Shoji Kurata,[1] *M.Sc.; Haruo Hirano,*[1] *B.Sc.; Masatoshi Nagai,*[2] *Ph.D.*

Development of Fluorescent Markers Using Polycyclic Aromatic Hydrocarbons with Vaseline

REFERENCE: Kurata S, Hirano H, Nagai M. Development of fluorescent markers using polycyclic aromatic hydrocarbons with Vaseline. J Forensic Sci 2002;47(2):244–253.

ABSTRACT: Identifiable fluorescent markers were developed as tracers to tail suspects using phenanthrene, anthracene, fluoranthene, pyrene, perylene, and coronene in vaseline. Vaseline was used as a carrier of the marker. Of the six compounds in the vaseline, perylene and fluoranthene were readily observed under ultraviolet (UV) light at a wavelength of 365 nm. All six compounds were identified selectively and sensitively without interference of vaseline using a high performance liquid chromatograph (HPLC) with a fluorescence detector. The detection limit was much less than 1 ng, corresponding to that of the observation behavior under UV light. The results showed that each component with vaseline was more effective than the individual component for the delay in degradation. The case examples of the fluorescent markers are shown.

KEYWORDS: forensic science, fluorescent marker, polycyclic aromatic hydrocarbons, vaseline, high performance liquid chromatography, fluorescence spectrophotometer, sublimation, photodegradation

Fluorescent compounds are utilized as invisible tracers under fluorescent light and sunlight to tail suspects in the forensic science field in Japan. The compounds stick to suspects, revealing crime evidence. The fluorescent substances dissolved in organic solvents can also be used to attach bar-codes and seals (1) detectable under UV light. However, the fluorescent substances alone would not be suitable for the tracers to tail the suspects because they were scarcely detected in crime scenes after adhering to the criminal's shoes. Most polycyclic aromatic hydrocarbons (PAHs) emit some light with a visible wavelength under UV light at 365 nm and then are used as fluorescent substances in a variety of industrial products. The UV light was used as a light source for exciting the PAHs' molecules because it usually came from portable UV lamps. In this study, the analytical methods of PAHs, with vaseline prepared as the fluorescent markers (2) and degradation after applying, are studied for the development of fluorescent markers that are more suitable and visually detectable for the tracers under UV light in a small amount of PAHs. Furthermore, the compounds mixed into vaseline were prepared as sticky gel samples in order to increase adhesive power of PAHs as markers. Since vaseline is harmless to the skin, it is suitable for a carrier of applied marker. For the scientific analyses of PAHs, many researchers reported that a small amount of PAHs were analyzed by gas chromatography (GC) (3,4), gas chromatography-mass spectrometry (GC-MS) (5,6), and HPLC (2,6–14). The detection methods of PAHs in vaseline have not been fully established. In this study, the fluorescent PAHs were analyzed by HPLC with a fluorescence and UV/Visible photodiode array detection in series. The detection limit of PAHs was also studied, compared to the observation behavior under UV light. Moreover, the degradation of the PAHs in vaseline applied as markers was studied for a short time (less than one hour) by a spectrofluorimeter and long term (several hours at four different places) by HPLC with a fluorescence detector. The three case examples of the fluorescent markers are also shown.

Methods

Chemicals

Phenanthrene (CP 98%) and coronene (CP 95%) were purchased from Tokyo Kasei Kogyo Co. (Tokyo, Japan). Perylene (CP 99%) was from Aldrich Chem. Co. (Milwaukee, WI). Anthracene (CP 99.5%), fluoranthene (CP 97%), pyrene (CP 98%), vaseline (EP), chloroform (CP 99%), methanol (HPLC grade), and distilled water (HPLC grade) were from Wako Pure Chemical Industries (Tokyo, Japan). Diethyl ether (CP 99.5%) was from Sanraku Finechem. Co. (Yamaguchi, Japan).

Ultraviolet Ray Lamp

The fluorescence measurements were carried out with a portable National Co. fluorescent lamp BF-661 (Matsushita Electric Appliance Industry Inc., Japan) and with a FL6.BLB (6W) light source (Toshiba Co., Japan) at an excitation wavelength of about 365 nm for the observation behavior.

Spectrofluorimeter

The fluorescence measurements were carried out with a Hitachi F-4500 fluorescence spectrophotometer equipped with the excitation source of a 150-W CW xenon lamp. The band pass for the excitation and emission monochromators were set to 5 nm. Solutions were placed in a 1 cm path-length quartz cell or powder cell fixed

[1] Forensic Chemist, Criminal Investigation Laboratory, Metropolitan Police Department, 2-1-1, Kasumigaseki, Chiyoda-ku, Tokyo 100-8929, Japan.
[2] Associate professor, Graduate School of Bio-applications and Systems Engineering, Tokyo University of Agriculture and Technology, 2–24 Nakamachi, Koganei-shi, Tokyo 184-8588, Japan.
Received 30 April 2001; and in revised form 6 July 2001; accepted 10 Aug. 2001.

with a solid holder. The solvent was methanol-water (7:3 and 9:1) or vaseline.

HPLC Analysis and Conditions

Six kinds of PAHs were separated in a reversed-phase HPLC system on a Finepak SIL C18S column (150 × 4.6mm i.d., 5μm particle size) obtained from Jasco Co. (Tokyo, Japan). The HPLC separation was carried out in an oven at 40°C using a low pressure gradient composed of mobile phase A (70% methanol in water, v/v) and mobile phase B (90% methanol in water, v/v). The gradient expressed as changes in the mobile phases was: 100% solution A was held for 0–27 min and 100% solution B after 27 min. The mobile phases were continuously degassed using a Gastorr GT-103 vacuum degasser (Tokyo Kasei Kogyo Co., Japan). A flow rate of 1 mL/min and an injection loop of 20 μL (Rheodyne injector) were used for all the samples. For the fluorescence and UV/Visible analyses, a PU-880/CO-860 liquid chromatograph (Jasco Co., Japan) coupled with a FP-820 fluorescence detector and a MULTI-340 photodiode array detector in the series was used. The fluorescence analysis was carried out using a time program method suitably changeable to the emission and excitation wavelengths detected as optimum. The UV/Visible photodiode array detector was used in the region of 230 to 450 nm.

Sample Preparation

Six kinds of PAHs with and without vaseline were the samples used without purification in this study. The solutions of the PAHs in a small amount of chloroform were added to vaseline (1%/99% and 0.1%/99.9%), then stirred into a water bath at 70°C. The vaseline solution of the PAHs was prepared by cooling gradually to room temperature. The PAHs without vaseline were prepared for comparison at the same concentration as those with vaseline were dissolving in chloroform. The samples without vaseline were analyzed after the chloroform solvent was volatilized.

