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HPLC of Some Polar Compounds on a Porous Graphitized Carbon HypercarbTM Column

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Abstract: The chromatographic performance of a HypercarbTM stationary phase was studied by using benzene, indene, and some polar compounds as probes. The effect of the mobile phase composition on the retention of the substances was investigated. The influence of organic modifier acetonitrile on the retention was also studied and interpreted on the basis of intermolecular interactions. Significant retention of these molecules was observed in acetonitrile-water eluents. The multitype nature of interaction of the solute on the reversed phase surface and its charge induced interactions with polar molecules were responsible for significant retention of these substances. Separation on the HypercarbTM column showed that the retention order of solutes generally does follow their polarity order. The dispersion and orientational interactions undoubtedly influence, but do not solely determine, the elution order of solutes. According to the chromatographic data, it can be concluded that the retention of the investigated substances is defined by the interaction of the polarized or polarizable functional groups in the samples with a graphite surface (charge-induced interactions), which is termed in the literature as the “polar retention” by HypercarbTM.

Keywords: HypercarbTM, Retention mechanism, HPLC, Nitrogen-heterocyclic compounds, Graphitized carbon, Polar retention

INTRODUCTION

In many respects, the progress of modern chromatographic science depends on the development of new sorbents with varied thermodynamic properties.

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Recently, research interest has focused on the search for new, highly effective and universal sorbents for liquid chromatography. In this regard, graphitized porous carbon has attracted special emphasis. Porous graphitic carbon is a relatively new material for high performance liquid chromatography (HPLC).^[1,2] The use of graphitic carbon for chromatography has been actively developed in the last ten years. Many subsequent studies have shown that the carbon columns have exclusive properties compared to silica based packing materials and ion exchangers, the matrixes of which are organic polymers.

In 1978, Knox and Gilbert patented a method for the production of porous carbon.^[3] In this methodology, a high-porosity HPLC silica gel is impregnated with a phenol-formaldehyde resin. The resin is carbonized slowly at 1173–1273 K in nitrogen or argon, and silica particles are dissolved out with alkali. They called this new material “porous glassy carbon”. Initial research confirmed that the material was microporous with poor chromatographic performance. On heating to 2273–3073 K, the micropores closed and the material became graphitized. This material was called porous graphitized carbon, and is now marketed under the trade name HypercarbTM. The typical physical characteristics of HypercarbTM are shown in Table 1. Further studies, carried out by the different authors, have shown that this sorbent possesses various interesting properties.

The carbon columns are physically and chemically stable. The chemical stability of the HypercarbTM column expands the application of polar compounds in reversed phase chromatography. They can resist strong acidic and alkaline conditions, and no swelling is observed in the various solvents including 100% water. The development of these graphitic carbon HPLC columns was also motivated by the fact that the application of silica or silica based supports in HPLC is limited by the low stability of silica at high pH values^[4] and, in the case of reverse phase silica based supports, by undesirable electrostatic interactions between the polar regions of solutes and the free silanol groups not covered by the hydrophobic ligand. Liem et al.^[5] demonstrated that a HypercarbTM column has a distinct advantage over C-18 reverse phase columns in terms of column stability over the complete pH range of 1–14. It is also well known that the use of 100% aqueous solution (as the HPLC mobile phase) may collapse the C-18 (alkyl)-bonded phase and result in a retention time shift. The HypercarbTM columns can be exploited by 100% aqueous phases for a long time, as

Table 1. Typical physical characteristics of HypercarbTM [3]

Particle size, μm	Pore size, \AA	Pore volume, cm^3/g	Spec. surface, m^2/g	Mech. stability, bar
5–7	250	0.7	120	>400

confirmed by the perfect reproducibility of retention times and the forms of the peaks.

