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# Chromatographic retention behaviour of monosubstituted benzene derivatives on porous graphitic carbon and octadecyl-bonded silica studied using molecular modelling and quantitative structure–retention relationships

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ABSTRACT

The retention behaviour of a series of 28 monosubstituted benzenes, representing a diverse range of functional groups and substituent shape, were investigated using porous graphitic carbon (PGC) and octadecyl-bonded silica (ODS) stationary phases. For the majority of analytes retention on PGC was greater than on ODS, and in most cases this effect occurred at both pH 2.5 and 7.0. The main trends observed on PGC (in comparison with ODS) were: (i) similar or reduced retention of low polarity molecules such as the hydrocarbon and halogenated analytes; (ii) increased retention of conjugated analytes with extended planarity; (iii) increased retention of polar and charged species; and (iv) substantial increases in retention for selected polar and negatively charged analytes, including some ionised and unionised acid analytes. Poor retention of positively charged analytes was observed on both stationary phases. Molecular modelling studies have explored the geometry of $\pi$–$\pi$ stacking interactions in retention on PGC and have highlighted the strong retention of large conjugated analytes, with extended planar conformations, which can interact with the graphite surface with cofacial geometry. Quantitative structure–retention relationships showed the importance of hydrophobic ($\pi$) and electronic factors (e.g. mean polarisability and LUMO energy) in retention on PGC, whilst retention on ODS was correlated to hydrophobicity ($\log P$ and $\pi$).

## 1. Introduction

Porous graphitic carbon (PGC) has received considerable interest as a stationary phase for high-performance liquid chromatography since it first became commercially available in 1988 [1–7]. Due to its unique chemical and structural properties, PGC is now established as an alternative stationary phase in HPLC, providing complementary capabilities to the conventional reversed-phase materials. PGC has proved especially valuable in the separation of highly polar and charged analytes [4,5] and structurally similar analytes, including structural isomers [4,5,8–11], two important applications where traditional reversed-phase materials show poor performance.

The original development of PGC was motivated in part by the need to identify an alternative to reversed-phase silica gel based materials, in which some of the limitations of these silica based materials could be alleviated (e.g. separations at extremes of pH) [1,3]. Whilst it was initially assumed that the mechanism of retention on PGC would be primarily based on dispersive forces and hence similar to that on reversed-phase columns [3,12], it soon became apparent that additional interactions were participating. An early review of the properties and characteristics of PGC highlighted the following chromatographic behaviours [2], which are now widely documented [4–6,13,14]: (i) a notable polar retention effect giving increased retention of polar analytes compared with reversed-phase silica based supports, indicating an unexpected affinity between the polar analytes and the graphite surface [15–17]; (ii) an increased retention of non-polar analytes compared with reversed-phase silica based supports [8]; and (iii) an increased selectivity for structurally similar analytes such as geometric/structural isomers [8–11].

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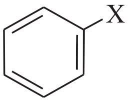
**Table 1**
Structures of monosubstituted benzene analytes.



| Analyte name | X |
|---|---|
| Hydrocarbons ($C_xH_y$) | |
| Benzene | H |
| Toluene | $CH_3$ |
| Ethylbenzene | $CH_2CH_3$ |
| *t*-Butylbenzene | $C(CH_3)_3$ |
| Styrene | $CH{=}CH_2$ |
| Biphenyl | $C_6H_5$ |
| Halogenated compounds ($C_xH_yX$) | |
| Chlorobenzene | Cl |
| Bromobenzene | Br |
| Iodobenzene | I |
| Benzylchloride | $CH_2Cl$ |
| Benzylbromide | $CH_2Br$ |
| Alcohols, ethers, aldehydes, ketones, esters ($C_xH_yO_z$) | |
| Phenol | OH |
| Anisole | $OCH_3$ |
| Benzyl alcohol | $CH_2OH$ |
| Benzaldehyde | CHO |
| Acetophenone | $COCH_3$ |
| Methyl benzoate | $CO_2CH_3$ |
| Phenyl acetate | $OCOCH_3$ |
| *trans*-cinnamaldehyde | $CH{=}CHCHO$ |
| Carboxylic acids ($C_xH_yCO_2H$) and benzenesulfonic acid | |
| Benzoic acid | $CO_2H$ |
| *trans*-cinnamic acid | $CH{=}CHCO_2H$ |
| Benzenesulfonic acid | $SO_3H$ |
| Phenylacetic acid | $CH_2CO_2H$ |
| Nitrogen containing compounds ($C_xH_yNO_z$) | |
| Nitrobenzene | $NO_2$ |
| Aniline | $NH_2$ |
| Benzonitrile | CN |
| Benzamide | $CONH_2$ |
| Phenyltrimethylammonium chloride | $^{+}N(CH_3)_3{}^{-}Cl$ |