Analytical Methods

The fluorescent PAHs were accurately weighed, dissolved in vaseline, and applied on a square filter paper (2 × 1 cm). The compounds without vaseline were also used for comparison. The light emitted from the samples was observed under UV light at a wavelength of about 365 nm. The light was identified by comparison with standard colors. The emission spectra of the compounds with and without vaseline were measured by excitation at 365 nm to compare with the results from the observation of the emitted fluorescence. Furthermore, 20 μL of the chloroform solution containing the samples were measured by HPLC. Each component was detected by both fluorescence and UV/Visible photodiode array detectors in series, based on the retention times and shape of the spectra of the standard PAHs. The samples were measured with the fluorescence detection of HPLC under optimization of the excitation and emission wavelengths. The detection limits were compared to that by observation under UV light.

Degradation of Fluorescent Compounds after Application

The short time degradation of the fluorescent compounds was studied for the photodegradation, sublimation, and solubility in water after application. The degradation of each PAH compound with and without vaseline due to UV photodegradation after application was studied as follows: a 10 μL of sample each containing 0.1% PAH compound with vaseline applied on a powder cell was fixed with a solid holder. Then the fluorescent intensities were measured for 40 min after application by a fluorescence spectrophotometer at 30°C. The chloroform solution without vaseline was also determined for comparison. The fluorescent measurement of the samples without vaseline started immediately after volatilizing chloroform used as the solvent at room temperature. The excitation wavelengths were selected as follows: 254 nm for phenanthrene and pyrene, 365 nm for fluoranthene and perylene, 254 and 365 nm for anthracene, and 300 nm for coronene. The samples were excited continuously for 40 min. The decay of fluorescent intensity of each compound was evaluated by means of the intensity of the strongest peak in the emission spectra measured.

The sublimation of each PAH compound with and without vaseline after application was studied. Ten μL of the vaseline mixtures or chloroform solutions were dropped on the bottoms of 3-mL vials, which were then capped with rubber stoppers backed with filter paper. The vials were kept in an oven at 60°C for 5, 15, 30, and 60 min. The filter papers were measured by the fluorescence spectrophotometer. The sublimation degree of the PAH compounds was estimated by the intensity of the strongest peak in the emission spectra measured.

The solubility in water of each PAH compound with and without vaseline was studied by the following experiment: after each of the 10 μL of the vaseline mixture and the chloroform solution was applied to the entire surface of one side of the slides (0.5 × 0.5 cm), each slide was dropped in the bottom of a cell containing 3 mL of water, with the face turned upward, and then the fluorescence intensity of the PAH compound eluted in water was measured continuously for 30 min by the fluorescence spectrophotometer.

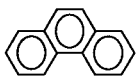
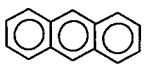
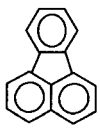
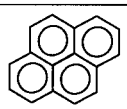
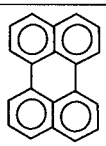
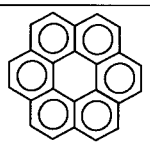
Furthermore, the long term degradation of the fluorescent compounds was studied for a variety of environments after application on a filter paper (2 × 1 cm). The samples were 1 mg in the chloroform solutions and the vaseline mixtures containing 0.1–1% fluorescent compounds applied on the filter paper. The samples were then kept for half a year in a refrigerator at 5°C, by a window (sun), in a drawer (shade), and in distilled water (10 mL) in a beaker. The latter three places preserving samples were at room temperature. The degeneration of each fluorescent component during the storage time was measured by HPLC with fluorescence detector.

Results and Discussion

Observation of Fluorescent Compounds

The observation of the compounds with the 1% concentration of vaseline under UV light at a wavelength of 365 nm is shown in Table 1. The results without vaseline are also shown for comparison. The color of the light emitted from the compounds with vaseline was different from those of the compounds alone. All the fluorescent colors of phenanthrene, anthracene, and pyrene with vaseline were purple, which was probably emitted from the vaseline. On the other hand, the fluorescence color of fluoranthene, perylene, and coronene with vaseline was pale greenish-blue, bright greenish-blue, and pale greenish-sky, respectively. The fluorescence of perylene and fluoranthene was easily observed even at concentrations of 0.01%. The detection limit for the observation of the two compounds was at a 10 ng level, but the compounds with vaseline were observed only at 1 ng. Therefore, the two compounds were very useful as fluorescent markers visible by the observation.

TABLE 1—*Observation of fluorescent compounds.*

Compound		Colors of fluorescences of components alone and those with vaseline observed under ultraviolet light of wavelength about 365 nm.	
Name	Structural Formula	Component	Component with vaseline
Phenanthrene		Not detected	Purple
Anthracene		Purple	Purple
Fluoranthene		White	Pale greenish-blue
Pyrene		White	Purple
Perylene		Vivid yellowish-orange	Bright greenish-blue
Coronene		Yellow	Pale greenish-sky

Emission Spectra

The emission spectra of the compounds with and without vaseline at 365 nm are shown in Fig. 1*a* to *f*. The shapes and intensities of the bands in the spectra of the compounds with vaseline were different from those without vaseline. The band fluorescence intensities of perylene and fluoranthene with vaseline were higher than those of the other compounds in the visible region over 450 nm. These two compounds with vaseline were more easily observed than the others. The emission spectra of phenanthrene, anthracene, and pyrene with vaseline at 365 nm were overlapped with that of vaseline in the visible region. However, the emission spectra of the compounds with vaseline at 254 or 300 nm were quite similar to those of the compounds dissolved in solvents such as methanol/water (7:3 and 9:1), shown in Fig. 2*a-2* to *f-2*. From the result, these compounds were probably soluble in vaseline. The addition of vaseline to these compounds changed the solid phase to the liquid phase and then shifted the fluorescence band of five compounds, except for fluoranthene, to the UV region, compared to the band for the compounds without vaseline.