The flat adsorptive surface of HypercarbTM shows extraordinary separating properties for structurally related compounds. The separation of enantiomers (chiral separation) can be achieved by the addition of optically active modifiers to the mobile phase.^[6-10] As a result, the HypercarbTM column has been extensively applied for the separation of ionized and highly polar compounds,^[11-14] geometric isomers,^[15,16] enantiomers, sugars, carbohydrates, and glucosides.^[17-19]

In many cases, chromatography is an alternative and successful approach for also estimating the various physicochemical properties. Data on the chromatographic behaviours of polar compounds, which is one of the most important fields in modern chemistry, gives useful information for physical, combinatorial, and medicinal chemistry. In addition, although liquid chromatography is a powerful separation method, the separation of very polar compounds remains difficult. Furthermore, from the point of view of the adsorption's theory of substances by surfaces, studying the chromatographic behavior of polar compounds has practical applications and theoretical interest.

Recently, nitrogen-containing heterocyclic compounds have been developed as medical and biological agents to combat the various diseases. During the late 20th century they were introduced as orally active compounds in medicine and the number of such heterocyclic compound drugs is likely to expand in the near future. They represent a successful strategy for chemotherapeutic development. For these reasons, recent research has concentrated on the synthesis and design of heterocyclic drugs. In particular, the class of azoles (imidazole and triazole derivatives) has supplied many effective drugs currently in clinical use, and newer azoles with an expanded spectrum of activity are at the moment in continuous development. An interdisciplinary approach to their chemistry is currently directed towards medicinal chemistry, supramolecular chemistry, and advanced organic materials.


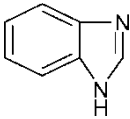
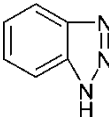
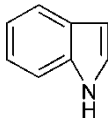
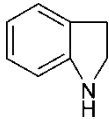

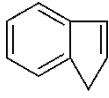

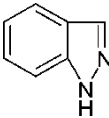
This paper studies the retention mechanism of benzene, indene, and some polar heterocyclic substances by the HypercarbTM column.

EXPERIMENTAL

Reagents

The standard compounds presented in Table 2 were obtained commercially from Sigma (St. Louis, MO, USA). HPLC grade acetonitrile used was purchased from Panreac Corp. (Spain), and trifluoroacetic acid (TFA) from Sigma (St. Louis, MO, USA). The water was filtered by a Millipore ultra pure water system (Millipore, Bedford, MA, USA).

Table 2. Structural formulas and molecular weights of the investigated substances

Substance	Structural formula	Molecular weight, M	Empirical formula
Benzene		78.11	C ₆ H ₆
Benzimidazole		118.14	C ₇ H ₆ N ₂
1,2,3-Benzotriazol		119.13	C ₆ H ₅ N ₃
Indole		117.15	C ₈ H ₇ N
Indoline		119.17	C ₈ H ₉ N
Imidazole		68.08	C ₃ H ₄ N ₂
Indene		116.16	C ₉ H ₈
1,2,4-Triazole		69.07	C ₂ H ₃ N ₃
Indazole		118.14	C ₇ H ₆ N ₂

Sample Preparation

Five milligrams of each substance were dissolved in 1 mL of the twice distilled water or in the mixture of the twice distilled water and acetonitrile. A constant injection volume (5 μ L) was used throughout for the individual solution.

Apparatus and Method

The database of the retention times used in this study was generated using a Spectra System P4000 liquid chromatography system equipped with a Rheodyne (Cotati, CA, USA) injector, a 50 μL sample loop, and a Spectra System UV3000 detector. The flow rate of the mobile phase was 1.0 mL/min and it was monitored at wavelengths of 210, 254, and 280 nm. The Hypercarb™ column was purchased from Thermo Hypersil-Keystone. The column size was 100.0 \times 3.0 mm and particle size was 5 μm . The dead time (t_M) was determined as the retention time of a 1% solution of sodium nitrite. The mobile phase flowed in isocratic mode. Various binary systems of 0.1% TFA in water and acetonitrile were used. All the experimental runs were performed at 293 K. The signal was acquired and processed by the ChromQuest software. The quantum chemical calculations were performed using the HyperChem software (Hypercube Inc., FL, USA, version 7.0) on a PC. Semi empirical AM1 calculations were used for geometry optimization (293 K) of the molecules by the software. The polarizability (α) and dipole moment (μ) are the physicochemical properties that were calculated for each molecule. The retention factor (k) can be calculated according to Eq. (1).

$$k = (t_R - t_M)/t_M \quad (1)$$

were t_M is the dead time, and t_R is the retention time. The retention factors and calculated physicochemical properties of the investigated substances are summarized in Table 3.

