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RETENTION MECHANISMS ON METALLIC STATIONARY PHASES

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SUMMARY

An electron-rich, porous graphitic carbon stationary phase for high-performance liquid chromatography has been shown to act as an electron-pair acceptor of solutes under non-polar conditions. The application of delocalized electron bands as chromatographic substrates was extended to other conductors, namely a palladium metal stationary phase, which also interacted by an electron donor–acceptor mechanism. Quantitative structure–retention relationships are presented as indicators of chromatographic retention mechanisms.

INTRODUCTION

Despite the widespread use of chromatography as an analytical tool, relatively little is known about chromatographic mechanisms at the molecular level. In the course of studying various non-polar stationary phases for use in retention prediction, porous graphitic carbon, which was expected to behave as an “ideal reversed phase”¹, produced retention behavior anomalous for classical reversed phases².

Upon closer examination, retention on graphite should not be governed by only dispersive (electrostatic) interactions, particularly under non-polar solvent conditions where solvophobic phenomena³ are minimized. The uniqueness of porous graphitic carbon compared to hydrocarbonaceous stationary phases is due to its delocalized band of electrons⁴, available for electronic interactions, specifically donor–acceptor (charge transfer) interactions and direct π -electron overlap^{5,6}. Using the tools of quantitative structure–retention relationships (QSRRs)⁷, *i.e.*, linear free-energy relationships, the chromatographic column becomes a “free-energy transducer”⁸, translating differences in chemical potentials of solutes, arising from differences in structure, to chromatographic retention. If such electronic interactions, in addition to the expected London forces, are governing retention on porous graphitic carbon, then solute descriptors reflecting electron donor–acceptor properties and/or orbital energies should be highly correlated to solute retention.

Furthermore, if the delocalized electron band of graphite contributes sub-

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stantially to its selectivity, then an entire class of substrates possessing similar electronic structures, *i.e.*, metals and conductive polymers, should also exhibit similar characteristics as chromatographic stationary phases. Metal ions have previously been used in chromatography to alter and improve retention properties of conventional stationary phases through the formation of discrete metal complexes⁹⁻¹⁵. However, reduced metal or metal-like surfaces have not been widely studied as chromatographic phases.

The experiments described in this report were designed to (1) determine whether the delocalized bands of electrons in porous graphitic carbon participate in solute-substrate interactions under non-polar solvent conditions, and (2) determine whether selected metals behave in a similar fashion, demonstrating a new class of stationary phases based on solute-[conduction-band] interactions.

EXPERIMENTAL

Materials

Porous graphitic carbon was obtained from Professor John Knox (University of Edinburgh, Edinburgh, U.K.). Palladium spheres (nominal diameter 8 μm) were lent by Platina Laboratories (Piscataway, NJ, U.S.A.). Solvents were of high-performance liquid chromatography (HPLC) grade and were purchased from Fisher (Pittsburgh, PA, U.S.A.). Solutes (Table I) of the highest available purity were purchased from various sources and used without further purification.

TABLE I
SOLUTE SET FOR CHROMATOGRAPHIC ANALYSES

1	Benzene	C_6H_6
2	Toluene	$\text{C}_6\text{H}_5\text{CH}_3$
3	Acetophenone	$\text{C}_6\text{H}_5\text{COCH}_3$
4	Chlorobenzene	$\text{C}_6\text{H}_5\text{Cl}$
5	Methyl benzoate	$\text{C}_6\text{H}_5\text{COOCH}_3$
6	Nitrobenzene	$\text{C}_6\text{H}_5\text{NO}_2$
7*	Aniline	$\text{C}_6\text{H}_5\text{NH}_2$
8	Anisole	$\text{C}_6\text{H}_5\text{COCH}_3$
9	Benzyl alcohol	$\text{C}_6\text{H}_5\text{CH}_2\text{OH}$
10	Phenol	$\text{C}_6\text{H}_5\text{OH}$
11	<i>p</i> -Cresol	$\text{C}_6\text{H}_4\text{OHCH}_3$
12	4-Ethylphenol	$\text{C}_6\text{H}_4\text{OHC}_2\text{H}_5$
13	4- <i>n</i> -Propylphenol	$\text{C}_6\text{H}_4\text{OHC}_3\text{H}_7$
14	4- <i>sec.</i> -Butylphenol	$\text{C}_6\text{H}_4\text{OHCH}(\text{CH}_3)(\text{CH}_2\text{CH}_3)$
15	4- <i>tert.</i> -Butylphenol	$\text{C}_6\text{H}_4\text{OHC}(\text{CH}_3)_3$
16	Carvacrol	$\text{C}_6\text{H}_3\text{OH-}o\text{-CH}_3\text{-}m\text{-CH}(\text{CH}_3)_2$
17	Thymol	$\text{C}_6\text{H}_3\text{OH-}m\text{-CH}_3\text{-}o\text{-CH}(\text{CH}_3)_2$
18	2-Isopropylphenol	$\text{C}_6\text{H}_4\text{OHCH}(\text{CH}_3)_2$
19	2- <i>tert.</i> -Butylphenol	$\text{C}_6\text{H}_4\text{OHC}(\text{CH}_3)_3$
<i>Additional solutes</i>		
20	Pyridine	$\text{C}_5\text{H}_5\text{N}$
21	3- <i>tert.</i> -Butylphenol	$\text{C}_6\text{H}_4\text{OHC}(\text{CH}_3)_3$
22	2- <i>sec.</i> -Butylphenol	$\text{C}_6\text{H}_4\text{OHCH}(\text{CH}_3)(\text{CH}_2\text{CH}_3)$