Separation and Detection by HPLC

The excitation and emission wavelengths of each component were measured by the spectrofluorimeter in the HPLC mobile phase (methanol-water (7:3 or 9:1)) in order to optimize the fluorescence detection. As shown in Fig. 2*a-1* to *f-2*, the optimum excitation wavelengths were 251 (emission wavelength; 365), 251 (400), 236 (462), 240 (392), 252 (438), and 301 (444) nm for phenanthrene, anthracene, fluoranthene, pyrene, perylene, and coronene, respectively. In Fig. 3, all the PAHs could be readily determined with high selectivities without interference of vaseline in the chromatogram of the extract of the six PAHs, with vaseline using chloroform by HPLC with a fluorescence detector and UV/Visible photodiode array detector. The detection limit for HPLC with a fluorescence detector under the measurement conditions was at a level of 1–10 picogram (pg). The HPLC with a fluorescence detector was more sensitive than the observation behavior under UV light for the detection of the compounds used in this study. It was reported that HPLC with a fluorescence detector could only selectively detect the components in the PAHs at fixed excitation wavelengths (360, 254, or 286 nm) and emission wavelengths in the visible region (8,9,14). This detection method was developed and applied to the time program method, fixed at the optimum wavelengths of excitation and emission, and switched suitably such that the peak intensity of the fluorescence in the compounds was maximized (6,10,11). The necessary amount of samples for the HPLC under a time program is smaller than that for the HPLC described above. The detection limit of the time program method in this experiment was several to several hundred times lower than that of the detection method described above. The detection limit by the time program method for the HPLC with a fluorescence detector was not contradictory to a previous report (10). When the solution

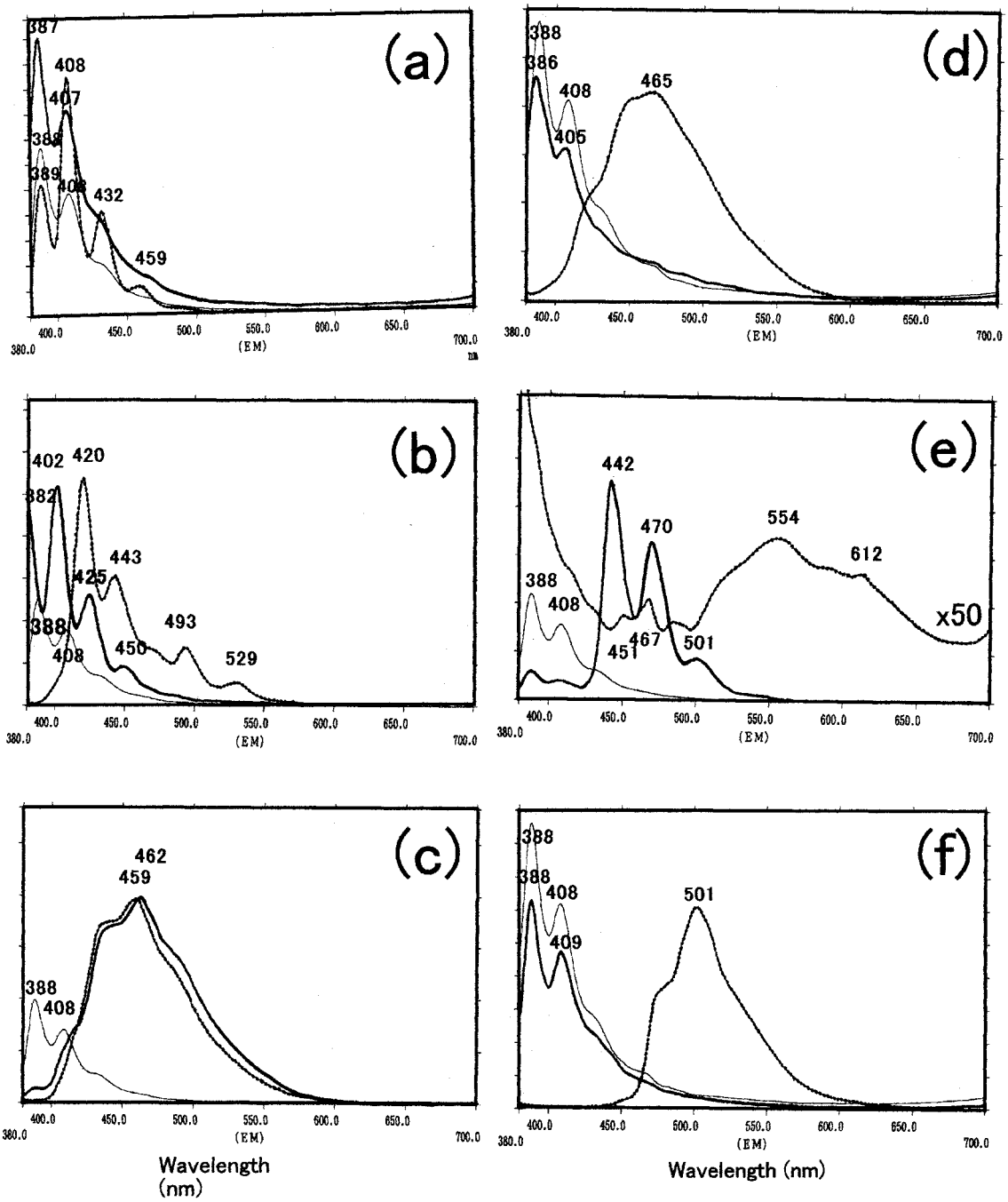


FIG. 1—*Emission spectra (bold solid curve) of phenanthrene (a), anthracene (b), fluoranthene (c), pyrene (d), perylene (e), and coronene (f) with vaseline, those (dotted curve) of each component without vaseline, and those (fine solid curve) of vaseline excited at 365 nm.*

of methanol-water (7:3) was selected as the mobile phase, two pairs of isomers were readily separated in a short time, i.e., a pair of phenanthrene and anthracene and another pair of fluoranthene and pyrene. The weight ratio for phenanthrene, anthracene, fluoranthene, pyrene, perylene, and coroneme was roughly 5:1:20:5:1:5 in the range of 100 pg to 100 ng. Furthermore, all the compounds were determined by HPLC using a UV/Visible detector (7,10–14). In this experiment, the compounds were measured by HPLC with a UV/Visible photodiode array detector at about 1 ng in the region of 230 to 450 nm. The UV/Visible absorption spectrum of each compound obtained by a UV/Visible photodiode array detector was just like the excitation spectrum as shown in Fig. 2*a-1* to *f-1*. Although this detection limit for the UV/Visible photodiode array detection was similar to that obtained by Müller and Rohbock (7), the PAHs were measured by the fluorescence detector in this study using much smaller amounts than those by the UV/Visible photodiode array detector. Therefore, the HPLC with a fluorescence detector was very suitable for the analysis of trace amounts of PAHs.

