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Note

Stability of benzo(*a*)pyrene on silica gel plates for high-performance thin-layer chromatography

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Since the introduction of high-performance thin-layer chromatography (HPTLC)[1], new possibilities for the application of TLC analysis have become available. The principle advantages of the new technique are better separations in shorter times and the fact that nanolitre spotting volumes, resulting in very small spots, enable larger numbers of spots to be applied on HPTLC plates. Thus, the ratio of the number of test spots to the number of standard spots can be increased considerably. These features, coupled with the use of analyzers for direct scanning of the developed plates, give HPTLC a great advantage over "classical" TLC. Unfortunately, the only coating material so far available with pre-coated HPTLC plates is silica gel. Thus, at the moment only those classical TLC separations initially developed for silica gel supports can be considered for HPTLC. A separation in which the advantages of HPTLC would be of particular interest is that of polycyclic aromatic hydrocarbons (PAH), the identification and determination of which in particulate matter, water, soil and other environmental samples has to be carried out routinely in many laboratories.

Despite the fact that several TLC methods for the separation of PAH on silica gel have been described[2–7], the results must be regarded with some reserve. As early as 1964, Inscoe[8] pointed out that some PAH are unstable when applied and run on silica gel plates. However, no quantitative evaluation of his results was given and the experiments had been carried out with very large amounts (about 10 μg) per spot. Recently, Rao and Vohra[7] also reported on the rapid fading of benzo(*a*)pyrene spots on silica gel plates and concluded that any *in situ* evaluation is impossible.

Benzo(*a*)pyrene (B*a*P), which often serves as an indicator of the total amount of carcinogenic PAH, was selected for the present investigation of the stability of such compounds on HPTLC silica gel plates and the possibility of their *in situ* evaluation.

EXPERIMENTAL

Materials

Solutions of 6–200 μg/ml of benzo(*a*)pyrene in cyclohexane were used. *n*-Hexane and methanol were pro analysi grade reagents. The silica gel plates used were "HPTLC Fertigplatten Kieselgel 60 für die Nano-DC", 10 × 10 cm (Merck, Darm-

stadt, G.F.R.), and were either untreated or impregnated with 5% of paraffin wax in light petroleum (b.p. 60–80°).

Equipment

An EVA Chrom-Applicator was obtained from W + W Electronic Scientific Instruments (Basle, Switzerland). A platinum–iridium capillary[1] of capacity 185 nl was used. A Fluotest UV visualizer was obtained from Quartzlampen GmbH (Hanau, G.F.R.), a Chromatogramm-Spektralphotometer KM 3 from Carl Zeiss (Oberkochen, G.F.R.) and a Servogor RE 647 recorder from Metrawatt (Nürnberg, G.F.R.).

Thin-layer chromatography

Aliquots (185 nl) of different B*a*P concentration equivalent to between 1.1 and 37 ng of B*a*P per spot were applied on untreated and paraffin wax-impregnated plates at a distance of 15 mm apart and 7 mm from the bottom of the plates. Untreated plates were developed with *n*-hexane and impregnated plates with methanol. In both instances the running distance was 6 cm. After development, each plate was viewed briefly (5 sec) under long-wavelength UV light (360 nm) while still wet in order to mark the positions of the spots. The plates were dried in a cold air stream and the fluorescent intensity of the spots was measured by repeated scanning of the plate at right-angles to the direction of development. For quantitative evaluation, the peak heights were measured.

In order to minimize the UV radiation falling on the B*a*P spots during the different scans, the scanner was used in the monochromator–sample mode[9]. A very quiet baseline was achieved by excitation with the mercury line at 296.5 nm[10]. For isolation of the fluorescent radiation, an FL 39 filter was used, which passes radiation of wavelength 390 nm and above to the extent of at least 65%.

