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Journal of Chromatography A, 793 (1998) 403–408

JOURNAL OF
CHROMATOGRAPHY A

Short communication

Thin-layer chromatographic separation of selected polyaromatic hydrocarbons on copper phthalocyanine dye impregnated plates

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Received 9 April 1997; received in revised form 3 September 1997; accepted 3 September 1997

Abstract

The separation of anthracene, phenanthrene, pyrene, benzo[*ghi*]perylene, triphenylene, perylene and fluoranthene on aluminium oxide thin-layer chromatography plates, coated with hydrolyzed copper phthalocyanine dye Ostazin turquoise V-G, has been studied. The elution order reflects planarity of the molecules or carcinogenicity. Based on the measurement of the adsorption isotherms of the hydrolyzed copper phthalocyanine dye, it has also been shown that different aluminium oxide surfaces have different affinities, a phenomenon important for the preparation of the thin-layer chromatography plates. © 1998 Elsevier Science B.V.

Keywords: Stationary phases, TLC; Polynuclear aromatic hydrocarbons

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) in the environment, biota, foods and food chains are of considerable interest to analytical chemists and toxicologists because of their mutagenic and carcinogenic properties. Since the concentration of PAHs in environmental samples is very low, a preconcentration step is usually necessary, using either classical sorbents or special, more selective ones. Efforts have been made to find more selective adsorbents using the fact that PAHs as π -electron donors form electron donor–acceptor (EDA) complexes with various electron attracting agents. Trinitrobenzene was first used as an EDA forming compound in thin-layer chromatography (TLC) for the separation of PAHs [1]. Using thin-layer plates impregnated with picric

acid, styphnic acid, 2,4,7-trinitrofluorenone, caffeine, urea, dimethylformamide and silver nitrate, a method for better separation of PAHs was developed [2–6]. Other authors [7] have also stated that PAHs and some naturally-occurring acetylenic substances are less decomposed on the thin-layer plates impregnated with caffeine due to interactions. For the separation of PAHs in TLC some compounds having solubility properties and forming EDA complexes, e.g. N-methylated cyclic ureides, purine derivatives and bile acid salts were added to the mobile phase [8]. In addition to the above mentioned compounds the TLC plates were also impregnated with pyromellitic dianhydride [5,9], chloranil, tetracyanoethylene and desoxycholate sodium [5] and 1,5,6,8-naphthalene tetracarboxylic acid dianhydride [9] for the chromatography of PAHs.

Recently, a very efficient method for selective adsorption of compounds with a planar structure

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consisting of at least three fused rings from aqueous solution has been found, based on copper phthalocyanine trisulphonate covalently linked to cellulose which is named 'blue cotton' after its colour [10]. This selective adsorption can be easily destroyed by a methanol–ammonia solution. Only a few PAHs, e.g. benzo[*a*]pyrene, have been studied for their ability to form complexes useful for their preconcentration and/or further separation or for their ability to be extracted from the aqueous solution on the 'blue cotton' [10]. Another sorbent, 'blue pearls', based on polymethacrylamide with covalently linked copper phthalocyanine trisulphonate, has been tested for the adsorption and recovery of benzo[*a*]pyrene and fluoranthene [11]. Fluoranthene, pyrene, chrysene, benzo[*a*]pyrene, triphenylene, 2,3-benzofluorene and *p*-terphenyl were almost completely adsorbed from their aqueous solutions on precipitated barium salt of copper sulphophthalocyanine and cobalt phthalocyanine [12]. Recoveries of these PAHs by thermal desorption gas chromatography were in the range of 71–95%.

Recent results have shown the potential usefulness of silica gel loaded with covalently bound copper phthalocyanine derivative for the separation of naphthalene, anthracene and its alkyl derivatives, phenanthrene, *o*-terphenyl and triphenylene in HPLC [10] and porous glass loaded with covalently bound copper phthalocyanine trisulphonic acid derivative [13]. Separation of several aromatic pairs, i.e. triphenylene/*o*-terphenyl, perylene/ α,α' -binaphthyl and chrysene/pyrene have been studied on a HPLC column packed with different, covalently bound metalloporphyrins [16]. No data are available for the separation of PAHs in TLC using the interaction with the derivative of copper phthalocyanine and the main goal of this paper was to investigate this separation.

