



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 π – π 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 (π) 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 π).

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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].

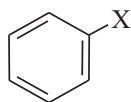
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Table 1
Structures of monosubstituted benzene analytes.



| Analyte name | X |
|--|---|
| Hydrocarbons (C _x H _y) | |
| Benzene | H |
| Toluene | CH ₃ |
| Ethylbenzene | CH ₂ CH ₃ |
| <i>t</i> -Butylbenzene | C(CH ₃) ₃ |
| Styrene | CH=CH ₂ |
| Biphenyl | C ₆ H ₅ |
| Halogenated compounds (C _x H _y X) | |
| Chlorobenzene | Cl |
| Bromobenzene | Br |
| Iodobenzene | I |
| Benzylchloride | CH ₂ Cl |
| Benzylbromide | CH ₂ Br |
| Alcohols, ethers, aldehydes, ketones, esters (C _x H _y O _z) | |
| Phenol | OH |
| Anisole | OCH ₃ |
| Benzyl alcohol | CH ₂ OH |
| Benzaldehyde | CHO |
| Acetophenone | COCH ₃ |
| Methyl benzoate | CO ₂ CH ₃ |
| Phenyl acetate | OCOCH ₃ |
| <i>trans</i> -cinnamaldehyde | CH=CHCHO |
| Carboxylic acids (C _x H _y CO ₂ H) and benzenesulfonic acid | |
| Benzoic acid | CO ₂ H |
| <i>trans</i> -cinnamic acid | CH=CHCO ₂ H |
| Benzenesulfonic acid | SO ₃ H |
| Phenylacetic acid | CH ₂ CO ₂ H |
| Nitrogen containing compounds (C _x H _y NO _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

π – π 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 μ m particle size and Hypercarb PGC column (100 mm × 3.0 mm i.d.) 5 μ m 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 μ g ml⁻¹) were injected as 10 μ l 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).

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

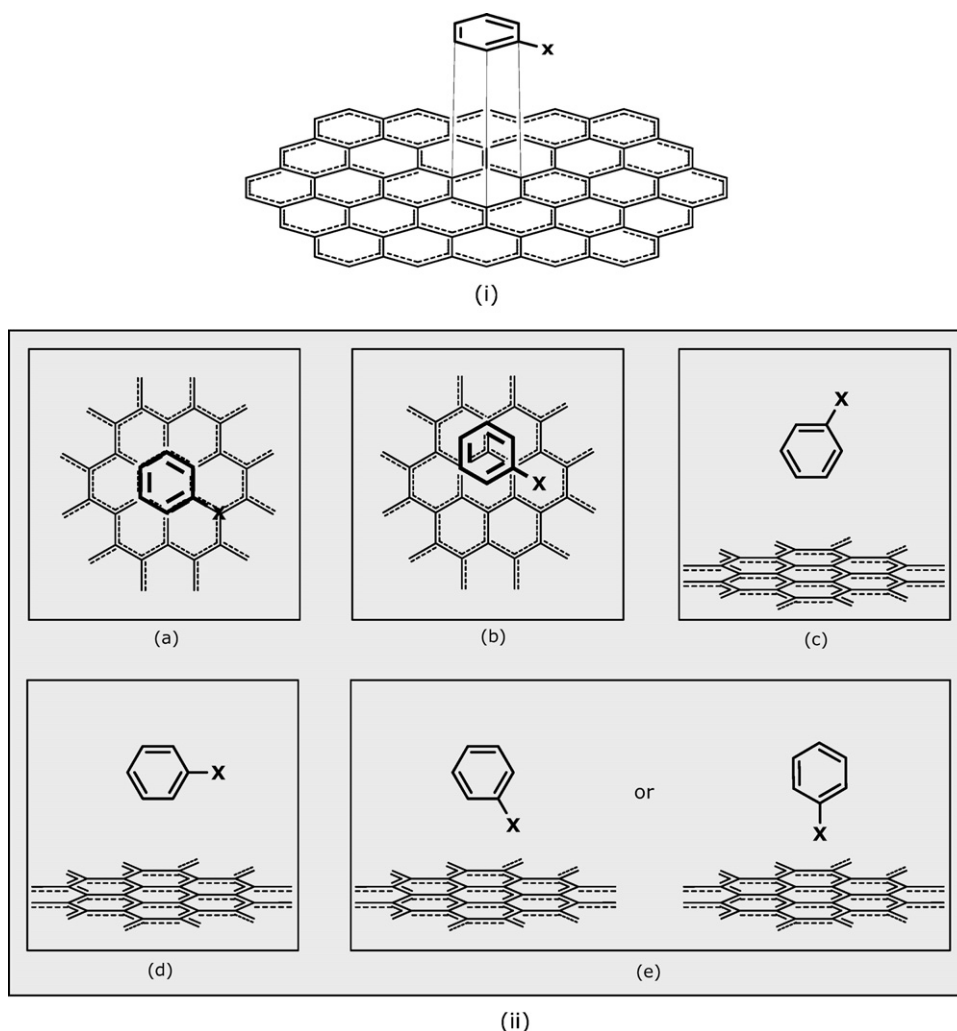


Fig. 1. Structure of the model graphite surface and geometries of alignment between the model surface and the monosubstituted benzene analytes, as used in the calculation of E_I values which are presented in Table 2. (i) The complete model graphite surface ($C_{78}H_{22}$), with a monosubstituted benzene analyte positioned in a cofacial geometry, and (ii) the five alternative geometries investigated in molecular modelling studies, showing part of the model surface with the analyte in bold; (a) cofacial geometry with no offset, (b) cofacial geometry with offset, (c) face-edge geometry with substituent directed away from the surface (x up), (d) face-edge geometry with substituent directed parallel to the surface (x side), (e) face-edge geometry with substituent directed towards the surface (x down).

where C is the percentage of organic modifier in the mobile phase and a is the slope of the graph produced. Each $\log k_w$ value was extrapolated using $\log k$ measurements from six different mobile phase compositions. $\log k$ values were calculated as the mean of three measurements.

The difference in retention of analytes on ODS compared to PGC was assessed quantitatively by calculating Δk_w for each analyte using Eq. (2).

$$\Delta k_w = 100 \left(\frac{k_w(\text{PGC})}{k_w(\text{ODS})} - 1 \right) \quad (2)$$

2.2. Molecular modelling

The energies of interaction between the benzene derivatives and a model graphite surface were calculated using semi-empirical molecular orbital methods. Adsorption of analytes onto the surface of PGC was simulated using the AM1 method within MOPAC [28]. An extended polycyclic aromatic molecule ($C_{78}H_{22}$) was used to represent the PGC stationary phase (Fig. 1).

Five alternative geometries for alignment of the analyte with the model graphite surface were considered, and are shown in Fig. 1. Energies of interaction (E_I (in kcal/mol)) were calculated

as previously described [21], and further details are provided in the supplementary material. The lowest value for the interaction energy, indicating the strongest interaction between analyte and surface, was used to represent the optimal interaction energy ($E_{I(\min)}$).