RESULTS AND DISCUSSION

In this paper the retention mechanism is discussed based on analysis of the chromatographic results of the studied compounds. Although the chromatographic retention on different carbonized stationary phases in HPLC has been described in many papers,^[3,4,11] an investigation of the chromatographic behavior of nitrogen containing heterocyclic compounds is a new area of research. The chromatographic analysis of molecular interactions should provide a foundation to analyze the simplest model compounds.

For studied substances, one of the most important factors in their chromatographic retention is the dispersion interactions between the surfaces of the solute and the stationary phase, which are related to the steric factors, molecular size, and branching. By analyzing the experimental chromatographic behavior of such molecules, we observed the influence of the nature of the substances on the chromatographic retention.

The evaluation of unspecific (dispersion) interactions with the sorbent can be conducted by analyzing the dependence of the retention factors of the sorbates on their molecular weights. The dependences of the common

Table 3. Retention factors and some physicochemical properties of the investigated substances

Substance	Dipole moment, μ (D)	Polarizability, α (Å ³)	Retention factor, k	
			Water + 0.1% TFA	Acetonitrile/Water + 0.1% TFA (20/80 vol%)
Benzene	0.000	10.43	1.33	0.11
Imidazole	1.467	7.60	0.44	0.18
1,2,4-Triazole	0.944	6.89	0.29	0.13
Benzimidazole	4.392	13.78	1.75	1.23
1,2,3-Benzotriazol	2.608	13.07	Not elute	20.87
Indazole	1.003	13.78	0.49	0.23
Indole	2.463	14.49	0.50	0.21
Indoline	2.719	14.68	0.51	0.29
Indene	0.402	14.97	0.43	0.22

logarithms of the retention factors of some substances on the molecular weights are represented in Fig. 1 for several different eluents. This figure clearly shows that the retention does not change with an increase in the molecular weight of the sorbates, which indicates the weak contribution of the dispersion interaction on the retention. Therefore, it is necessary to take into consideration the permanent physicochemical characteristics of substances, which should provoke the specific interactions with any apolar phase.

Chromatographic retention results from the solvation and partition of the individual compounds in a stationary phase. The chromatographic behavior is affected by intermolecular forces which include van der Waals, ion–dipole, dipole–dipole, dipole induced dipole interactions, etc. The chromatographic partition, in turn, results from adsorption–desorption of the compounds on the stationary phase, during which the degree of freedom of the molecule changes. The entropic effects also play important roles. As a result, chromatographic retention depends mainly on the physicochemical properties, and the size and shape of the solute molecule.

The polarizability (α) of a molecule, an important physical property which is currently attracting research attention in the description of chemical interactions, is a measure of the overall electronic charge distribution that can be distorted by an external electric field and also depends on the molecular volume. The influence of the polarizability on the chromatographic retention with different eluents is shown in Figs. 2 and 3. It is very evident that on the HypercarbTM column with the purely aqueous mobile phase, the retention factor decreased appreciably with increasing polarizability value. The modification of the eluent by the addition of 20% acetonitrile

