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Electrospray mass spectrometric determination of polycyclic aromatic hydrocarbons by detecting the π–π complexes with tropylium cation

Communication

Hiroshi Moriwaki,*[a] Akira Imaeda[b] and Ryuichi Arakawa[b]

[a] *Osaka City Institute of Public Health and Environmental Science, 8–34, Tojo-cho, Tennoji, Osaka 543-0026, Japan. E-mail: moriwaki@mxp.mesh.ne.jp*

[b] *Department of Applied Chemistry, Faculty of Engineering, Kansai University, 3-3-35 Yamate-cho, Suita, 564-8680, Japan*

Received 8th December 1998, Accepted 21st January 1999

Published on 01 January 1999 on http://pubs.rsc.org | doi:10.1039/A809573F
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A method is presented for the determination of polycyclic aromatic hydrocarbons (PAHs) by detecting the PAH–tropylium (π donor–π acceptor) complexes using liquid chromatography-electrospray ionization-mass spectrometry. The complexes were formed by mixing with the tropylium cation after the separation of PAHs through reversed phase liquid chromatography. The detection limits, defined as three times the noise, in the selected-ion monitoring mode were 25–165 ng of the injected PAHs.

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants whose carcinogenicity and estrogenicity have been extensively studied.[1–3] In order to evaluate the risk of PAHs in the environment, various methods for the determination of PAHs have been used. Gas chromatography-mass spectrometry (GC-MS) is an excellent and powerful tool for the identification of PAHs because of its high separation efficiency.[4] However, PAHs whose molecular mass lies beyond the range (~300 u) are difficult to characterize and detect by GC-MS.[5] In addition, it was found that the partial decomposition of unstable species of PAH, such as nitrated PAHs, occurs in the injector, the column and interface of the GC-MS.[6] This effect makes the identification and quantification of PAHs difficult. These problems are overcome by using high performance liquid chromatography (HPLC), because it can be carried out at room temperature. Therefore, the methods based on HPLC coupled with a UV detector have been extensively used for the analysis of PAHs in environmental samples.[7,8]

The LC-MS method would supply more useful information for the analysis of PAHs because of the relatively high specificity of an MS detector in comparison with a UV detector. However, very few examples are known based on the detection of PAHs by LC-MS using several commercially available interfaces, including thermospray,[9] liquid ionization[10] and atmospheric pressure chemical ionization.[5] Furthermore, there are few reports about the detection of PAHs using electrospray as the interface due to the difficulty of ionizing low polar compounds.[11]

In this study, a novel approach to detecting and identifying PAHs by liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS) is demonstrated.

The tropylium cation (TR^+) has been known as a strong π-acceptor, and it recognizes PAHs by π–π interactions, and almost quantitatively forms the [PAH–TR^+] 1 : 1 cation complex.[12] On the other hand, ESI is a very powerful method for the characterization and identification of non-covalently bound polar species, because the technique is a very soft ionization method,[13] and it is well known that ESI achieves the best sensitivity for compounds that are precharged in the solution.[14] Therefore, the [PAH–TR^+] complexes are expected to show high responses using ESI-MS.

By utilizing the characteristics of TR^+ and ESI, we determined PAHs by detecting the [PAH–TR^+] complexes, which were formed by mixing with TR^+ after separation of the PAHs through reversed phase liquid chromatography using ESI-MS.

Experimental

Materials

All solvents were of HPLC grade and other chemicals were of analytical-reagent grade. Three PAHs, pyrene, benzo(*a*)pyrene and coronene, were used as target compounds. Pyrene was obtained from Wako Pure Chemical (Osaka, Japan). Coronene and tropylium tetrafluoroborate were purchased from Tokyo Kasei (Tokyo, Japan) and benzo(*a*)pyrene was from Nacalai tesque (Kyoto, Japan).

HPLC conditions

Liquid chromatography was carried out on an HPLC apparatus equipped with a Model L-7100 chromatograph (Hitachi, Tokyo, Japan) and Thermo Minder SM-05 (Taitec, Tokyo, Japan). A GL Science Inertsil ODS-2 semi-micro column (5 μm particle size, 150 × 1.0 mm id) was used for the LC separation of the PAHs. The column temperature was 30 °C. Methanol was used as the mobile phase and the flow rate was 0.02 ml min^{-1}. In LC-ESI-MS, post-column addition of 1.0 mM of the tropylium cation acetonitrile solution at 0.1 ml min^{-1} was carried out using a Shimadzu LP-6A liquid delivery pump. The mobile phase was mixed in a tee (Yokogawa 0100-0782, stainless) with the tropylium cation solution.