* Not included in correlations.

Chromatographic apparatus and conditions

The chromatographic system was comprised of a Model 8500 syringe pump (Varian, Walnut Creek, CA, U.S.A.), a Model 834 autosampler (DuPont, Wilmington, DE, U.S.A.), a Model 7013 injection valve with 1- μ l loop (Rheodyne, Cotati, CA, U.S.A.) and a Model 757 UV detector, 210 nm, with 0.5- μ l flowcell (Kratos Analytical, Ramsey, NJ, U.S.A.). A temperature-controlled chamber, made from R-10 foam insulation and thermostated with 3 m of 1 cm diameter copper tubing containing circulating temperature-controlled water, housed 0.5 m of mobile phase equilibration tubing, the injection valve and the column. A 30 cm \times 1 mm glass-lined, stainless-steel column blank (previously silanized with trimethylchlorosilane) was packed with a slurry of porous graphitic carbon in heptane at 3000 p.s.i. for 1 h. A 50 cm \times 1 mm glass-lined, stainless-steel column blank (also silanized) was dry-packed with palladium spheres. Heptane-dissolved solutes were chromatographed with neat heptane eluent at 2 ml/h for porous graphitic carbon or 4 ml/h for palladium and 23°C with detection at 210 nm. The column void volume was determined by refractive index change of an injection of pentane in heptane. Chromatographic data were collected with MAXIMA™ chromatography software (Dynamic Solutions, Ventura, CA, U.S.A.). The first statistical moments (retention times) of the peaks were calculated by summation of at least 50 time slices, by downloading the data from MAXIMA™ to Lotus 123™ (version 2.01; Lotus Development, Cambridge, MA, U.S.A.) where the moment analyses were completed.

Molecular descriptors and statistical analysis

QSRRs were constructed by statistical correlation of matrices⁷: (1) $m \cdot n$, where m = solute and n = various numerical molecular descriptors, and (2) $m \cdot l$, where l = solute retention, expressed as $\log k'$. Molecular descriptors [heat of formation (H_f), total energy (E_{tot}), E_{homo} , E_{lumo} , dipole moment and excess atom charge] were calculated for each solute by using MNDO3¹⁶ on molecular coordinate files obtained from the Cambridge Crystallographic Database¹⁷. Calculations were performed on a VAX 780 computer with an array processor.

Submolecular polarity parameters, Δ , were calculated from excess atomic charges¹⁸. Values of pK were obtained from the literature¹⁹. Statistical models were constructed by stepwise linear regression by means of Asystant +™ software (version 1.0; Macmillan Software, New York, NY, U.S.A.). The suitability of retention models was determined by correlation coefficients and statistical significance.

Surface analysis of phases

Scanning Auger spectroscopy was performed on the palladium powder to determine surface characteristics (presence of oxidation or contaminants).

RESULTS AND DISCUSSION

Retention mechanisms under normal-phase conditions

Experimental conditions were chosen (heptane eluent, substituted aromatic solutes, see Table I) to maximize the occurrence of chemical interactions that might be masked under the solvophobic environment conditions normally encountered in reversed-phase chromatography. Typical chromatograms are shown in Figs. 1 and 2.