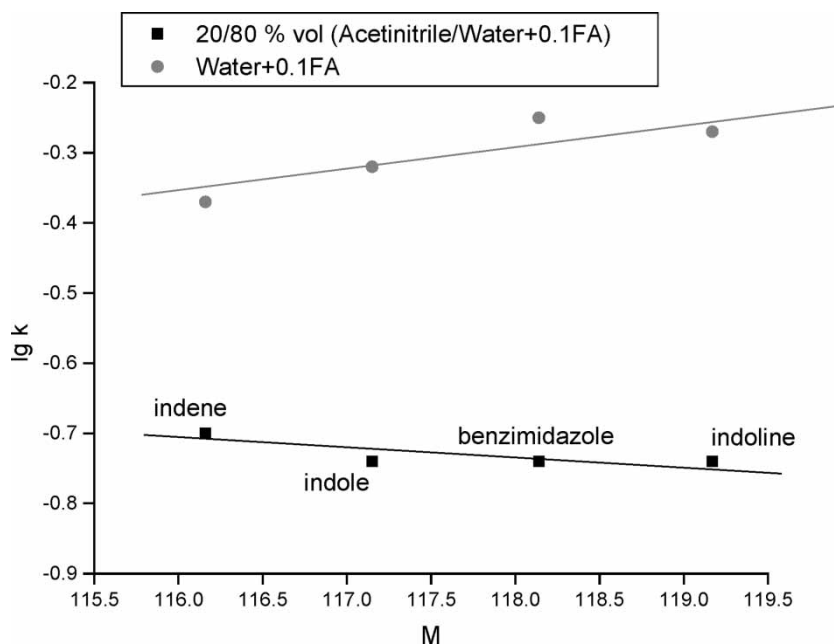


Figure 1. Retention factor (k) of the sorbates as a function of the molecular weight (M) on the Hypercarb™ column.

decreased the chromatographic retention for all sorbates. Furthermore, the polarizability did not influence the retention. This phenomenon has been shown to influence the contribution of specific interaction “sorbate-eluent”, leading to the conclusion that dipole–dipole and van der Waals intermolecular interaction with the mobile phase are more important than nonspecific interaction in retention of the compounds. An increase in the eluting force of the mobile phase leads to the decrease of the contribution of unspecific dispersion interactions, due to the growth of the contribution of the specific interactions of sorbates with the eluent.

However, it is necessary to note also that the elution order of the sorbates does not change with variation of the mobile phase composition. From these results, one can deduce that the physicochemical interactions of the substances with the mobile and stationary phase Hypercarb™ are similar for all the investigated substances. The parameter of the polarity of the sorbates, taking into account the field and resonance effects, was analyzed using total dipole moment (μ). This parameter is one of the fundamental conformation dependent physical properties of a polar molecule.^[20] Even though the total charge on a molecule is zero, the nature of chemical bonds is such that the positive and negative charges do not completely overlap in most molecules. To obtain more information about the interaction of compounds with both stationary phases, the correlation between the retention factor measured at

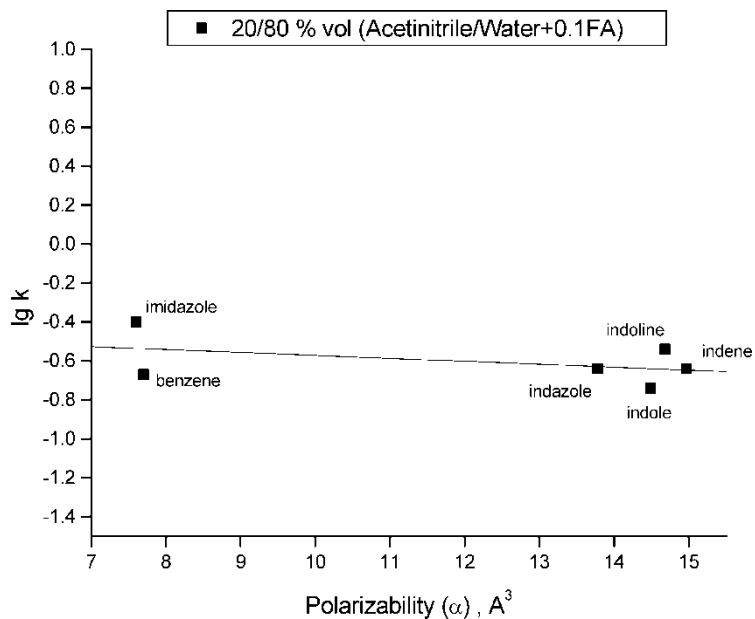


Figure 2. Retention factor (k) of the sorbates as a function of the polarizability (α) on the HypercarbTM column with the aqueous/organic mobile phase.

