Missouri, Columbia, MO 65201.

benzo[a]pyrene (BaP) in a sample of petroleum crude oil which is being certified as a Standard Reference Material (SRM). During the course of preliminary studies we have confirmed the large degree of selectivity for the detection of BaP over benzo[e]pyrene (BeP) noted by others (*7, 8*). We have observed the molecular anion of BaP to be more than 1000 times more abundant than that of BeP under selected source conditions in the NICI mode using methane as the reagent gas. Our observations, reported here, show that the ion source pressure and temperature play an important role in the selectivity of detection for BaP. We have also observed excellent absolute sensitivity for the detection of BaP and are able to detect quantities as low as 1 pg in the selected ion monitoring mode.

EXPERIMENTAL SECTION

Negative ion chemical ionization mass spectra were recorded on a Hewlett-Packard 5985B quadrupole GC/MS system (Hewlett-Packard Co., Palo Alto, CA) with a dual EI/CI ion source and electronics capable of detecting negative ions. Chromatographic separations were carried out on a 30 m × 0.25 mm i.d. fused silica capillary column coated with a 0.25-μm film of a nonpolar liquid phase. Samples were injected in either the split or splitless modes with an injection port temperature of 300 °C and the column temperature was programmed from 200 to 300 °C at a rate of 4 °C/min. The column was interfaced directly to the ion source by inserting it through a 30 cm length of 0.16 cm o.d. stainless steel tubing. The stainless steel tubing also served as a conduit for introduction of the methane reagent gas (Matheson Ultra High Purity 99.97%) which was brought in coaxially with the capillary column. The pressure in the ion source was adjusted by varying the methane flow into the source via a flow controller. An ionization gauge, which was mounted approximately 15 cm from the source, was used to monitor the ion source manifold pressure. The pressure in the ion source itself was measured with a thermocouple gauge. Spectra were recorded under conditions optimized empirically for the detection of BaP. The ion source was normally operated at 200 °C with a filament emission current of 300 μA and a primary electron beam energy of 60 eV. The mass spectrometer was calibrated in the NICI mode using ions at *m/z* 414, 452, and 633 from perfluorotributylamine and ions at *m/z* 233 and 235 from rhenium oxide generated by the filament. The ReO₃⁻ isotopes provide a good source of ions at low mass for tuning the mass spectrometer in the negative ion mode.

The PAH were obtained commercially: BaP (Community Bureau of Reference, BCR, Brussels, Belgium); BaP-*d₁₂* 98.6 atom % D (MSD Isotopes, St. Louis, MO); and BeP (Pfaltz and Bauer, Inc., Stamford, CT). The standards were analytical grade or higher and were used without further purification. Methylene chloride solutions of the PAH were prepared gravimetrically. The Wilmington crude oil sample was obtained from the Department of Energy and is one of the oils being stored in the EPA Repository