The carboxylic acid compounds and aniline were modelled as both ionised and unionised species, to represent the different experimental pH conditions used. Benzenesulfonic acid was modelled as the ionised form, since this would be expected to be the predominant form at both experimental pH values used. Phenyltrimethylammonium chloride (PTMAC) was modelled as the charged phenyltrimethylammonium cation.

2.3. QSRR methods

QSRR analysis was performed on retention data obtained for the monosubstituted benzene derivatives on the ODS and PGC phases. Analyte structural descriptors were selected to assess topology, geometry, polarity, size, hydrophobicity and physicochemical properties and were calculated using the TSAR 3.0, VAMP and COSMIC software packages (Oxford Molecular Ltd). More details are provided in the supplementary material. Correlations between the chromatographic $\log k_w$ measurements and the

structural descriptors were assessed using bivariate and multivariate linear regression (MLR) analysis.

3. Results and discussion

3.1. Chromatographic retention data

The values of $\log k_w$ obtained at pH 2.5 and pH 7.0, for the 28 analytes on PGC and ODS, are given in Table 2, together with values of Δk_w . Graphs showing the relationships between $\log k$ and mobile phase composition are given in Figs. 2 and 4 (and Figs. S2–S5). Retention data were reproducible to better than 1% from run to run.

3.1.1. Low polarity analytes

For the six hydrocarbon analytes, the alkylbenzene compounds were all more strongly retained on ODS than PGC over the entire range of mobile phases used, and provided a retention order which was in accordance with the length of the alkyl substituent on both phases, as has previously been observed [8,21] (Figs. 2 and S2). Zhang et al. have also reported the increased retention of *n*-alkylbenzene analytes with increasing alkyl chain length on PGC [29], with the exception of toluene and ethylbenzene, where retention was essentially the same. Again, in keeping with previous results, *t*-butylbenzene showed lower retention on PGC than might be expected from the number of carbon atoms in its substituent, reflecting the bulky nature of this functional group [10,21]. Retention of both styrene and biphenyl were stronger on PGC than ODS at pH 2.5 for the range of mobile phase compositions used (Fig. 2). At pH 7.0 the retention of styrene on both phases was similar, whilst biphenyl retention on ODS was greater than on PGC. Biphenyl showed the highest retention on PGC of the six hydrocarbon analytes. Comparing graphs of $\log k$ versus mobile phase composition for the six analytes, biphenyl showed unusual behaviour on PGC at pH 2.5, where higher methanol composition was required. Considering the difference in retention of the hydrocarbon analytes on ODS and PGC, as shown by Δk_w values (Table 2), in all cases except for biphenyl at pH 2.5, retention was greatest on ODS. Larger decreases in retention on PGC compared to ODS were observed at pH 7.0 than at pH 2.5.

For the five halogenated benzene derivatives, retention on ODS was generally greater than on PGC at a given pH, although on occasions retention was greatest on PGC (e.g. for iodobenzene at all but the lowest methanol concentrations (Figs. 2 and S3)). For the halobenzenes, retention orders, as defined by $\log k_w$ values, were in accordance with the atomic mass and volume of the halogen atoms on both stationary phases. The values of $\log k_w$ for the benzyl halides, whilst also increasing with increasing atomic mass of the halogen atom, were lower than those of the corresponding halobenzenes on both phases. Considering the Δk_w values for the halogenated analytes (Table 2), the most significant decreases in retention on PGC compared to ODS were again observed at pH 7.0 compared to pH 2.5, except for benzyl chloride where retention was strongest on PGC.

The larger decrease in retention of the majority of these low polarity analytes (all except benzylchloride) on PGC compared to ODS at pH 7.0 compared to pH 2.5 may be explained by the presence of an acidic functionality on the graphite surface. At neutral pH these functionalities may become ionised, reducing the overall hydrophobicity of the phase, and hence diminishing the possible interaction of these low polarity analytes with the graphite surface. Potentiometric titration studies by Patterson [30] have demonstrated the presence of acidic groups on the surface of PGC stationary phase material. As PGC is manufactured by a template process [31], the presence of any residual silica on the PGC surface is likely to account for these observations. Whilst a similar effect

may occur on ODS, where any surface silanol groups that have not been effectively capped with hydrocarbon may ionise at neutral pH, it would appear that the prevalence of this effect is less than that seen in PGC.

3.1.2. Polar analytes

Considering the eight oxygen-containing polar analytes, for all compounds except phenyl acetate, retention was greater on PGC than on ODS (excluding two data points for anisole at pH 7.0 at high methanol concentration, Figs. 2 and S4). In all cases $\log k_w$ values were greater on PGC than ODS except for phenyl acetate at pH 7.0. Given the polar nature of all compounds in this series, this could indicate the presence of a polar retention effect on graphite. For all analytes, retention on ODS increased with increasing pH (considering $\log k_w$), whilst on PGC an increase in retention with increasing pH was seen for phenol, anisole and methyl benzoate; for acetophenone no difference was seen between the two pH values; and for all other analytes a decrease in retention was seen when pH was increased.

The chromatographic behaviour of phenyl acetate was unusual in the series since retention was very similar for all conditions studied, and this analyte was the only compound for which retention on PGC was less than on ODS (at pH 7.0). Phenyl acetate also showed the lowest $\log k_w$ on PGC for the series at pH 7.0 and the second lowest $\log k_w$ on PGC at pH 2.5. The highest values for $\log k_w$ on PGC for this series was seen with methyl benzoate and *trans*-cinnamaldehyde. This may be connected with the high degree of conjugation in both these molecules, which will extend the planar nature of these molecules, and increase the potential for delocalization of charge (Fig. 3).

Visual inspection of graphs of $\log k$ versus mobile phase composition showed slight curvature in the cases of phenol, benzyl alcohol and benzaldehyde for retention on ODS (Figs. 2 and S4). The minor non-linearity in the relationship between $\log k$ and mobile phase composition may affect the extrapolated $\log k_w$ values and hence represent a source of error in these values. In the case of retention on PGC no deviation from linearity was observed. *Trans*-cinnamaldehyde showed unusual behaviour on PGC at pH 2.5 (Fig. 2), where higher methanol composition was required. Similar behaviour was also observed for biphenyl.

Of the eight compounds in this series, *trans*-cinnamaldehyde showed the largest Δk_w value (at pH 2.5) and hence the largest increase in retention on PGC compared to ODS. Benzaldehyde also showed a large Δk_w value at pH 2.5. Both of these analytes show conjugation which extends beyond the aromatic ring into the substituent, an effect which may in part explain their enhanced retention on PGC.

