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Note

Liquid chromatography of aromatic hydrocarbons on a chemically bonded stationary phase of the charge-transfer type

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Formation of charge-transfer complexes has been often employed for the separation of aromatic hydrocarbons in column chromatography and thin-layer chromatography (TLC)^{1,2}. Silica gel impregnated with various electron acceptors, e.g. picric acid, 2,4,6-trinitrobenzene or 2,4,7-trinitrofluorenone, has been recommended by many authors³⁻⁷ for this purpose. This simple method, however, has many disadvantages, the main one being the solubility of the electron acceptors in usual mobile phases. This difficulty may be overcome by chemical bonding of the electron acceptors. Preparation of a chemically bonded stationary phase is frequently performed via synthesis of a reactive silane, which is then bonded on to the silica surface⁸⁻¹⁰. If synthesis of the silane is difficult or impossible, some reactive groups primarily bonded on the surface of silica can be chemically transformed. Thus, phenyl groups have been sulphonated¹¹ and amino groups have been converted into amidic groups¹²; stationary phases incorporating polymers¹³, ion-exchange groups¹⁴ and peptide chains¹⁵ have also been prepared. The reactivity of chemically bonded amino groups has recently been used for the preparation of a 3-(2,4,5,7-tetranitrofluorenimino)propyldiethoxysiloxane stationary phase. This compound, which functions as a chemically bonded strong electron acceptor, has been applied to the separation of alkyl-substituted polynuclear aromatic hydrocarbons¹⁶. Recently, Porath and Caldwell¹⁷ reported the preparation of a charge-transfer ligand, $-O-CH_2-CHOH-CH_2-S-C_6H_3(NO_2)_2-2,4$, obtained by treating thiolated Sephadex G-25 or thiolated Sepharose 6B gels with 2,4-dinitrochlorobenzene. The new π -electron-acceptor gels (DNP-S-Sephadex G-25; DNP-S-Sepharose 6B) have been found to be suitable for the separation of some neurophysiologically important aromatic nitrogen compounds.

The objective of this communication is to describe the preparation and, briefly, the characterization of the chromatographic properties of a silica gel (Silpearl) modified by chemically bonded 3-(2,4-dinitroanilino)propyl (DNAP) groups.

EXPERIMENTAL

Materials

Silica gel, Silpearl, for TLC (Kavalier, Votice, Czechoslovakia) had a surface area of 280 m²/g. A fraction having a particle diameter of 30-40 μ m, which was ob-

tained by elutriation and chosen as a basic support for the experiments, was dried at 100° for 5 h before use. 2,4-Dinitrofluorobenzene (Eastman Organic Chemicals, New York, N.Y., U.S.A.) and 3-aminopropyltriethoxysilane (Pierce, Rockford, Ill., U.S.A.) were used without further purification. Aromatic hydrocarbons (Fluka, Buchs, Switzerland; Institute for Organic Synthesis, Rybitví, Czechoslovakia; Aldrich-Europe, Beerse, Belgium), all reagent grade chemicals, were used as obtained. Chloroform, acetone, methanol, ethanol, toluene and *n*-heptane were purified by rectification prior to use.

Preparation of DNAP-Silpearl

A 250-ml round-bottomed flask equipped with a mechanical stirrer was charged with 10 g of dry Silpearl (30–40 μm particle diameter), 100 ml of toluene and 2.5 ml of 3-aminopropyltriethoxysilane. The mixture was refluxed under stirring for 6 h and allowed to cool to room temperature. The silylated Silpearl was filtered off, washed with hot toluene, acetone and methanol and dried at 100° for 1 h. The concentration of amino groups, determined by potentiometric titration, was 0.7 mequiv./g.

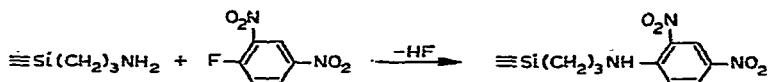
A 100-ml round-bottomed flask was charged with a solution of 0.5 g of sodium hydrogen carbonate in 10 ml of distilled water. Then 3 g of the silylated Silpearl and a solution of 0.5 g of 2,4-dinitrofluorobenzene in 20 ml of ethanol were successively added. The mixture was shaken at room temperature for 2 h, and the bright-yellow DNAP-Silpearl was filtered off, washed with distilled water, methanol and acetone and dried at 100° for 1 h. No unchanged amino groups were detected by potentiometric titration. Elemental analysis showed a carbon content of 8.25–8.27%.