Degradation of Fluorescent Compounds after Application

The degradation of fluorescent peak intensities of six PAHs in vaseline due to UV photodegradation at 30°C after application is

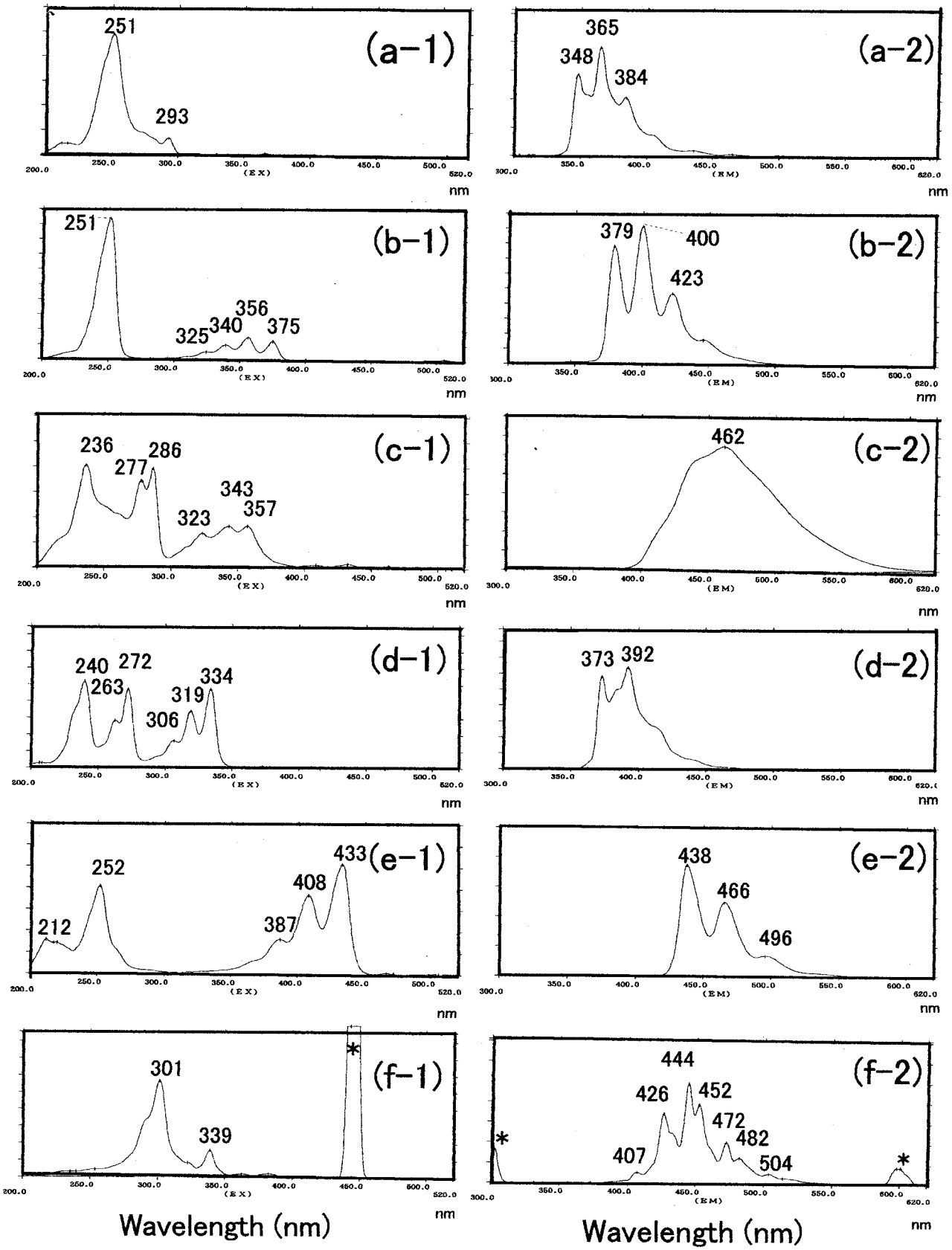


FIG. 2—*Excitation spectra of phenanthrene (a-1), anthracene (b-1), fluoranthene (c-1), pyrene (d-1), perylene (e-1), and coronene (f-1) and emission spectra of each compound (a-2~f-2). Solvents are methanol-water (7:3) for phenanthrene, anthracene, fluoranthene, and pyrene, and methanol-water (9:1) for perylene and coronene. The peaks with an asterisk are scattering lines.*