298 K, while using dissimilar eluents and the dipole moment, is demonstrated in Fig. 4. Figure 4 clearly shows that for the set of investigated substances such dependences have a multifaceted character.

Knox and Ross^[1-3] summarized the retention by graphite from aqueous/organic eluents, determined by a balance of several factors that was termed the “polar retention” of HypercarbTM. In this case, the elution’s order coincides with increasing of polarity by contract to the expectation of a reversed phase column. More polar samples were more strongly retained than less polar samples.

The k value of the samples, except 1,2,3-benzotriazol, showed an exponential dependence with polarity parameters (dipole moment). This substance had a high retention factor (20.87) when using the modified eluent. The mobile phase, consisting of water only (water + 0.1% TFA), did not elute this substance in all cases (Table 3) because of the very low solubility of 1,2,3-benzotriazol in water. Therefore, 1,2,3-benzotriazol is not shown in Fig. 4 at all.

The chromatographic retention of benzene is also determined by its solubility in water. The retention time of nonpolar benzene grew sharply when using water with TFA as the mobile phase. In this case, benzene was eluted directly before polar benzimidazole. The treatment of eluent with 20% acetonitrile sharply reduced the retention time of the nonpolar benzene because

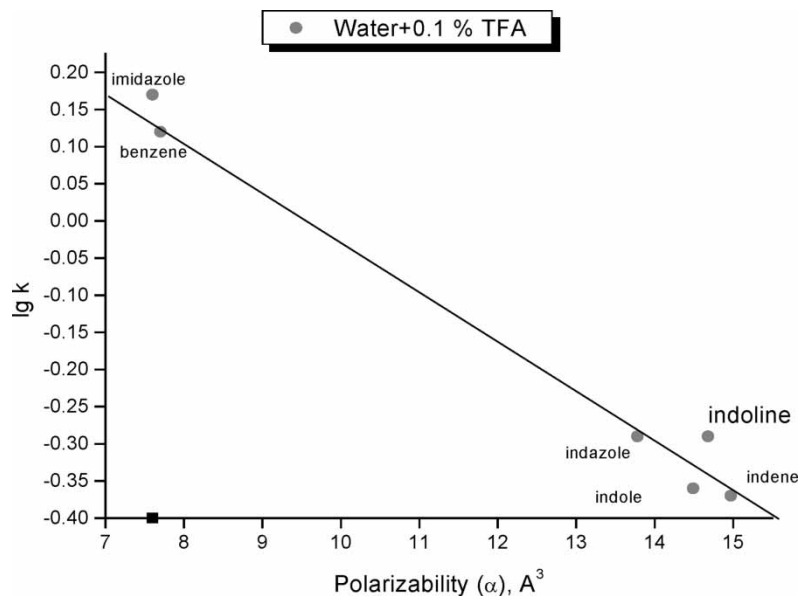


Figure 3. Retention factor (k) of the sorbates as a function of the polarizability (α) on the Hypercarb™ column with the aqueous mobile phase.

benzene's solubility in the mobile phase composition was increased. As expected, nonpolar benzene was eluted from the column first, as a result of the "polar retention".

The "polar retention" phenomena can be estimated by comparing the chromatographic behavior of the following sorbates. Thus, imidazole and 1,2,4-triazole, that, in effect, have identical molecular weights, were eluted at different times. This can be explained by the large contribution of specific interactions of 1,2,4-triazole with the sorbent Hypercarb™. The appearance in 1,2,4-triazole of the additional nitrogen atom, in comparison with imidazole, increases its polarity and, as a result, increases the chromatographic retention.