A - BENZO[b]FLUORANTHENE   D - BENZO[a]PYRENE

B - BENZO[k]FLUORANTHENE   E - PERYLENE

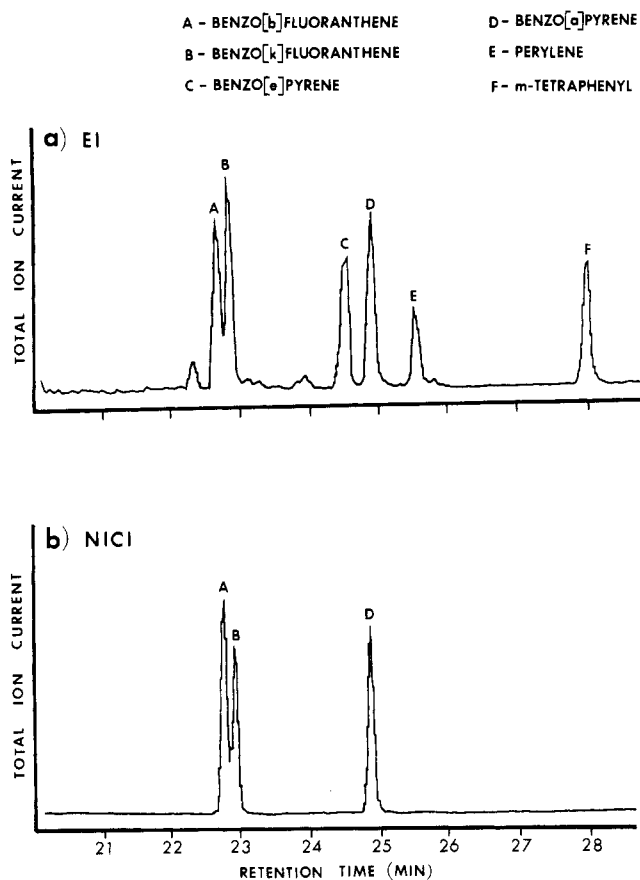C - BENZO[e]PYRENE   F - m-TETRAPHENYL

Figure 1. Total ion chromatograms of PAH mixture analyzed using (a) electron impact ionization and (b) methane negative ion chemical ionization mass spectrometry.

Figure 2. Methane NICI spectra for benzo[a]pyrene and benzo-[e]pyrene. Source manifold pressure was 3.0 × 10⁻⁴ torr.

at the Oak Ridge National Laboratory, Oak Ridge, TN. Approximately 0.5-g samples of the oil were diluted 1:10 with methylene chloride, BaP-d_{12} was added as an internal standard, and 1–2 μL aliquots of the samples were injected onto the capillary column.

RESULTS AND DISCUSSION

Benzo[a]pyrene and benzo[e]pyrene are structural isomers with empirical formula $C_{20}H_{12}$ which exhibit similar chromatographic properties as well as essentially identical mass spectra in the EI and positive ion CI modes. Because of the large differences in their mutagenicities, it is important to distinguish between the two isomers and to be able to measure small amounts of BaP in the presence of BeP. Figure 1 shows two total ion chromatograms of a solution of polycyclic aromatic hydrocarbons run in the EI and NICI modes. Approximately 10 ng of each compound was injected on column. While all of the compounds give a similar response in the EI mode, the only compounds with a significant response in the NICI mode are the benzofluoranthenes and BaP. The methane NICI spectrum for BaP, shown in Figure 2, has a strong molecular anion at m/z 252 and a small (<1%) adduct ion at m/z 266. Stockl and Budzikiewicz (10) have shown that alkyl radical species present in the CH_4 plasma can react with substrate molecules to produce $(M + C_nH_{2n})^{-\cdot}$ ions and we believe this process yields the $(M + CH_2)^{-\cdot}$ species at m/z 266. In comparison, the NICI spectrum of BeP (see Figure 2) shows a base peak at m/z 266 and a much less intense molecular anion at m/z 252. Therefore, an enhanced selectivity for the detection of BaP in the presence of BeP is possible by monitoring m/z 252.