2. Experimental

2.1. Materials

Reactive copper phthalocyanine dye (Ostazin turquoise V-G: C.I. Reactive Blue 21) was purchased from Spolek pro chemickou a hutnří výrobu (Ústř nad Labem, Czech Republic), methanol from Lachema (Brno, Czech Republic), hexane from Merck (Darm-

stadt, Germany). PAHs were obtained from Fluka (Buchs, Switzerland) with the exception of fluoranthene which was obtained from Aldrich (Milwaukee, WI, USA). Thin-layer ready-made foils Alufol based on neutral aluminium oxide bound with starch on aluminium foil were obtained from glassworks Kavalier (Votice, Czech Republic). From the same source we obtained ready-made foils Silufol based on silica gel and containing starch as a binder and ready-made foils Lucefol based on cellulose without any binder. TLC plates with aluminium oxide 60 F₂₅₄, layer thickness 0.2 mm, 20×20 cm were obtained from Merck. Pre-coated TLC plates with aluminium oxide ALOX-25, layer thickness 0.25 mm, without fluorescent indicator were obtained from Macherey–Nagel (Düren, Germany). Aluminium oxide for chromatography, type 507C neutral, 100–125 mesh, was obtained from Fluka.

2.2. Modification of the dye by hydrolysis

A 2.4-g amount of Ostazin turquoise V-G (C.I. Reactive Blue 21) was dissolved in 150 ml of 5% (w/w) solution of sodium carbonate and the reaction mixture was kept at constant temperature 70°C and constant volume for 6 h in order to hydrolyze the reactive group of the dye. The reaction mixture was occasionally stirred. After that time water was allowed to evaporate slowly. After evaporation of almost all the water, the remaining viscous dye solution was withdrawn from the beaker and dried at elevated temperature. The yield of hydrolyzed dye (HPh) was 1.48 g.

2.3. Determination of sorption capacity of the sorbents

For sorption capacity experiments neutral aluminium oxide (Fluka) and aluminium oxide scratched directly from the Alufol plate, both activated at 120°C for 2 h, were used. Aluminium oxide (10 mg) was suspended in 2 ml of water or methanol solution of HPh and the mixture was shaken at the ambient temperature for 4 h. The total amounts of HPh were 25, 50, 100, 150, 200, 300, 400, 500 and 600 μg in 2 ml of water and 5, 25, 50, 100, 150, 200, 300, 400, 500, 600 and 800 μg in 2 ml of methanol. The concentration of the free (unbound) dye in the

supernatant (C_{eq}), calculated to the unit mass, was determined from the calibration curve and the amount of the dye bound to the unit mass of the sorbent (q_{eq}), was calculated from the difference. The absorbance of supernatant was measured on a Varian DMS 300 (Varian Techtron, Mulgrave, Victoria, Australia) double beam spectrophotometer.

2.4. Thin-layer chromatography

TLC of PAHs was performed on the Alufol chromatographic plate developed in a glass beaker covered with a glass plate and saturated with the solvent. A 0.5- μg amount of each PAH was applied to the spot, but 0.25 μg , 1 μg and 1.5 μg were used for fluoranthene, anthracene and phenanthrene, respectively. The total developing path was 10 cm and the distance of the spots was measured manually with the final illumination of the spots using a UV lamp with two wavelength 254 nm and/or 366 nm.

The plates with the hydrolyzed copper phthalocyanine (HPh) were prepared by dipping half of the plate into the solution of HPh in methanol (0.1 mg HPh/ml). The prepared plates were washed with methanol before application of the spots. The amount of HPh (0.46 $\mu\text{g cm}^{-2}$) on the blue part of the plate was estimated from the content of copper (0.0224 $\mu\text{g cm}^{-2}$), after mineralization with concentrated nitric acid and elemental analysis for copper using a PU 7450 ICP spectrometer (Pye–Unicam, UK).

3. Results and discussion

HPh dye which was only adsorbed on the aluminium oxide was used in our study unlike the procedure described earlier for the preparation of ‘blue cotton’ or ‘blue pearls’ [10,11] based mainly on covalently bound phthalocyanine dye either on cellulose or on aminopropylsilica gel particles.

The investigation of the interaction between PAHs and Ostazin turquoise V-G or HPh could be carried out in two ways. Originally we studied the chromatographic separation of the mixture of HPh with the selected PAHs in methanolic solution, applied on the different plates and developed in different solvent systems. We used silica gel plates Silufol, aluminium oxide plates Alufol and plates with cellulose Lucefol.

We expected the spots of the complexes could be seen, but they were not visible under these conditions.

When we mixed three adsorbents from Alufol, Silufol and Lucefol in three test tubes with solution of HPh, it was found that aluminium oxide had the strongest adsorption for the dyes independently of the solvent system used, because once the dye was adsorbed, it was not possible to wash it out with methanol. Therefore we started dipping or spraying half of the plate in methanolic HPh solution in order to get a better, immediate and quite clear comparison of PAH interaction (Fig. 1). The mechanism of interaction is not quite clear yet, but generally the more planar the molecule is, the stronger is the interaction. We have studied three different solvent systems. The measured R_F values are summarized in Table 1. Practically no interaction was observed in hexane after double development, while only small shifts in R_F values for benzo[ghi]perylene, triphenylene and perylene were measured.

