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SEPARATION OF POLYNUCLEAR AZA-HETEROCYCLICS BY HIGH-SPEED LIQUID CHROMATOGRAPHY ON A CHEMICALLY BONDED STATIONARY PHASE

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SUMMARY

A high-speed liquid chromatographic method for analysis of polynuclear aza-heterocyclics found in airborne particulate matter is described. A column-packing material (chromatographic brush) has been prepared by reacting *p*-nitrophenyl isocyanate with surface silanol groups of Corasil I. The separation requires less than three minutes for the six compounds tested. The method is suitable for the quantitative determination of these compounds, with relative standard deviation of approximately 2% in a concentration range of *ca.* 50–1000 ng. An ultraviolet detector was used and detection limits of 1 ng were obtained for most components.

INTRODUCTION

The necessity to control air pollution has led to an increased interest in sensitive and specific methods of analysis for air contaminants. Much of the recent work has been concerned with detection and estimation of polynuclear aromatic hydrocarbons[1], several of which are known to be carcinogenic, such as benzo[*a*]pyrene and related compounds. SAWICKI *et al.*[2,3] have established the presence of aza-heterocyclics in airborne particulate matter and identified a number of them. The amount of aza-heterocyclics in urban air is relatively small[3] (sub-p.p.b. range) compared to the total aromatic hydrocarbon content, and very sensitive analytical techniques are required to monitor these potentially hazardous components. Carcinogenic aza-heterocyclics have been found in car-exhaust fumes[4], cigarette smoke[5,6] and other air pollution source effluents[2].

The existence of a large variety of other organic substrates in association with polycyclic aza compounds necessitates a preliminary analytical separation. The final separation and identification of the aza-arenes have been attempted by column chromatography (CC) and thin-layer chromatography (TLC)[7,8], gas chromatography (GC)[9], paper chromatography (PC)[10], and paper electrophoresis[11]. SAWICKI[1] has

used either ultraviolet or fluorescence measurements after final separation on TLC plates in his extensive work on aza-heterocyclics. However, the analysis time is long, sometimes requiring two-dimensional development in two different solvent systems. The detection limits are of the order of 0.05 μg.

The use of high-speed liquid chromatography (HSLC) for the separation of aza-aromatics has been reported earlier[12]. Silver ion impregnated on Zipax® support was specifically developed in this work to separate this class of compounds. Donor–acceptor complex formation was the determining factor in the separation process.

Recently, considerable attention has been drawn to the possibility of organic stationary phases (brushes) chemically bonded to the active surface (free silanol groups) of the column support material such as silica gel, Porasil, Corasil, Zipax®, etc. to overcome inherent disadvantages of regularly loaded stationary phases in liquid–liquid chromatography. The advantages of chemically bonded stationary phases in HSLC have been well documented[13]. Several of such packing materials for HSLC have been available commercially for some time. KIRKLAND AND DE STEFANO[14] prepared permaphase supports by chemically reacting silane reagents containing various functional groups and then polymerizing the silanes to desired limits. DU PONT[15] has recently reported another material with an octadecyl group incorporated in it. These materials can be made with diverse selectivities by properly choosing the physicochemical properties of the bonded silicones and can be "tailor-made" for specific needs. The Durapak[16] packings marketed by Waters Associates Inc. (Framingham, Mass., U.S.A.) are made by reacting the surface silanol groups with different alcohols. These materials also show unusual selectivity in the separation of a number of compounds. Recently, LOCKE *et al.*[17] have reported the incorporation of phenyl and naphthyl moieties containing different functional groups into Porasil, which resulted in a wide range of substrates. It is obvious that the principal advantage of these brushes lies in their adaptability to physicochemical modification to solve selective separation problems. This latter property is especially important since it can be used to overcome deficiencies in column characteristics.

In this study a new brush-type column-packing material made by bonding aromatic isocyanates to the surface silanol groups of porous glass beads has been studied. This column was specifically developed for the separation of electron-donor compounds such as polynuclear aza-heterocyclics.

EXPERIMENTAL

Apparatus

A schematic diagram of the liquid chromatograph is given in Fig. 1. The pump was a Haskel air-driven piston pump, Model No. 17082-3 (Haskel Engineering and Supply Co., Burbank, Calif., U.S.A.) rated for a maximum pressure of 7000 p.s.i. A ball valve (Whitey Research Tool Co., Emeryville, Calif., U.S.A.) was put in the system behind the liquid pressure gauge for stop flow injection. A Laboratory Data Control ultraviolet detector was used for monitoring the column effluent. The photometer detects absorbance at 254 nm (with provision for 280 nm) using a low-pressure mercury source. A Beckman 10-in. strip-chart recorder was used for recording the chromatographic bands in normal scale. Evaluation of the peak areas was done with a Gelman planimeter.