The reasons for the increased production of BaP molecular anion over that of BeP are unclear at this time. A direct correlation between experimentally determined electron affinities and observed NICI sensitivities has yet to be estab-
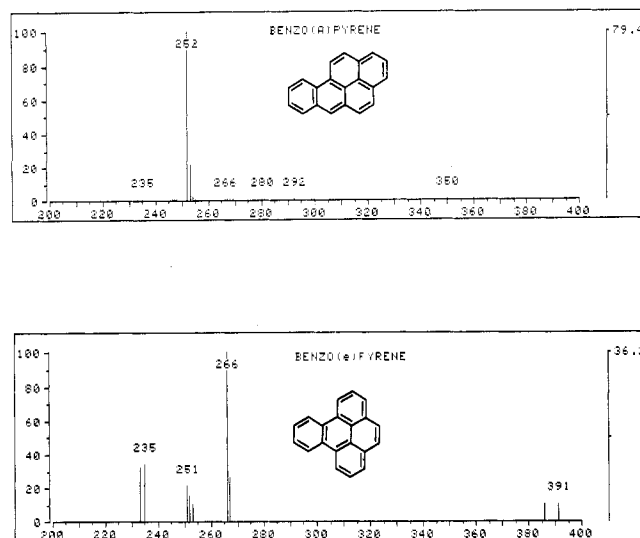
lished. Reliable sources of molecular electron affinities are lacking as this is a difficult parameter to measure experimentally. However, a recent compilation of electron affinity data for some PAH (11) shows no correlation between electron affinity and NICI response. The electron affinities for BaP and BeP are reported to be 0.67 eV and 0.51 eV, respectively. The large difference in the NICI sensitivities of BaP and BeP cannot be explained by the slight difference in their electron affinities alone. Similarly, the electron affinities of fluoranthene and pyrene are reported to be 0.63 eV and 0.59 eV, respectively; yet fluoranthene was 30 times more sensitive than pyrene in the NICI mode (12). There is also a sizable discrepency between the ratios of BaP to BeP molecular anions reported by Ilda and Daishima (50:1) (7) and the value reported here (1000:1) under optimized conditions. We show that the ion source pressure and temperature affect the observed selectivity between BaP and BeP.

The variation in the selectivity of BaP to BeP was measured as a function of the ion source pressure. The pressure was measured both in the ion source manifold, using an ionization gauge mounted approximately 15 cm from the source, and in the ion source itself using a thermocouple gauge. Measurement of pressure in a range that spanned 1 order of magnitude with both gauges showed a roughly linear relationship between the two values. However, we feel that the values determined with the ionization gauge were much more sensitive to slight changes in pressure and that relative changes in pressure could be determined more precisely with the ionization gauge. A methylene chloride solution of BaP and BeP was analyzed in the NICI mode over a range of ion source manifold pressures from 0.8 to 8.0 × 10⁻⁴ torr at a constant source temperature of 200 °C. The molecular anions (m/z 252) for BaP and BeP were monitored as a function of pressure and the results of these experiments are illustrated in Figure 3. The selectivity of detection is defined as (peak area of BaP/ng of BaP)/(peak area of BeP/ng of BeP). In going from low to high pressure in this range, the selectivity of detection between the two isomers decreases by an order of magnitude, but with BaP always favored.

The absolute sensitivity for the detection of BaP was also measured as a function of the ion source pressure (Figure 3b). The absolute sensitivity for a species depends, in part, on the extent to which the newly formed and excited molecular anion can be stabilized by interactions with reagent gas molecules, i.e., collisional stabilization. Collisions can also lead to fragmentations, however, with a resulting decrease in the abundance of molecular anions and sensitivity. We measured an
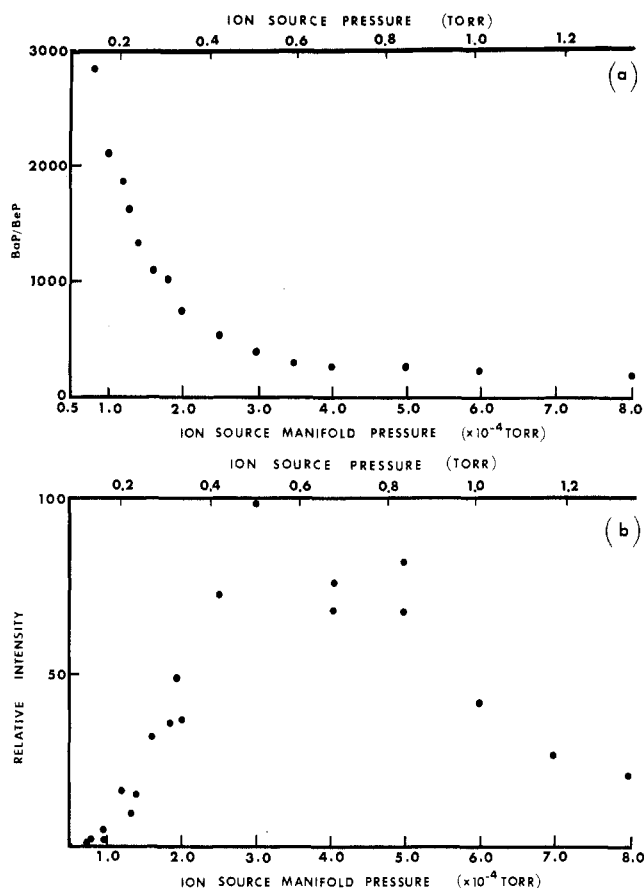
Figure 3. Variation of (a) selectivity of BaP to BeP and (b) sensitivity of detection of BaP as a function of ion source pressure.