ESI-MS

ESI-MS was performed using a Hitachi M-1200AP (Tokyo, Japan) mass spectrometer connected to a Hitachi L-7100 chromatograph. The multiplier and focus voltage were set at 2.5 kV and 120 V, respectively. The desolvator temperature was set at 400 °C. The vaporizer temperature was set at 300 °C. Flow injection analyses (FIA) of the PAHs were carried out in the presence or absence of TR^+. In the case of FIA, methanol was used as the mobile phase and the flow rate was 0.05 ml min^{-1}. The drift voltage was set at 70 V, unless otherwise noted. For LC-ESI-MS, the drift voltage was fixed at 20 V. The injection volumes of the sample solutions in ESI-MS were 5 μl. When working in the selected ion monitoring mode (SIM), the $[M]^+$ and $[M + TR]^+$ ions were monitored depending on the target analyte.

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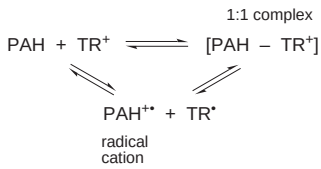
Results and discussion

Detection of PAHs

In the case of the mass spectral investigation by ESI through flow injection of the PAH acetonitrile solution (1.0 mM of pyrene, benzo(*a*)pyrene and coronene), there were no peaks assignable to the PAHs. On the other hand, with the injection of the solution of PAHs containing the same molar amount of TR^+ (1.0 mM), both the M + 91 peaks correspond to the [PAH–TR^+] complex and the M^+ peaks attributed to the PAH radical cations were observed as the main peaks. The intensities of the M + 91 peaks were stronger than those of the M^+ peaks. In the case of coronene, in particular, the peak of [coronene–TR^+] was about four times stronger than that of the coronene radical cation. Hence, [PAH–TR^+] cation complexes were selected as the monitor ion to detect the PAHs.

In order to establish the optimum drift voltage for the detection of the [PAH–TR^+] complex, the signals of the complex and radical cation *vs.* drift voltage for pyrene were studied. When higher drift voltages were used, more of the pyrene radical cation peak was observed and a decrease in the intensity of the [pyrene–TR^+] occurred. This fact indicates that charge transfer may potentially compete with complex formation and dissociation in the electrospray interface as shown in Scheme 1. Such charge transfer reactions are often significant.[11,12] The [pyrene–TR^+] complex showed a maximum at 20 V as can be seen in Fig. 1. From this result, the optimum drift voltage was determined to be 20 V for the present method. Under this condition, the mass spectra of coronene (1.0 mM) in the presence of the same molar amount of TR^+ by FIA showed the [coronene–TR^+] complex (*m/z* 391), but there were no peaks corresponding to the radical cation species (*m/z* 300) as shown in Fig. 2.

Next, it was investigated how the molar ratio (PAH *vs.* TR^+) affects the response of the [PAH–TR^+] complex. Injections of 5 μl 1.0 mM solutions of pyrene containing different concentrations of TR^+ were performed. At 0.5 mM TR^+ (TR^+ : pyrene = 0.5 : 1), the response was half that of 1.0–5.0 mM. However, no significant increase and decrease in the responses of [pyrene–TR^+] were observed with 1.0–5.0 mM TR^+ in the injection solution (Fig. 3). From these results, the molar ratio was adjusted in the range of TR^+/PAH > 1 under the LC-ESI-MS condition.