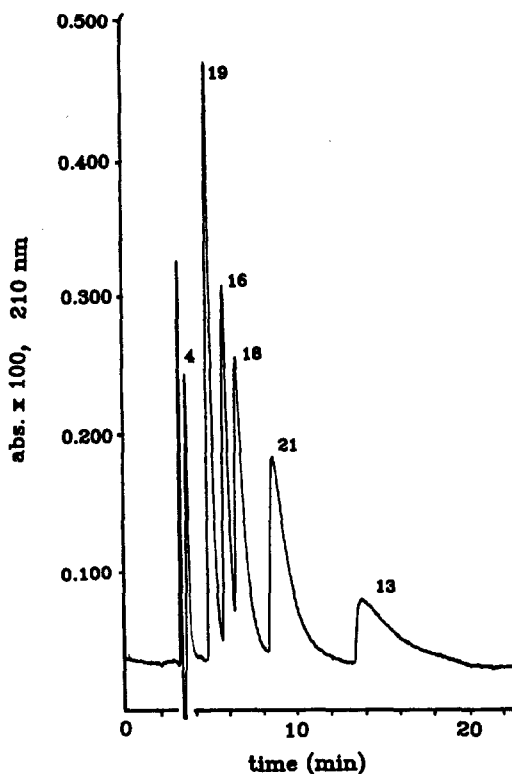


Fig. 1. Typical retention of substituted benzenes on porous graphitic carbon eluted with heptane. Solute labels correspond to Table I.

Our initial hypothesis pictured the substrate electron band participating in direct π - π orbital overlap between the aromatic ring of the solutes and the substrate (stationary phase). Had this idea been correct, good correlation between solute retention and the energies of the available π -bonding orbitals, E_{homo} and E_{lumo} , would

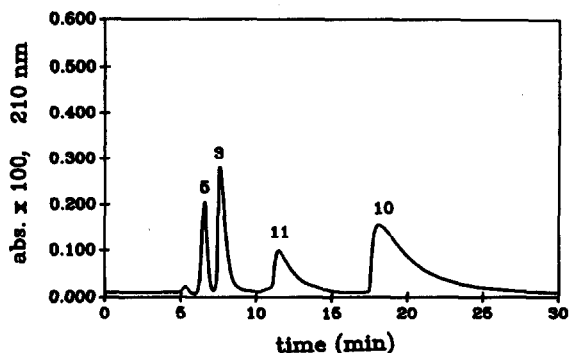


Fig. 2. Typical retention of substituted benzenes on palladium spheres eluted with heptane. Solute labels correspond to Table I.

have been expected. However, in the case of retention on porous graphitic carbon, we found the solute retention to be correlated only weakly to E_{homo} and E_{lumo} , with a stronger correlation to the submolecular polarity parameter, Δ , a measure of the localized or concentrated dipole of a molecule. The strongest correlation observed is that shown in eqn. 1:

$$\begin{aligned} \log k' &= 2.10\Delta - 1.01 & (1) \\ n = 21 \quad r &= 0.800 \quad F\text{-value} = 33.7 \quad \text{significance} = 1.37 \cdot 10^{-5} \end{aligned}$$

It was found that improved correlation equations could be obtained by separating the phenols from other substituted benzenes as shown in eqns. 2 and 3:

For substituted benzenes

$$\begin{aligned} \log k' &= 1.57\Delta - 0.277 E_{\text{homo}} - 3.49 & (2) \\ n = 11 \quad r &= 0.972 \quad F\text{-value} = 69.3 \quad \text{significance} = 8.86 \cdot 10^{-6} \end{aligned}$$

For phenols

$$\begin{aligned} \log k' &= -39.4\Delta - 2.45 E_{\text{lumo}} + 17.9 & (3) \\ n = 10 \quad r &= 0.907 \quad F\text{-value} = 16.3 \quad \text{significance} = 0.00230 \end{aligned}$$

While the majority of the correlations was attributed to the submolecular polarity parameter, Δ , alone ($r = 0.923$ and -0.747 , sig. = $5.02 \cdot 10^{-5}$ and 0.0131 , for benzenes and phenols, respectively) better models were formed by including the contributions of E_{homo} and E_{lumo} (above).