Figure 4. Variation of (a) selectivity of BaP to BeP and (b) sensitivity of detection of BaP as a function of ion source temperature.

optimum pressure of approximately 3.0×10^{-4} torr in the ion source manifold (0.45 torr in the source) for the detection of BaP as seen in Figure 3b. A manifold pressure of 2.0×10^{-4} torr was chosen as the best compromise for optimizing both selectivity for BaP and absolute sensitivity.

The variation in the selectivity of detection for BaP was also measured as a function of the ion source temperature. A solution of BaP and BeP was measured in the NICI mode over a range of source temperatures from 100 to 250 °C at a constant ion source manifold pressure of 3.0×10^{-4} torr. The relative abundances of the BaP and BeP molecular anions and adduct ions at m/z 266 were monitored as a function of ion source temperature. The selectivity of detection for BaP, as defined above, was determined as a function of source temperature and is shown in Figure 4. As the source temperature was increased from 100 to 200 °C the abundance of the BaP molecular anion increases relative to that for BeP. Above 200 °C the opposite effect is observed, and the abundance of the BaP molecular anion decreases relative to that for BeP. Interestingly, as the temperature is increased from 100 to 200 °C the abundance of the m/z 266 adduct ion relative to the m/z 252 molecular anion is approximately constant for BaP, but increases by a factor of 50 for BeP. This increase in abundance of adduct ion for BeP with increasing source temperature, with a corresponding decrease in abundance of BeP molecular anion, accounts for the increased selectivity of detection of BaP to BeP fom 100 to 200 °C. The absolute sensitivity of detection for BaP was also monitored as a function source temperature and goes through a maximum at a source temperature of approximately 150 °C.