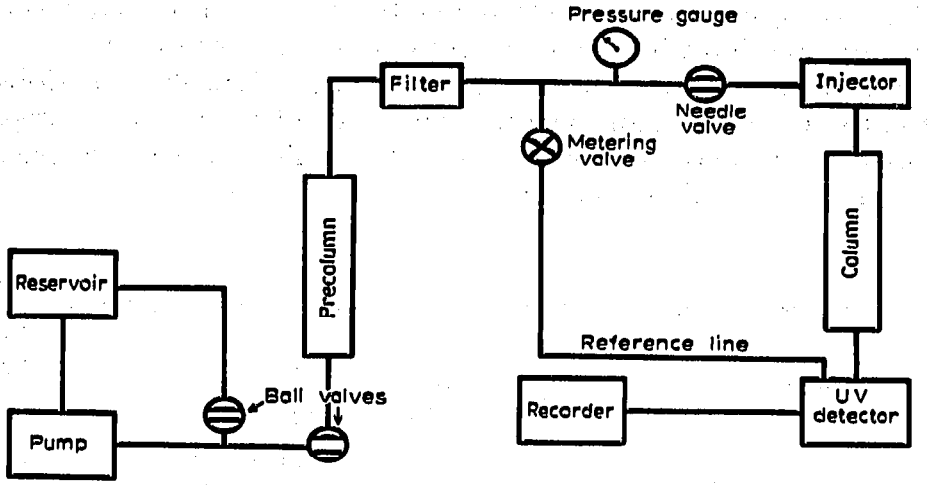


Fig. 1. Schematic diagram of a liquid chromatograph.

Chemicals

Spectral-grade *n*-hexane and acetonitrile were used as mobile phases. The hydrocarbon solvents were dried over sodium and freshly distilled under anhydrous conditions before use. All other solvents were dried with appropriate drying agents. The polynuclear aza-aromatic compounds were obtained in the purest form available and were used without further purification. The *p*-nitrophenyl isocyanate (Eastman-Kodak Co., Rochester, N.Y., U.S.A.) used for preparing the brush material was distilled under reduced pressure before use.

Column and support

The column tubes used in this study are commercially available and are of 1000 × 2.2 mm precision bore stainless steel. All fittings are of stainless steel (Swagelok, Crawford Fitting Co., Solon 39, Ohio, U.S.A.). Before packing the column material the tubes were washed as described by KARGER *et al.*[18].

Preparation of brush material

Corasil I (solid-core, controlled-surface porosity beads) available commercially from Waters Associates, Inc. was used to prepare the brush material. The support was treated with a 1:1 mixture of concentrated nitric and sulphuric acid, washed free of acid with doubly distilled water and finally dried overnight at 110°. To 18 g of Corasil in 50 ml of benzene 7.0 g of *p*-nitrophenyl isocyanate were added and heated under reflux conditions for 24 h in a nitrogen atmosphere. After cooling, the supernatant liquid was decanted and the support was washed several times with 50-ml portions of benzene. It was then transferred to a Soxhlet apparatus and extracted successively with benzene, *n*-hexane, and methylene chloride, until only a negligible amount of ultraviolet-absorbing material was found in the extracts. The brush material was then taken out and dried overnight in a vacuum oven at 60° and stored in a desiccator.

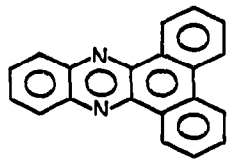
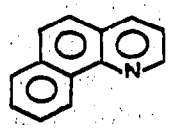
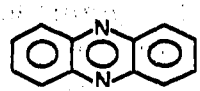
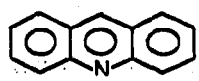
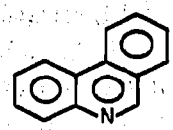
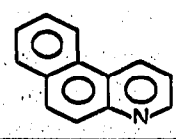
Packing of columns and sample injection

The columns were packed by a dry packing technique which consists of small

incremental additions while applying indirect vibration to the column stand and light tapping of the column on the floor. The bottom support of the column packing was of Dacron sailcloth and glass wool was used for a top plug. Sample injections were made directly into the top of the column packing with a 10-μl Hamilton syringe (No. 701N) through a rubber septum covered with Teflon tape. Typical sample sizes were several microlitres of *ca.* 100 μg/ml concentration.