Retention of the nitrogen containing analytes followed similar trends. Nitrobenzene, benzonitrile and benzamide all exhibited stronger retention on PGC when compared with ODS, and at both pH values (Figs. 2 and S4). Consequently, values of $\log k_w$ were also larger on PGC than on ODS. This can be seen as a further example of a polar retention effect on graphite. On ODS, retention of nitrobenzene, benzonitrile and benzamide increased as the pH was increased. On PGC, the retention of nitrobenzene also increased as the pH was increased, whilst for benzonitrile and benzamide retention decreased. Benzamide showed the largest Δk_w value (at pH 2.5) of the three analytes, perhaps suggesting the presence of a very strong PREG for this analyte, and/or the role of enhanced planarity in the retention of this analyte.

For all eleven polar analytes an increased retention on ODS was observed at pH 7 compared to pH 2.5. A possible explanation for this is that as pH increases, any accessible silanol groups that have not been capped with a C_{18} hydrocarbon may ionise and may then interact with polar analytes, resulting in an increase in retention. Equally, since all of these analytes are likely to become protonated

Table 2
Retention data and calculated interaction energy (E_i) values for analytes.

| Analyte | Chromatographic retention data | | | | | | | | | | Calculated E_i values (kcal/mol) ^a | | | | | | |
|--|--------------------------------|--------|-----------|--------|-----------|--------|-----------|--------|--------------|--------------|---|---------------------|---------------------|---------------------|-------------------|--------|---------------|
| | ODS | | | | PGC | | | | | | Cofacial | | | Face-edge | | | $E_{i(\min)}$ |
| | pH 2.5 | | pH 7.0 | | pH 2.5 | | pH 7.0 | | pH 2.5 | pH 7.0 | no offset ^b | offset ^b | x down ^b | x side ^b | x up ^b | | |
| | log k_w | a | log k_w | a | log k_w | a | log k_w | a | Δk_w | Δk_w | | | | | | | |
| Hydrocarbons | | | | | | | | | | | | | | | | | |
| Benzene | 2.058 | -0.025 | 2.289 | -0.027 | 1.945 | -0.025 | 1.778 | -0.026 | -22.8 | -69.1 | 0.068 | -0.038 | -0.119 | -0.119 | -0.119 | -0.119 | |
| Toluene | 2.627 | -0.031 | 2.917 | -0.033 | 2.396 | -0.030 | 2.454 | -0.030 | -41.3 | -65.6 | 0.226 | -0.246 | 1.153 | -0.109 | 0.682 | -0.246 | |
| Ethylbenzene | 3.254 | -0.037 | 3.641 | -0.040 | 2.946 | -0.036 | 3.102 | -0.037 | -50.9 | -71.1 | -0.262 | -0.216 | -0.259 | -0.121 | 0.175 | -0.262 | |
| <i>t</i> -Butylbenzene | 4.230 | -0.044 | 4.739 | -0.047 | 3.032 | -0.037 | 3.030 | -0.036 | -93.7 | -98.0 | 0.081 | 1.268 | 0.843 | -0.108 | 1.032 | -0.108 | |
| Styrene | 3.071 | -0.036 | 3.429 | -0.038 | 2.896 | -0.031 | 2.945 | -0.031 | -33.2 | -67.2 | -0.005 | -0.120 | -0.216 | 0.371 | 1.356 | -0.216 | |
| Biphenyl | 4.070 | -0.042 | 4.557 | -0.046 | 4.172 | -0.034 | 4.066 | -0.035 | 26.4 | -67.7 | -0.385 | -0.277 | 0.790 | 0.341 | 0.790 | -0.385 | |
| Halogenated compounds | | | | | | | | | | | | | | | | | |
| Chlorobenzene | 2.602 | -0.031 | 2.924 | -0.033 | 2.520 | -0.030 | 2.430 | -0.028 | -17.2 | -67.9 | -0.229 | -0.201 | 0.454 | -0.107 | -0.067 | -0.229 | |
| Bromobenzene | 2.773 | -0.033 | 3.074 | -0.035 | 2.777 | -0.032 | 2.568 | -0.028 | 0.92 | -68.8 | -0.535 | -0.504 | 0.344 | -0.003 | 0.088 | -0.535 | |
| Iodobenzene | 2.975 | -0.034 | 3.358 | -0.037 | 3.026 | -0.032 | 2.786 | -0.028 | 12.3 | -73.2 | -0.780 | -0.581 | 0.210 | 0.665 | 0.294 | -0.780 | |
| Benzylchloride | 1.853 | -0.024 | 2.071 | -0.026 | 1.264 | -0.015 | 2.284 | -0.028 | -74.2 | 63.4 | -0.003 | 0.129 | -1.148 | 0.259 | -1.260 | -1.260 | |
| Benzylbromide | 2.495 | -0.031 | 2.788 | -0.033 | 2.684 | -0.033 | 2.336 | -0.028 | 54.5 | -64.7 | -1.520 | -1.424 | -1.371 | -0.219 | 0.178 | -1.520 | |
| Oxygen containing polar compounds | | | | | | | | | | | | | | | | | |
| Phenol | 1.118 | -0.020 | 1.364 | -0.022 | 1.705 | -0.024 | 1.889 | -0.026 | 229 | 235 | -0.275 | -0.023 | 0.581 | 0.027 | 0.823 | -0.275 | |
| Anisole | 1.947 | -0.025 | 2.209 | -0.027 | 2.295 | -0.027 | 2.661 | -0.032 | 123 | 183 | 0.241 | 0.217 | 2.385 | 0.016 | 0.016 | 0.016 | |
| Benzyl alcohol | 1.142 | -0.042 | 1.388 | -0.022 | 2.087 | -0.028 | 1.789 | -0.024 | 782 | 152 | 0.283 | 0.215 | 0.948 | 0.872 | 0.605 | 0.215 | |
| Benzaldehyde | 1.324 | -0.020 | 1.620 | -0.024 | 2.406 | -0.028 | 2.099 | -0.023 | 1110 | 202 | -0.412 | -0.605 | 1.216 | -0.168 | 1.162 | -0.605 | |
| Acetophenone | 1.441 | -0.022 | 1.657 | -0.023 | 2.434 | -0.026 | 2.434 | -0.025 | 884 | 499 | -0.583 | -0.863 | 1.742 | -0.673 | 0.042 | -0.863 | |
| Methyl benzoate | 1.999 | -0.026 | 2.241 | -0.028 | 2.794 | -0.027 | 3.179 | -0.032 | 525 | 765 | -0.141 | -0.980 | 0.188 | 0.312 | 0.144 | -0.980 | |
| Phenyl acetate | 1.637 | -0.024 | 1.830 | -0.026 | 1.720 | -0.024 | 1.588 | -0.022 | 21.2 | -42.8 | -0.177 | -0.150 | 0.301 | 0.635 | 0.668 | -0.177 | |
| <i>trans</i> -Cinnamaldehyde | 1.720 | -0.025 | 1.926 | -0.026 | 2.971 | -0.024 | 2.548 | -0.033 | 1680 | 319 | -1.439 | -1.659 | -0.356 | -1.499 | -1.259 | -1.659 | |
| Nitrogen containing polar compounds | | | | | | | | | | | | | | | | | |
| Nitrobenzene | 1.760 | -0.025 | 1.952 | -0.026 | 2.545 | -0.027 | 2.807 | -0.030 | 509 | 617 | -0.494 | -0.268 | 2.052 | 0.452 | -0.386 | -0.494 | |
| Benzonitrile | 1.435 | -0.023 | 1.538 | -0.023 | 2.332 | -0.029 | 2.124 | -0.025 | 690 | 286 | -0.536 | -0.374 | 0.805 | -0.280 | -0.515 | -0.536 | |
| Benzamide | 0.640 | -0.016 | 0.796 | -0.018 | 1.881 | -0.023 | 1.506 | -0.019 | 1640 | 413.0 | -0.877 | -1.375 | 2.265 | 1.919 | 0.403 | -1.375 | |
| Charged compounds | | | | | | | | | | | | | | | | | |
| Benzoic acid ^c | 1.435 | -0.021 | 0.387 | -0.016 | 3.058 | -0.039 | 0.803 | -0.010 | 4100 | 161 | 4.732 | 4.496 | 5.232 | 5.264 | 4.414 | 4.414 | |
| <i>trans</i> -cinnamic acid ^c | 1.841 | -0.026 | 0.684 | -0.015 | 3.089 | -0.023 | 2.193 | -0.017 | 1670 | 3130 | -0.282 | -0.372 | 2.441 | 0.039 | 2.309 | -0.372 | |
| Benzenesulfonic acid ^c | 0.228 | -0.021 | 0.243 | -0.021 | 1.604 | -0.024 | 1.494 | -0.027 | 2270 | 1680 | 4.681 | 5.453 | 6.713 | 4.583 | 4.755 | 4.583 | |
| Phenylacetic acid ^c | 0.144 | -0.009 | 0.210 | -0.010 | 2.223 | -0.029 | 2.360 | -0.028 | 11,900 | 14,000 | 3.778 | 4.454 | 5.129 | 5.854 | 4.911 | 3.778 | |
| Aniline ^c | 0.478 | -0.028 | 1.048 | -0.019 | 0.443 | -0.005 | 1.186 | -0.018 | -7.71 | 37.3 | 0.842 | 0.684 | 0.711 | 0.894 | 0.725 | 0.684 | |
| PTMAC ^c | -0.271 | -0.008 | -0.140 | -0.009 | -0.203 | -0.006 | -0.114 | -0.011 | 6.20 | 16.9 | 10.532 | 10.868 | 11.348 | 9.523 | 6.958 | 6.958 | |
| Unionised form of charged analytes | | | | | | | | | | | | | | | | | |
| Benzoic acid ^d | | | | | | | | | | | -0.519 | 0.153 | 1.329 | 1.399 | 0.271 | -0.519 | |
| <i>trans</i> -Cinnamic acid ^d | | | | | | | | | | | -1.514 | -1.705 | -0.498 | -0.525 | 1.209 | -1.705 | |
| Phenylacetic acid ^d | | | | | | | | | | | -0.295 | 0.100 | 1.421 | 1.622 | 0.847 | -0.295 | |
| Aniline ^d | | | | | | | | | | | 0.636 | 0.257 | 0.425 | 0.770 | 0.579 | 0.25 | |