The selectivity of detection for BaP in the presence of BeP is also demonstrated in Figure 5. Solutions representing approximately 1:1.3, 1:130, and 1:1300 of BaP:BeP concentration ratios were prepared and analyzed by GC/NICIMS in the selected ion monitoring mode at an ion source manifold
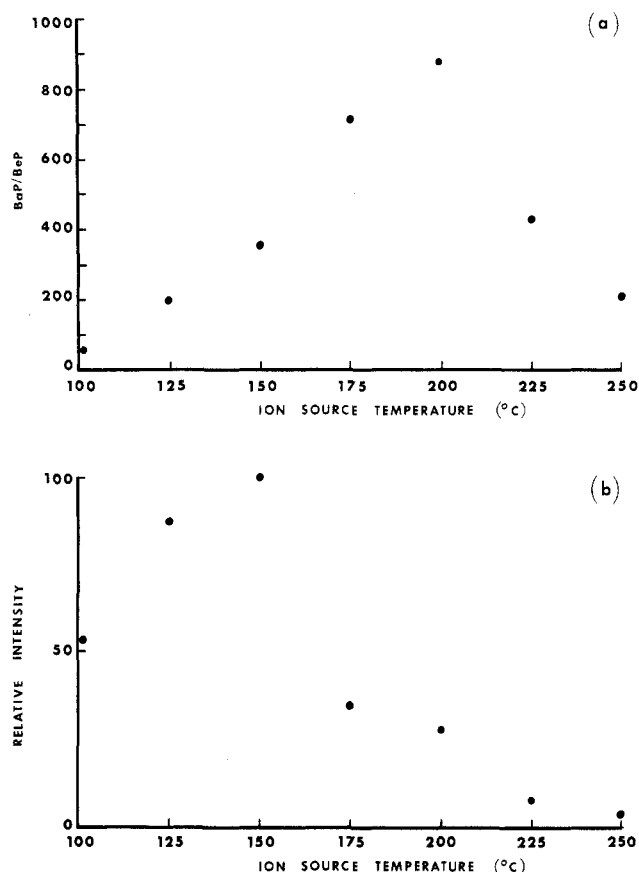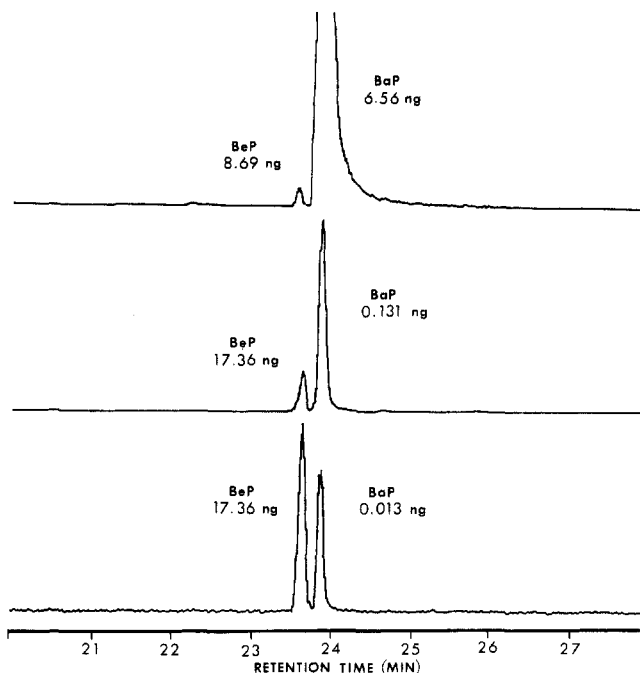

Figure 5. Methane NICI single ion records (m/z 252) for three solutions representing BaP:BeP concentration ratios of (a) 1:1.3, (b) 1:130, and (c) 1:1300.

pressure of 1.4×10^{-4} torr and an ion source temperature of 200 °C. The molecular anions at m/z 252 were monitored with a dwell time of 100 ms yielding at least 20 data points across each peak. The molecular anion of BaP is over 1000 times more abundant than that of BeP at this pressure and temperature.

In addition to the selectivity of detection of BaP, we have also observed excellent absolute sensitivity for BaP in the

Table I. Concentrations of Selected Polynuclear Aromatic Hydrocarbons in Petroleum Crude Oil SRM 1582

	concentration,[a] µg/g		
compound	GC/MS-PICI	GC/MS–NICI	HPLC
benzo[a]pyrene	1.1 ± 0.23 (n = 15)	0.95 ± 0.05 (n = 15)	1.2 ± 0.1 (n = 10)
perylene	30.2 ± 1.7 (n = 15)	30.0 ± 1.1 (n = 15)	32.6 ± 1.2 (n = 10)
benzo[ghi]perylene		1.7 ± 0.1 (n = 15)	
indeno[1,2,3-cd]pyrene		0.17 ± 0.04 (n = 14)	

[a] Values represent the mean ± one standard deviation.

selected ion monitoring mode. A study of the response in the methane NICI mode vs. the amount of BaP on column showed a linear relationship (correlation coefficient = 0.999+) over the range from 20 pg to 20 ng. Quantities of BaP as low as 1 pg could be detected in the selected ion monitoring mode with a signal/noise ratio of 10. In comparison, the lower limit of detection using electron impact ionization was between 5 and 10 pg for BaP on our instrument.