These and other analyses indicated that a localized polar segment of the molecule was responsible for retention rather than the π -orbitals of the aromatic ring, reflected by E_{homo} . Equivalent studies on the palladium metal substrate produced similar results, in that retention was best correlated to a localized segment of the solute molecule, reflected by Δ , rather than to the π -orbital energies alone. The equations observed for palladium are shown in eqns. 4 and 5:

For the entire solute set

$$\begin{aligned} \log k' &= -2.79\Delta + 1.05 & (4) \\ n = 15 \quad r &= 0.610 \quad F\text{-value} = 7.70 \quad \text{significance} = 0.058 \end{aligned}$$

For phenols only

$$\begin{aligned} \log k' &= -45.0\Delta - 2.75 E_{\text{lumo}} + 20.2 & (5) \\ n = 10 \quad r &= 0.826 \quad F\text{-value} = 7.53 \quad \text{significance} = 0.018 \end{aligned}$$

Again, primary correlation was achieved with Δ alone ($r = -0.685$, significance = 0.895), with the complement of E_{lumo} .

Furthermore, alkyl- and halogen-substituted benzenes were unretained on the palladium stationary phase and only very weakly retained on porous graphitic carbon. Solutes containing oxygen or nitrogen (n-electron donors) were retained more strongly

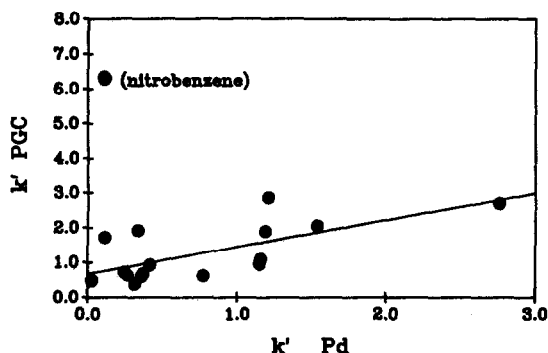


Fig. 3. Comparison of retention, k' , of substituted benzenes and phenols between porous graphitic carbon (PGC) and palladium (Pd). Regression line does not include nitrobenzene.

on both phases. With the exception of nitrobenzene, good correlations between the retention properties of palladium and porous graphitic carbon were observed under non-polar solvent conditions (Fig. 3).

On a "perfect reversed-phase", where only dispersive interactions could take place, very little solute retention would be expected with non-polar eluents. It was difficult to find such a material to serve as the control stationary phase. An exhaustively coated alumina/cross-linked polybutadiene phase²⁰, used under similar non-polar conditions, exhibited strong retention towards a number of solutes, presumably due to unshielded accessible aluminol sites or oxidized sites on the butadiene. However, no similarity in retention properties to the metallic phases was observed. Polystyrene-based materials were not suitable as control stationary phases, due to their aromatic nature.

Upon closer examination of solute retention on porous graphitic carbon and palladium, the polar substituent on the solute ring was further implicated in retention. Positional isomers of substituted phenols showed a decrease in retention for *tert.*-butyl-substituted phenol when the substituent was moved from the *para*- to the *meta*- and finally to the *ortho*-position. Inductive and resonance effects on the solute ring could be ruled out due to the dissimilar behavior of the *ortho*- and *para*-substituents. In addition, as the bulkiness of the *ortho*-substituent increased, retention decreased on both porous graphitic carbon and palladium, further highlighting the activity of the hydroxyl group in the retention of phenols on porous graphitic carbon and palladium (Fig. 4).

	k'	
	PGC	Pd
R = <i>i</i> -Pr	0.68	0.37
= <i>s</i> -Bu	0.60	0.36
= <i>t</i> -Bu	0.38	0.31
R' = <i>t</i> -Bu	0.93	0.42
R'' = Methyl	2.9	1.2
= Ethyl	1.9	1.2
= <i>s</i> -Bu	1.1	1.2
= <i>t</i> -Bu	0.95	1.1

Fig. 4. Retention of phenol isomers on porous graphitic carbon (PGC) and palladium (Pd) as a function of substituent size and ring position. *i* = iso; *s* = *sec.*; *t* = *tert.*; Bu = butyl; Pr = propyl.

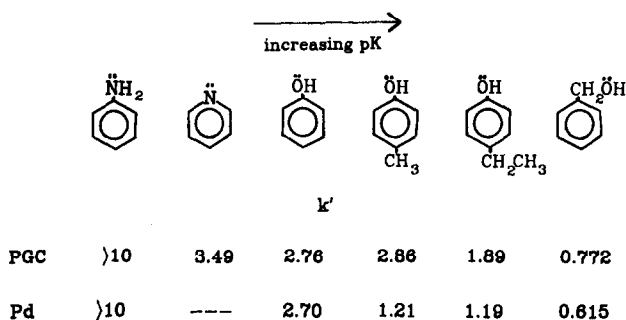


Fig. 5. Solute basicity and retention. PGC = Porous graphitic carbon; Pd = palladium.

