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Sorption and Retention Behaviours of Benzene and Some Five-Membered Heterocycles on a Porous Graphitized Carbon Column

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The sorption on a porous graphitized carbon column was tested by using benzene and some five-membered heterocycles as a sorbates. The performance of five solutes with two types of eluents was examined. The influence of mobile phase modifier on the retention was discussed. The Langmuir isotherm was applied to describe the adsorption of substances on the surface of the graphitized adsorbent. The parameters of the adsorption isotherms were compared with different mobile phase compositions. The exponential curves of benzene, imidazole, indole and indoline are logarithmically increased in pure aqueous eluent, but 1,2,4-triazole had showed linear adsorption isotherm. The solubility, solvophobic effects and selective interactions between solute polarizable or polarized functional groups with graphite affect the sorption of substances.

Key Words: Sorption, Retention, Five-membered heterocycles, Porous graphitized carbon.

INTRODUCTION

It is well-known that the evolution of modern separation science depends on the development of original sorbents with various properties. In recent times, research activity has focused on the search for new, highly effective and universal sorbents for separation. Knox and Gilbert[1] patented a method for making a robust porous carbon that possessed the required properties for use in high-performance liquid chromatography. These authors showed that a porous graphitic carbon does not have the true graphite structure and composed of layers of hexagonally arranged carbon atoms in the sp^2 hybridization[2,3]. It is established that a porous graphitic carbon is available as 5 and 7 μm particles. The media have a specific surface area of 120 m^2/g, a 250 Å median pore diameter and a 75 % porosity. The packing material can withstand pressures of at least 400 bar and the packing material is stable over the entire pH range of 0-14[4]. This sorbent demonstrate mechanical rigidity and chemical stability, that is why provides the evergrowing use in separations. The development of graphitic carbon sorbents specially for HPLC was motivated by the fact that the application of silica or silica-based supports is limited by the low stability of silica at high pH values and in the case of reverse-phase silica-based supports, by undesirable electrostatic interactions between the polar regions of solutes and the free silanols groups not covered by the hydrophobic groups. It is no wonder that a porous graphitized carbon, a.k.a. Hypercarb^{TM}, has extensive application for separation of the ionized and highly polar compounds[5-9], geometric isomers[10,11] and enantiomers[12-16].

Large families of aromatic and five-membered heterocycles are important for modern chemistry, biology and medicine. It is beyond any doubt that adsorption behaviours of such solutes have practical and theoretical interest and gives useful information for adsorption's theories[17]. An additional point to emphasize is that the rapid development of applications of preparative and simulated moving bed (SMB) separations in the industry has led to a recent renewal of interest in the fundamentals of non-linear chromatography[18,19]. Under such conditions, the equilibrium isotherms between the two phases of the separation system are rarely linear[20,21]. Most models of single-component adsorption isotherm applied in liquid chromatography are semi-empirical extensions of models derived for gas-solid equilibria. This extension remains valid so long as the solute concentration in the liquid phase is sufficiently low and the solute is strongly adsorbed[22,23]. In general, adsorption isotherms can be determined only experimentally. Despite of the fact that there are currently a quite large number of experimental methods available, their appropriate and efficient application is still far away from being a routine job. In the meantime, the importance and the benefit of these isotherms have been widely realized. In particular for this reason, in the last years many equilibrium studies have been performed[24]. It is generally recognized that the nature of solid and liquid phases plays dominant role in HPLC[25], although the mechanism of its influence on the analyte retention is a subject of intense researches. The important part of this mechanism is the adsorption on a surface. The fundamental mechanisms of cyclic compounds adsorption have been determined *i.e.*, π-π interactions and donor-acceptor complex formation, taking into account the influence of the so-called solvent effect. Adsorption behaviours has been studied for many years and many experimental adsorption isotherms have been reported[26,27]. The application of these isotherms in regards to the interpretation of the retention of different analytes is quite satisfactory. On the other hand, the difficulties in interpreting adsorption data expressed in surface specific still exist[27]. The porous graphitized carbon Hypercarb^{TM} is quite new sorbent for the separation area and one is forced to accept the fact that an absorptions mechanism is far from fully explored.

In this work, we investigated the effect of eluent on the retention of benzene and some five-membered heterocycles and Langmuir-type isotherm was applied to describe the adsorption of solutes on the porous graphitized carbon column.