The accurate quantitative determination of toxic organic compounds such as BaP in environmental samples often requires that extensive sample cleanup techniques such as solvent extraction, column, or HPLC chromatography be used to isolate the BaP free of interferences prior to the final quantitative measurement step by GC/MS. In addition to being time consuming, extensive sample pretreatment steps introduce the potential for errors in the analysis. The use of sample cleanup steps can be avoided by using a measurement technique which is highly selective for the analyte(s) of interest. We have observed the chromatograms of complex samples run under NICI conditions to be much simpler than the corresponding EI or positive ion CI chromatograms. GC/NICIMS was used for the determination of BaP, indeno[1,2,3-cd]pyrene, and benzo[ghi]perylene in a sample of Wilmington crude oil which is being certified as NBS SRM 1582, Organics in Petroleum Crude. Sample preparation prior to the GC/MS measurement step consisted of simple dilution of the oil with methylene chloride (0.5 g of oil dissolved in 5 mL of methylene chloride). Attempts to determine BaP in the oil by GC/MS using either electron impact (EI) or positive ion chemical ionization (PICI) were unsuccessful due to the low concentration of the analyte in the oil and the many interferences encountered in the selected ion monitoring analysis of the diluted oil. Figure 6 shows the selected ion monitoring analysis of the Wilmington crude oil using EI, PICI, and NICI techniques. The single ion record at m/z 252 in the EI mode (Figure 6a) is complex and shows coeluting interferences for the BeP, BaP, and perylene. Monitoring the ^{13}C isotope at m/z 253 does not lead to any improvement in the selectivity of detection of BaP. In the PICI mode (Figure 6b) the BaP is barely detectable above the base line in either the m/z 252 (M+) or 253 (M + H)+ single ion records. Indeed, the m/z 253 single ion record, which yields the most sensitivity, shows coeluting interferences for the BaP and the perylene. The interferences in the single ion records made quantitation of BaP in the diluted oil very imprecise. Values for the BaP determined by EI and PICI GC/MS fell in a range of 0.95–2.3 µg/g (ppm), with coefficients of variation of 15–30%.

Quantitation of BaP in the NICI mode, Figure 6c, was facilitated by the selectivity of detection of BaP. The single ion record at m/z 252 (M⁻·) shows a response for the benzofluoranthenes, BaP, and perylene only. The BaP peak was free of interferences and was easily integrated as was the BaP-d_{12} internal standard shown by the single ion record at m/z 264. The results of the GC/NICIMS determination of BaP, indeno[1,2,3-cd]pyrene, and benzo[ghi]perylene are shown in Table I. Note that there was essentially no signal observed for the BeP which was present at approximately
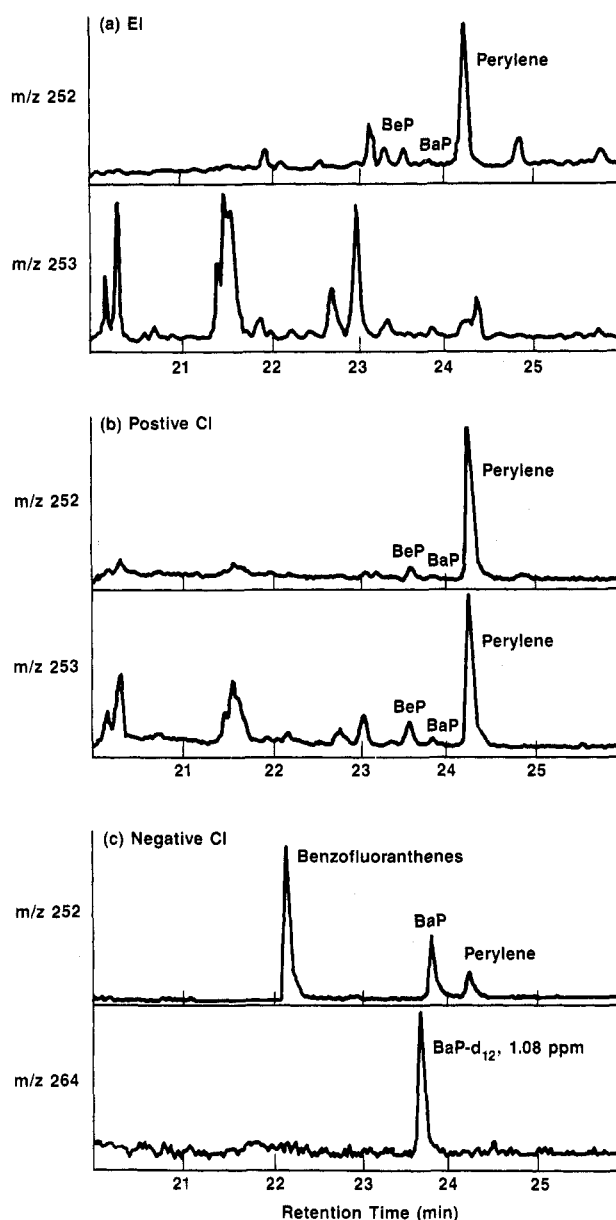