Scheme 1

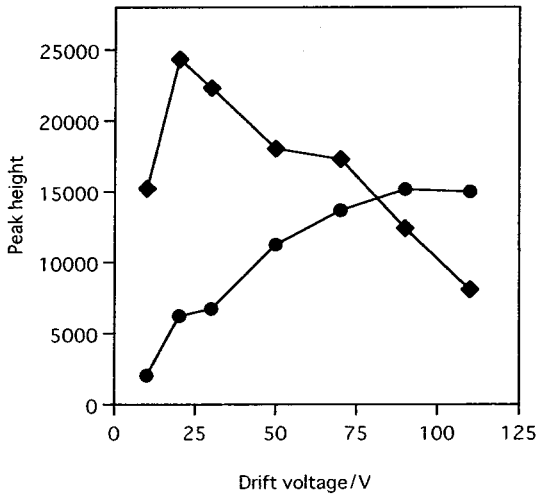


Fig. 1 Variation in peak height of M–TR^+ and M^+ ions *vs.* the drift voltage (V) for pyrene.

LC-ESI-MS

[PAH–TR^+] complexes were detected by ESI-MS with the addition of TR^+ solution after the separation of PAHs through a semi-micro ODS column. Fig. 4 shows the chromatograms of pyrene, benzo(*a*)pyrene and coronene using the following selected ion modes: *m/z* 293, 343 and 391, each peak of PAHs being detected separately by different ion modes. Thus, the combination of HPLC and ESI-MS provides further high specificity.

The calibration equations were obtained for pyrene, benzo(*a*)pyrene and coronene using a series of standard solutions over the concentration range 0.10–0.50, 0.01–0.50 and 0.10–0.50 mM, respectively (Fig. 5 for pyrene). The detection limits and calibration equations are summarized in Table 1. The detection limits, defined as three times the noise, in the selected-ion monitoring mode were 25–115 ng of the PAHs injected as shown in Table 1.

Conclusions

The determination of PAHs by LC-ESI-MS has, in the past, not been particularly well reported. The present method makes electrospray mass spectrometric determination of PAHs possi-

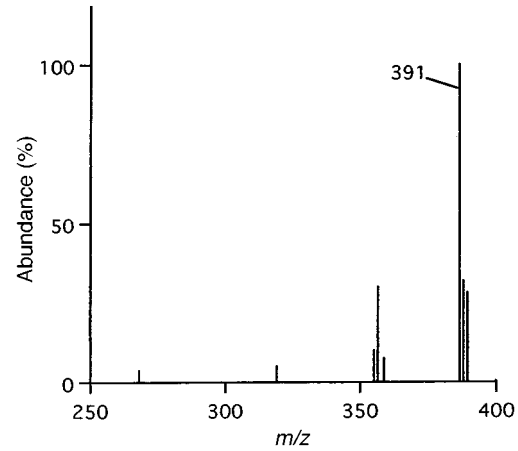


Fig. 2 ESI-MS spectrum of the [coronene–TR^+] ion at drift voltage of 20 V.

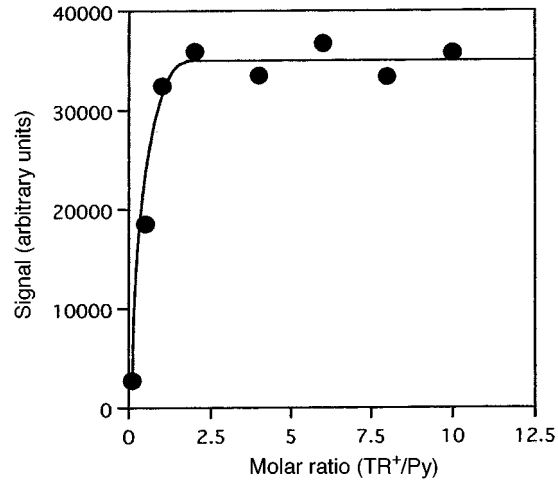


Fig. 3 Effects of molar ratio (pyrene 1.0 mM, TR^+ 0.50–5.0 mM) on the ion signal of [pyrene–TR^+].