^a Interaction energies (E_i in kcal/mol) for a complex between the given analyte and a model graphite surface molecule. E_i with the most negative value, representing $E_{i(\min)}$, is highlighted in bold.

^b Correspond to the geometries shown in Fig. 1.

^c E_i values calculated for the ionised form of the analytes.

^d E_i values calculated for the unionised form of the analytes.

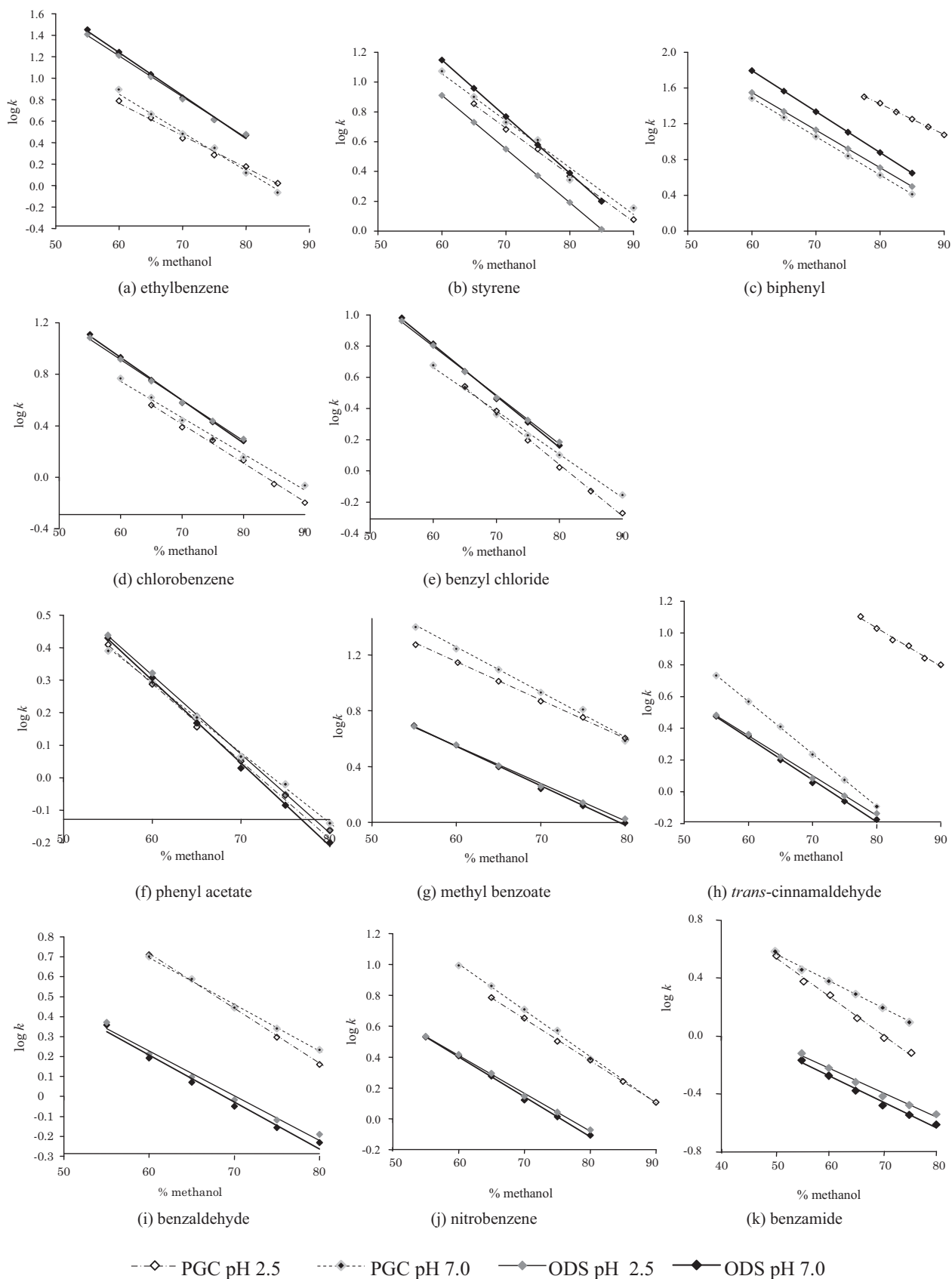


Fig. 2. The relationship between retention ($\log k$) and mobile phase composition for low polarity and polar analytes on ODS and PGC at different pH values.