Figure 6. Single ion records for GC/MS analysis of Wilmington crude oil sample using (a) electron impact, (b) positive ion chemical ionization, and (c) negative ion chemical ionization.

three times the BaP concentration. The coefficient of variation for the BaP determinations (n = 15) was 5.0%. The GC/NICIMS value for the BaP concentration was also in good agreement with the value of 1.2 µg/g determined at NBS using a multidimensional HPLC procedure.

CONCLUSIONS

The specificity of NICI mass spectrometry, coupled with a high-resolution separation technique such as fused silica capillary column gas chromatography, provides a highly selective means for the determination of NICI-sensitive compounds in complex environmental samples.

We have shown that NICI mass spectrometry can be used to provide accurate quantitative data for PAH in a crude oil sample with a minimum amount of sample cleanup or pretreatment.

The variations in response factors for different PAH in the NICI mode reported by other investigators can be linked to differences in operating conditions. This report has shown how the selectivity of detection for isomeric PAH can change as a function of ion source pressure and temperature. It is possible by carefully adjusting the ion source pressure and temperature to change both the selectivity of detection of BaP to BeP as well as the absolute sensitivity of detection for BaP. Source pressure and temperature and the manner in which they are monitored should be taken into account when comparing NICI results from different instruments. As noted earlier, the reasons for the NICI sensitivity of PAH are not completely understood. Our observed differences in NICI sensitivities for BaP and BeP do not correlate well with reported electron affinities. Other factors such as the reagent gas and electron energy may also show an effect on the selectivity. The qualitative selectivity of NICI mass spectrometry for certain PAH seems to be a general observation in the recent literature.

Registry No. Benzo[a]pyrene, 50-32-8; benzo[e]pyrene, 192-97-2; indeno[1,2,3-cd]pyrene, 193-39-5; benzo[ghi]perylene, 191-24-2; perylene, 198-55-0; methane, 74-82-8.