The greatest interaction or shifts in R_F values on the plates covered with HPh were observed in

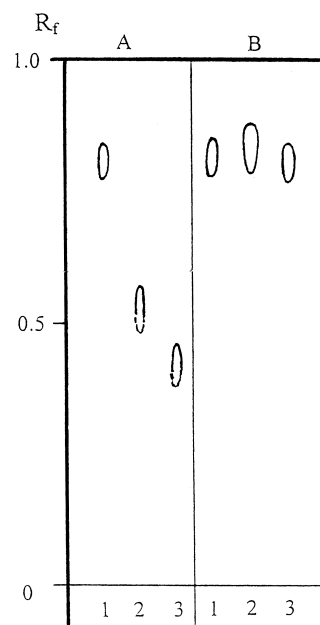


Fig. 1. TLC separation of selected PAHs: 1=anthracene, 2=perylene, 3=benzo[ghi]perylene) on Alufol plate coated with HPh (A); the second half of the plate is without any coating (B). The plate was developed in 80% methanol.

Table 1
Comparison of R_F values on Alufol aluminium oxide plates in different solvent systems with and without HPh

Compound	No. of rings	R_F					
		Methanol		80% Methanol		Hexane	
		+HPh	Plain	+HPh	Plain	+HPh	Plain
Anthracene	3	0.86	0.86	0.84	0.85	0.92	0.92
Phenanthrene	3	0.86	0.86	0.85	0.86	0.92	0.92
Pyrene	4	0.86	0.88	0.84	0.90	0.88	0.88
Triphenylene	4	0.82	0.82	0.81	0.85	0.71	0.73
Fluoranthene	4 ^a	0.92	0.94	0.88	0.92	0.85	0.85
Perylene	5	0.67	0.92	0.57	0.88	0.60	0.64
Benzo[ghi]perylene	6	0.57	0.84	0.46	0.84	0.52	0.55

^a One five carbon ring in the molecule.

methanol and even more in 80% methanol for perylene and benzo[ghi]perylene. To get a deeper insight into the interaction mechanism, we studied separation of some additional PAHs in 80% methanol (*o*- and *p*-terphenyl, 2,3-benzanthracene and picene), in which the largest shift in R_F values had been observed. For PAHs with three rings (anthracene, phenanthrene and additional *o*- and *p*-terphenyl) no shift in R_F values was observed. For PAHs with four rings (pyrene, triphenylene, fluoranthene and 2,3-benzanthracene), the planarity starts to influence the R_F values. While more linear 2,3-benzanthracene does not show any shift of R_F value, the other, more planar four rings PAHs show a difference in R_F values from 0.04 to 0.06 (see Table 1). For five ring PAHs (perylene and picene), the difference is also higher for the more planar perylene molecule; the exact shift is very difficult to measure owing to problems with solubility and detection of the above mentioned PAHs, especially picene. These findings reveal that the shift in R_F value is in accordance with the number of rings in the molecule (see Table 1) and with the planarity of the molecule which corresponds to the calculated index of 'carcinogenicity' [14] (anthracene 1.71, 2,3-benzanthracene 1.88, pyrene 4.80, picene 4.86, phenanthrene 4.89, triphenylene 4.89 and benzo[ghi]perylene 26.42, no data for *o*- and *p*-terphenyl, perylene and fluoranthene) and to the electronic density as well and with the findings of Hayatsu [10] that 'blue cotton' preferentially adsorbs mutagenic compounds with three or more rings in a

molecule. The highest shift for benzo[ghi]perylene is very interesting also from the point of view of the complex stability in methanolic solution. Similar observations were reported in [10], where the authors observed the separation of some PAHs and therefore the complexes formed must be stable, or interactions took place even in pure methanol or acetonitrile used as mobile phase.

As coverage of the plate surface with HPh increased, the strength of the interaction also increased in such way that even so far unresolved spots of anthracene, phenanthrene, fluoranthene and pyrene could be efficiently separated. But the higher the coverage of the plates with HPh is, the poorer is the detection of the spots, which is a disadvantage of the described procedure. The fluorescence of the benzo[ghi]perylene and perylene spots in methanolic solvent systems is weakest on the plates covered with HPh, which is in accordance with the strongest interaction observed. One has to be careful observing the spots under UV light after applying the PAHs on the plate or after the first development in hexane because of possible transformation of PAHs to corresponding quinones or hydroxyquinones, which could lead to false identification, especially in the case of anthracene [15].