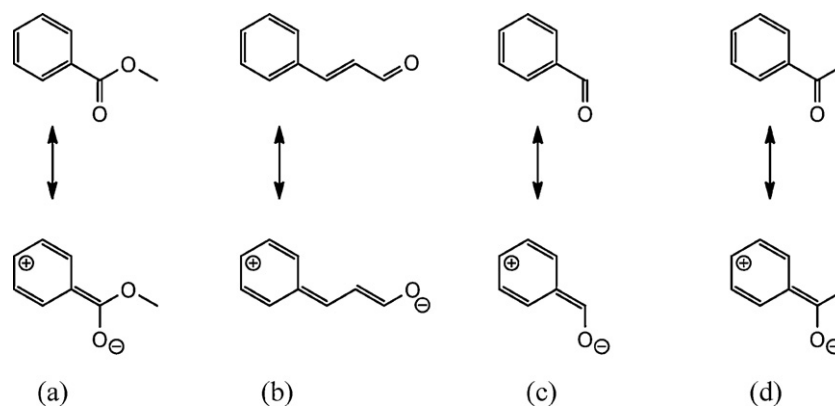


Fig. 3. Analytes with resonance structures which result in a more rigid and planar molecule. Only two resonance structures are shown here for simplicity. (a) methyl benzoate; (b) *trans*-cinnamaldehyde; (c) benzaldehyde; (d) acetophenone.

to some degree in acidic media, as protons form coordinate covalent bonds to oxygen and nitrogen lone pairs, this would result in a reduced retention of these analytes on ODS at pH 2.5 (where silanol groups are not ionised).

The increase in retention on PGC at pH 7.0, as seen with a number of analytes, could support the presence of an acidic functionality on the PGC surface [30]. The increased retention of phenol, anisole, methylbenzoate and nitrobenzene on PGC at higher pH, may be explained as a favourable charge/dipole based interaction between the highly polar analytes and a charged PGC surface. Equally, since these analytes will show some degree of protonation at low pH values, when the PGC surface will not be ionised, this may also serve to reduce retention at pH 2.5. The reason why other analytes are retained less strongly on PGC at pH 7.0, despite their expected polarity, is unclear.

3.1.3. Charged analytes

The charged analytes studied included three acid analytes, one basic analyte and phenyltrimethylammonium chloride (PTMAC). Of these, both PTMAC and benzenesulfonic acid would be expected to be charged at both pH values used in this study, whilst for the carboxylic acids and aniline a dominant charged form is expected at only one of the two pH values, this being pH 7.0 for the acids and pH 2.5 for aniline.

For all ionisable and charged compounds except PTMAC (analysed as the phenyltrimethylammonium cation in these studies) at pH 2.5, retention was greater on PGC than on ODS (Figs. 4 and S5). Considering $\log k_w$ values, retention on PGC was higher than on ODS in all cases except for aniline where retention at pH 2.5 was slightly lower on PGC than ODS. For PTMAC all $\log k_w$ values were the smallest of all the analytes, setting this molecule apart from the twenty-seven other analytes in this study.

Of note is the wide range of slopes visible in the $\log k$ versus mobile phase composition plots for these analytes (Fig. 4), and reflected in the a values (Table 2). In comparison to the a values obtained with the low polarity and polar analytes, there appears to be much greater variability in the values obtained with the ionisable analytes. Also of interest is the observation that for the majority of the low polarity and polar analytes, a values decrease on ODS with increasing pH, whilst on PGC a values can increase or decrease with increasing pH. In the case of the ionisable analytes on ODS three analytes show large increases in a values with increasing pH. No significant deviations from linearity were observed for these plots, supporting the validity of extrapolated $\log k_w$ values. In conditions where the analyte is appreciably ionised, the use of varying methanol percentages in the mobile phases, where differing effective pH values may be experienced by the analyte, could be a source of error in the calculation of $\log k_w$ values.

Benzoic acid and *trans*-cinnamic acid showed appreciably stronger retention at pH 2.5 compared to pH 7.0, on both ODS and PGC. This is likely to reflect the formation of the carboxylate anions of these acids at higher pH, which will reduce any reversed-phase type interaction on both phases, and may result in electrostatic repulsive interactions with any negatively charged functionality on either the ODS or PGC phases. The reduction in retention on PGC at pH 7.0 was greatest for benzoic acid. A possible explanation for this lies in differences in the conjugation of the two analytes. *trans*-Cinnamic acid is a highly conjugated molecule and the negative charge arising at higher pH can be distributed throughout its structure, with a lower unit of charge per atom than for benzoic acid. This may result in a smaller decrease in retention for *trans*-cinnamic acid on PGC.

Benzenesulfonic acid was poorly retained on ODS at both pH 2.5 and 7.0. Retention was greater on PGC than on ODS, with a slightly larger retention at pH 2.5. Again, this may be explained by the presence of a weakly acidic group on the PGC surface, which may be ionised at pH 7.0 and therefore produce an electrostatic repulsive effect. It is of note that both benzenesulfonic acid and *trans*-cinnamic acid anions showed appreciable retention on PGC.

All of the acid analytes showed large values of Δk_w , which were amongst the highest of the twenty-eight analytes studied here. This indicates substantially greater retention on PGC than ODS, potentially arising from a very strong PREG for these analytes. Of note are the high values of Δk_w observed at both pH values, indicating a larger retention on PGC compared to ODS for both charged and uncharged forms of the acids. Benzoic acid and benzenesulfonic acid had substantially higher Δk_w values at pH 2.5 than at pH 7.0, whilst for *trans*-cinnamic acid Δk_w was highest at pH 7.0. An explanation for the enhanced retention of these acids on PGC at lower pH has already been provided, whilst the enhanced retention of *trans*-cinnamic acid at higher pH may arise from the extended conjugation in this molecule as discussed above.