EXPERIMENTAL

The standards (analytical grade) were obtained commercially from Sigma (St. Louis, MO, USA). The acetonitrile (HPLC grade) was purchased from Panreac Corp. (Spain). Trifluoroacetic acid (TFA) was purchased from Sigma (St. Louis, MO, USA). The water was obtained from a Millipore ultra pure water system (Millipore, Belford, MA, USA).

The database of the retention times was generated using a Spectra System P4000 liquid chromatography system equipped with a Rheodyne injector (Cotati, CA, USA), a 50 μL sample loop and a Spectra System UV3000 detector at 210 nm. The Hypercarb^{TM} column was purchased from Thermo Hypersil-Keystone. The column size was 100.0 × 3.0 mm i.d. and particles size was 5 μm. The flow rate of mobile phase was 0.7 mL/min in isocratic mode. Various systems of 0.1 % trifluoroacetic acid in water/acetonitrile were used. Injected samples of test compound solutions varied in volume from 1 to10 mL. All experimental runs were performed at 293 K. The signal was acquired and processed by the ChromQuest software.

Sample preparation: Each substance was dissolved in distilled water or in a mixture of distilled water and acetonitrile to make the appropriate solution (benzene 0.40 mol/L, imidazole 1.77 mol/L, indole 1.07 mol/L, indoline 1.04 mol/L and 1,2,4-triazole 0.45 mol/L). These solutions were sonicated for 0.5 h and were then stored at 277 K. It should be emphasized that the working solutions were re-prepared after every two days, so as to avoid potential errors arising from decomposition.

Adsorption equilibrium: The retention factor (k) is calculated by the following equation:

$$k = (t_R - t_0)/t_0 \tag{1}$$

where t_R is the retention time of the sample and t_0 is the retention time of the non-retained peak, a.k.a. the dead time (taken as the first deviation of the baseline following injection of 4 μL KNO_3). The retention factors reported in this study are the averages of at least three determinations. The accuracy of the determination of retention time was no higher than 1 %. Evaluation of the results of the chromatographic experiment was carried out using mathematical statistic techniques. The phase ratio (F) can be calculated from the eqn. 2.

$$F = (1 - \varepsilon)/\varepsilon \tag{2}$$

where ε is the total void fraction of column. The Langmuir isotherm is probably the mist widely applied adsorption isotherm. A basic assumption of this model is that adsorption takes place at specific homogeneous sites within the adsorbent. The material balance and Langmuir adsorption isotherm[21,28-30] were used as eqn. 3 and 4. For explanation of eqn. 3 and 4, finite element method (FDM) was used as eqn. 5.

$$u\frac{\partial C_i}{\partial z}+\varepsilon\frac{\partial C_i}{\partial t}+(1-\varepsilon)\frac{\partial q_i}{\partial t}=0 \qquad (3)$$

$$q_i=\frac{aC_i}{1+bC_i} \qquad (4)$$

$$u\Delta t(C_{z+Dz,t}-C_{z,t})+\varepsilon(C_{z+\Delta z,t+\Delta t}-C_{z+\Delta z,t})+(1\text{-}\varepsilon)\,(q_{z+\Delta z,t+\Delta t}-q_{z+\Delta z,t}) \qquad (5)$$

where u is the interstitial velocity (m/min) and Δ_z is the theoretical plate number of the solute according to the length direction changes. The parameter a of Langmuir isotherm was obtained from the assumption of equilibrium constant at the low injection concentration using relation equation a = k/F. Then parameter b was obtained from eqn. 5 with repetition calculation until the value of calculated retention time is same with experimental one[31].

RESULTS AND DISCUSSION

In this paper, the chromatographic performance has been investigated and adsorption isotherm of benzene and five-membered heterocycles was discussed. The Langmuir isotherm was applied to indicate the adsorption-desorption of solutes on the porous graphitized carbon column.

