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Note

Detection and characterization of nitro derivatives of some polycyclic aromatic hydrocarbons by fluorescence quenching after thin-layer chromatography: application to air pollution analysis

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Polycyclic aromatic hydrocarbons (PAHs) emitted into the atmosphere are rapidly degraded by photo-oxidation, by reaction with atmospheric oxidants and by reactions with other chemical pollutants. So far we have studied under laboratory conditions possible reactions of PAHs adsorbed on solid carriers with sulphur dioxide[1,2] and free radicals[3]. PAHs can also react with nitrogen oxides (NO_x) or nitric acid, which are present in polluted air in relatively high concentrations. NO_x or dilute nitric acid can either add to, substitute or oxidize PAHs. Research on these possible reactions is being carried out at present. To follow the probable formation of nitro derivatives of PAHs, we had to develop an analytical method for their detection and characterization. For this purpose, the fluorescence quenching technique on a thin-layer plate was employed. The utilization of this technique in air pollution analysis was first proposed by Sawicki and co-workers[4–6].

EXPERIMENTAL

All nitro derivatives of PAHs were synthesized by known procedures by direct nitration with concentrated nitric acid.

For thin-layer chromatography (TLC), silica gel coated aluminium foil (Silufol) and cellulose plates (Lucefol) (Kavalier, Votice, Czechoslovakia) were used, with cyclohexane–chloroform (1:1) and N,N-dimethylformamide–water (2:3), respectively, as developing solvents. The developed plates were first sprayed with reducing reagent and the resulting fluorescent spots examined under UV light. After drying, the plates were either sprayed or fumed with quenching reagents and the changes in the fluorescence of the spots were observed under a 365-nm light source.

The following reagents were employed: reducing reagent [0.1 g of potassium borohydride was dissolved in a mixture of 15 ml of methanol, 3 ml of water and 1 ml of 0.05 % copper(II) chloride solution], which must be freshly prepared directly before each detection; aniline (20 % methanolic solution); phenylhydrazine (20 % methanolic solution); and carbon disulphide (fumes were used).

RESULTS AND DISCUSSION

The R_F values obtained in the separation by silica gel and cellulose TLC,

together with the fluorescence properties and detection limits of seven nitro derivatives of PAHs, are presented in Table I. No nitro derivatives of PAHs have natural fluorescence and, in order to detect them on a plate they were reduced *in situ* with potassium borohydride solution to polycyclic amines, which are intensely fluorescent. All of the amines remained fluorescent after spraying with aniline and phenylhydrazine, except 1-aminonaphthalene, which was quenched with phenylhydrazine. Carbon disulphide quenched the fluorescence of 1-aminonaphthalene,9-aminoanthracene and 6-aminobenzo[a]pyrene.

TABLE I

CHARACTERIZATION TESTS AND R_F VALUES FOR POLYNUCLEAR AROMATIC NITRO DERIVATIVES ON THIN-LAYER PLATES ·

Compound	R_F value		Fluorescence colour[*]				Detection limit (ng)
	Silufol plate	Lucefol plate	KBH₄	Aniline	Phenylhydrazine	CS₂	
1-Nitronaphthalene	0.59	0.91	B	B	Q	Q	500
9-Nitroanthracene	0.66	0.80	Y	Y	Y	Q	50
6-Nitrochrysene	0.58	0.53	B	B	B	1B	100
3-Nitrofluoranthene	0.53	0.71	Y	Y	Y	G	1
1-Nitropyrene	0.52	0.64	B	B	B	B	1
6-Nitrobenzo[a]pyrene	0.61	0.44	G	G	G	Q	5
1,6-Dinitropyrene	0.27	0.52	B	B	B	B	1

[*] B = blue; Y = yellow; G = green; lB = light blue; Q = quenched.

Table II compares the R_F values and the fluorescence properties of polynuclear carbazoles, polynuclear aza heterocyclic compounds, polycyclic aromatic hydrocarbons and some compounds that contain other functional groups. Silica gel plates with cyclohexane–chloroform as the developing solvent are the best for the general separation of nitro derivatives from other compounds present in atmospheric samples. In this chromatographic system, the PAHs are found close to the solvent front, the aza compounds at the origin and the carbazoles in the R_F range 0.1–0.4. Except for dinitropyrene, the R_F values of nitro derivatives of PAHs are in the range 0.5–0.7.

With cellulose plates and dimethylformamide–water as the developing solvent, the nitro derivatives are more efficiently separated from each other, with R_F values ranging from 0.44 for 6-nitrobenzo[a]pyrene to 0.91 for 1-nitronaphthalene. The effects of two different quenching reagents (aniline and phenylhydrazine) on the fluorescence of these compounds were investigated. All PAHs, benzanthrone, 1-acetylpyrene and polynuclear sulphonic acids were quenched by aniline; most of the carbazoles and aza compounds were not quenched. Only dibenzo[c,g]carbazole, benzo[b]acridine and 1-azapyrene remain weakly fluorescent after treatment with phenylhydrazine.