The ability of the polar group to donate a lone pair of electrons to the substrate was indicated by observing retention trends and basicity of the polar group lone pair (Fig. 5). Specifically, where pK values were available for solutes, a linear trend of pK to solute retention was apparent (Fig. 6), even though the number of data points was limited.

The correlation of retention with basicity and the loss of retention when access to the unshared pair of electrons on basic solutes was hindered, in addition to the poor retention characteristics of solutes unable to function as n -electron donors on porous graphitic carbon and palladium, offered strong evidence that the dominant retention mechanism of the substituted aromatic molecules studied was an electron-pair donor-acceptor interaction.

To a first approximation, ultra-high vacuum studies of metallic surfaces support the types of solute-substrate interactions encountered in HPLC on electron-band substrates. For example, studies of solute adsorption on palladium metal in ultra-high vacuum²¹ support the idea of an n - σ donor-acceptor interaction of the lone electron pair governing adsorption of pyridine, as opposed to the adsorption of benzene or xylene which is dominated by π -electron transfer. As a result, the aromatic ring, which is normally adsorbed parallel to the metal surface, is adsorbed polar-end-down when a lone electron pair, as in pyridine, phenol, or aniline, is present.

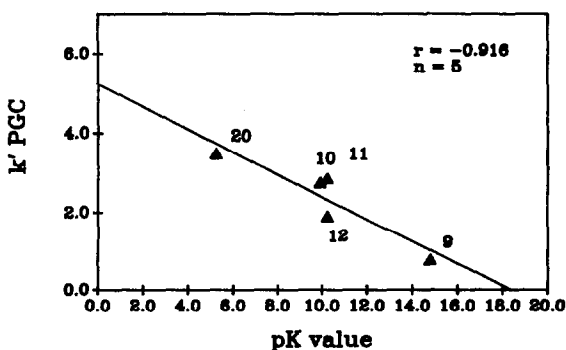


Fig. 6. Dependence of retention on solute pK on porous graphitic carbon (PGC). Solute labels correspond to Table I.

Additional support of such a mechanism is found in argentation chromatography, where silver ions are incorporated into the mobile or stationary phase of a reversed-phase system to increase selectivity of various unsaturated hydrocarbons and azaheterocycles^{9,10,12,13}. While the delocalized electron bands of porous graphitic carbon and palladium do not form the same discrete metal–ligand complexes found with metal ions, their selectivity and bond strength follow the same order under similar solvent conditions¹², based on the basicity and spatial environment of solute lone-electron pairs.

Surface analysis of palladium

Scanning Auger spectroscopy showed the palladium spheres used as HPLC substrates to be essentially free of surface oxide and foreign metal contamination, as demonstrated by the characteristic shape and separation of the palladium $3d_{3/2}$ and $3d_{5/2}$ peaks. Extensive analyses indicated that the palladium surface was chemically clean.

CONCLUSION

A porous graphitic carbon stationary phase for HPLC was demonstrated by statistical approaches to behave primarily as an electron pair acceptor for substituted aromatic solutes capable of n-donation under non-polar solvent conditions. The retention order of the solutes closely followed the basicity of the lone electron pair on various solutes. Diminished retention of solutes with sterically hindered lone pairs was also consistent with this interpretation. Palladium metal exhibited trends qualitatively similar to graphite under normal-phase conditions.

Due to strong adsorption of water to the palladium surface, it was not possible to compare porous graphitic carbon materials with palladium under reversed-phase conditions. It was also found that palladium was easily “poisoned” by solutes which had very high affinities for the stationary phase. Work is in progress to examine other metals, in particular gold, to determine whether changes in retention levels and orders follow that predicted from the known band properties of these two metals. It is possible that the electronic properties observed can be attenuated through coverage of the metal with a thin aromatic polymer film, and/or by the use of semi-conductive polymers.

Regardless of these practical problems, the development of efficient, electrically conductive chromatographic surfaces should permit several interesting new experiments, such as the use of a chromatographic sorbent as an electrode, in an attempt to modulate retention as a function of potential^{22,23}. They may also function as improved charge-transfer-type materials. Research is currently underway to investigate these applications.

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