Aniline was the only molecule in this series of ionisable analytes for which lower retention on PGC compared to ODS was seen, as reflected in $\log k_w$ values; this was observed at pH 2.5 when aniline would be expected to be completely ionised. An explanation for this effect might be the interaction of the protonated amine with residual acidic silanols on the surface of the ODS silica, an electrostatic interaction which would be present only when aniline is ionised. The $\log k_w$ values for aniline at pH 2.5 were very small on both phases and in the case of PGC was the second smallest of all twenty-eight analytes in this study. PTMAC (analysed as the cation in these studies) showed the weakest retention of all analytes on both stationary phases. These results suggest that retention of positively charged analytes on PGC appears to be less favourable than for negatively charged analytes as seen with the ionised acid analytes. For

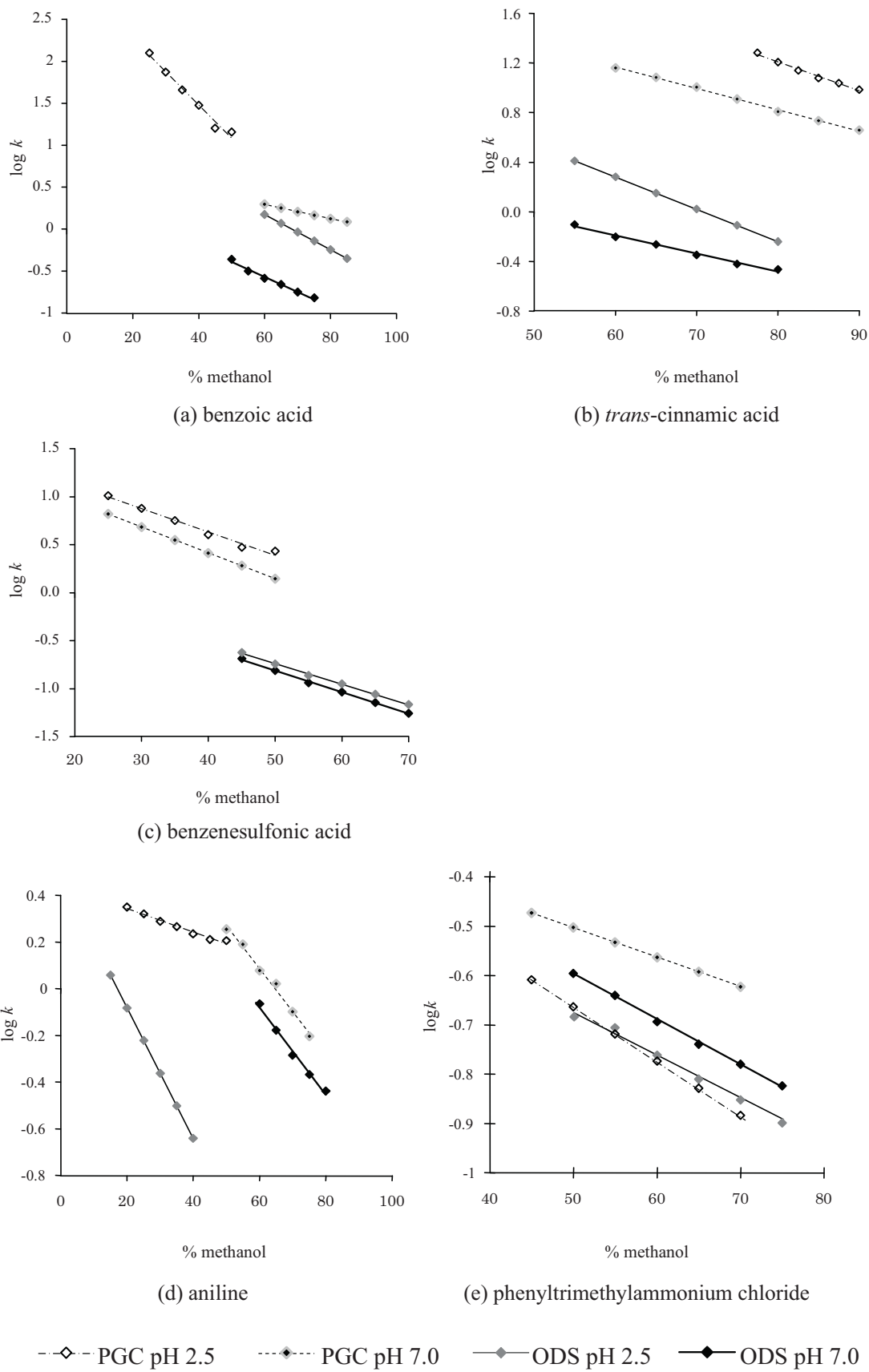


Fig. 4. The relationship between retention ($\log k$) and mobile phase composition for charged analytes on ODS and PGC at different pH values.

both aniline and PTMAC, retention on both phases increased with increasing pH. For aniline this is likely to reflect the change in ionisation state of this analyte with pH, where the neutral form is better retained. For PTMAC, which is positively charged at both pH values, increased interaction would be expected with a negatively charged stationary phase, as would be present at higher pH.

3.2. Molecular modelling

The energies of interaction between the monosubstituted benzene analytes and a model graphite surface (E_I) are presented in Table 2. The relationship between $E_{I(\min)}$ (the optimal interaction energy) and $\log k_w$ on PGC is given in Fig. 5.

3.2.1. Low polarity analytes

Considering all hydrocarbon analytes in this series, there appears to be some correlation between $E_{I(\min)}$ and $\log k_w$ on PGC (Fig. 5), with $E_{I(\min)}$ generally becoming more negative and $\log k_w$ becoming larger, with increasing size of the substituent (i.e. number of carbon atoms). The *t*-butyl group departs from this trend, showing a larger $E_{I(\min)}$, and hence less favourable interaction with the surface than might be expected from its size, and this mirrors its relatively low $\log k_w$ values compared to the other alkylbenzenes. These results probably reflect the bulky and branched nature of the substituent, which produces a lower association with the surface, and similar results have been reported previously [10,21]. Calculation of $E_{I(\min)}$ values for alkylbenzenes and their correlation with $\log k$ values have been previously reported and hence are not discussed here further [21]. Biphenyl was found to have the most attractive association with the model surface of all the hydrocarbons, an observation that is mirrored in its stronger retention on PGC compared to the other hydrocarbon analytes.

The $E_{I(\min)}$ values for the halobenzenes showed increasing associative interactions with the surface with increasing size of halogen atom (atomic mass and volume), mirroring the increase in retention with increasing atom size. Similar results were observed for the benzyl halide analytes. For all halogenated analytes, the preferred orientation of association was in the cofacial geometry with no offset, except for benzyl chloride where the face-edge *x*-up geometry was favoured.

3.2.2. Polar analytes

Considering the eight oxygen containing polar analytes, the lowest values of $E_{I(\min)}$ were seen with those analytes which contain an sp^2 hybridised carbon atom adjacent to the aromatic ring. Each of these analytes showed high values of $\log k_w$ on PGC, providing experimental support for the modelling results. This suggests an increased retention on graphite for molecules with the ability to attain an extended planar geometry, or to increase their polarity through electron delocalisation (Fig. 3). No agreement between experimental and modelling results were seen for the other analytes. For all compounds in this series a cofacial geometry was most favoured for association, except in the case of anisole where the face-edge *x*-side and *x*-up geometries were the most favoured.