The procedure was also tested on other plates, e.g. aluminium oxide plates Merck 60 with a fluorescent indicator and Macherey Nagel aluminium oxide plates without a fluorescent indicator. The aluminium oxide plates Merck 60 with fluorescent indicator

have R_F values for tested PAHs in the opposite order when a low concentration of HPh was applied to the plate. The R_F values were similar to the Alufol plates as the concentration of the HPh on the plate increased.

The strength of the HPh adsorption on the plates depends primarily on the nature of the surface, mostly on the acidity or basicity of aluminium oxide and most probably also on the binder used or on another organic compounds already present on the surface (a fluorescent dye). Thus comparison of different types of aluminium oxide is very useful for further evaluation of this interaction. We have studied adsorption quality of the neutral aluminium oxide Fluka and aluminium oxide scratched directly from the Alufol surface. Equilibrium sorption isotherms for aqueous and methanolic solutions of HPh and aluminium oxides as sorbents are shown in Fig. 2. Sorption isotherms represent the equilibrium distribution of the dye molecules between the aqueous or methanolic and solid phases as the HPh concentration is increased. As can be seen from Fig. 2, the adsorbed amount of HPh is higher in methanol,

but equilibrium is reached at lower concentrations of HPh in water solution. In both solvent systems the adsorbed amount of HPh is higher on Alufol. These interesting results will be the subject of further investigations.

4. Conclusions

The hydrolyzed copper phthalocyanine dye Os-tazin turquoise V-G is adsorbed readily from water or methanolic solution to the aluminium oxide surface either in the form of the plain sorbent or to the TLC plates covered with an aluminium oxide layer. Copper phthalocyanine dye on the aluminium oxide TLC plates interacts with PAHs according to their number of rings and their planarity. The separation of selected PAHs, with different structures and planarity and with different number of the rings, using this interaction was studied. The strength of interaction could be controlled by amount of dye on the plate. The method seems to be suitable for separation of PAHs prior to their further analysis.

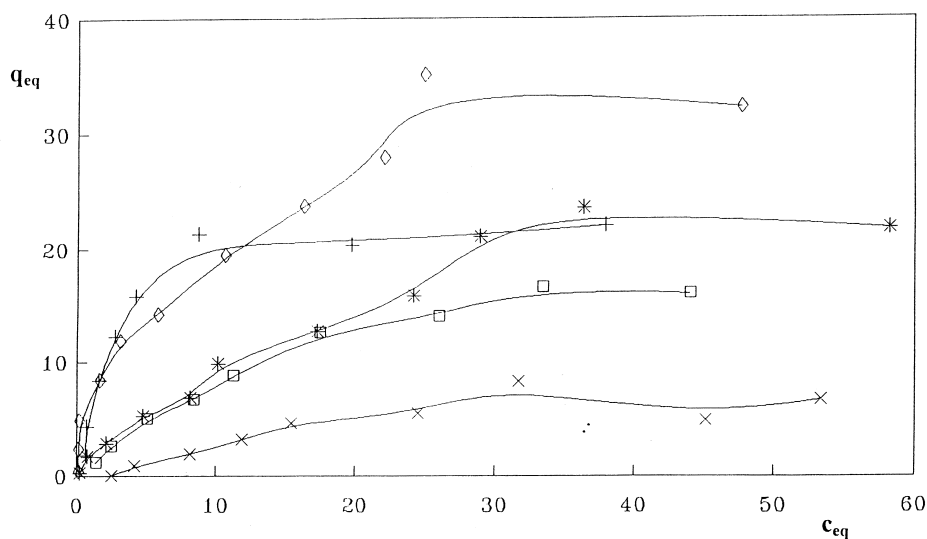


Fig. 2. Equilibrium sorption isotherms of HPh on aluminium oxide as a sorbent: C_{eq} =equilibrium liquid phase concentration of the free HPh ($\mu\text{g mg}^{-1}$), q_{eq} =equilibrium solid-phase concentration of the adsorbed dye ($\mu\text{g mg}^{-1}$). The sorbents used: aluminium oxide Fluka ($\square, *$), aluminium oxide scratched from Alufol plate ($+, \diamond$), starch (\times). The sorption isotherms were studied in water ($\square, +, \times$) and in methanol ($*, \diamond$).

Acknowledgements

The work was supported by the Grant Agency of the Czech Republic, grant No. 511/95/1060. The authors thank Associate Professor I. Čepčiansky from the Technical University Pardubice for helpful discussions.

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