The fluorescence resulting from the reduction of nitro derivatives of PAHs to their amino derivatives on thin-layer chromatograms and their subsequent treatment with the quenching reagents are of practical value in the detection and characterization of trace amounts of these compounds in very complicated mixtures, such as extracts of airborne particulates.

TABLE II

R_F VALUES AND FLUORESCENT COLOURS OF SOME CARBAZOLES, POLYNUCLEAR AZA HETEROCYCLIC COMPOUNDS AND POLYCYCLIC AROMATIC HYDROCARBONS ON THIN-LAYER PLATES UNDER DIFFERENT CONDITIONS

Compound	R_F value		Fluorescence colour*				
	Silufol plate	Lucefol plate	Untreated		KBH₄	Aniline	Phenyl-hydrazine
			Silufol plate	Lucefol plate			
Carbazole	0.39	0.87	V	V	V	V	Q
Benzo[a]carbazole	0.23	0.85	V	V	V	V	Q
Dibenzo[b,g]carbazole	0.20	0.75	V	V	IB	BV	Q
Dibenzo[c,g]carbazole	0.17	0.72	V	V	V	V	IV
Dibenzo[a,i]carbazole	0.21	0.75	V	V	V	V	Q
1-Azacarbazole	0.00	0.71	B	V	B	Q	Q
2-Azacarbazole	0.00	0.17	B	B	B	Q	Q
Benzo[a]acridine	0.00	0.51	B	B	B	B	Q
Dibenzo[a,j]acridine	0.00	0.35	BV	V	BV	BV	Q
Benzo[b]acridine	0.00	0.73	YG	V	B	B	IB
Dibenzo[a,h]phenazine	0.33	0.00	B	Y	Y	Y	Q
1-Azafluoranthene	0.00	0.82	O	YG	O	Q	Q
1-Azapyrene	0.00	0.77	YG	IB	YG	B	IB
Pyrene	0.91	0.87	B	B	B	Q	Q
Fluoranthene	0.88	0.84	B	IB	B	Q	Q
Perylene	0.87	0.67	B	B	O	Q	Q
Benzo[g,h,i]perylene	0.86	0.57	V	V	Y	Q	Q
Anthanthrene	0.89	0.51	V	V	V	Q	Q
Benzo[a]pyrene	0.88	0.65	V	V	V	Q	Q
Benzo[k]fluoranthene	0.88	0.65	V	V	V	Q	Q
Coronene	0.87	0.45	V	V	Y	Q	Q
Benzanthrone	0.19	0.87	GY	GY	G	Q	Q
1-Acetylpyrene	0.22	0.86	B	B	B	Q	Q
1-Aminopyrene	0.00	0.85	B	B	B	B	B
1-Pyrenesulphonic acid	0.00	1.00	V	V	V	Q	Q
6-Benzo[a]pyrenesulphonic acid	0.00	1.00	V	V	V	Q	Q

* B = blue; Y = yellow; G = green; V = violet; O = orange; IB = light blue; IV = light violet; Q = quenched.

Analysis of atmospheric samples

A 0.1017-g amount of the dichloromethylene extract of airborne particulates collected on glass-fibre filters from the Prague atmosphere was pre-chromatographed on a silica gel column, and the benzene fraction was further separated by TLC. After reduction and spraying with aniline and phenylhydrazine, there only three spots remained fluorescent on the plate. On fuming with carbon disulphide, the yellow fluorescence colour of one spot changed to green, and the green and light-blue fluorescences of the other two spots were quenched. On the basis of both the R_F values on the thin-layer plates and the fluorescence quenching effects, two of the three unknown substances were characterized as 3-nitrofluoranthene and 6-nitrobenzo[a]pyrene.

REFERENCES

1 J. Jäger, *Cesk. Hyg.*, 18 (1973) 59.
2 J. Jäger and M. Raković, *J. Hyg. Epidemiol. Microbiol. Immunol.*, 18 (1974) 137.
3 J. Jäger, *Cesk. Hyg.*, 22 (1977) 11.
4 E. Sawicki, T. W. Stanley and W. C. Elbert, *Talanta*, 11 (1964) 1433.
5 E. Sawicki, T. W. Stanley and H. Johnson, *Mikrochim. Acta*, (1965) 178.
6 E. Sawicki, H. Johnson and K. Kosinski, *Microchem. J.*, 10 (1966) 72.