The three nitrogen containing polar analytes did not display any clear correlations between $E_{I(\min)}$ and $\log k_w$. For these analytes conjugation between the aromatic ring and substituent would again be expected. Although $E_{I(\min)}$ values were comparably favourable to those seen for the oxygen containing analytes with extended conjugation, these values did not correlate with their retention on PGC. The most favourable geometry of association was again in the cofacial geometry.

3.2.3. Charged analytes

When considering the carboxylic acid analytes at pH 2.5, the lowest values of $E_{I(\min)}$ were again seen with those analytes which

contain an sp^2 hybridised carbon atom adjacent to the aromatic ring, and again these analytes showed high values of $\log k_w$ on PGC, indicating a qualitative agreement between experimental and modelling results for these compounds. The strongest association with the model surface was in a cofacial geometry for these analytes. The carboxylate anion models had noticeably higher values of $E_{I(\min)}$ suggesting a weaker association with the model surface. In the case of the benzoate anion, $E_{I(\min)}$ was strongly positive, suggesting a repulsive interaction with the model surface. $E_{I(\min)}$ for the *trans*-cinnamic acid anion was negative but was significantly larger than for the unionised *trans*-cinnamic acid. For benzenesulfonic acid all E_I values were large and positive suggesting very poor associations between this anion and the PGC surface in all interaction geometries, and in agreement with the very poor retention on PGC.

The values of $E_{I(\min)}$ for the cationic and neutral aniline species were both positive and the difference between these values was far smaller than for the different carboxylic acid species studied. This suggests a repulsive interaction for both aniline species with the PGC surface, and a smaller difference in interaction with PGC on ionisation. For the phenyltrimethylammonium cation all values of E_I were very large and positive, suggesting strongly repulsive interactions in all geometries. The most favourable geometry of association with the model surface, or least unfavourable, was in the face-edge *x*-up geometry. $E_{I(\min)}$ for the PTMA cation was the largest positive value of all the twenty-eight analytes studied, and the $\log k_w$ values were the smallest obtained, indicating a very poor association between this charged analyte and the PGC surface.

3.2.4. Molecular modelling conclusions

Previously published work has shown a strong correlation between chromatographic $\log k$ values and calculated $E_{I(\min)}$ values for *n*-alkylbenzenes and amylbenzene structural isomers on a model PGC surface [21]. For the twenty-eight monosubstituted benzenes in this study no correlation between $E_{I(\min)}$ and $\log k_w$ was observed across the complete series of analytes, although for some subsets of analytes interesting structure–retention relationships have emerged, where experimental chromatographic results have supported modelling results. This suggests that the simple molecular model used in these calculations is not valid for all analytes in this study. In particular, the absence of solvent molecules in these calculations is a likely weakness, and the results for the charged analytes suggest this is particularly the case for very polar species, where the values of $E_{I(\min)}$ are overestimated, representing an overestimation of the degree of repulsion with the PGC surface. The idea of modelling retention whilst neglecting the presence of solvent was first proposed by Knox and Ross [32], who suggested that the analyte–solvent interaction was of little importance when compared with the analyte–stationary phase interactions. This may not, however, be the case; charged analytes will be highly solvated in aqueous media, which is likely to reduce repulsive interactions with the PGC surface.

3.3. QSRR analysis

3.3.1. Bivariate linear regression analysis

The correlations obtained between $\log k_w$ values and a variety of structural descriptors are given in Table S1. The regression coefficients (r^2) for all correlations were below 0.5, except for correlations involving $\log P$, π and mean polarizability.

On ODS the linear relationship between $\log P$ and $\log k_w$ gave correlation coefficients of 0.755 and 0.786 at pH 2.5 and pH 7.0, respectively. Although these were not strong linear correlations, they suggest that there is some correlation between retention