The effect of polar confinement also appeared in examples of other studied substances. Therefore, indoline, indazole, and benzopyrrole, which have close molecular weights but different dipole moments, were eluted from the column at different times. It is obvious that these sorbates underwent a characteristically specific interaction with the surface area of the Hypercarb™, caused by the presence of the electron lone pair of the nitrogen atoms in the heterocycles. It was demonstrated by a statistical approach that the Hypercarb™ primarily behaves as an electron pair acceptor for substituted heterocyclic sorbates, which are capable of electron donation under non polar condition. The retention order of the samples closely followed the basicity of their lone-electron pair.

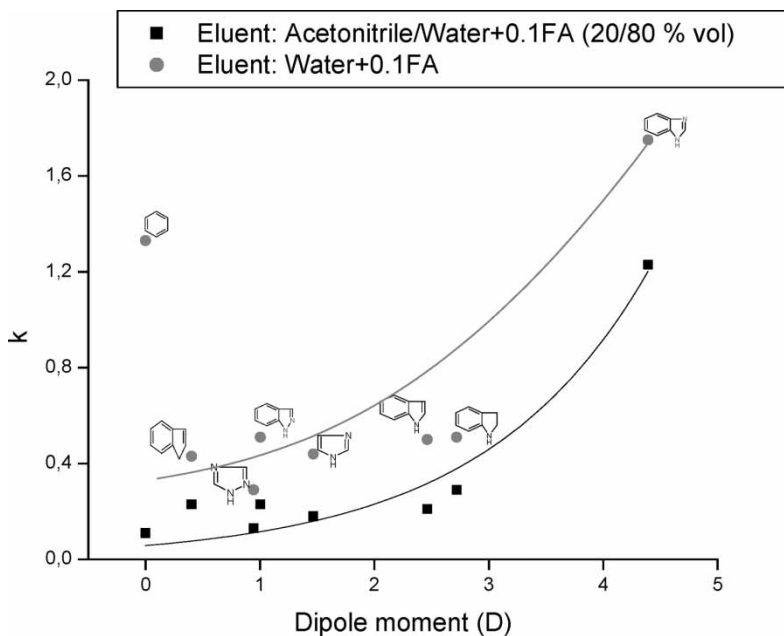


Figure 4. Retention factor (k) of the sorbates as a function of the dipole moment (D) on the Hypercarb™ column.

However, the chromatographic retention of the molecules by the sorbent Hypercarb™ is determined not only by the polarity of the sorbates, but also gains a contribution from the orientational interaction confinement. Thus, from Fig. 4 it is obvious that the low-polarity molecule of indene is retained practically (eluent water/acetonitrile 80/20%), just as in the case of indazole, which has a mean polarity and a molecular weight that is similar to that of indene. In this case, the effect of the stabilization of its electron structure, caused by delocalization of the π -electrons of the benzene ring and five-term nitrogen containing heterocycles, influenced the retention of indene.

Consequently, the chromatographic retention of the investigated substances by Hypercarb™ from the aqueous/organic eluent system can be determined by a balance of the multifarious interactions. Dispersive interactions (London type) are present between the graphite surface and the sample. They have an unspecific nature and become determined mainly by the molecular weight of sorbates. The hydrophobic interactions, which tend to drive samples out of solution, may have an important effect on the selectivity of the Hypercarb™. There are similar interactions between the Hypercarb™ surface and the eluent, which is displaced by the sample. Finally, it is a special interactions mode, which determines the extraordinary chromatographic characteristics of the Hypercarb™. In the literature these interactions are

termed “polar retention” by Hypercarb™. They consist of the interaction of polarized or polarizable functional groups of the samples with a graphite surface (charge-induced interactions), and this stereochemistry of the sample molecule forces the polar group close to the graphite surfaces.

CONCLUSION

The Hypercarb™, as a porous graphitized carbon support, demonstrated unique retention characteristics. Chromatographing on the Hypercarb™ column indicated that the retention order of the solutes generally does not follow their hydrophobicity order. The molecular hydrophobicity undoubtedly exerts an influence, but does not determine the elution order of the sorbates. The chromatographic behaviors of the investigated molecules are resolved by a balance of the varied interactions, among which “polar retention” has the largest responsibility.

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