Liquid chromatography

The chromatograph consisted of a Micropump VCM 150 (Development Workshops, Czechoslovak Academy of Sciences, Prague, Czechoslovakia), a 20 cm \times 6.0 mm O.D. \times 3.5 mm I.D. glass column with a water-jacket, a two-beam UV analyser (254 nm) equipped with 10- μm flow cells (Development Workshops) and a TZ 21S linear recorder (Laboratory Instruments, Prague, Czechoslovakia). The water-jacket was connected with a water thermostat maintaining the column temperature to $\pm 0.2^\circ$. The column was packed with Silpearl or DNAP-Silpearl using a "tap and bounce" procedure. Samples of aromatic hydrocarbons were injected as 2% solutions in the mobile phase with a microsyringe through a septum. Degassed *n*-heptane containing 1% (v/v) of chloroform was used as the mobile phase.

RESULTS AND DISCUSSION

The stationary phase (DNAP-Silpearl) was prepared by treating free silanol groups of Silpearl with ethoxy groups of 3-aminopropyltriethoxysilane¹⁶ and by quantitative reaction of 2,4-dinitrofluorobenzene with the primary amino groups (0.7 mequiv./g) chemically bonded to Silpearl surface.



The selective formation of the chemically bonded DNAP groups, which is analogous to the Sanger reaction¹⁵, proceeded under very mild conditions.

The values of capacity factors measured for several non-condensed and condensed aromatic hydrocarbons on untreated Silpearl (k'_0) and DNAP-Silpearl (k'_D) are listed in Table I. Heats of adsorption, ΔH_{ads} (Table I), were obtained from van 't Hoff plots of $\ln k'_D$ against $1/T$ in the temperature range 20–60°.

The relatively high values of k'_D observed on DNAP-Silpearl clearly demonstrate an increasing strength of the interaction between the solute and the modified stationary phase, attributable to the formation of chemically bonded charge-transfer

TABLE I

CAPACITY FACTORS FOR LIQUID CHROMATOGRAPHY OF AROMATIC HYDROCARBONS ON SILPEARL (k'_0) AND DNAP-SILPEARL (k'_D), AND HEATS OF ADSORPTION (ΔH_{ads}) MEASURED ON DNAP-SILPEARL

Solute	k'_0 *	k'_D *	ΔH_{ads} (kcal/mole)
Benzene	0.08	0.10	-0.5
Biphenyl	0.10	0.52	—
Naphthalene	0.20	0.82	-1.0
Acenaphthene	0.12	1.29	—
Fluorene	0.26	1.45	—
Anthracene	0.35	3.57	-1.3
Phenanthrene	0.35	3.71	-1.4
Fluoranthene	0.65	7.50	—
Pyrene	0.70	8.05	-1.7

* Mobile phase, 1% (v/v) chloroform in *n*-heptane; flow-rate, 1.5 ml/min; temperature, 20°.

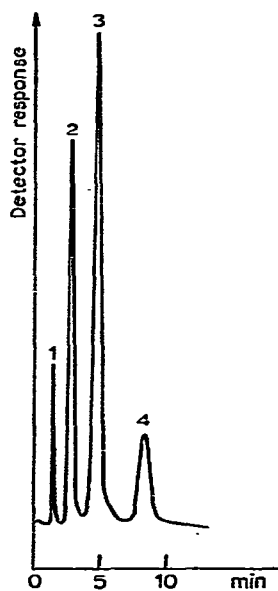


Fig. 1. Liquid chromatography of the mixture of benzene (1), naphthalene (2), fluorene (3) and anthracene (4) on DNAP-Silpearl stationary phase using *n*-heptane with 1% (v/v) of chloroform as the mobile phase.

complexes. Porath and Caldwell¹⁷ have interpreted the strong adsorption of some aromatic nitrogen compounds on DNP-S-Sephadex G-25 and DNP-S-Sepharose 6B gels as due to the formation of π,π electron-transfer complexes; however, they have suggested that, at the same time, molecular sieving, ionic adsorption, hydrogen-bond formation, hydrophobic interaction and Van der Waals-London forces may be responsible for the observed adsorption effect. The use of the modified silica gel (DNAP-Silpearl) and the non-polarity of the mobile phase as well as of the solutes, mean that only Van der Waals-London forces might be operative, in addition to electronic charge transfer, in our chromatographic system.

The values of ΔH_{ads} , which are comparable with those of enthalpies of formation of charge-transfer complexes arising from interactions of some polynitrobenzenes with aromatic hydrocarbons¹⁹, indicate that the electronic charge transfer prevails in the observed adsorption effect.

In spite of the relatively large particle diameter of the DNAP-Silpearl used in our experiments, successful separations of non-condensed and condensed aromatic hydrocarbons were obtained. A typical chromatogram of a hydrocarbon mixture is shown in Fig. 1. Symmetrical peaks were obtained in all experiments.

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