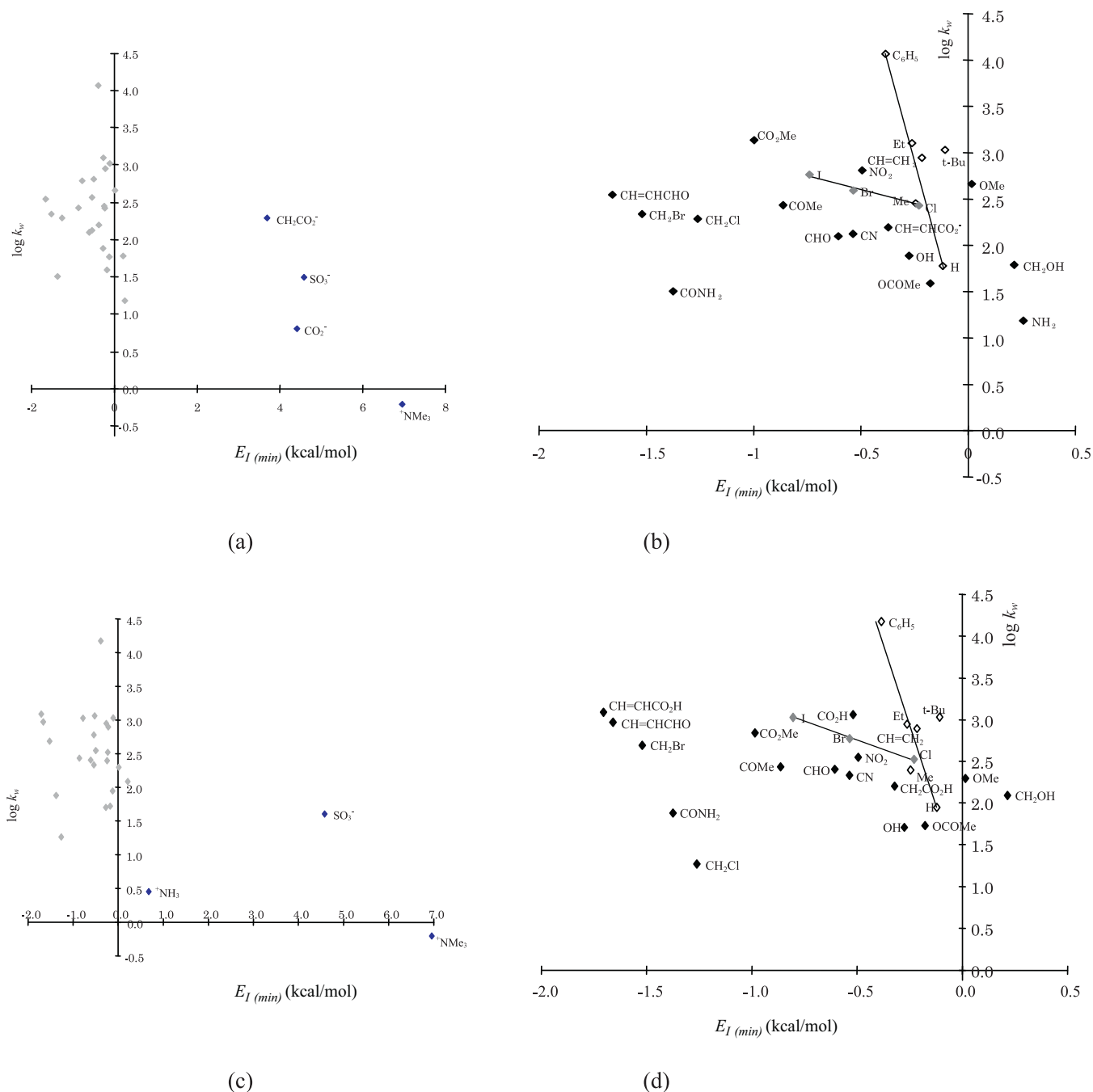


Fig. 5. The relationship between $E_{I(\text{min})}$ and $\log k_w$ on PGC at pH 7.0 (a & b) and pH 2.5 (c & d). (b) and (d) are expansion of the grey data points from (a) and (c). Open diamond data points represent hydrocarbon analytes.

on ODS and hydrophobicity. Correlations between retention on ODS and hydrophobicity for simple aromatic analytes have been reported previously [33–35]. On PGC however, there was a much lesser correlation between $\log P$ and $\log k_w$ at either pH value ($r^2 = 0.435$ at pH 7.0 and 0.333 at pH 2.5). This observation was mirrored by Hennion et al. [25] whilst studying the retention behaviour of polar compounds on PGC. Hennion concluded that hydrophobic interactions were not the main interaction in the retention mechanism of the polar compounds under study. Studies by Poole and co-workers [22] on neutral organic molecules, and using the solvation parameter model, concluded that dispersive interactions between the analyte and PGC, and hydrophobic

effects were both significant interactions in the retention on PGC with water/methanol mobile phases, with electron lone pair and dipole-type interactions also favouring retention. On both ODS and PGC some correlation was observed between π (the Hansch–Fujitsa hydrophobicity substituent constant) and $\log k_w$ at both pH values, with correlation coefficients between 0.714 and 0.804. The correlation, although limited, between retention on PGC and mean polarizability is of interest ($r^2 = 0.604$ at pH 2.5). The relatively poor correlation coefficients obtained could partly reflect errors in the calculation of $\log k_w$ values, although there is no evidence of such errors for the vast majority of the experimental conditions used in this study.

3.3.2. Multivariate linear regression analysis

The MLR analyses giving the highest statistical significance for the two different phases and at the two pH values under study are given in Eqs. (3)–(5), where P_E is the mean polarisability, π is the Hansch–Fujitsa parameter and E_{lumo} is the lowest unoccupied molecular orbital (LUMO) energy. Eq. (3) describes PGC at pH 2.5, and indicates that retention is dependent upon a hydrophobic descriptor (π) and two electrostatic type descriptors (P_E and E_{lumo}).

$$\log k_w = 0.0692P_E + 0.432\pi - 0.359E_{lumo} + 1.24 \quad (3)$$

$$n = 28, r^2 = 0.821, F\text{-value} = 29.0, \text{significance} = 7.88 \times 10^{-8}$$

Eq. (4) describes PGC at pH 7.0, and suggests that retention is dependent on both hydrophobic (π) and electrostatic (E_{lumo}) properties of the analytes.

$$\log k_w = 0.636\pi - 0.358E_{lumo} + 2.35 \quad (4)$$

$$n = 28, r^2 = 0.856, F\text{-value} = 59.2, \text{significance} = 1.39 \times 10^{-9}$$

For ODS at pH 2.5, no MLR results were found which explained the observed retention with more significance than the bivariate analysis results. Eq. (5) describes ODS at pH 7.0, and shows that retention is dependent solely on hydrophobic properties (π and $\log P$) of the analytes.

$$\log k_w = 0.454\pi - 0.118 \log P + 2.63 \quad (5)$$

$$n = 28, r^2 = 0.641, F\text{-value} = 17.9, \text{significance} = 2.48 \times 10^{-5}$$

When PTMAC was removed from the dataset used to create Eq. (5), 97.0% of the total variance within the dataset could be described by π and $\log P$. This improvement suggests that the retention of PTMAC on ODS is different to the other analytes, this observation potentially being explained by the positive charge of this analyte at neutral pH, and also by its bulky molecular structure. This finding could be explained as a result of the PTMA cation interacting with acidic silanols present on the surface of the ODS column, although the extreme bulkiness of the quaternary ammonium cation would render the charged nitrogen atom difficult to access and hence may limit its ability to interact with the ODS surface silanols.

Solvation parameter model studies on neutral organic molecules, including some monosubstituted benzenes, in methanol/water mobile phases, have also shown the importance of hydrophobic effects, and electrostatic-type interactions in retention on PGC, together with dispersive interactions between the analyte and PGC. Specifically, lone pair and dipole-type interactions were found to be favourable to retention, whilst hydrogen bond interactions reduced retention [22].

4. Conclusions

Retention studies have shown that for the majority of analytes and at both pH values under study, retention on PGC was greater than on ODS, with the exception of the hydrocarbon and halogenated analytes. 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. The largest increase in retention on PGC was seen for uncharged benzoic acid and the negatively charged benzenesulfonic acid and *trans*-cinnamic acid analytes. A lesser retention of positively charged analytes was observed on both stationary phases, with the anilinium cation being the only polar analyte where retention on ODS was greater than on PGC.

Molecular modelling calculations have provided some insights into the geometry and energetics of association between the analytes and PGC surface. The strong retention of large conjugated analytes such as *trans*-cinnamic acid, biphenyl and a number of conjugated polar molecules was explained by the ability of these molecules to adopt extended planar conformations and enhanced polarity due to electron delocalisation, and to interact with the graphite surface in a cofacial geometry. The inclusion of solvent molecules in the simulation of surface–analyte interactions would be likely to improve the model, particularly when considering charged analytes.

Bivariate (linear regression) analysis showed that there is some correlation between retention on ODS and hydrophobicity ($\log P$ and π), whilst for PGC some correlation was observed with π , and polarizability also appears to have some effect. The lack of any definitive linear correlation (with high correlation coefficient) suggests that retention is not dependent on a single structural descriptor parameter alone. MLR analysis produced some interesting correlations for retention on PGC, this particularly being the case given the very diverse range of molecules studied. On PGC at pH 2.5, $\log k_w$ was found to be dependent on hydrophobic (π) and electronic (P_E and E_{lumo}) parameters. This is in accordance with the observed retention behaviour which is clearly based to some degree on reversed-phase or hydrophobic interactions, but which also exhibits an additional dependence on polar interactions. On PGC at pH 7.0, $\log k_w$ was also found to be dependent on hydrophobicity (π) and a single electronic parameter (E_{lumo}).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2011.12.090.

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