TABLE I

CAPACITY FACTORS (k') ON AN ISOCYANATE COLUMN AND pK_a'S OF SOME POLYNUCLEAR AZA-AROMATIC COMPOUNDS STUDIED

No.	*Name*	*Structure*	*Physical properties*	*pK_a*	*k'*
1	Dibenzo[*a*,*c*]phenazine		m.p. 220°	—	0.09
2	Benzo[*h*]quinoline		338/719 mm	4.25	0.45
3	Phenazine		b.p. >360°	1.23	1.58
4	Acridine		b.p. 345–346°	5.60	2.45
5	Benzo[*c*]quinoline		b.p. 360°	4.52	4.21
6	Benzo[*f*]quinoline		350/721 mm	5.15	5.96

RESULTS AND DISCUSSION

Table I shows the compounds studied, pK_a values and their capacity factor on the isocyanate column. The chromatographic separation of the mixture of six compounds on the bonded stationary phase column is shown in Fig. 2. The separations obtained under identical conditions with plain Corasil are shown in Fig. 3. Only three of the compounds were eluted in 7 min, with phenazine and dibenzophenazine appearing close to the solvent peak. It has been observed earlier that the aza-aromatics have a strong attraction for silica gel. This can be attributed to hydrogen bonding of the lone pair of electrons on the nitrogen to the hydroxyl group. However, this hydrogen bonding depends on the relative accessibility of the lone pair and

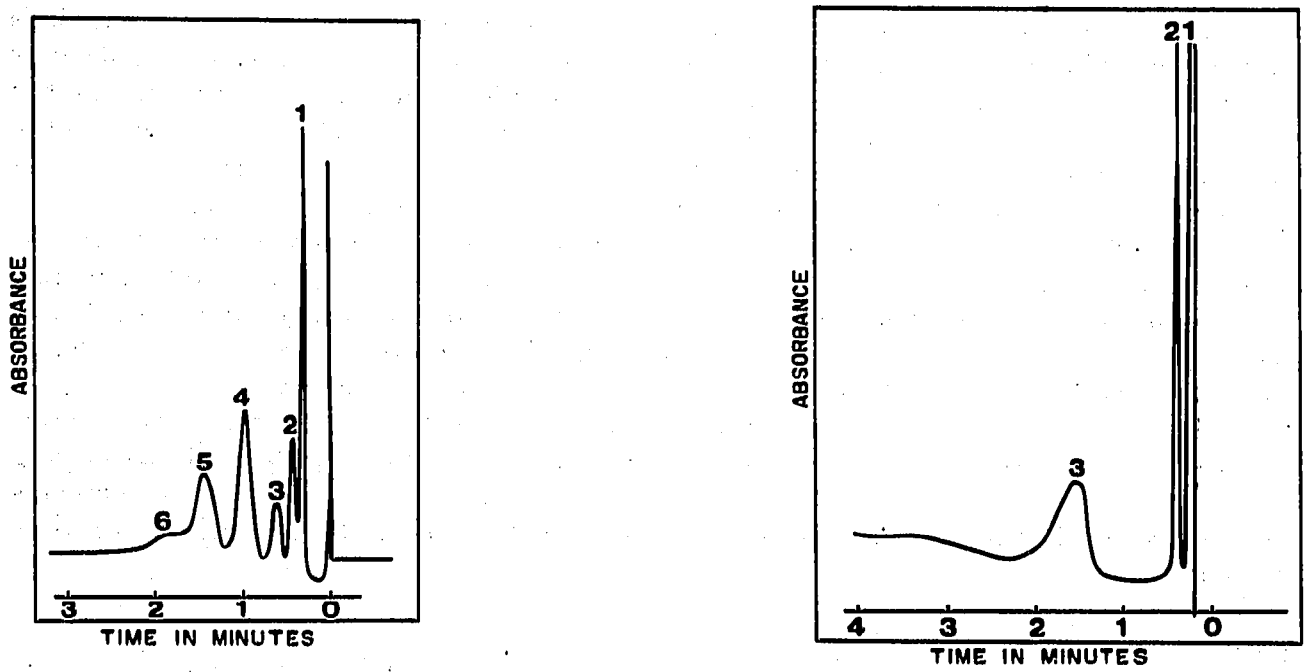


Fig. 2. Separation of polynuclear aza-aromatics on a chemically bonded stationary phase column. Column: length 1 m, I.D. 2.2 mm; support: *p*-nitrophenyl isocyanate bonded to Corasil I. Mobile phase: 1% acetonitrile in *n*-hexane; pressure: 1000 p.s.i. Peaks in the order of elution are: (1) dibenzo[*a*,*c*]phenazine; (2) benzo[*h*]quinoline; (3) phenazine; (4) acridine; (5) benzo[*c*]quinoline; (6) benzo[*f*]quinoline.