Table-1 shows the chemical structures, solubility in water[32], logarithm of partition coefficients (log $P_{oct/water}$) and polar surface area (PSA) of benzene, imidazole, indole, indoline and 1,2,4-triazole. The calculation of log $P_{oct/water}$ and PSA was performed using the ChemOffice program (Cambridge Soft, MA, USA). These characteristics are important physical properties that are currently attracting research attention in the description of chemical interactions. The values of retention factors are listed in Table-2. For all test compounds, retention times with the two mobile phases are close to each other; however, for the eluent modified with acetonitrile, the retention factors are significantly smaller than those for the 100 % water eluent. For sorbates, one of the most important factors in their chromatographic retention is the interactions between the solute and the surfaces of stationary phase, which are related to the steric factors, molecular size, branching, *etc*. The chromatographic retention results from the solvation and partition of the individual compounds in a stationary phase. The chromatographic behaviour is affected by intermolecular forces which include vander Waals, ion-dipole, dipole-dipole, dipole-induced dipole and dispersions interactions. The chromatographic partition results from adsorption-desorption of the sorbate on the stationary phase, during which the degree of freedom of the molecule changes. As a result, retention mechanism is very complex and depends mainly on the physico-chemical properties of sorbates and sorbents.

TABLE-1
STRUCTURAL FORMULAE, SOLUBILITY IN WATER, LOGARITHM OF PARTITION COEFFICIENTS (log $P_{oct/water}$), POLAR SURFACE AREA (PSA) OF THE SUBSTANCES

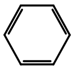
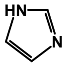
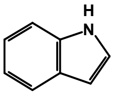
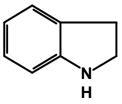
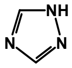
Substance	Structural formula	Solubility in water, g/100 mL, 298 K (Ref. 32)	log $P_{oct/water}$	PSA
Benzene		0.18	1.8660	0.0
Imidazole		66.30	-0.6690	24.1
Indole		0.19	1.8120	12.8
Indoline		0.50	1.5089	12.8
1,2,4-Triazole		125.00	-0.3138	35.4

TABLE-2
RETENTION FACTORS (k) OF THE INVESTIGATED SUBSTANCES

Substance	k	
	Water + 0.1 % TFA	Acetonitrile/water + 0.1 % TFA (20/80 % vol)
Benzene	1.33	0.11
Imidazole	0.44	0.18
Indole	0.50	0.21
Indoline	0.51	0.29
1,2,4-Triazole	0.29	0.13

The logarithm of partition coefficients (log $P_{oct/water}$) is used to predict adsorption of organic molecules on hydrophobic media such as graphitized carbon. In Fig. 1, the log $P_{oct/water}$ values are plotted *vs.* the retention factors with different eluents. It is evident that the modification of the eluent by the addition of 20 % acetonitrile decreased the chromatographic retention for all sorbates. We examined the log $P_{oct/water}$ and the retention on the graphitized carbon stationary phase but were unable to see a statistical correlation. Furthermore, the logarithm of partition coefficients almost did not influence the retention all sorbates, except benzene. This phenomenon may be explained by the solvent-free components in the eluents used and also benzene will be weakly solvated in aqueous conditions. It also suggests that in practice the water solvation may be an important factor in retention of hydrophilic sorbates. Besides it is necessary to stress that the strength of interaction between a hydrophobic analyte, such as benzene and the porous graphitized carbon surface largely depends on how well the molecule fit onto the flat graphite surface. Some authors[33,34] concluded that highly structured and rigid molecules generally would be less retained on Hypecrarb™ than flexible molecules of the same molecular weight.