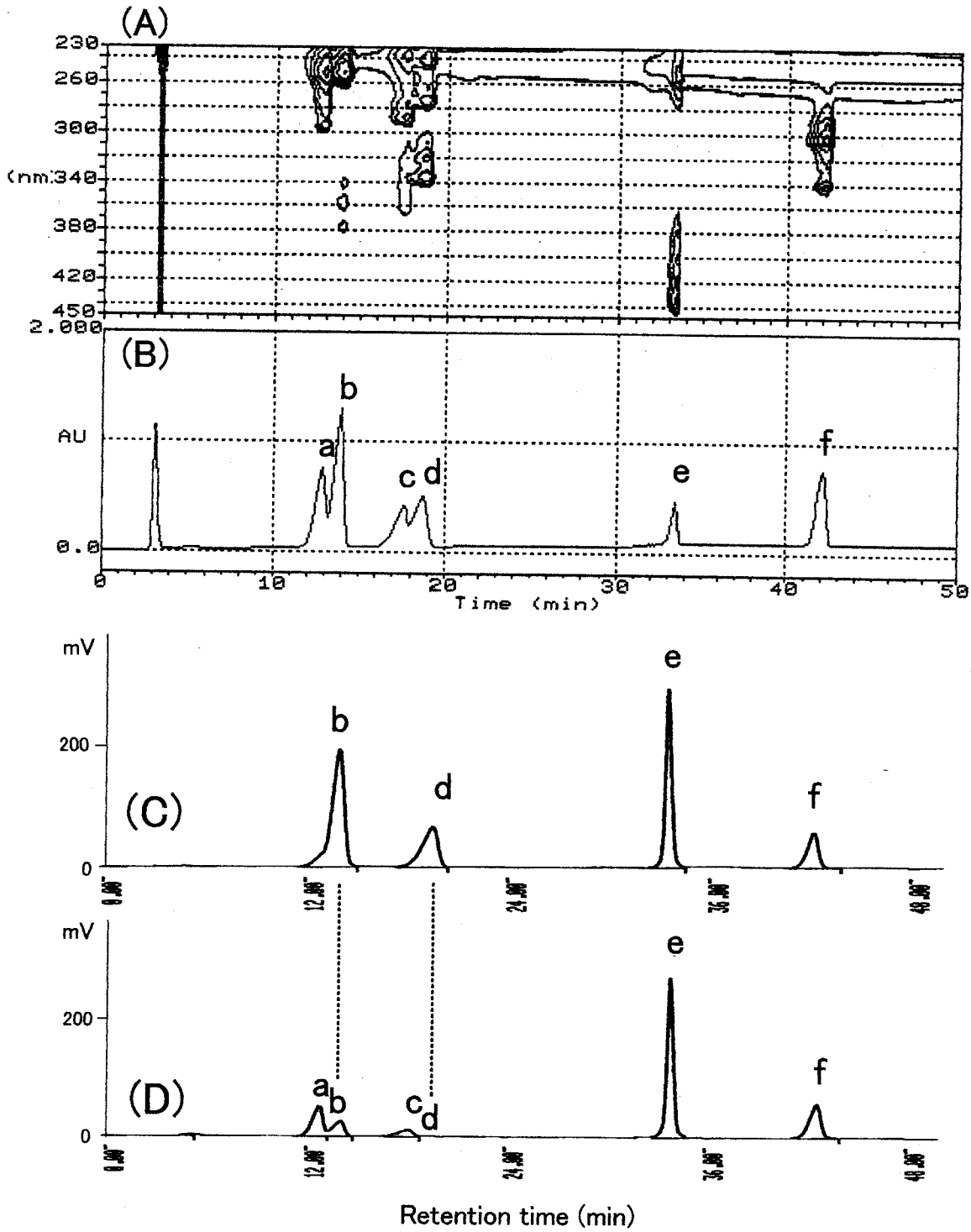


FIG. 3—*A contour plot of UV/Visible absorption spectra to retention time (A) and a chromatogram (B) showing the separation of 1000 ng of phenanthrene (a), anthracene (b), fluoranthene (c), pyrene (d), perylene (e), and coronene (f) using HPLC with UV/Visible photodiode array detector. Chromatograms (C) and (D) showing the separation of 10 ng of each compound using HPLC with fluorescence detector. Excitation and emission wavelengths used in the time program method are as follows: 251 and 400 nm for 0–16 min, 240 and 392 nm for 16–25 min, 252 and 438 nm for 25–38 min, and 301 and 444 nm after 38 min in a chromatogram (C), respectively. 251 and 365 nm for 0–16 min, 236 and 462 nm for 16–25 min, 252 and 438 nm for 25–38 min, and 301 and 444 nm after 38 min in a chromatogram (D), respectively.*

shown in Fig. 4A. Coronene in vaseline was degraded strikingly and became another unknown fluorescent compound, the emission peak of which was obtained at about 414 nm, by UV light. Anthracene was also photodegraded. The other four compounds, phenanthrene, pyrene, perylene, and fluoranthene, decayed a little.

For comparison with the PAH compound without vaseline, the decay of fluorescent peak intensities of phenanthrene, anthracene, and coronene, which were degraded clearly by UV light, is shown in Fig. 4*B*. From the comparison between Fig. 4A and B, the addition of vaseline promoted the photodegradation of coronene and

decreased oppositely the decay of phenanthrene and anthracene. There is no difference in the decay of pyrene, perylene, and fluoranthene with and without vaseline, the degradation of which was observed a little.

The result of the experiment of sublimation of the PAH compounds alone at 60°C after dropping the chloroform solutions in vials is shown in Fig. 5*A*. The fluorescent peak intensities of four compounds, except for perylene and coronene, detected on filter papers fixed at vial caps increased due to the gradual sublimation. Perylene and coronene were not detected from the filter paper. Consequently, the decay rate of the compound decreased in the following order: phenanthrene ~ anthracene > fluoranthene ~ pyrene > perylene ~ coronene. The result of the experiment of sublimation of the PAH compounds with vaseline at 60°C after ap-

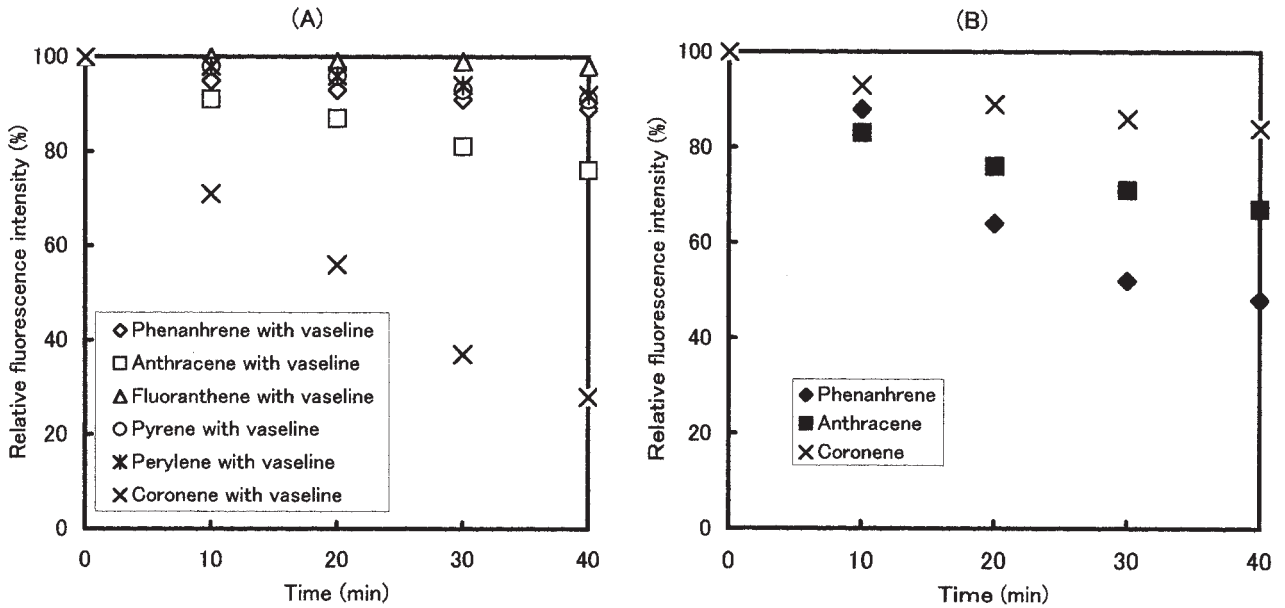


FIG. 4—*(A): The degradation of the fluorescence peak intensity of the PAH compound with vaseline under UV light at 30°C. The excitation and emission wavelength of each compound measured: 254 and 366 nm for phenanthrene, 365 and 402 nm for anthracene, 365 and 463 nm for fluoranthene, 254 and 385 nm for pyrene, 365 and 443 nm for perylene, and 300 and 446 nm for coronene. (B): That of the PAH compound alone under UV light at 30°C. The excitation and emission wavelength of each compound measured: 254 and 386 nm for phenanthrene, 365 and 446 nm for anthracene, and 300 and 499 nm for coronene.*