One clear difference between PGC and traditional reversed-phase silica gel based material is the rigid, planar nature of the PGC surface compared with the more flexible alkyl chains bonded to the silica surface. The PGC surface comprises flat graphite sheets, containing repeating and co-joined $sp^2$ hybridised carbon-based six-membered rings, and as such is structurally similar to polycyclic aromatic molecules [2,18]. Several studies have shown the importance of analyte shape in retention on PGC, including the very strong retention of large planar molecules [8,15,19,20] and the reduction in retention of highly branched molecules where contact with the PGC surface is sterically limited [10,21,22]. Aromatic molecules are known to associate and stack in two preferred orientations, these being cofacial and face-edge geometries [23,24], but an understanding of the role of these associations in the separation of analytes on PGC is still in its infancy [21].

Other factors which have been reported to influence retention behaviour on PGC include hydrophobicity, polarity, size and topology [2,8–10,16,19,25–27]. Whilst a number of studies have explored the role of key aspects of analyte structure on separation on PGC, less is known about the shared contribution of these structural features to analyte retention. The aim of this work was to use both chromatographic and computational chemistry approaches to investigate the mechanisms of retention of a congeneric series of monosubstituted benzenes on PGC. Twenty-eight benzene derivatives with a structurally diverse range of substituents were chosen (Table 1) to allow the contribution of a number of different structural features, including aromatic stacking interactions, to analyte retention to be explored, and an assessment to be made of their combined contribution to retention. The benzene ring provides a common aromatic backbone to all twenty-eight analytes, where $\pi$–$\pi$ stacking interactions with the PGC surface may occur, and the substituents were chosen to explore different molecular topologies, hydrophobicities and polarities. Of particular note in this work, is the use of a number of highly polar and charged analytes, thus allowing the polar retention effect on graphite (PREG) [2,27] to be explored.

The retention characteristics of the monosubstituted benzene derivatives were measured on PGC and ODS. Molecular modelling calculations were carried out to identify the optimal geometry of interaction between the analytes and a model PGC surface, and to explore the role of aromatic–aromatic stacking interactions in the separations on PGC. Quantitative structure–retention relationship (QSRR) studies were used to explore the structural features, and their combinations, of most importance to retention. This combined experimental/computational approach has previously been used to study a series of *n*-alkylbenzenes and amylbenzene structural isomers [21], and the work described here extends those studies to include a more diverse range of analytes, and hence more complex mechanisms of retention.

## 2. Materials and methods

### 2.1. Chromatography

#### 2.1.1. Materials

Methanol (HPLC grade) was supplied by Fisher Chemicals (Loughborough, U.K.). All water used was supplied by an Elgastat very high purity unit (Elga Ltd., High Wycombe, U.K.). All analytes were purchased from Sigma–Aldrich (Poole, U.K.).

#### 2.1.2. Instrumentation

HPLC analysis was performed on an Integral Micro-Analytical 100Q Workstation (PerSeptive Biosystems, now part of Applied Biosystems, Foster City, U.S.A.) with a variable wavelength UV detector set at 220 nm.

#### 2.1.3. Analysis conditions

HPLC was performed using a Hypersil ODS column (150 mm × 4.6 mm i.d.) 5 μm particle size and Hypercarb PGC column (100 mm × 3.0 mm i.d.) 5 μm particle size (ThermoQuest, Runcorn, UK). Conditions used were methanol:water (v/v, 90–15%) mobile phase with 5 mM tris(hydroxymethyl)-aminomethane buffer at pH 7.0 and pH 2.5, and flow rates of 1.0 ml $min^{-1}$ and 0.42 ml $min^{-1}$ for ODS and PGC systems, respectively. The two pH values were chosen to be within the operational limits of the ODS column, whilst still providing environments where the ionisation of the acidic and basic functional groups of the analytes could be explored. Buffer solutions at the required pH values were prepared in the aqueous phase, and were then mixed with methanol. The flow rates were set to maintain comparable column linear flow velocities. Chromatography was performed at ambient temperature. Samples (100 μg $ml^{-1}$) were injected as 10 μl volumes, in triplicate.

#### 2.1.4. Data treatment

The chromatographic retention factor, $k$, was calculated by the integration software within the Integral Workstation. The retention time of the unretained analyte (solvent) peak was taken as the time interval from the moment of injection to the time when the trace for the solvent disturbance crossed the baseline.

The logarithm of the chromatographic retention factor extrapolated to 100% water ($\log k_w$) was calculated for all analytes using Eq. (1).

$$\log k = \log k_w + aC \qquad (1)$$