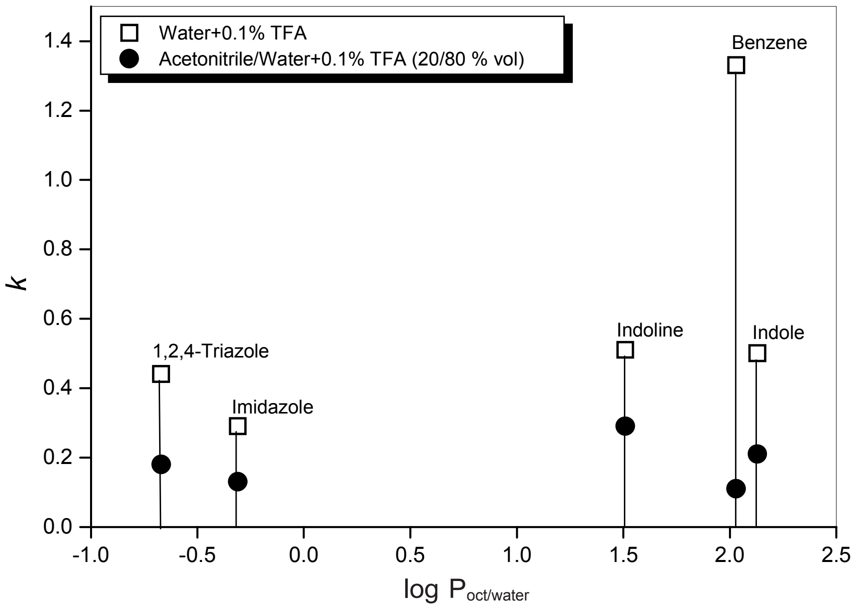


Fig. 1. Retention factors (k) of the sorbates as a function of the logarithm of partition coefficients (log $P_{oct/water}$) on the porous graphitized carbon column with the mobile phases

The interaction of the molecules with the sorbent is determined not only by the polarity of the sorbates, but also gains a contribution of size of molecules and orientation components. The polar surface area (PSA) is defined as the surface sum over of polar atoms, (usually oxygen and nitrogen), including hydrogens[35] is a very useful parameter for prediction of molecular transport and sorption properties. The relationships between k and PSA when using of dissimilar eluents are shown in Fig. 2. It is not difficult to show that retention factors (k) decrease with increasing of PSA values. Bigger size and/or larger number of groups of an adsorbate lead to a higher heterogeneity for the same surface. The 1,2,4-triazole was eluted from the column first. Imidazole and 1,2,4-triazole which, have close molecular weights but different PSA, were eluted at different times. The appearance in 1,2,4-triazole of the additional nitrogen atom, in comparison with imidazole, increases its PSA, as a result, increases its retention on Hypercarb^{TM}. This may be caused by the large contribution of nitrogen atom with the surface of porous graphitized carbon. The effect of PSA also appeared with other substances. Effects of different substituents are considered too. It is interesting to compare the retention of indole and indoline. The PSA values of these sorbates are identical; however, the molecular weights are differing by a factor of two. But on all occasions, the retention factors (Table-2) of these two sorbates were close. This fact substantiates our assumptions of the weak contribution of dispersion interactions to the overall retention of the investigated solutes.

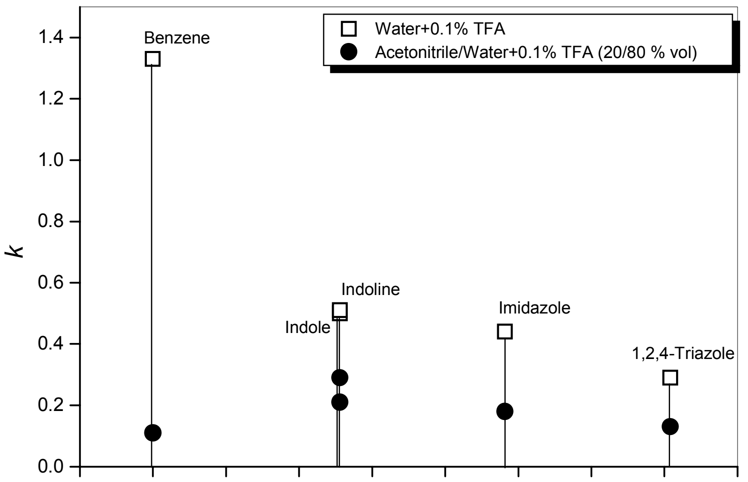


Fig. 2. Retention factors (k) of the sorbates as a function of the polar surface area (PSA) on the porous graphitized carbon column with the mobile phases

Table-3 shows the column parameters of this experiment. To calculate parameters of the adsorption isotherm, some of the kinetic parameters should be learned first. The total porosity of the column was obtained by measuring the dead volume of the column. Under the same mobile phase composition and flow rate, a dead time of the column was determined by injection of a 1 % solution of sodium nitrate and then multiply with flow rate to obtain dead volume. The total porosity of the column was obtained by dividing the dead volume by total empty volume of the column. Single component adsorption isotherm was obtained using the material balance eqn. 3 with no consideration of axial dispersion coefficient. The parameters a and b of Langmuir isotherms estimated by eqn. 4 and shown in Table-4. According to this table, we can see that the value of these parameters was decreased when acetonitrile was added into the mobile phase, but except one sorbate, 1,2,4-triazole. Its coefficients was a little increased with acetonitrile modified mobile phase. The adsorption isotherms of benzene, imidazole, indole, indoline and 1,2,4-triazole on columns packed with porous graphitized carbon with water as the eluent are presented in Fig. 3. The concentration of each substances in the solid (q*) and mobile (C) phases was abtained using Langmuir isotherm approach. From the Fig. 3, we can see that the