Fig. 3. Separation of polynuclear aza-aromatics on plain Corasil I column. Column: length 1 m, I.D. 2.2 mm; support: Corasil I, 37–50 μ. Mobile phase: 1% acetonitrile in *n*-hexane; pressure: 1000 p.s.i. Peaks in the order of elution are: (1) dibenzo[*a*,*c*]phenazine; (2) phenazine; (3) benzo-[*h*]quinoline.

consequently on the stereochemistry of the molecule. Where the effective hydrogen bond formation does not take place, the compound is not expected to be retained in the chromatographic column and comes out with or immediately after the solvent front. This behaviour is observed in the case of dibenzo[*a*,*c*]phenazine.

A study of HETP *(H) vs.* velocity *(v)* for phenazine shows that after a critical velocity of *ca.* 3 cm/sec the efficiency changes very little. Pressures above 700 p.s.i. should be used to obtain optimal separation conditions.

Factors affecting the separation

The elution order of the compounds studied can be explained on the basis of donor–acceptor complex formation which again is affected by the electronic and steric effects of the molecules. The brush material formed by reaction of *p*-nitrophenyl isocyanate with the surface silanol groups should behave as a good charge acceptor due to the presence of several acceptor sites in the molecule, and the aza-arenes should be good donors due to the presence of non-bonded electrons (lone pairs) available for coordination. However, this expected behaviour of donor–acceptor complex formation can be affected (see pK_a values, Table I) if the lone pairs of the nitrogen become inaccessible due to the stereochemistry of the molecule. As indicated in Fig. 2 the largest molecule is eluted first from the column since the nitrogen atoms are most sterically hindered, thus preventing any effective complex formation.

This steric hindrance is also apparent in the benzo[*h*]quinoline molecule which is eluted immediately after dibenzo[*a*,*c*]phenazine. A comparison of the molecular models of the three benzoquinolines studied provides a qualitative picture of the relative steric hindrance in the molecules, which is supported by their behaviour on the columns. In the absence of steric hindrance relatively strong electron donor–acceptor complexes are formed and the retention time is high (see Table I).

The relatively short retention time of the phenazine is probably due to the decreased basicity as compared to acridine (see Table I). The pK_a value for phenazine is 1.23 compared to 5.60 for acridine, which is about 1000 times stronger and hence forms stronger complexes. This phenomenon has also been observed by VIVILECCHIA *et al.*[12] and by ENGEL AND SAWICKI[10] in their work on aza-heterocyclics.

In order to prove that donor–acceptor complex formation plays the major role in the separation process, a number of analogous aromatic hydrocarbons having no donor properties were tested under identical conditions. All these hydrocarbons were eluted with the solvent front indicating absence of any significant complex formation.

Reproducibility of the column

The chemically bonded stationary phase as described was found to be stable for months under actual operating conditions. The capacity factor [k'] values for phenazine at mobile phase velocities ranging from 3.2–9.1 cm/sec have been found to be practically constant ($k' = 1.6 \pm 0.1$). Another batch of support under identical conditions gave similar values.

Quantitative analysis

Quantitative analysis of aza-aromatics can be performed by this method. The reproducibility of quantitative analysis of three compounds with widely different k' values was studied. Quantitative data were obtained by plotting either peak area or peak height *vs.* concentration. Relative standard deviations in peak area and peak height measurement of seven replicate samples were *ca.* 2%. Linear calibration plots were obtained over a range of 50–1000 ng.

Detection limit

Five of the six compounds studied could be detected to a *ca.* 0.5 ng level at a signal-to-noise ratio of 3:1. The relatively poor detection limit for benzo[*f*]quinoline (*ca.* 10 ng) may be due to peak broadening (high k' value).

CONCLUSIONS

A chromatographic support such as the one discussed, which has electron acceptor properties, can be used to advantage for the selective separation of donor molecules of the nature of aza-arenes. Other electron donor compounds such as polynuclear aromatic amines or polychlorohydrocarbons (PCBs) could also be separated on such a column. The advantages of chemically bonded stationary phases (brushes) under high liquid pressure conditions are well recognized and are demonstrated by the uniform baselines that can be obtained with an ultraviolet detector, since no stationary phase is being lost. The columns can be used for a large number

of separations without change or loss in separation performance and can therefore be utilized for quantitative routine monitoring of aza-arenes at the nanogram or even subnanogram concentration level.

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