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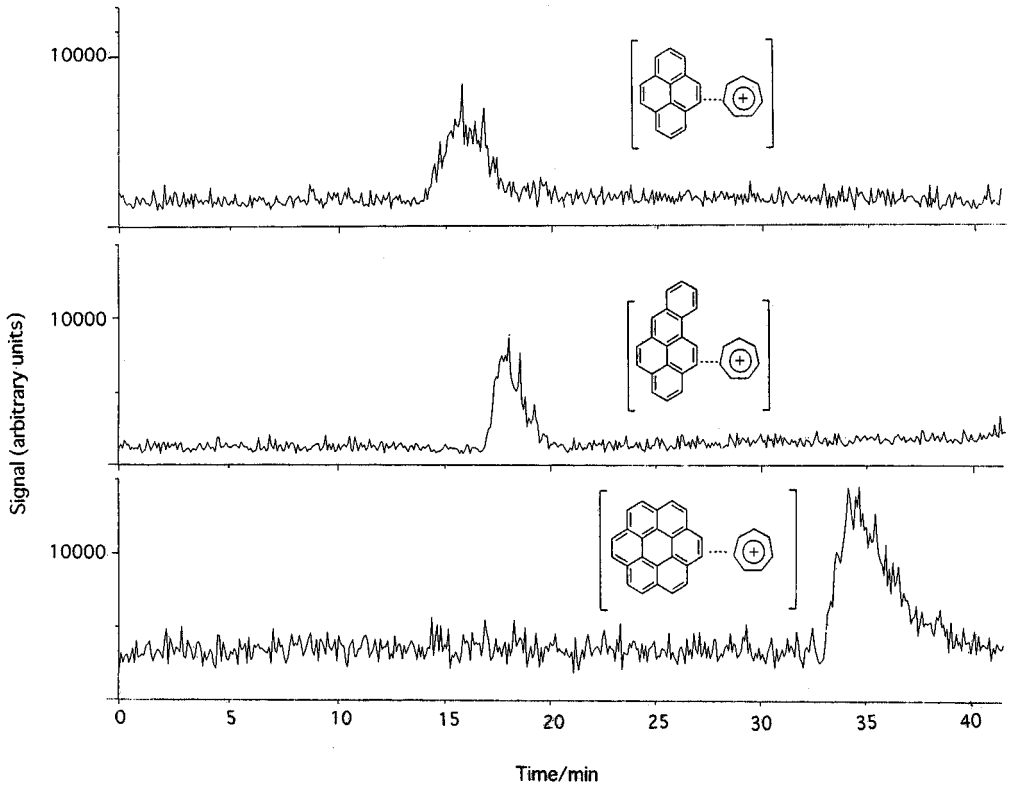


Fig. 4 LC-ESI-MS chromatograms of pyrene, benzo(*a*)pyrene and coronene for the injection (5 μl) of a standard solution of PAHs (0.2, 0.05 and 0.25 mM) by selected-ion monitoring (*m/z* 293, 343 and 391, respectively).

Table 1 Detection limits and calibration equations for PAHs obtained using LC-ESI-MS

Analyte	Monitor ion (*m/z*)	Retention time/min	Detection limit[a] Concentration/ mM	Detection limit[a] Injection/ng	Calibration equation	Correlation coefficient (R^2)
Pyrene	293	15.8	0.1	101	$y = 28737x - 138$	0.997
Benzo(*a*)pyrene	343	18.0	0.02	25	$y = 158800x - 82$	0.992
Coronene	391	34.6	0.2	225	$y = 45173x - 251$	0.990

[a] Calculated as three times the baseline noise.

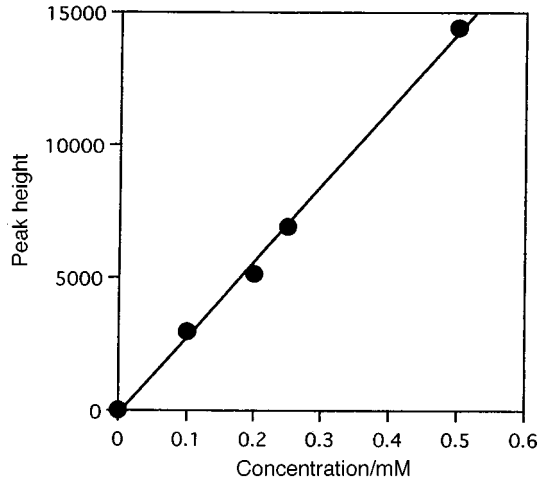


Fig. 5 Calibration graph for pyrene in the range 0.1–0.5 mM.

ble by the complexation with TR^+, a strong π-acceptor and ionic group.

The detection of PAHs by monitoring host–guest complexes is a new application of ESI, and this method would contribute to the analysis of low volatile or thermally unstable species of PAHs that are difficult to detect by GC-MS.

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Paper 8/09573F

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