LITERATURE CITED

(1) Dougherty, R. C. *Biomed. Mass Spectrom.* **1981**, 9 (7), 283.
(2) Hass, J. R.; Friesen, M. D.; Hoffman, M. K. *Pract. Spectrosc.* **1980**, 3, Part B, 316.
(3) Cavallaro, A.; Bandi, G.; Invernizzi, G.; Luciani, L.; Mongini, E.; Gorni, G. *Pergamon Ser. Environ. Sci.* **1982**, 5, 55.
(4) Dougherty, R. C.; Wander, J. D. *Biomed. Mass Spectrom.* **1980**, 7 (9), 401.
(5) Stan, H.-J.; Kellner, G. *Biom. Mass Spectrom.* **1982**, 9 (11), 483.
(6) Ramdahl, T.; Urdal, K. *Anal. Chem.* **1982**, 54, 2256.
(7) Iida, Y.; Daishima, S. *Chem. Lett.* **1983**, 273.
(8) Oehme, M. *Anal. Chem.* **1983**, 55, 2290.
(9) Zackett, D.; Clupek, J. D.; Cooks, R. G. *Anal. Chem.* **1981**, 53, 723.
(10) Stockl, D.; Budzikiwlecz, H. *Org. Mass Spectrom.* **1982**, 17 (8), 376.
(11) Chen, E. C. M.; Wentworth, W. E. *J. Chromatogr.* **1981**, 217, 151.
(12) Hilpert, L. R.; Byrd, G. D., NBS, June 1983, unpublished results.

Quaternary Ammonium Salts as Calibration Compounds for Fast Atom Bombardment Mass Spectrometry

A. J. DeStefano*[1] and T. Keough

The Procter & Gamble Company, Miami Valley Laboratories, P.O. Box 39175, Cincinnati, Ohio 45247

The fast atom bombardment (FAB) mass spectra of the quaternary-ammonium-salt mixture methylpoly(oxyethylene)(15)octadecylammonium chloride (Ethoquad 18/25) have been used to generate nominal-mass computer calibrations to 1200 amu. Using derivatives formed by the reaction of Ethoquad 18/25 with perfluorinated anhydrides, we were able to extend the mass calibration to ∼1800 amu. The FAB spectra of organic salt mixtures contain high-mass ions (600–1600 amu) of significantly greater intensity than the FAB spectra of inorganic salts.

Fast atom bombardment (FAB) mass spectrometry (1–3) is a rapidly emerging technique for the analysis of polar (4, 5), labile (6–8) compounds. To date, most of the FAB studies reported in the literature have been performed in the analog mode, with the data being output onto UV-sensitive paper. For most studies in which the mass spectrometer has been operated under computer control, calibration of the mass axis was accomplished in the FAB mode using either Ultramark compounds (9) or alkali halide salts dissolved in glycerol (9). Alternatively, calibration was done in the electron ionization (EI) mode and this EI calibration was used for FAB analyses (10).

We felt it important to operate our spectrometer under computer control for a number of reasons. First was the obvious problem of storing and archiving large quantities of

UV-sensitive paper. Second, we wanted to take advantage of some of the capabilities afforded by the modern data systems including background subtraction, spectral averaging, and the displaying of desorption profiles. The latter feature was especially important in light of reports of significant changes in some FAB spectra as a function of sample analysis time (11, 12). Since we anticipated performing a large number of FAB analyses, and since switching from EI to FAB required a source change on our instrument, our interest was in developing a FAB-oriented mass-calibration procedure. Our calibration attempts using inorganic salts dissolved in glycerol were, in general, unsuccessful. We were unable to consistently produce high-mass cluster ions of sufficient intensity to calibrate the mass axis to beyond 1000 amu. In addition, the gaps between reference ions in the spectra of the pure alkali halides were sufficiently large that mass calibration often failed with our standard (low field) magnet when the instrument was operated at full accelerating voltage.

The inconsistent calibration results we obtained with the inorganic salts led us to examine a new approach involving organic salts. We reasoned that high-molecular-weight organic salts could provide an increase in sensitivity over inorganic salts since the need to form large cluster ions would be eliminated. For these studies, we chose the mixture methylpoly(oxyethylene)(15)octadecylammonium chloride (A),

$$(CH_2CH_2O)_yH$$
$$|$$
$$C_{18}H_{37}\text{-}N\text{-}CH_3 Cl$$
$$|$$
$$(CH_2CH_2O)_xH$$
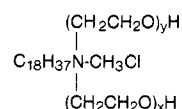
A, $x + y = 15$ (average)

[1] Current address: Norwich Eaton Pharmaceuticals, P.O. Box 191, Norwich, NY 13815.