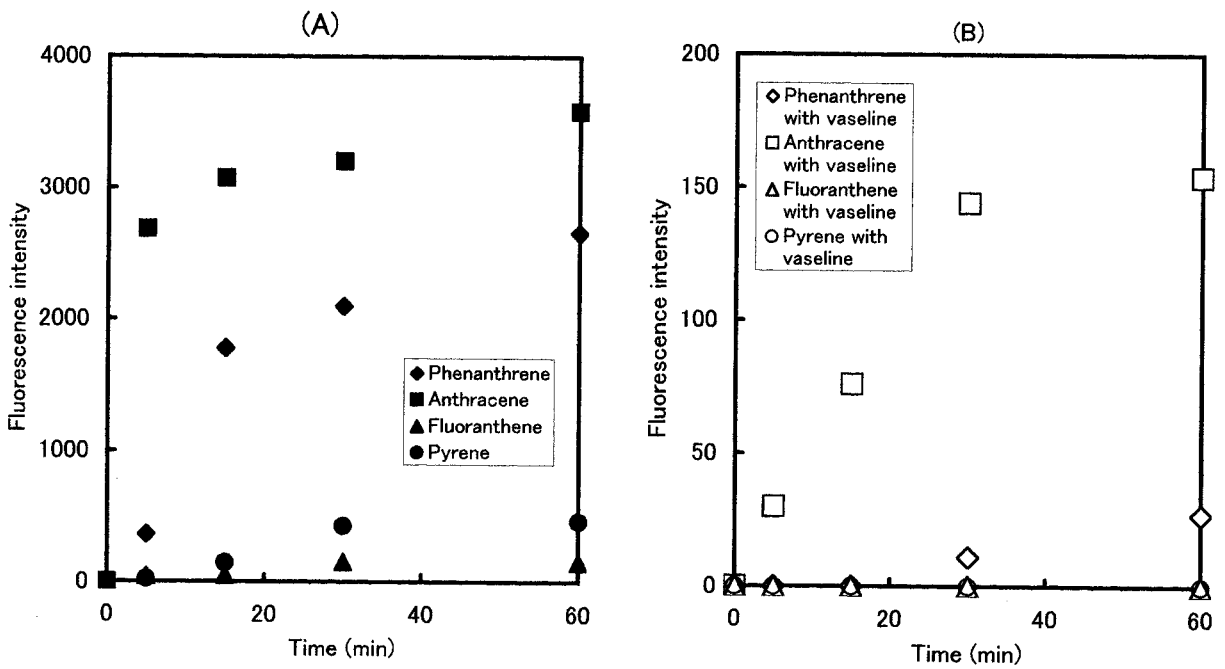


FIG. 5—*The increase of the fluorescence peak intensity of the PAH compound transferred from the bottom of the vial to the filter paper fixed on the back of the cap due to the sublimation to the passage of time at 60°C. Samples of (A) and (B) are the PAHs with and without vaseline stuck on the bottoms of the vials, respectively. The excitation and emission wavelength of each compound measured: 254 and 367 nm for phenanthrene, 254 and 384 nm for anthracene, 365 and 466 nm for fluoranthene, 254 and 394 nm for pyrene.*

plying the vaseline solutions in vials is shown in Fig. 5*B*. Only small amounts of anthracene and phenanthrene were detected from the filter paper fixed at the caps of vials. The other compounds were not detected. Accordingly, the addition of vaseline as a matrix was effective for the delay of decrease in the compounds due to prevention of the sublimation.

The results of the experiment of the solubility in water of each of the PAH compound with and without vaseline are shown in Fig. 6*A* and *B*. Phenanthrene, anthracene, pyrene, and fluoranthene were detected from both samples of water that was soaking each compound with and without vaseline. Although these compounds were slightly soluble in water, the addition of vaseline obviously prevented the solubility in water of the four PAHs. Perylene and coronene were not detected in both samples with and without vaseline.

For the long term degradation of the fluorescent compounds, the chloroform solution (1 mg) and the vaseline solution (1 mg) containing 0.1% anthracene, 1% fluoranthene, and 0.1% perylene applied on the filter paper were used as samples. Since perylene degraded most slowly of three components, the peak of the compound was selected as a reference peak for HPLC measurement. The degradation of the other components was evaluated by the ratio of the peak intensity to that of perylene. The peak intensity ratios of anthracene and fluoranthene to perylene in the samples with decay time measured by the HPLC are shown in Fig. 7*A*. The samples were stored for half a year in a refrigerator at 5°C after the samples were measured by HPLC and then applied on the filter papers. The degradation rate of the fluorescent compounds increased with decreasing molecular weight. The degradation rate of the fluorescent compounds with vaseline was much slower than that in the chloroform solution. Fig. 7*B* shows the time dependence of the peak intensity ratio of fluoranthene to perylene in the samples kept under the different storage conditions. The ratio of fluoranthene to perylene with vaseline in a refrigerator at 5°C maintained the highest peak intensity during the decay time. The ratio in water in a beaker was higher than those in the shady and sunny rooms after one month but the ratio became zero after six months. The ratio of the compounds without vaseline in water became zero after one month as shown in Fig. 7*B*. The decay rate of the PAH compounds in the sunny place was faster than that in the shady place because of photodegradation as shown in Fig. 7*B*. The degradation of the fluorescent compounds six months after application increased in the following order: in a refrigerator (5°C) $<$ in shade $<$ in water in a beaker $\sim$ in sun. Thus, the PAHs degraded due to the sublimation and the photodegradation. The components in this study were detectable for more than half a year, except for those in water. The filter paper in water was damaged and faded in six months. The decrease in most of the PAHs in water was reported to be decayed below 10% in five days due to photodegradation (5). As shown in Fig. 6*A*, the PAH compounds eluted in water in a short time could be detected easily by the fluorescence spectrophotometer. However, the PAHs with vaseline in water did not decay below 10% after one month because the PAHs in vaseline were barely soluble in water. Therefore, the addition of vaseline is advantageous to preserving the PAHs for a long period after application in both air and water. The fluorescent compounds such as the PAHs with vaseline are useful as markers under UV light, even for long periods after application.

Operational Uses and Scenarios

The fluorescence markers are the creamy mixture of several PAHs with vaseline at 1% concentration each. The 200 mL of trial products are packed in squeeze tubes (Coghlan's Ltd., Canada).

