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Note

High-performance liquid chromatographic behavior of some chemically bonded nitroaromatic type stationary phases

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An examination of the recent literature shows that the use of charge-transfer interactions continues to play an important role in high-performance liquid chromatography (HPLC) separations[1–5]. The emphasis of many of these investigations has been aimed at either the preparation and characterization of coated or chemically-bonded charge-transfer type stationary phases and/or the applicability of these stationary phases to several types of separation problems.

A recent study by Lochmüller and Ryall[6] of HPLC stationary phases having bonded nitrophenyl groups showed that the degree of nitration of the bonded groups had an apparent effect on the magnitude of charge-transfer interaction with the solute. In order to examine this behavior in greater detail, a systematic study was undertaken of several bonded phases of the nitroaromatic type. Of special interest were the effects of the degree of nitration and, to a limited extent, the molecular position of the nitro group on chromatographic retention and selectivity for several polyaromatic hydrocarbons (PAH). Bonded nitrofluorenimine[1] type stationary phases were prepared and used for this study since these would provide a suitable range of stationary phases having varying degrees of nitration.

EXPERIMENTAL

Instrumentation

The liquid chromatograph and column slurry-packing procedure have been described earlier[1].

Materials

All of the bonded stationary phases were formed on a matrix of LiChrosorb Si 60 10-μm median particle size silica gel having a reported surface area of approximately 500 m^2/g (E.M. Laboratories, Elmsford, N.Y., U.S.A.). Prior to chemical modification, the silica gel was dried in air at 160°. The nitrofluorenones and the 3-aminopropyltriethoxysilane (Aldrich, Milwaukee, Wisc., U.S.A.) as well as the

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methyltriethoxysilane (PCR, Gainsville, Fla., U.S.A.) were used without further purification. Polyaromatic hydrocarbons, which were gifts from other investigators, were used as obtained. Spectrograde acetonitrile was used as the HPLC mobile phase.

Preparation of bonded phases

Preparation of the bonded tetranitrofluorenimine stationary phase has been described previously[1]. The current study involved the preparation of additional nitrofluorenimine phases wherein the number and the position of nitro groups were varied. In general, the rate of reaction between the nitrofluorenone reagent and the aminated silica gel decreased with a decrease in the number of nitro groups on the fluorene nucleus. Thus, reaction times of up to 20 h were used to insure complete reaction of the mono- and dinitrofluorenones with aminated silica. The freshly prepared phases were subjected to exhaustive Soxhlet extraction with acetonitrile.

Characterization of the bonded phases and determination of the "phase mass" were accomplished by wet chemical methods[1]. Determination of the bonded amine phase mass was accomplished through non-aqueous acid-base titrimetry with perchloric acid dissolved in acetic acid. Determination of bonded fluorenimine phase mass was achieved through reduction of the nitro groups with excess titanous chloride reagent (Fisher Scientific, Raleigh, N.C., U.S.A.) followed by back-titration of the excess titanous ions with a solution of ferric ammonium sulfate.

DISCUSSION

Fig. 1 illustrates the loss in solute retention with a decreasing number of nitro groups on the bonded fluorene moiety. The capacity factor, k', normalized for fluorenimine phase mass, for each of the three cholanthrene solutes, varies linearly with the number of nitro groups in inductively equivalent positions of the fluorene nucleus.

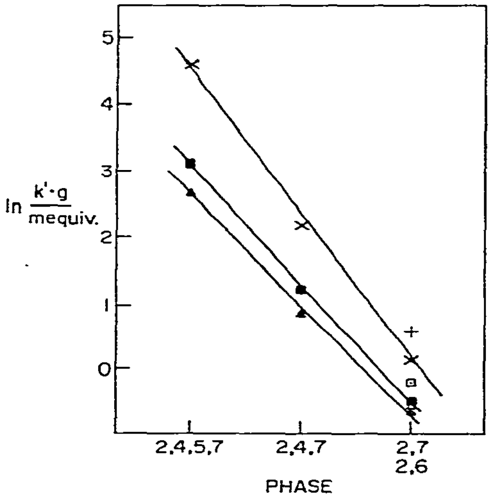
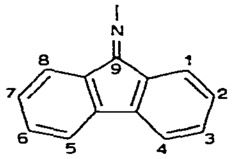