RESULTS AND DISCUSSION

The most satisfactory procedure for determining possible time-dependent decomposition of B*a*P on a silica gel plate is by means of repeated discrete scans over a long period of time. During these scans, the unavoidable successive exposures to UV light effectively simulate the exposures that would have taken place if the plate had been subjected to more prolonged initial inspection during UV localization of the spots.

Fig. 1 shows the changes in fluorescent intensity recorded in successive scans of different spots from different plates, expressed as the percentage change in intensity relative to the first scan (0%). The time for each scan was about 100 sec. Curve 1 is typical for all spots on plate A. For an amount of 1.5 ng of B*a*P spotted, the readings remained constant for more than 10 min, then abruptly decreased to about one tenth of that value, for no obvious reason. The shape of this curve implies the possibility of determining B*a*P exactly within the first 10 min after the run. Unfortunately, this state of affairs did not hold for all of the plates examined. For other plates from the same batch, the decrease in fluorescent intensity did not occur with the same time period. There were even plates with variations from one track to another. The most critical situation was encountered with one track on plate B where the scan (curve 2 in Fig. 1) indicates a loss of up to 10% during the first 100 sec.

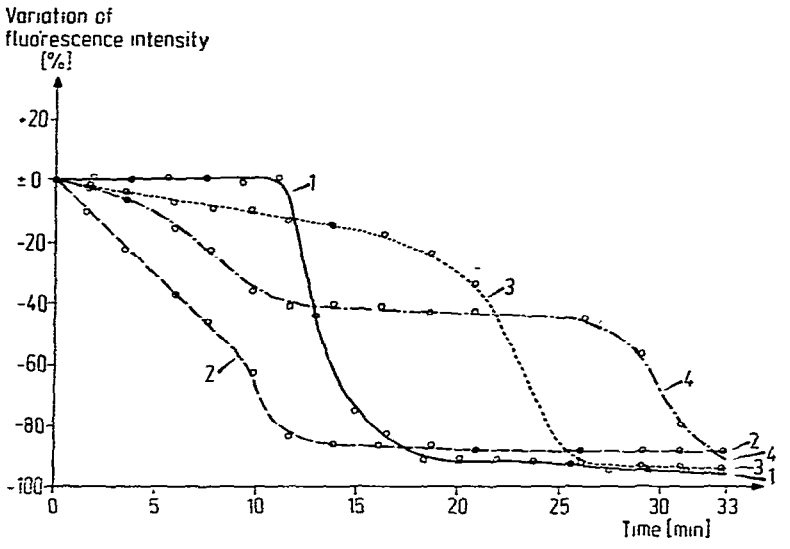

Fig. 1. Stability of benzo(*a*)pyrene on untreated HPTLC silica gel plates. Curve 1, 1.5 ng of B*a*P per spot (plate A); curve 2, 3 ng of B*a*P per spot (plate B); curves 3 and 4, 30 ng of B*a*P per spot (plate C).

As is shown by curves 3 and 4 in Fig. 1, the shape of a curve apparently does not depend on the amount of B*a*P applied. However, it is interesting to note that all of the curves exhibit the same horizontal range at about 10% of the initial amount of B*a*P.

So far, no explanation can be offered for the differences in the rate of decrease in the intensity of the B*a*P spots. Supposing, however, that the activated sites on a silica gel plate possess different available free energy contents, then a possible explanation could be that different amounts of energy (*i.e.*, different times of exposure to UV light) would be needed for each site to induce the photochemical modification of the B*a*P molecule and the resulting decrease in fluorescent intensity. If this hypothesis is accepted, it would appear that a decrease in the energy content of the activated sites of a silica gel plate should result in the ability of the plate to accept much more energy (*i.e.*, exposure to light) before any decay of the B*a*P molecule takes place. Thus, B*a*P should be more stable on such "deactivated" plates.