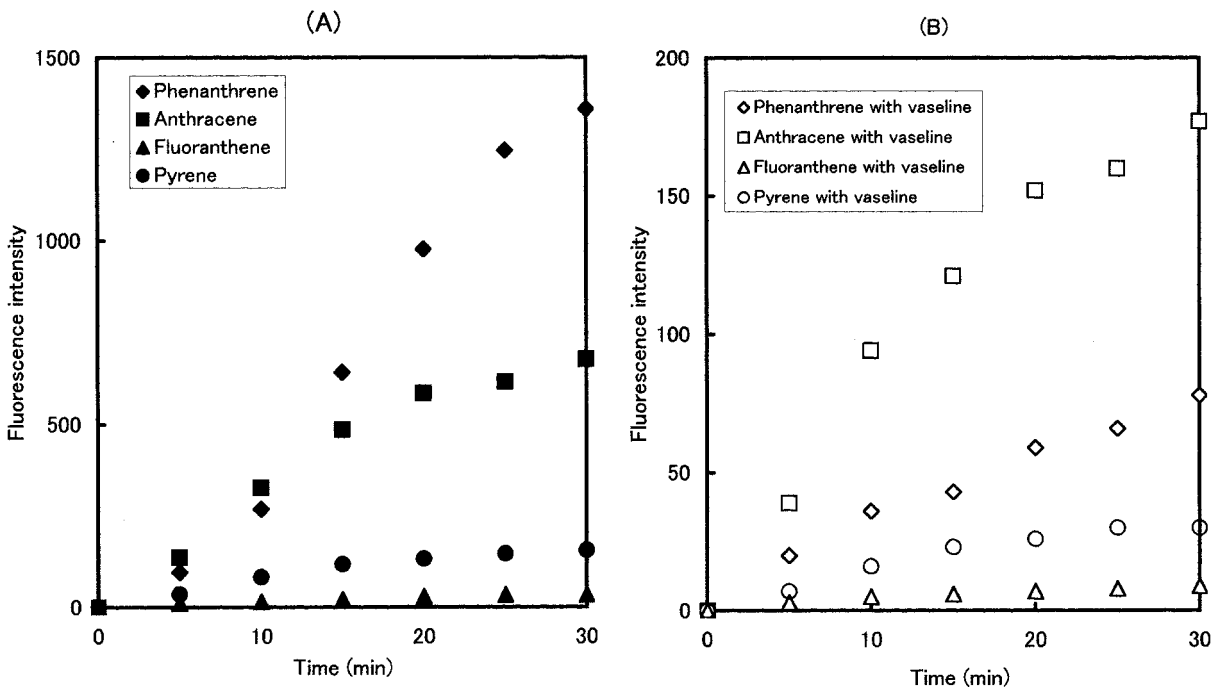


FIG. 6—*The increase of the fluorescence peak intensity of the PAH compound eluted from the slide to 3 mL of water in a cell due to the solubility to the passage of time. Samples of (A) and (B) are the PAHs with and without vaseline stuck on the slides, respectively. The excitation and emission wavelength of each compound measured: 254 and 365 nm for phenanthrene, 254 and 381 nm for anthracene, 365 and 461 nm for fluoranthene, 254 and 392 nm for pyrene.*

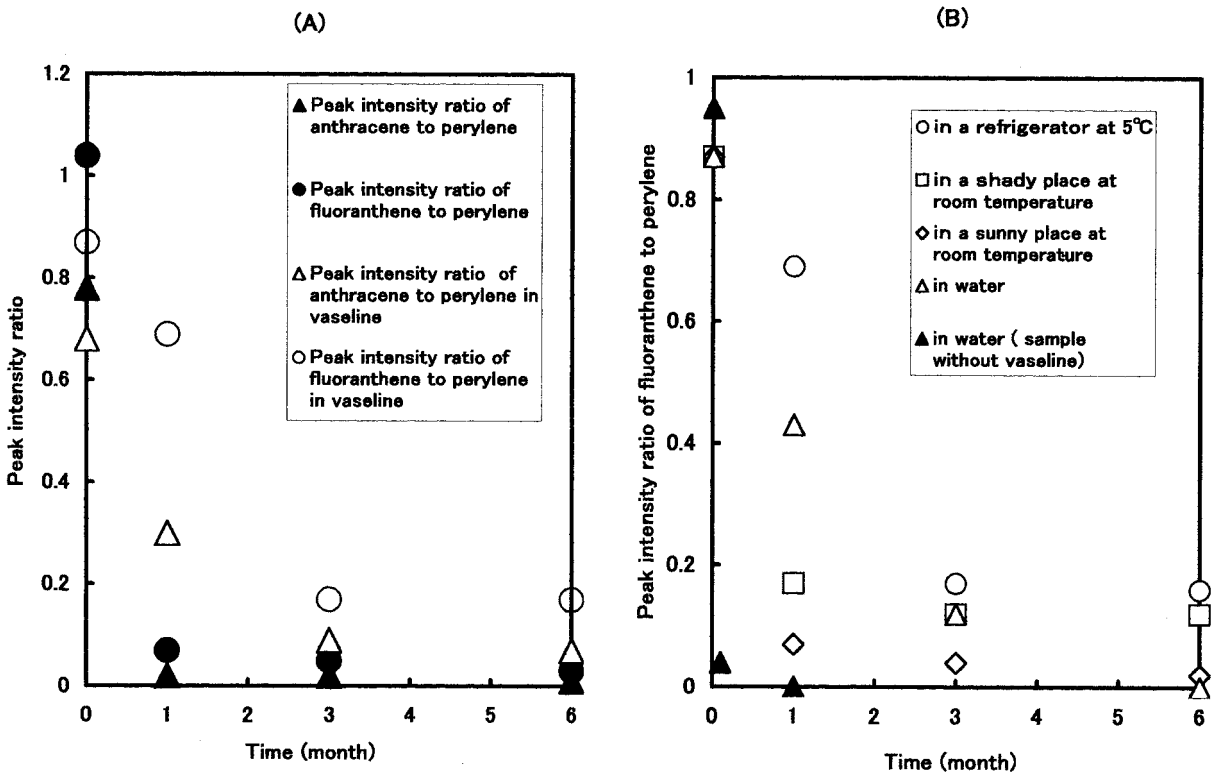


FIG. 7—*(A): The peak intensity ratio of anthracene and fluoranthene to perylene kept in refrigerator at 5°C measured by HPLC with the fluorescence detector. Samples: chloroform solution (1 mg) and vaseline (1 mg) containing 0.1% anthracene, 1% fluoranthene, and 0.1% perylene applied on the filter paper. (▲) and (●): the peak intensity ratio of anthracene and fluoranthene to perylene, respectively. (□) and (○) : the peak intensity ratio of anthracene and fluoranthene to perylene with vaseline, respectively. (B): The peak intensity ratio of fluoranthene to perylene with vaseline measured by HPLC with the fluorescence detector. Samples: vaseline (1 mg) containing 0.1% anthracene, 1% fluoranthene, and 0.1% perylene applied on filter paper in a refrigerator at 5°C (○), in a shady place at the room temperature (□), in a sunny place at the room temperature (□), in water in beakers (□), and 0.1% anthracene, 1% fluoranthene, and 0.1% perylene without vaseline in water (▲).*