TABLE-3
COLUMN PARAMETERS

Parameter	Value
Column length, L (m)	0.1
Column diameter, D (m)	0.003
Particle diameter, D_P (m)	5×10^{-6}
Specific surface, (m^2/g)	120
Column volume (m^3)*	0.707×10^{-6}
Dead time, t_M (min)*	0.921
Total column porosity, ε (-)*	0.912

*Calculated value.

TABLE-4
PARAMETERS OF LANGMUIR ISOTHERM

Mobile phase	Substance	a (-)	b (mL/mg)
Water + 0.1 % TFA	Benzene	3.455	1.172×10^{-1}
	Imidazole	29.376	2.292
	Indole	3.558	3.741×10^{-2}
	Indoline	4.471	7.145×10^{-2}
	1,2,4-Triazole	0.923	1.500×10^{-8}
Acetonitrile/water + 0.1 % TFA (20/80 % vol)	Benzene	1.364	5.000×10^{-9}
	Imidazole	0.867	5.000×10^{-10}
	Indole	1.205	3.325×10^{-3}
	Indoline	1.974	2.956×10^{-2}
	1,2,4-Triazole	1.133	5.000×10^{-9}

exponential curves of benzene, imidazole, indole and indoline are increased logarithmically but one substance, 1,2,4-triazole, was increased linearly. 1,2,4-Triazole have higher solubility in mobile phase than other sorbats and the variation of the solubility has an impact on the equilibrium constant. For porous graphitic carbon column, it has been demonstrated that sorption depends on a combination of solubility and solvophobic effects that tend to drive solutes out of solution and selective interactions between solute polarizable or polarized functional groups with graphitized carbon, which are in addition to normal dispersion interactions.

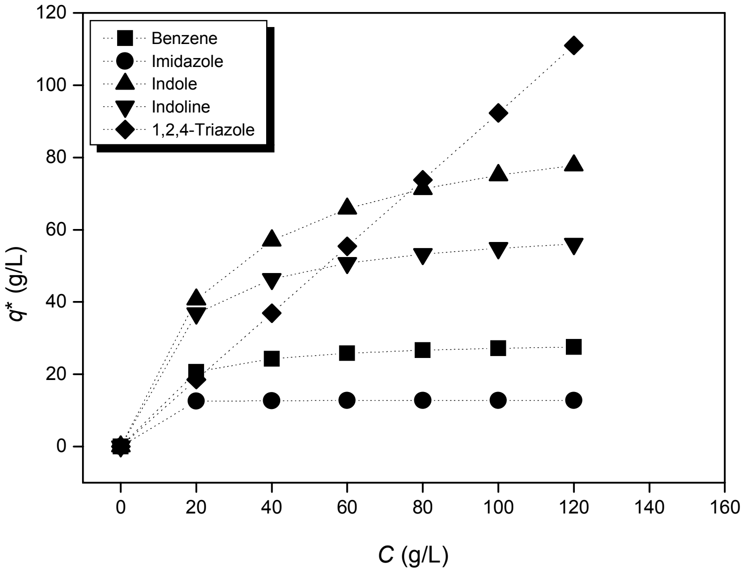


Fig. 3. Adsorption isotherms of benzene, imidazole, indole, indoline and 1,2,4-triazole on columns packed with porous graphitized carbon with water as the eluent

Conclusion

The Langmuir adsorption isotherms of benzene and five-membered heterocycles on the porous graphitized carbon with pure aqueous and aqueous /acetonitrile eluents were investigated. With Langmuir isotherm, the exponential curves of benzene, imidazole, indole and indoline are logarithmically increased in aqueous solution, but 1,2,4-triazole had showed linear adsorption isotherm. The coefficients of Langmuir isotherm were compared with different mobile phase compositions. According to the experimental results, the solubility, solvophobic effects and selective interactions between solute polarizable or polarized functional groups with graphite affect the sorption of substances.

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