Fig. 1. Plot of ln (k'/meq./g) *vs.* number of nitro groups for 1-, 2- and 3-methylcholanthrene, symbolized by ▲, ■ and ×, respectively. 2,6-Dinitrofluorenimine phase results are plotted as △, ⊡ and +, for 1-, 2- and 3-methylcholanthrene, respectively.

Since the same mobile phase was used throughout the study, this result is indicative of a linear, free-energy relationship



Bonded fluorenimine moiety

between the number of equivalent nitro groups and the charge-transfer complex stability constants. Of particular interest are the following observations which can be made concerning these data. Capacity at constant phase mass is clearly dependent upon the degree of nitration of the bonded fluorenimine whereas the selectivity behavior is less reflective of such a clear trend. Table I lists the selectivity values, α, for several alkyl-substituted PAH on the different stationary phases. The selectivity for the 1- and 2-methylcholanthrenes does not change between the 2,4,5,7-tetranitrofluorenimine phase and the 2,4,7-trinitro analog whereas the α value for 3- to 2-methylchloanthrene is significantly reduced in the 2,4,7-system as is the value for the 7-, 12-methylbenz[*a*]anthracene pair. In the stationary phase system with nitro groups at the 2,7-position selectivity is essentially lost for the 1-, 2-methylcholanthrenes but the 3-methyl derivative is separable from 1- and 2-methyl derivatives ($\alpha = 2.00$). The 2,6-dinitrofluorenimine bonded phase, differing from the 2,7-system in that a nitro substituent is placed farther from the imino linkage, does resolve the 1-,2-methylcholanthrene pair ($\alpha = 1.38$). In all cases, a 3-nitrofluorenimine bonded phase provided no measurable capacity for any of the solutes under the same conditions.

TABLE I

RETENTION AND SELECTIVITY RELATION FOR NITROFLUORENIMINE BONDED PHASES AND SELECTED BENZ[*a*]ANTHRACENES AND CHOLANTHRENES

All phases are listed in terms of the positions of the nitrofunctions on the fluorene entity. The phase mass is reported as milliequivalents of nitrofluorene per gram. Solvent (mobile phase) in all cases is acetonitrile. k' values reported as "—" were less than 0.05 units and all k values were calculated assuming k' (benzene) = 0.

Compound	*2,4,5,7 Phase (0.17 mequiv./g)*		*2,4,7 Phase (0.14 mequiv./g)*		*2,7 Phase (0.10 mequiv./g)*		*2,6 Phase (0.14 mequiv./g)*	
	k'	α	k'	α	k'	α	k'	α
3-methyl	17.4		1.35		0.12		0.25	
		4.48		2.68		2.00		2.27
2-methyl	3.90		0.50		<0.10		0.11	
		1.55		1.56		<1.05		1.38
1-methyl	2.50		0.32		—		0.08	
Benz[a]anthracenes								
7-methyl	5.72		0.55		—		—	
		3.71		2.04		<1.05		1.00
12-methyl	1.54		0.27		—		—	

Selectivity, therefore, at least for the solute systems studied here appears to be sterically controlled; *i.e.*, nitro substitution at the 5- or 6-position of the fluorenimine nucleus is important to separation as well as retention. The series 1-, 2-, 3-methylcholanthrene cannot be considered in any simple manner. It is obvious from the present data that methyl substitution on the aromatic ring has a far greater stabilizing effect on the charge-transfer complex than substitution on the saturated "aca" ring. The good linear relationship observed in Fig. 1 is indicative of the inductive nature of the stabilization process in that nitro groups in inductively equivalent positions appear additive in their contribution to ln k'.

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