When the PAHs with vaseline are stored in a refrigerator, they are good for a few years. Vaseline carries the marker and delays the degradation of the fluorescent substances after application. If fingers touch objects thinly spread or sprayed in a solvent such as ethanol by the marker, they do not feel the marker or any slipperiness. The markers used outdoors probably remain identifiable for a few months after being applied, as shown in the experiment. After the marked object is touched, fingers will consecutively transfer the marker at least several times. Once the marker adheres to a porous surface like paper or cardboard, only part of it can be wiped off. Excessive and unnecessary marker can be wiped off with detergents and organic solvents.

The following are the three cases where the markers have been used successfully in actual operations. In Case 1 of the operational uses, a marker was squeezed out from a tube and then applied by hand on gravel in front of the house of thieves as a tracer. After the marker was stepped on by the thieves, it was carried to the crime scene on their shoes. The marker was searched for using a UV lamp. The marked bath mats or tissues used for wiping the marker off of floor boards were taken as samples for laboratory analyses such as HPLC. The identification result of the marker was used as crime evidence. Case 2: the marker was injected into extremely thin rubber tubes (about 2 mm i.d.), which were then cut into short 5 mm tubes. The tubes were scattered in front of the thief's house entrance where he stepped on them. The marker was transferred from the tubes to his shoes and was detected at the scene by using a UV lamp. The markers on a mat and his shoes, taken as samples, were confirmed by HPLC in the laboratory. This result became a piece of evidence for the support of his crime. In Case 3, the marker was used in order to apprehend a criminal who rummaged habitually through office desks at midnight. The ethanol solution of the marker was sprayed on the drawers of the desks and the surrounding floors. The suspect went out from the office immediately after the crime. The marker could be detected on his shoes and hands under ultraviolet light. The markers taken as samples were identified by HPLC in a laboratory. The use of the marker succeeded in proving his crime. Consequently, it was proved that the fluorescence marker can be used in various cases.

Conclusions

Perylene and fluoranthene with vaseline emitted a strong pale light under UV light at about 365 nm. Perylene and fluoranthene of the six PAHs with vaseline were readily visible by observation as the fluorescent substances under UV light. All six PAHs were readily detected without interference of vaseline as a matrix by HPLC with a fluorescence detector. The detection limits were much less than those by observation under UV light. Each fluorescent component after application had a different rate of degradation. However, the addition of vaseline to the components decreased the decay of the components due to sublimation and photodegradation. The time on the applied substances was significantly lengthened compared with the components alone. Even if the fluorescent components were diluted to a concentration of 1% with vaseline, they emitted

fluorescence under UV light. Several markers can be manufactured by the combination of the components. The PAHs with vaseline in a refrigerator can be preserved for long periods. Thus, the samples containing the six PAHs with vaseline can be produced as fluorescent markers satisfactory to our purpose of use, applied to the materials for investigation, and identified. The fluorescent markers with vaseline as the carrier in this study were more effective than the fluorescent substances alone, as shown in three successful cases.

References

1. Kurata S. Discrimination of cinnabar seal inks affixed to paper-Color test and Fourier-transform infrared spectroscopy. Act Crim Japon [Japanese] 1990;56:19–29.
2. Kurata S, Hirano H. Study on fluorescent substances for development of multipurpose markers. Act Crim Japon [Japanese] 1994;60:95–100.
3. Xing J, Wu CY, Li T, Zhong ZL, Chen YY. Separation of aromatic isomers by capillary gas chromatography with two calix[4]arene polysiloxane stationary phase. Anal Sci 1999;15:785–9.
4. Lee WS, Chang-Chien GP. All-hydrocarbon liquid crystalline polysiloxanepolymer as stationary phase in gas chromatography capillary column for separation of isomeric compounds of polynuclear aromatic hydrocarbons. Anal Chem 1998;70:4094–9
5. Kenmotsu K. Multiresidue determination of polycyclic aromatic hydrocarbons (PAHs) in environmental samples. J Resource and Environ [Japanese] 1998;34:1361–9
6. Miege C, Bouzige M, Nicol S, Dugay J, PichonV, Hennion MC. Selectiveimmunoclean-up followed by liquid or gas chromatography for the monitoring of polycyclic aromatic hydrocarbons in urban waste water and sewage sludges used for soil amendment. J Chromatogr. A 1999;859: 29–39.
7. Müller J, Rohbock E. Method for measurement of polycyclic aromatic hydrocarbons in particulate matter in ambient air. Talanta 1980;27:673–5.
8. Johnson E, Abu-Shumays A, Abbot SR. Use of fluorescence detection in high-performance liquid chromatography. J Chromatogr 1977;134: 107–19.
9. Kasiske D, Klinkmüller KD, Sonneborn M. Application of high-performance liquid chromatography to water pollution analysis. J Chromatogr 1978;149:703–10.
10. Brouwer ER, Hermans ANJ, Lingeman H, Brinkman UAT. Determination of polycyclic aromatic hydrocarbons in surface water by column liquid chromatography with fluorescence detection, using on-line micelle-mediated sample preparation. J Chromatogr A 1994;669:45–57.
11. Wise SA, Sander LC, May WE. Determination of polycyclic aromatic hydrocarbons by liquid chromatography. J Chromatogr 1993;642:329– 49.
12. Pino V, Ayala JH, Afonso AM, Gonzalez V. Determination of polycyclic aromatic hydrocarbons in marine sediments by high-performance liquid chromatography after microwave-assisted extraction with micellar media. J Chromatogr A 2000;869:515–22.
13. Jinno K, Ohta H, Hirata Y, Sasaki S, Abe H. Microcolumn liquid chromatography combined with computer-assisted retention prediction system for polycyclic aromativ hydrocarbons in extract from diesel particulate matter. Anal Lett 1984;17(A10):905–13.
14. Pensado L, Casais C, Mejuto C, Cela R. Optimization of the extraction of polycyclic aromatic hydrocarbons from wood samples by the use of microwave energy. J Chromatogr A 2000;869:505–13.

Additional information and reprint requests:
Shoji Kurata
Criminal Investigation Laboratory
Metropolitan Police Department
2-1-1, Kasumigaseki
Chiyoda-ku Tokyo 100-8929, Japan