Deactivation of the silica gel layer is possible by covering the surface of the layer with an inert material, *e.g.*, by impregnating it with paraffin wax. The resulting paraffin wax-coated silica gel layer behaves like a support for reversed-phase chromatography. According to this, the developer must be polar. Methanol was chosen as it leads to approximately the same R_F value for B*a*P as does *n*-hexane with the untreated plates. Further experiments to find a developer capable of separating several PAH on impregnated HPTLC plates are in progress.

For a detailed examination of the characteristics of paraffin-impregnated HPTLC plates, the same experiments were performed as for the untreated plates. The results of the investigation of different tracks on four plates (A, B, C and D) are presented in Fig. 2. It can be seen that for amounts of B*a*P between 1.1 and 37 ng per spot, there is no significant decrease in fluorescent intensity. Even after 15 min, the

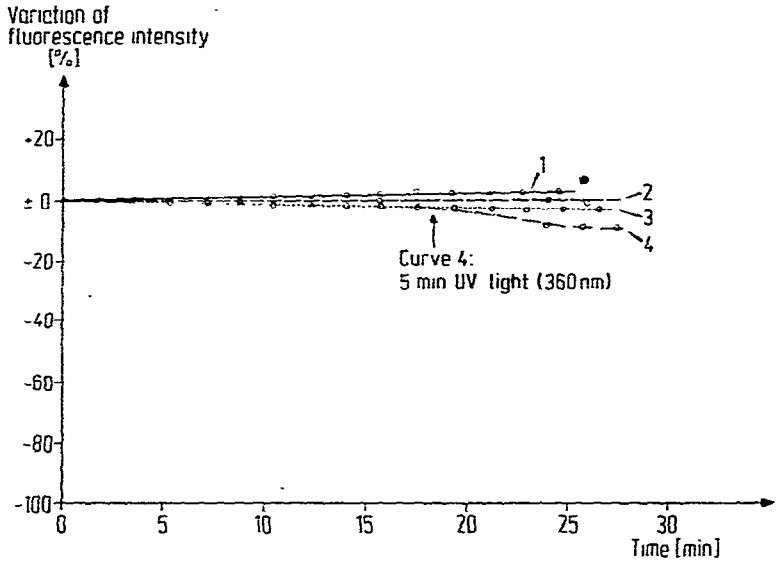

Fig. 2. Stability of benzo(*a*)pyrene on paraffin-impregnated HPTLC silica gel plates. Curve 1, 37 ng of B*a*P per spot (plate A); curve 2, 1.9 ng of B*a*P per spot (plate B); curve 3, 1.5 ng of B*a*P per spot (plate C); curve 4, 1.1 ng of B*a*P per spot (plate D).

changes do not exceed 1.5% and increase only by about 3% after 30 min. As the curves in Fig. 2 were obtained with repeated scans, *i.e.*, repeated exposure to UV light, the error for a single scan about 15 min after the development of a plate will certainly be less than 1.5%.

Even under extremely unfavourable conditions, simulated by exposing one plate to 360-nm radiation for 5 min, the decrease in fluorescent intensity did not exceed 10% for a spot containing 1.1 ng (*cf.*, curve 4 in Fig. 2).

The slight increase in fluorescent intensity that was observed for larger amounts of B*a*P (*cf.*, curve 1 in Fig. 2) cannot be explained at the moment; possibly a secondary product with slightly stronger fluorescence is formed on the paraffin wax-impregnated silica gel plate.

CONCLUSION

The results show that nanogram amounts of B*a*P are unstable on HPTLC silica gel plates. The losses, which may reach 50% after a few minutes, prevent any *in situ* evaluation. In order to take advantage of HPTLC in PAH analysis, it is recommended that the separation be carried out with HPTLC silica gel plates impregnated with 5% paraffin wax in light petroleum. This pre-treatment produces plates on which losses of B*a*P by photochemical reactions are considerably reduced, thus permitting a correct *in situ* determination of B*a*P. The paraffin wax-impregnated HPTLC silica gel plates should also be suitable for HPTLC separations of other unstable compounds, *e.g.*, aflatoxins.

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